Black Sea Journal of Agriculture

Volume 7 | Issue 5

ISSN: 2618 - 6578

BS Journals

BLACK SEA JOURNAL OF AGRICULTURE

(BSJ AGRI)

Black Sea Journal of Agriculture (BSJ Agri) is a double-blind peer-reviewed, open-access international journal published electronically 6 times (January, March, May, July, September, and November) in a year since January 2018. It publishes, in English, full-length original research articles, innovative papers, conference papers, reviews, mini-reviews, rapid communications or technical note on various aspects of agricultural science like agricultural economics, agricultural engineering, animal science, agronomy, including plant science, theoretical production ecology, horticulture, plant breeding, plant fertilization, plant protect and soil science, aquaculture, biological engineering, including genetic engineering and microbiology, environmental impacts of agriculture and forestry, food science, husbandry, irrigation and water management, land use, waste management etc.

ISSN: 2618 - 6578
Phone: +90 362 408 25 15
Fax: +90 362 408 25 15
Email: bsjagri@blackseapublishers.com
Web site: http://dergipark.gov.tr/bsagriculture
Sort of publication: Periodically 6 times (January, March, May, July, September, and November) in a year
Publication date and place: September 15, 2024- Samsun, TÜRKİYE
Publishing kind: Electronically

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doi: 10.47115/bsagriculture.1509098

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
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GENETIC DIVERSITY OF WHITE CABBAGE (*Brassica oleracea* var. *capitata* subvar. *alba*) INBREED LINES USING SRAP MARKERS

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Abstract: Genetic diversity assessment is crucial for effective breeding programs and the conservation of plant genetic resources. This study aimed to characterize the genetic diversity of 24 cabbage (*Brassica oleracea* var. *capitata* subvar. *alba*) inbred lines using Sequence-Related Amplified Polymorphism (SRAP) markers. A total of 45 SRAP primer combinations were employed, resulting in the amplification of 258 bands, of which 194 (75.2%) were polymorphic. The polymorphism information content (PIC) values ranged from 0.03 to 0.42, with a mean value of 0.20, indicating relatively low genetic diversity among the studied inbred lines. The major allele frequency (MAF) values varied between 0.54 and 0.99, with an average of 0.83, further confirming the limited genetic diversity. The effective allele number (NE), gene diversity (H), and Shannon information index (I) averaged 1.40, 0.23, and 0.35, respectively. Principal component analysis (PCA) revealed that the first seven principal component axes accounted for 90.59% of the total variance among the cabbage lines, demonstrating that the genetic diversity could be largely explained along a few dimensions. STRUCTURE analysis identified three major genetic clusters, with Cluster 3 exhibiting the highest proportion of genetic composition (40.3%) and the highest level of genetic differentiation (mean Fst = 0.4080). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering analysis, based on the Dice similarity method, produced a dendrogram depicting the genetic relationships among the inbred lines. The Mantel test value r for the UPGMA clustering was 0.78, indicating a good fit between the dendrogram and the original similarity matrix. The study highlights the utility of SRAP markers in assessing genetic diversity and relationships among cabbage inbred lines, providing valuable information for breeding programs and genetic resource management. The identification of genetically distinct clusters and the quantification of genetic variation within and among these clusters can guide future breeding efforts and facilitate the development of improved cabbage varieties with desirable traits.

Keywords: *Brassica oleracea*, Genetic diversity, Inbred lines, SRAP markers, STRUCTURE analysis, UPGMA clustering

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Received: July 02, 2024
Accepted: July 12, 2024
Published: September 15, 2024

Cite as: Ekbiç E, Tırınk C. 2024. Genetic diversity of white cabbage (*Brassica oleracea* var. *capitata* subvar. *alba*) inbreed lines using SRAP markers. BSJ Agri, 7(5): 429-436.

1. Introduction

White cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable species cultivated and consumed worldwide. This plant, which belongs to the Brassicaceae family, is of great importance for health and nutrition due to its high nutritional value, containing various antioxidants, vitamins (especially vitamin C and vitamin K), minerals and fiber (Fahey et al., 2001). In addition, studies have suggested that white cabbage may have protective effects against several types of cancer (Verhoeven et al., 1996). Cabbages are categorized into white cabbage (*B. oleracea* var. *capitata* subvar. *alba*), red cabbage (*B. oleracea* var. *capitata* subvar. *rubra*), and savoy cabbage (*B. oleracea* var. *capitada* subvar. *sabauda*) (Nieuwhof, 1969). White cabbage constitutes the most consumed group of cabbage, utilized either as a table vegetable or in the pickling industry. In Türkiye, the total production of white cabbage is 597910 tons. The provinces with the highest production are listed as Samsun (143241 tons), Niğde (135495 tons), Bursa (34830 tons), Antalya (25116 tons), Mersin (22534 tons), and Afyonkarahisar (22465 tons) (Anonymous, 2021).

Plant breeding involves genetic studies aimed at developing more productive and resilient plant varieties. While traditional breeding methods rely on phenotypic selection and hybridization techniques, the use of molecular markers has increased significantly in modern breeding studies. Molecular markers are used for various purposes, including detection of genetic variation, gene mapping, pedigree analysis, and improving the efficiency of breeding programs (Collard and Mackill, 2008).

Molecular marker techniques play a major role in revealing genetic diversity and relationships by detecting differences at the DNA level. Commonly used molecular marker techniques include RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats), RAPD (Random Amplified Polymorphic DNA), and SRAP

(Sequence-Related Amplified Polymorphism). Each marker has unique advantages and limitations. For example, SSR markers exhibit a high degree of polymorphism, while AFLP markers provide high reproducibility and comprehensive genetic information (Powell et al., 1996). SRAP markers are often preferred in plant genetics and breeding studies. SRAP typically targets exon regions to obtain polymorphic bands, thereby providing high accuracy in determining genotypic differences (Li and Quiros, 2001). The simplicity, speed, low cost and high reproducibility of SRAP markers make this technique ideal for genetic diversity analysis. A study by Ferriol et al. (2003) reported that the SRAP marker technique provided more information than AFLP, while another study by Liu et al. (2008) indicated that the SRAP marker system produced relatively more informative bands and was more efficient compared to ISSR and RAPD marker systems. SRAP technology, which was initially detailed by Li and Quiros (2001) in Brassica, is a marker system that preferentially amplifies open reading frames (ORFs). This technique utilizes two types of primers: forward primers, which are 17 base pairs long and have 14 nucleotides rich in C and G with 3 selective bases at the 3' end, and reverse primers, which are 18 base pairs long with 15 nucleotides rich in A and T (Ferriol et al., 2003).

This study aims to determine the molecular diversity of white cabbage breeding lines using SRAP markers. Detection of genetic diversity is essential for plant breeding because plant populations with a broad genetic base are more resilient to environmental changes and diseases. In this context, our study aims to provide important data for the conservation of white cabbage genetic resources, to guide breeding efforts and to develop new lines. Our study will provide a better understanding of the genetic diversity of cabbage and integrate this knowledge into practical breeding applications. As a result, we aim to make a significant contribution to the conservation of white cabbage genetic resources and the support of sustainable agricultural practices.

2. Materials and Methods

A total of 24 white cabbage inbred lines, derived from the cabbage genetic pool at the Black Sea Agricultural Research Institute, were used for this study (Table 1).

2.1. SRAP Analysis of Inbred Lines

The mini-prep method of Haymes (1996) was used for DNA isolation and DNA extraction from young leaves of cabbage seedlings. Cabbage inbred lines were characterized using 45 SRAP primer combinations (Table 2). Polymerase chain reaction (PCR) experiments were carried out using a total reaction mixture volume of 15 µL. This mixture consisted of 7.5 µL PCR master mix (Dream Taq Green Master Mix, ThermoScientific), 1 µL forward (ME) and 1 µL reverse (EM) primers (10 pmol each), 3 µL genomic DNA (15-20 ng), and 2.5 µL deionized water. The PCR protocol was performed as follows: An initial predenaturation step was performed at 94 °C for 2 minutes. This was followed by 5 cycles of denaturation at 94 °C for 1 minute, annealing at 35 °C for 1 minute, and extension at 72 °C for 1 minute. This was followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 50 °C for 1 minute, and extension at 72 °C for 1 minute. Finally, a post extension step at 72 °C for 5 minutes was performed (Yildiz et al., 2011).

2.2. Gel Electrophoresis

The PCR products obtained from the SRAP evaluation were separated by gel electrophoresis. A 3% agarose gel (Fisher BioReagents) containing 1X TAE (Tris-Acetic Acid-EDTA) buffer was prepared, and the samples were loaded onto the gel. Electrophoresis was performed on a SCIE-PLAS system (Hu20) at 100 V and 300 mA for 4 hours. After electrophoretic separation, the gel was stained with ethidium bromide (10 mg/ml) for 20 minutes and then washed with distilled water. The stained gel was then visualized under a UV transilluminator (Syngene-Ingenius). The resulting gel image was analyzed, with distinct bands scored as 1 (present) and 0 (absent). This scoring system was used to generate a binary data matrix for further data analysis.

Table 1. White cabbage inbred lines used in the study

Inbred lines	Consumption purposes	Precocity
40	for stuffing	Mid-season
501	for pickling	Mid-season
518	for pickling	Mid-season
22-1	for pickling	Mid-season
A119	for stuffing	Early-maturing
A126	for stuffing	Early-maturing
A145	for pickling	Early-maturing
A168	for pickling	Early-maturing
A322	for stuffing	Early-maturing
A387	for stuffing	Early-maturing
A62	for pickling	Mid-season
BLMY-4	for stuffing	Early-maturing
BY27-2	for stuffing	Late-maturing
EXT	for pickling	Early-maturing
FG	for stuffing	Early-maturing
P43-1	for stuffing	Mid-season
P59-1	for stuffing	Early-maturing
P91	for pickling	Mid-season
P95	for stuffing	Early-maturing
TAR	for pickling	Early-maturing
W37	for pickling	Mid-season
WEİ	for pickling	Mid-season
YBB-35	for stuffing	Late-maturing
ZL-3	for pickling	Late-maturing

Table 2. Genetic diversity of cabbage inbred lines revealed by SRAP markers

Primer	TBN	PBN	PIC	MAF	NE	H	I
Me01Em02	7	5	0.26	0.78	1.36	0.21	0.32
Me01Em03	4	2	0.08	0.95	1.15	0.11	0.19
Me01Em04	3	2	0.14	0.90	1.35	0.20	0.30
Me01Em05	3	2	0.22	0.86	1.49	0.27	0.40
Me01Em08	5	5	0.28	0.75	1.87	0.46	0.66
Me02Em09	3	3	0.31	0.65	1.80	0.44	0.64
Me02Em10	2	2	0.23	0.81	1.36	0.26	0.43
Me02Em11	4	2	0.20	0.82	1.37	0.21	0.30
Me02Em12	4	3	0.21	0.88	1.08	0.08	0.15
Me02Em13	6	3	0.16	0.85	1.29	0.17	0.26
Me03Em02	4	4	0.19	0.84	1.36	0.21	0.33
Me03Em03	2	2	0.42	0.54	1.82	0.45	0.64
Me03Em04	3	2	0.09	0.94	1.26	0.16	0.26
Me03Em05	4	3	0.19	0.86	1.52	0.29	0.42
Me03EM10	10	10	0.25	0.80	1.41	0.26	0.42
Me03Em13	4	4	0.21	0.82	1.54	0.31	0.46
Me04Em10	5	4	0.19	0.88	1.41	0.26	0.40
Me05Em02	5	5	0.27	0.78	1.74	0.41	0.59
Me05Em03	9	5	0.12	0.90	1.21	0.13	0.21
Me05Em04	12	8	0.14	0.88	1.28	0.18	0.29
Me05Em05	7	6	0.24	0.77	1.49	0.30	0.46
Me05Em08	5	3	0.24	0.77	1.30	0.19	0.29
Me06Em09	5	4	0.06	0.97	1.15	0.12	0.21
Me06Em10	6	3	0.11	0.90	1.36	0.20	0.29
Me06Em11	7	4	0.15	0.89	1.39	0.21	0.31
Me06Em12	8	7	0.25	0.79	1.48	0.28	0.43
Me14Em02	9	7	0.19	0.84	1.44	0.26	0.39
Me14Em03	3	1	0.03	0.99	1.01	0.01	0.03
Me14Em04	6	6	0.24	0.78	1.61	0.36	0.54
Me14Em05	4	2	0.15	0.86	1.26	0.15	0.23
Me14Em08	10	10	0.35	0.67	1.62	0.36	0.53
Me16Em10	9	8	0.26	0.76	1.47	0.27	0.41
Me17Em05	5	5	0.24	0.82	1.46	0.26	0.41
Me18Em09	7	5	0.16	0.88	1.32	0.18	0.28
Me18Em10	5	4	0.28	0.74	1.46	0.26	0.38
Me18Em11	2	1	0.19	0.77	1.32	0.20	0.29
Me19Em02	11	8	0.23	0.81	1.23	0.17	0.28
Me19Em03	11	10	0.32	0.73	1.44	0.27	0.41
Me19Em04	5	4	0.20	0.84	1.39	0.25	0.38
Me19Em05	7	5	0.22	0.80	1.33	0.21	0.32
Me21Em09	5	4	0.20	0.78	1.49	0.28	0.40
Me21Em10	6	4	0.20	0.83	1.38	0.23	0.35
Me21Em11	6	2	0.07	0.94	1.08	0.06	0.10
Me21Em12	6	3	0.14	0.88	1.39	0.22	0.31
Me21Em13	4	2	0.11	0.91	1.32	0.18	0.27
Total	258	194	-	-	-	-	-
Average	5.73	4.31	0.20	0.83	1.40	0.23	0.35

TBN= total band number, PBN= polymorphic band number, MAF= major allele frequency, NE= effective allele number, H= gene diversity (Nei, 1973), I= Shannon information index

2.3. Data Analysis

The polymorphism rate (Pr) was calculated for each primer. PopGene v.1.32 software (Yeh et al., 2000) was used to calculate genetic diversity parameters, including the effective number of alleles (Ne) (Kimura and Crow, 1964), gene diversity (H) (Nei, 1973), and Shannon's information index (I) (Lewontin, 1972), for the inbred cabbage lines under study. Polymorphism information content (PIC) and major allele frequency (Maf) values were obtained using PowerMarker v.3.25 software (Liu and Muse, 2005). Cluster analysis was performed using the unweighted pair-group method with arithmetic mean

(UPGMA) (Sneath, 1979), and a correlation matrix was generated using the Dice module of the NTSYSpc v.2.02 package (Rohlf, 2000). This analysis elucidated the genetic relationships among the cabbage lines. Additionally, multivariate analysis was performed using Principal Component Analysis (PCA) based on the correlation matrix in NTSYSpc, and a PCA scatterplot was generated using Past 3 statistical software. In further, a STRUCTURE analysis was performed on the cabbage lines using the Bayesian clustering algorithm implemented in the STRUCTURE 2.3.4 software tool, as introduced by Pritchard et al. (2000). The analysis was performed with a burn-in period of 10.000 iterations and 100.000 Markov Chain Monte Carlo (MCMC) repetitions after burn-in, with five iterations.

3. Results

3.1. Characterization of Cabbage Lines by SRAP Markers

A total of 258 bands were obtained from 45 combinations of SRAP primers, of which 194 (75.2%) showed a polymorphism among the cabbage breeding lines (Table 2). Based on primers, the highest total number of bands was observed in Me05Em04 primer at 12 and the lowest number of bands was observed in Me02Em10, Me03Em03 and Me18Em11 primers at 2. Polymorphism information content (PIC) values ranged from 0.03 to 0.42. The mean PIC value was calculated as 0.20. The highest PIC value was determined in the Me03Em03 primer with 0.42 and the lowest in the Me14Em03 primer with 0.03. The major allele frequency (MAF) values changed between 0.54-0.99 (average 0.83) indicating relatively low genetic diversity among cabbage lines. The effective allele number (NE) ranged from 1.01 to 1.87 with an average NE value of 1.40. Gene diversity (H) ranged from 0.01 to 0.46 and Shannon information index (I) ranged from 0.03 to 0.66. The mean values of H and I were calculated as 0.23 and 0.35, respectively. These results indicate that the genetic diversity of cabbage breeding lines was successfully characterized using SRAP markers.

The results of the principal component analysis (PCA) showed that the data from the SRAP markers could be largely represented by the first seven principal component axes (Table 3). The eigenvalues associated with these axes were 19.76, 0.47, 0.38, 0.38, 0.32, 0.29, and 0.27, respectively. These values indicate that the first seven principal component axes account for 90.59% of the total variance among the cabbage lines, demonstrating that the genetic diversity within these lines can be largely explained along a few dimensions.

Two-dimensional scatter plot in Figure 1 represents the genetic variation among cabbage inbred lines based on PCA. The plot reveals distinct clusters of inbred lines. The lines such as BLMY-4, FG, and WEI cluster closely together, indicating high genetic similarity. In contrast, lines such as 501, W37, and BY27-2 are positioned far apart from the main cluster, suggesting significant genetic differences.

Table 3. Principal components revealed by SRAP primers

PC axises	Eigen values	Variation (%)	Cumulative variation (%)
1	19.76	82.35	82.35
2	0.47	1.98	84.32
3	0.38	1.59	85.91
4	0.32	1.32	87.23
5	0.29	1.20	88.43
6	0.27	1.13	89.57
7	0.25	1.03	90.59

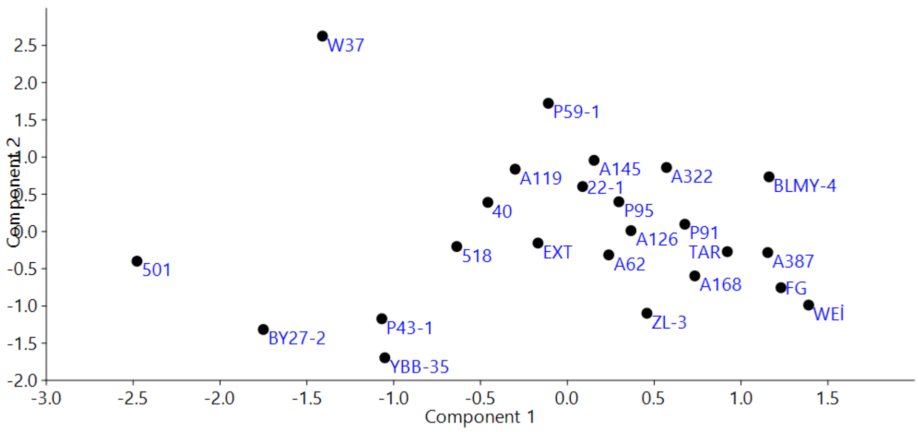

Figure 1. Scatter plot of inbred lines by PCA.

The STRUCTURE analysis of the SRAP marker data revealed the genetic structure of the inbred lines, identifying three major clusters based on the K-means method (Figure 2). The Q matrix represents the individual membership coefficients, indicating the proportion of an individual's genome that belongs to each of the inferred genetic clusters (Figure 3). Further assessment of the genetic diversity among the cabbage inbred lines using STRUCTURE, as summarized in Table 4, revealed distinct patterns among the three identified clusters. Cluster 1 exhibited an average distance (AD) of 0.273 between individuals within the same cluster, indicating a moderate level of genetic variation. The proportion of the genetic composition (PGC) in this cluster was 0.311, suggesting that approximately 31.1% of the genetic material of the inbred lines was represented in this cluster. The mean value of Fst for Cluster 1 was 0.0122 indicating minimal genetic differentiation from other clusters. Cluster 2 demonstrated a slightly lower average distance (AD) of 0.264 compared to Cluster 1, reflecting slightly reduced genetic variation within the cluster. The proportion of the genetic composition (PGC) in Cluster 2 was 0.286, showing that 28.6% of the genetic material was encompassed in this cluster. The mean Fst value for Cluster 2 was 0.0774, indicating a moderate level of genetic differentiation. Cluster 3 showed the lowest average distance (AD) of 0.178, indicating the least genetic variation among individuals within this cluster. However, it had the highest proportion of genetic composition (PGC) at 0.403, signifying that 40.3% of the genetic material of the inbred lines was present in this cluster. Notably, Cluster 3 exhibited a mean Fst value of 0.4080, which is substantially higher than those of the other clusters, indicating a significant level of genetic differentiation.

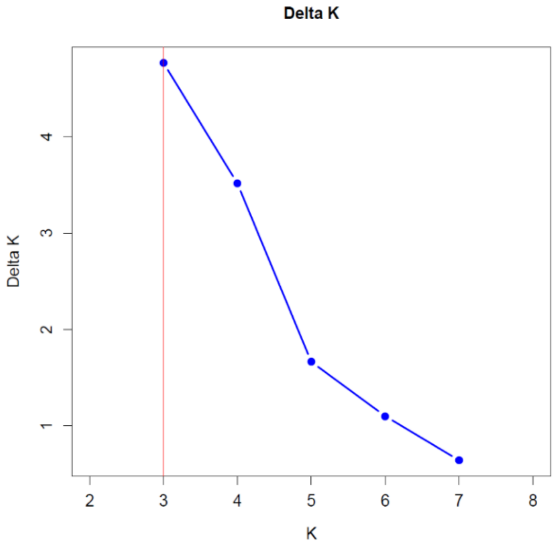

Figure 2. Optimum cluster number obtained by STRUCTURE analysis.

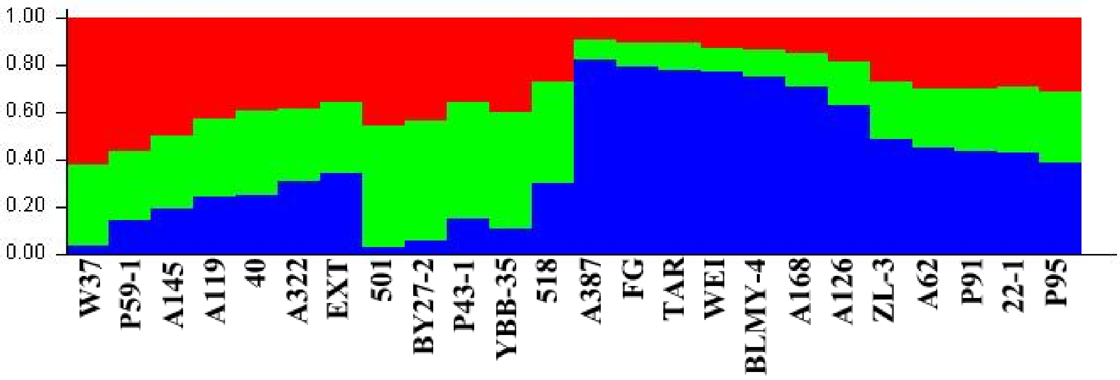

Figure 3. The assignment probabilities of each individual obtained by STRUCTURE.

Table 4. Genetic diversity of cabbage inbred lines revealed by SRAP markers

Clusters	AD	PGC	Mean values of Fst
1	0.273	0.311	0.0122
2	0.264	0.286	0.0774
3	0.178	0.403	0.4080

AD= average distance between individuals in same cluster, PGC= proportion of the genetic composition of inbred lines in each cluster.

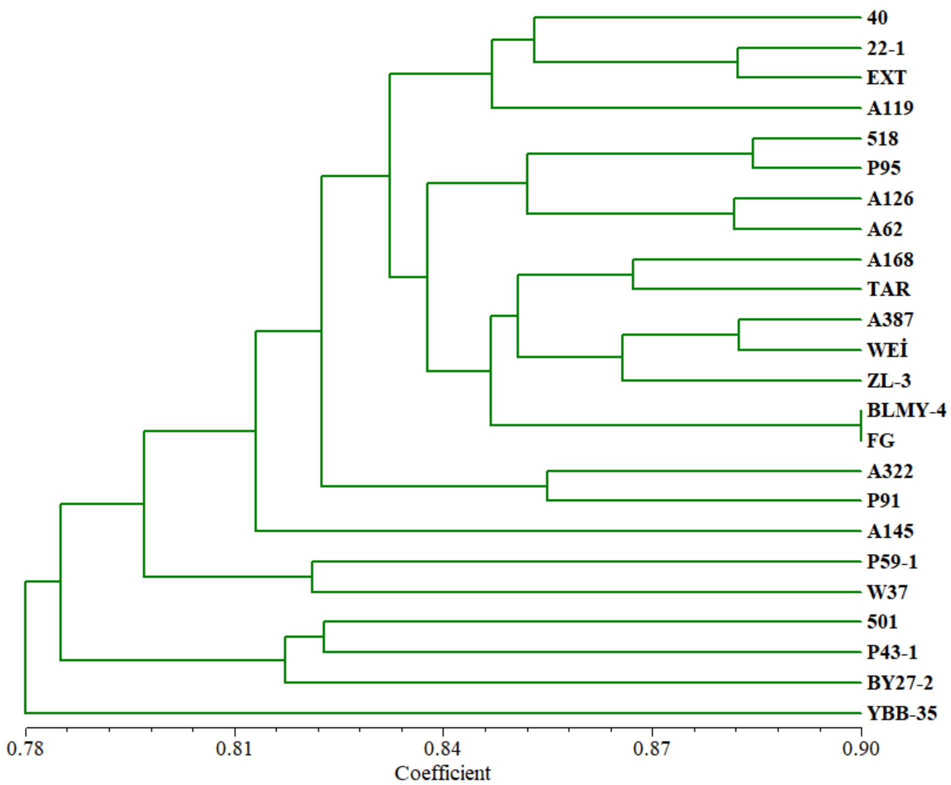

Figure 4. UPGMA cluster constructed from SRAP marker data.

The Mantel test value r for the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering analysis using the Dice similarity method was calculated to be 0.78. The dendrogram shown in Figure 4 represents the genetic relationships among 24 cabbage inbred lines based on SRAP marker data. The inbred lines BLMY-4 and FG are the most genetically similar, as indicated by their tight clustering towards the top right of the dendrogram. Similarly, lines 22-1 and EXT, A126 and A62, and W37 and 501 also show high genetic similarity due to their close proximity in the dendrogram. On the other hand, the inbred lines 501 and P91 are the most genetically distant from each other, as indicated by their positions on opposite ends of the dendrogram branches. BY27-2 and YBB-35 are also relatively genetically distant from the other lines, forming their own separate cluster at a lower similarity coefficient. Several clusters of inbred lines exhibit moderate genetic similarity. For example, the cluster including 40, 22-1, and EXT, as well as the cluster including A126, A62, A168, TAR, and A387, show intermediate levels of genetic relatedness. The cluster including A322, P91, and A145 also indicates moderate genetic similarity among these lines.

4. Discussion

Hybrid vegetable varieties are preferred by producers for their high yield, disease resistance, and uniform product quality. White head cabbage can be cultivated in almost every region of Turkey, with provinces such as Samsun, Amasya, Tokat, Niğde, and Nevşehir standing out in production. While the proportion of local hybrid varieties was significantly high in the hybrid varieties of the main vegetable crops grown in Türkiye, such as tomato, pepper and melon, this proportion remained at a very low level in cabbage. To increase this rate, some agricultural research institutes and universities in Turkey are conducting breeding studies on cabbage. The Black Sea Agricultural Research Institute is prominent in developing hybrid varieties of white head cabbage in Turkey. The findings of our study will greatly contribute to the breeding efforts carried out at this institution. The effectiveness of breeding programs planned for developing hybrid varieties with high adaptability to global climate change depends on selecting the most suitable parents (Schnable and Springer, 2013; Kadam and Lorenz, 2018). Choosing genetically different parents in hybrid variety development contributes to a high heterosis effect (Labroo et al., 2021). Therefore, the genetic characterization of breeding lines is of great importance. In our study, the genetic relatedness among 24 cabbage inbred lines in the genetic resource of the Black Sea Agricultural Research Institute was screened using SRAP markers. Our findings indicated that SRAP markers were effective in differentiating cabbage inbred lines. The genetic similarity among the inbred lines ranged from 78 to 90, indicating relatively low variability. In a genetic diversity study conducted on Brassicas (*Brassica napus*), it was reported that 20 SRAP primer combinations successfully differentiated 60 accessions (Ahmad et al., 2014). Researchers reported that the genetic similarity among accessions ranged from 40 to 100. In a genetic diversity study conducted with SRAP markers on another Brassica species (*Brassica juncea* L.), a total of 286 bands were obtained, with an average number of bands per primer combination being 9.23 (Li et al., 2018). The similarity among the 44 accessions was determined to range from 0.61 to 0.89. In a diversity study comparing SSR and SRAP markers among different Brassica species, it was reported that 36 SSR primers produced 133 polymorphic bands, while 43 SRAP primer combinations produced 268 polymorphic bands. SRAP primers were reported to be more successful than SSR primers in determining variation among Brassica species (Zhang et al., 2017). Another study investigating the effectiveness of SRAP and SSR markers for the diversity of elite *Brassica napus* breeding lines found that 8 SSR primers explained 96.08% of the variation among the breeding lines, while 12 SRAP primer combinations explained 98.72%, indicating that SRAP primers were nearly as successful as SSR primers (Zang et al., 2019). In the PCA applied to the SRAP marker data for the characterization of cabbage breeding lines, the first component explained 82.35% of the variation among the lines. This finding is consistent with the results obtained from the diversity study of Brassica napus varieties with SRAP markers (Framarzpour et al., 2021). STRUCTURE analysis showed that cabbage breeding lines were genetically divided into three groups. The findings revealed significant genetic variability among the breeding lines we have. Different cabbage species have been successfully differentiated with SRAP (Wu et al., 2009) and SSR (Pipan et al., 2024; Malik et al., 2024) markers in Brassica germplasms. The SRAP markers we used, were also successful in differentiating elite inbred cabbage lines.

5. Conclusion

The study of the genetic diversity of inbred cabbage lines using SRAP markers has provided comprehensive information on their genetic composition. Key genetic diversity metrics, including PIC, MAF, NE, H, and I, were evaluated. The mean PIC value of 0.20 (range: 0.03 to 0.42) indicates moderate genetic diversity, with primers like Me03Em03 showing high discriminatory power (PIC=0.42). An average MAF of 0.83 suggests a predominance of certain alleles, reflecting lower overall genetic diversity. The mean NE, H and I values further confirm the presence of genetic variability among the inbred lines. Principal component analysis showed that genetic diversity could be largely explained along a few dimensions. The cluster analysis using STRUCTURE and UPGMA showed distinct genetic groupings, with three major clusters identified. Cluster 3 showed the highest genetic differentiation, indicating significant divergence within this group. The Mantel test value (r=0.78) from the UPGMA clustering analysis underscores the robustness of the genetic relationships observed among the cabbage lines. The dendrogram further supports these findings, showing clear genetic differences and similarities among the lines. In conclusion, SRAP markers have been found to be a valuable tool for characterizing the genetic diversity of inbred cabbage lines. The moderate level of genetic diversity observed suggests potential for selective breeding and genetic improvement programs.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	E.E.	C.T.
C	70	30
D	90	10
S	100	
DCP	30	70
DAI	50	50
L	20	80
W	80	20
CR	80	20
SR	80	20
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

This study was summarized from the second authors MSc thesis under the supervision of the first author.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1489757

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 437-450 / September 2024

VERMICOMPOST EFFECTS ON SOIL CHEMISTRY AND BIOLOGY: CORRELATIONS WITH BASIL'S (*Ocimum basilicum* L.) TOTAL PHENOLIC CONTENT AND PHENOLOGICAL TRAITS

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Abstract: This study investigates the effects of vermicompost on the chemical and biological properties of soils, their nutrient content, and the effects on the growth and phenolic content of basil (*Ocimum basilicum* L.). Using a controlled experimental setup, we tested five dosages of vermicompost (0%, 4%, 12%, 20%, and 24%, w/w) to evaluate their influence on soil biological activity by measuring basal respiration (CO_2-C), microbial biomass C (MBC-C), and dehydrogenase activity (DHA) as well as basil's growth parameters and total phenolic content (TPC). The results show that vermicompost addition to soil enhanced soil microbial activity in direct proportion to the dose of vermicompost. The application of lower dosages of vermicompost (4% and 12%) significantly enhanced both fresh and dry weights. However, higher dosages (20% and 24%) were associated with reduced growth metrics. Notably, the highest vermicompost concentration (24%) led to a substantial increase in total phenolic content (TPC) in basil leaves, correlating with decreased growth metrics. The values for CO_2-C, MBC-C, and DHA were determined as 0.135, 20.756, and 12.806, respectively, at the highest solid vermicompost application dose of 24%. Fresh and dry weight were determined at 12% vermicompost application, and plant height and leaf length were also determined at 12% vermicompost application. The TPC showed a remarkable increase at the 24% application dose. This response indicates a defense mechanism of the plant against oxidative stress caused by excess nutrients or salinity from the vermicompost. A multiple regression analysis following a correlation analysis also revealed an inversely proportional relationship between phosphorus content in the soil and total phenolic content in basil leaves. Our findings illustrate that while moderate vermicompost dosages optimize plant growth and health, higher concentrations can strategically enhance phenolic content due to nutrient overload or salt-induced stress. These results offer critical insights for tailoring organic amendment applications to balance plant growth and biochemical properties in agricultural practices.

Keywords: *Ocimum basilicum* L., Phenolic content, Soil biology, Soil chemical parameters, Soil microbial activity, Vermicompost

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Received: May 25, 2024
Accepted: July 28, 2024
Published: September 15, 2024

Cite as: Hepşen Türkay FŞ. 2024. Vermicompost effects on soil chemistry and biology: Correlations with basil's (*Ocimum basilicum* l.) Total phenolic content and phenological traits. BSJ Agri, 7(5): 437-450.

1. Introduction

Vermicompost, derived from the breakdown of organic material through earthworm activity, has been increasingly recognized for its potential to enhance soil quality and plant growth. Research indicates that vermicompost enriches soil with nutrients, improves its structure, and enhances microbial activity, all of which contribute to better plant health and productivity (Edwards and Burrows, 1988; Atiyeh et al., 2000; Kizilkaya et al., 2012). Studies have shown that plants grown in vermicomposted soil exhibit increased growth rates, yield, and resistance to pests and diseases compared to those grown in non-vermicomposted soils (Arancon et al., 2005; Bachman and Metzger, 2008). Moreover, vermicompost has been found to influence the synthesis of plant secondary metabolites, including phenolic compounds, which play a crucial role in plant defense mechanisms (Szczech, 1999). Given the global push towards sustainable agriculture, understanding the specific effects of vermicompost on crop species such as basil (*Ocimum basilicum* L.), known for its economic and medicinal value, is particularly relevant.

Additionally, the interactions between compost, vermicompost, and earthworms have been found to significantly influence plant growth and yield, emphasizing the importance of integrating these natural processes into greenhouse and field settings (Doan et al., 2013). The positive effects of vermicompost on nutrient dynamics and the reduction of heavy metals in soil further illustrate its role in creating healthier, more productive soil environments (Kannadasan et al., 2013). Earthworms, often described as "ecosystem engineers", contribute to these improvements by enhancing soil structure, nutrient cycling, and plant growth, which are essential for robust agricultural systems (Wang et al., 2021). Moreover, the research by Samaranayake and Wijekoon (2011) highlights the ability of specific earthworm species to boost soil fertility and enhance the growth of crops such as maize, showcasing the practical applications of vermicompost in agriculture. Vermicomposts are commonly referred to as biological fertilizers due to their high microbial content. These organic fertilizers are known to promote soil microbial biodiversity by introducing a diverse range of beneficial microbes such as Arbuscular Mycorrhizal (AM) fungi, Azotobacter, Agrobacterium, and Rhizobacteria into the soil (Domínguez et al., 2014; Broz et al., 2016). Vermicomposts contain much larger populations of bacteria (5.7×10^7), fungi (22.7×10^4), and actinomycetes (17.7×10^6) compared to those in conventional thermophilic composts (Edwards et al., 2010). This superiority of vermicompost is due to its very high microbial activity. Additionally, vermicompost has a higher concentration of beneficial microbial populations, readily available plant nutrients, and plant growth regulators compared to conventional compost (Türkay, 2023). This study seeks to build upon these findings by specifically investigating the impact of varying vermicompost concentrations on basil, aiming to optimize plant health and soil quality while mitigating potential negative effects associated with excessive organic amendments.

In recent years, there has been a growing interest in studying the effects of vermicompost on plant growth and the phenolic content of various plants, including basil. Vermicompost, which is organic fertilizer produced from earthworms, has been found to significantly impact the growth, chemical composition, and oil yield of plants like basil. Studies have shown that vermicompost applications at different concentrations can lead to increased fresh and dry weight, leaf area, essential oil compounds, and nutrient levels in plants like basil (Türkay and Öztürk, 2019; Massoud et al., 2022; Türkay and Öztürk, 2023; Türkay et al., 2024).

The phenolic content of plants, including basil, has been a subject of interest due to its antioxidant properties. Basil extracts have been reported to possess a higher total phenolic acid content and greater antioxidant activity, making them valuable for potential pharmacological effects (Mintas et al., 2021). Furthermore, studies have highlighted the rich source of phenolic compounds in basil leaves, including various phenolic acids, flavonol-glycosides, and anthocyanins (Zlotek et al., 2016). These compounds contribute to the antioxidant capacity of basil and are essential for its health benefits (Türkay et al., 2024).

Research has also explored the antioxidant capacity of basil extracts, comparing different extraction methods and studying the antioxidant properties of various parts of the plant. Studies have investigated the antioxidant properties of different extracts of *Ocimum basilicum* and *Origanum vulgare*, emphasizing the importance of these plants as potential sources of antioxidants (Kaurinovic et al., 2011). Additionally, investigations into the effects of different drying methods on the proximate composition and antioxidant activities of *Ocimum basilicum* leaves have provided insights into preserving the bioactive compounds in basil (Mahirah et al., 2018).

Moreover, the influence of environmental factors on the phenolic composition of basil plants has been studied. Factors such as nitrogen starvation, different soil types, water stress, and pre-harvest UV-B supplementation have been found to affect the phenolic content of basil leaves, highlighting the importance of environmental conditions in determining the phytochemical profile of plants like *Ocimum basilicum* (Luna et al., 2015; dos Santos Nascimiento et al., 2020; Prinsi et al., 2020). Additionally, the use of humates from vermicompost to mitigate the effects of salinity on basil growth further underscores the potential of organic amendments in enhancing plant resilience (Reyes-Pérez et al., 2017).

Furthermore, the potential allelochemical effects of basil on weed control in other crops have been investigated, shedding light on the broader ecological implications of basil cultivation (Kamel et al., 2021). Additionally, studies have explored the biofortification of basil leaves with selenium to enhance their quality and shelf life, demonstrating innovative approaches to improving the nutritional value of basil plants (Puccinelli et al., 2020). The role of mycorrhizal fungi and microalgae in modulating the antioxidant capacity of basil plants further emphasizes the intricate interactions between plants and their symbiotic partners in influencing phenolic content (Hristozkova et al., 2018).

In conclusion, the research on vermicompost applications, phenolic content, and antioxidant properties of basil and other plants provides valuable insights into the factors influencing plant growth, chemical composition, and bioactive compound levels. Understanding the effects of vermicompost, environmental conditions, and extraction methods on plant phenolics is crucial for maximizing the nutritional and medicinal potential of plants like basil. These studies contribute to the broader knowledge of plant science and offer practical implications for sustainable agriculture and herbal medicine.

The primary objective of this study is to investigate the impact of different concentrations of vermicompost on the growth characteristics and total phenolic content of basil (*Ocimum basilicum* L.) due to the high microbial activity content of vermicompost. Specifically, the research aims to delineate how varying vermicompost dosages influence plant height, leaf length, and fresh and dry biomass, thereby providing insights into the optimal vermicompost concentration for enhancing plant growth and health. Additionally, the study seeks to explore the relationship between changes in soil microbial activity caused by applied vermicompost dosages and the production of phenolic compounds, which are vital for plant defense mechanisms. By analyzing these relationships, the study aims to provide practical guidelines for the application of vermicompost in agriculture, particularly in enhancing soil quality and plant health while minimizing potential adverse effects related to nutrient overload or salt stress. This comprehensive analysis is intended to aid farmers and agricultural practitioners in making informed decisions regarding the use of organic amendments like vermicompost in sustainable farming practices.

2. Materials and Methods

Before the experiment, the soil was air-dried to standardize moisture content and sieved through a 2 mm sieve to remove debris and ensure a uniform texture. This was crucial for accurate vermicompost integration. The soil underwent a detailed characterization to establish its baseline chemical properties (Table 1), including pH, electrical conductivity (EC), organic matter content, and essential nutrients like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn). These analyses were conducted using standard soil testing techniques, such as pH and EC measurements in a soil-water suspension, organic matter by the modified Walkley-Black method (Nelson and Sommers, 1996), and nutrient quantification through spectrometric or titrimetric methods. This initial characterization provided a crucial understanding of the soil's nutrient status and fertility, important for assessing the impacts of vermicompost on soil health and plant growth during the experiment.

The vermicompost used in this study was produced in-house at the Kırşehir Ahi Evran University, Faculty of Agriculture, using 100% barnyard manure and *Eisenia fetida* earthworms, which are effective composters. The earthworms were housed in plastic containers filled with manure under controlled conditions suitable for vermicomposting. After completion, the vermicompost was passed through a 2-mm sieve to ensure consistency and remove larger uncomposted particles, crucial for maintaining quality. To preserve its microbiological properties, the vermicompost was refrigerated at +4°C for a day before use, maintaining microbial integrity vital for its effectiveness as a soil amendment. Chemical and biological analyses of the vermicompost included pH, electrical conductivity (EC), organic matter content, and concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and trace elements like iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn). These analyses ensured the vermicompost's quality met the experimental needs, establishing a baseline for assessing its impact on soil health and plant growth in the study.

For the production of basil seedlings, the experiment began with the germination of basil seeds, which were bought from a local agricultural products supplier, in a controlled environment. The seeds were sown in 50 cc pots, three seeds per pot, across 10 replicates, ensuring an adequate number of samples for the study. At the end of the second week of germination, an evaluation was conducted to select the healthiest seedlings for continuation in the experiment. In each pot, one healthy individual was selected based on its overall health and developmental progress compared to its peers, and the remaining seedlings were carefully removed.

2.1. Experimental Design

The experiment assessed the impacts of vermicompost on basil through a structured treatment setup under controlled conditions, aiming for high scientific standards and replicability. Five vermicompost doses were prepared: 0% (control), 4%, 12%, 20%, and 24%, representing the proportion mixed with base soil to explore a spectrum of effects. The results of each treatment was obtained from the samples which taken from three pots in each group, totaling 15 experimental pots. The experiment took place in a plant growth cabinet, enabling precise control over temperature, humidity, and light. The temperature was consistently held at 25 °C, optimal for basil growth, humidity was consistently held at 55-60% and lighting (16h light: 8h dark photoperiods) was adjusted to promote full photosynthesis without causing photobleaching or heat stress.

Before planting, thorough mixing of soil and vermicompost for each treatment ensured uniformity, crucial for eliminating growth variability due to nutrient distribution differences. Soil moisture was closely monitored to maintain field capacity by weighing the pots daily and adjusting water levels accordingly.

Table 1. Analyses and methods used to determine the chemical properties of the soil used in the experiment (Kacar, 1994).

Analysis	Method
Soil Texture (% sand, silt, clay sized particles)	Hydrometer method
Soil reaction (pH)	Measured with a pH meter in a 1:1 (w/v) soil:distilled water mixture
Electrical Conductivity (EC, dS m^{-1})	Measured with an EC meter in a 1:1 (w/v) soil:distilled water mixture
Organic Matter, %	Modified Walkley-Black method
Total N, %	Kjeldahl method
Available P, $mg.kg^{-1}$	Olsen method
Exchangeable K, Ca, Mg, me100 g^{-1}	Ammonium acetate extraction method
Available Fe, Cu, Zn, Mn, $mg{\cdot}kg^{-1}$	DTPA extraction method

At the end of the experiment, critical growth parameters such as plant height, leaf length, and biomass (both fresh and dry weights) were measured for each individual in application groups. Additionally, the phenolic content in the basil leaves was analyzed by the Folin-Ciocalteu method to determine the effects of the vermicompost treatments.

2.2. Data Collection Methods

2.2.1. Description of plant growth measurements

In the study, detailed phenological and growth metrics were recorded to evaluate vermicompost's influence on basil. Plant height was measured from the stem base to the apex of the tallest shoot using a ruler, with measurements noted in millimeters. Similarly, leaf length was assessed from the base to the tip of the longest leaf on each plant, also in millimeters. At the end of the 10-week growth period, the fresh weight of each plant was promptly recorded post-harvest to prevent dehydration. For dry weight assessments, plants were placed in a drying area away from direct sunlight and kept at room temperature until thoroughly dried, after which dry weights were noted to evaluate biomass accumulation across different vermicompost treatments. Additionally, 15 grams of the top leaves from each plant were collected, dried as described, and analyzed for total phenolic content (TPC) using the Folin-Ciocalteu method. This spectrophotometric technique measures phenolic compounds, offering insights into the plants' antioxidant potential.

2.2.2. Soil and vermicompost sampling methods

Soil samples were collected at two critical times: before vermicompost application to establish a baseline, and after the 10-week growth period to evaluate treatment effects. The representative soil samples, which taken from the pots, were split into two portions; one was refrigerated at +4 °C to preserve biological properties for microbial analysis (Table 2), and the other was air-dried, sieved, and analyzed for chemical properties. The samples underwent detailed analysis for pH, electrical conductivity, nutrient content and microbial activity using standard laboratory methods, ensuring thorough evaluation of vermicompost's effects on soil health and plant growth.

This detailed approach to sampling and analysis ensures that the data collected will provide a comprehensive insight into how vermicompost affects soil properties over the course of the experiment, thereby facilitating a better understanding of the interactions between soil amendments and plant responses.

2.2.3. Total phenolic content analysis method

To evaluate the impact of vermicompost on the total phenolic content (TPC) of basil, we employed the Folin-Ciocalteu method, a standard for quantifying phenolic compounds in plant tissues and assessing antioxidant capacity. At the conclusion of the 10-week growing period, top leaves from each basil plant were uniformly harvested, with approximately 15 grams of leaf material collected from similar positions on each plant to ensure consistency. These leaves were then dried in the shade at room temperature to preserve the phenolic compounds and maintain consistent dry weight. The dried leaves were weighed, and a specific amount was used for extraction with an 80% methanol solution, chosen for its effectiveness in extracting phenolic substances. The extraction involved macerating the leaves in methanol under controlled conditions to ensure thorough extraction of phenolic compounds. The Folin-Ciocalteu reagent, prepared following standard laboratory protocols, reacts with the phenolic compounds to form a blue complex, the intensity of which was measured spectrophotometrically at 765 nm.

Table 2. The microbiological analyses used to determine the biological properties of soil

Analysis	Protocol
Basal Respiration (CO_2-C)	50 g of soil is moistened with distilled water until it reaches 55% of its maximum water holding capacity and placed into 1-liter Isermeyer jars. 25 mL of 0.05 M NaOH is added to the alkaline tube of the jar, and the jars are incubated at 25°C for 3 days. The CO_2 released by microbial respiration is trapped by the alkali, and the remaining OH- is titrated with standardized HCl in the presence of phenolphthalein indicator. The result is expressed as µg CO_2-C g^{-1} dry soil.
Microbial Biomass Carbon (MBC)	50 g of soil is moistened with distilled water until it reaches 55% of its maximum water holding capacity, then 200 mg of glucose is added and placed into 1-liter Isermeyer jars. The amount of CO_2 released from the soil is determined hourly as described in section 3.5.1. The maximum respiration at the end of 4 hours is calculated using the equation 40.04 µg CO_2 g^{-1} + 3.75 and the result is expressed as µg CO_2-C g^{-1} dry soil.
Dehydrogenase Activity (DHA)	After adding 30 mg glucose, 1 mL of 3% 2,3,5-triphenyltetrazolium chloride (TTC) substrate solution, and 2.5 mL of distilled water to a 6 g soil sample, the mixture is incubated at 37 °C for 24 hours. At the end of the incubation, the released 1,3,5-triphenylformazan (TPF) is determined spectrophotometrically at a wavelength of 485 nm, and the result is expressed as µg TPF g^{-1} dry soil.

Absorbance readings are directly proportional to the phenolic content in the samples. A calibration curve using known concentrations of gallic acid enabled the accurate calculation of the TPC, expressed as mg of gallic acid equivalents (GAE) per gram of dry weight.

2.3. Data Analysis

To evaluate the effects of varying vermicompost concentrations on basil growth parameters and TPC, we performed an Analysis of Variance (ANOVA) followed by Tukey post-hoc test (Genç and Soysal, 2018). This combination identifies significant differences between groups, denoted by distinct letters, indicating statistically significant results at $P<0.05$. Additionally, Principal component analysis (PCA) was employed to reduce data dimensionality and highlight the main variables affecting plant and soil traits (Kurnaz et al., 2022). This method uncovered patterns in how vermicompost dosages influence growth and soil characteristics. Pearson correlation coefficients were calculated to explore the relationships between growth parameters, soil properties, and total phenolic content (TPC), providing insights into the linear interactions within the dataset. Multiple regression analysis (Kurnaz et al., 2021) was used to examine the impact of changes in soil parameters, notably organic matter and phosphorus content, on TPC. This analysis provided a comprehensive understanding of the interactions between soil and phenolic compounds. All statistical tests were conducted with a 95% confidence interval, ensuring the robustness of our findings. The statistical analyses were conducted using the provided calculators on the Social Science Statistics (2024).

3. Results and Discussion

3.1. Characteristics of Soil and Vermicompost in Basil Cultivation

Tables 3 and Table 4 provide detailed chemical analyses of the soil and vermicompost used in our experiments, crucial for understanding the initial conditions affecting basil growth. Table 3 focuses on the soil used as a growing medium, documenting key parameters like pH, electrical conductivity, organic matter content, and essential nutrients such as nitrogen, phosphorus, and potassium. These parameters are vital for assessing the soil's fertility and its ability to support healthy plant growth. The data serves as a baseline, reflecting the soil's condition prior to any experimental treatments, and acts as a control for evaluating the impacts of subsequent vermicompost additions.

Table 4 describes the chemical properties of the vermicompost utilized in the experiments, providing insights into its quality and effectiveness as a soil amendment. This table outlines various characteristics of the vermicompost, including its nutrient content and biological properties, which are important for judging its capacity to enhance plant health and growth. The information is instrumental in assessing how vermicompost influences not only the phenological and biochemical traits of the basil plants but also the overall biological quality of the soil after its application.

Together, both tables are essential for establishing a scientific framework for the experimental treatments. The soil evaluated in our study has a slightly alkaline pH of 7.32 which is suitable for many plants, including basil, which thrive in a pH range of 6.0 to 7.5 (Couto, 2018; Neina, 2019). The electrical conductivity (EC) of 529.5 µS cm^{-1} indicates a moderate salt content, which should not significantly affect basil's water uptake (Rao et al., 2020). However, the low organic matter content of 1.151% suggests limited nutrient reserves and microbial activity, which are important for robust plant growth and soil structure (Tisdall and Oades, 1982; Chang et al., 2014). Essential nutrients like nitrogen, phosphorus, and potassium are present but in modest amounts, indicating basic soil fertility sufficient for basic plant needs but likely requiring supplementation for optimal growth.

Table 3. Chemical properties of the soil used in the experiment

Chemical properties	Values
pH	7.32
EC, µS cm^{-1}	529.5
Organic matter, %	1.151
Total N, %	0.100
Available P, ppm	1.681
Exchangeable K, me100 g^{-1}	0.427
Exchangeable Ca, me100 g^{-1}	33.711
Exchangeable Mg, me 100 g^{-1}	3.189
Available Fe, $mg.kg^{-1}$	3.41
Available Cu, $mg.kg^{-1}$	1.01
Available Zn, $mg.kg^{-1}$	0.32
Available Mn, $mg.kg^{-1}$	3.20

Table 4. Chemical properties of vermicompost used in the preparation of plant growing medium in the experimental setup

Chemical properties	Values
pH	7.10
EC, dS m^{-1}	12.8
Organic matter, %	41.6
Total N,%	2.08
C/N	11.59
NO_3-N $mg.g^{-1}$	713.05
NH_4-N $mg.g^{-1}$	518.2
Total P, %	0.81
Total K, %	1.72
Total Ca, %	1.64
Total Mg, %	0.73
Fe, $mg.kg^{-1}$	1146.90
Cu, $mg.kg^{-1}$	9.95
Zn, $mg.kg^{-1}$	25.47
Mn, $mg.kg^{-1}$	137.92

The vermicompost used in our study is slightly more acidic with a pH of 7.10, falling within a beneficial range for basil cultivation. This acidity level is advantageous as vermicompost contains a combination of macro and micro-nutrients that positively impact plant nutrition, growth, photosynthesis, and chlorophyll content, all of which are essential for basil cultivation (Moustafa et al., 2022). It has a high EC of 12.8 dS m^{-1}, suggesting significant salt content that could cause salinity issues if over-applied. The vermicompost is rich in organic matter (41.6%), enhancing soil structure, moisture retention, microbial life, and plant health. It contains high levels of essential nutrients and is abundant in micronutrients like iron, copper, zinc, and manganese, crucial for plant enzymatic processes and disease resistance.

Using vermicompost can significantly enhance soil nutrient levels and improve soil structure, benefiting water retention, aeration, and microbial activity. This helps in nutrient cycling and increases nutrient uptake by plants. Studies have shown that vermicompost applications lead to improvements in soil microbial biomass, soil porosity, water holding capacity, nutrient content, and plant growth (Sim and-Wu, 2010; Tejada et al., 2010; Pramanik et al., 2010; Lim et al., 2014; Pereira et al., 2014; Akhzari et al., 2015; Yadav and Garg, 2015; Rekha et al., 2018; Jahanbakhshi and Kheiralipour, 2019; Rivier et al., 2022). However, the high EC of vermicompost requires careful management to avoid salinity stress. Proper application rates and methods are crucial to maximize benefits and prevent adverse effects in the soil-plant system, ensuring enhanced soil fertility and support for plant growth.

3.2. The Effects of Vermicompost Applications on Soil Properties and Microbial Activity

The addition of vermicompost to soil at increasing doses (0%, 4%, 12%, 20%, and 24%) significantly enhances various chemical and biological properties of the soil (Table 5). As vermicompost doses increase, soil microbial activity, represented by CO_2-C and DHA, and microbial biomass, indicated by MBC-C, show marked improvements. Specifically, CO_2-C rises from 0.054 μg.g^{-1} at control (0%) to 0.135 μg.g^{-1} at 24%, and MBC-C increases from 7.759 μg.g^{-1} to 20.756 μg.g^{-1} over the same range. Additionally, dehydrogenase activity (DHA) increases from 2.416 μg TPF g^{-1} at 0% to 12.806 μg TPF g^{-1} at 24% (Table 5). These changes reflect enhanced soil microbial health and activity due to the addition of organic material from vermicompost.

Moreover, vermicompost application leads to significant improvements in soil fertility indicators, including organic matter (OM), total nitrogen (N Total), and phosphorus (P). OM content rises dramatically from 1.151% at 0% to 15.262% at 24%, while total nitrogen and phosphorus increase from 0.100% to 0.537% and 1.681 ppm to 13.876 ppm, respectively. Although soil pH decreases with higher vermicompost doses, from 7.315 to 7.212, electrical conductivity (EC), which measures soluble salt content, increases from 0.529 dS m^{-1} to 0.981 dS m^{-1}. These changes highlight the substantial benefits of vermicompost in enriching soil nutrients and enhancing soil structure, making it an effective amendment for sustainable soil management.

Overall, the addition of vermicompost at increasing doses positively affects the chemical properties of the soil, enhancing microbial activity, organic matter content, and nutrient availability. However, the increase in electrical conductivity indicates a potential risk of salinity stress at higher vermicompost doses.

3.3. Observations on Basil Growth and TPC under Different Vermicompost Treatments

Figure 1 and Figure 2 offer insights into the influence of varying doses of vermicompost on the phenological traits and total phenolic content (TPC) of basil, respectively.

Figure 1 reveals that applying vermicompost at concentrations of 4% and 12% significantly increased the fresh weights of basil to 21.78 grams and 22.93 grams, respectively, compared to 9.72 grams in the control group. However, fresh weight declined at a concentration of 20% and decreased further at 24%, suggesting that lower doses are more effective for biomass accumulation. The dry weight peaked at a dosage of 4% (1.83 grams) and decreased slightly at higher dosages but remained above control levels, indicating that moderate vermicompost levels effectively support biomass retention. Plant height was highest at 373.30 mm with 4% vermicompost, compared to 314.90 mm in the control, and decreased with higher dosages, though all treated plants were taller than the control. The maximum leaf length (108.40 mm) was observed at 12% dosage, with significant lengths also noted at 4% and 24% dosages, suggesting that mid-range vermicompost dosages might optimize leaf development.

Table 5. Chemical and biological properties of the soils of vermicompost (VC) application groups

VC Dose	CO_2-C μg.g^{-1}	MBC-C μg.g^{-1}	DHA μg TPF g^{-1}	pH	EC Ds m^{-1}	OM %	Total N %	P ppm
0%	0.054^{e}	7.759^{e}	2.416^{e}	7.315^{a}	0.529^{e}	1.151^{e}	0.100^{e}	1.681^{e}
4%	0.067^{d}	11.423^{d}	10.456^{d}	7.290^{b}	0.612^{d}	3.574^{d}	0.227^{d}	2.487^{d}
12%	0.096^{c}	15.035^{c}	12.007^{c}	7.264^{c}	0.830^{c}	7.987^{c}	0.360^{c}	6.889^{c}
20%	0.124^{b}	18.943^{b}	12.556^{b}	7.237^{d}	0.934^{b}	12.288^{b}	0.477^{b}	11.316^{b}
24%	0.135^{a}	20.756^{a}	12.806^{a}	7.212^{e}	0.981^{a}	15.262^{a}	0.537^{a}	13.876^{a}

The data are reported as means of 3 replicates. Means followed by the different letters are statistically significant at $P<0.05$.

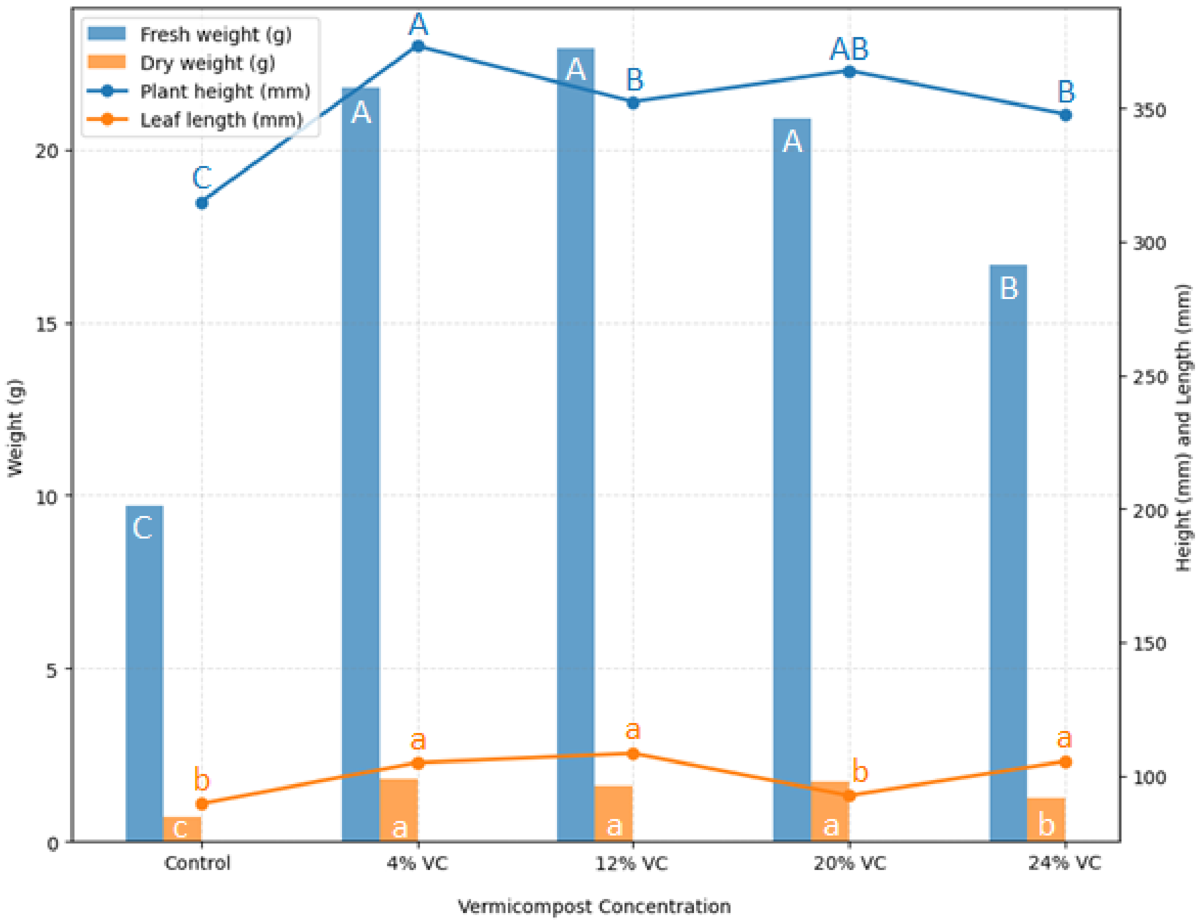


Figure 1. Effects of varying vermicompost doses on the phenological traits of basil.

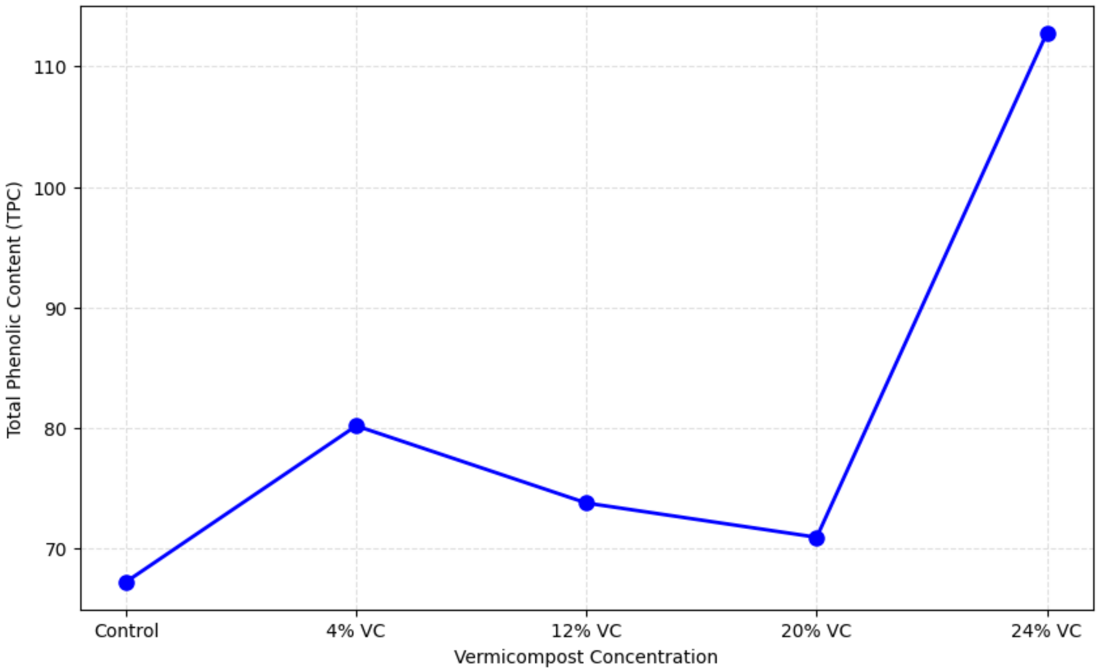


Figure 2. Impact of different vermicompost dosages on the total phenolic content (TPC) of basil.

TPC significantly increased with vermicompost application, with the control group having the lowest value at 67.25 mg GAE/g (Figure 2). TPC rose to 80.19 mg GAE/g at a 4% dosage but slightly decreased at 12% and 20%. The highest phenolic content (112.77 mg GAE/g) was observed at the 24% dosage, indicating a potential dose-response relationship where phenolic content might increase substantially at higher vermicompost concentrations.

The results suggest a nonlinear relationship between vermicompost dosage and both phenological traits and TPC. Moderate vermicompost applications (around 4% to

12%) generally enhanced growth parameters more effectively than no vermicompost. Very high dosages (24%), however, seemed to induce stress, reflected in reduced growth parameters but increased phenolic content, possibly as a stress response mechanism in basil. This increase in phenolics at high vermicompost levels could be due to enhanced secondary metabolite production, which plants often increase under stress conditions (Akula and Ravishankar, 2011; Kumar and Sharma, 2018; Tikoria et al., 2022; Sharma et al., 2023; Türkay and Öztürk, 2023; Türkay et al., 2024).

These findings suggest that while moderate vermicompost applications can enhance growth and overall biomass, higher concentrations might be used strategically to boost phenolic content, potentially enhancing the medicinal and nutritional value of basil. Thus, the choice of vermicompost dosage could be tailored depending on the desired outcome, whether it is maximizing growth or enhancing specific phytochemicals.

Overall, the experiment underscores how organic amendments like vermicompost can influence plant growth and secondary metabolite production, particularly phenolic compounds. This aligns with existing research suggesting that vermicompost not only improves soil fertility and plant growth but also enhances the production of plant secondary metabolites due to increased nutrient availability and improved soil health (Atiyeh et al., 2000; Nunes et al., 2018; Nurhidayati et al., 2018).

This study demonstrates that lower vermicompost dosages (4% and 12%) enhance fresh and dry weights of plants, suggesting improved nutrient uptake and soil structure which boost root growth and nutrient absorption, consistent with findings from Gill et al. (2018). Conversely, higher dosages (20% and 24%) may cause nutrient overload or salt stress, potentially impeding plant growth despite the rich nutrient content of vermicompost. Optimal growth in plant height and leaf length at mid-range dosages confirms that moderate levels provide a balanced nutrient mix, avoiding physiological stress. A study by Ibrahim et al. (2022) found that higher rates of vermicompost, especially when combined with high concentrations of ascorbic acid, resulted in optimal growth and yield of tomato plants grown in saline soil, suggesting that very high or low dosages could potentially lead to nutrient overload or salt stress. Pérez-Gómez et al. (2017) reported that higher dosages of vermicompost could lead to salt stress, impacting potato plants' growth negatively, whereas mid-range dosages minimized these stress effects and were optimal for growth. Adamipour et al. (2016) observed that mid-range doses of vermicompost produced the highest growth values for *Festuca arundinacea* Schreb. under salinity stress conditions, suggesting that higher doses may cause detrimental effects due to nutrient overload or exacerbation of stress conditions.

A notable increase in total phenolic content (TPC) at the highest vermicompost dosage (24%) correlates with reduced growth metrics, indicating a stress-induced enhancement in phenolic synthesis. Plants likely increase phenolics as a defense mechanism against oxidative stress caused by high nutrient levels or salinity from the vermicompost. Phenolics are crucial in plant defense against abiotic stresses such as nutrient and salt stress, which is consistent with the increased phenolic content observed. Aliyar et al. (2021) discuss the effect of vermicompost application on the growth and water relationships of quinoa plants under salinity stress conditions, suggesting that higher vermicompost concentrations lead to an increase in phenolic content as a response to oxidative stress. Beykkhormizi et al. (2016) noted that the application of vermicompost under salinity stress conditions led to significant changes in phenolic content in bean plants, pointing to a stress response mechanism involving phenolic synthesis due to high nutrient levels or salinity.

The results highlight a nonlinear relationship between vermicompost dosage and the resulting phenological and biochemical traits, suggesting that vermicompost is beneficial up to a threshold; beyond this, it may induce stress that compromises growth while boosting secondary metabolite production. This duality offers strategic opportunities for agricultural applications and enhancing phytochemical properties. For crops focusing on growth and yield, maintaining moderate vermicompost applications (around 4% to 12%) is ideal for optimal plant growth and health. For medicinal and aromatic plants, where secondary metabolites like phenolics are valued, higher vermicompost dosages can be utilized to enhance the concentration of bioactive compounds, increasing their therapeutic and nutritional value.

In conclusion, vermicompost is a valuable soil amendment for improving plant growth and soil properties, but its application must be carefully managed to prevent adverse effects at higher concentrations. Tailoring application rates to meet specific crop needs and desired outcomes allows growers to maximize the benefits of vermicompost, supporting sustainable agricultural practices and enhancing the nutritional and medicinal value of crops like basil.

3.4. Statistical Insights: Understanding the Data through PCA, Correlation Coefficients, and Multiple Regression Analysis

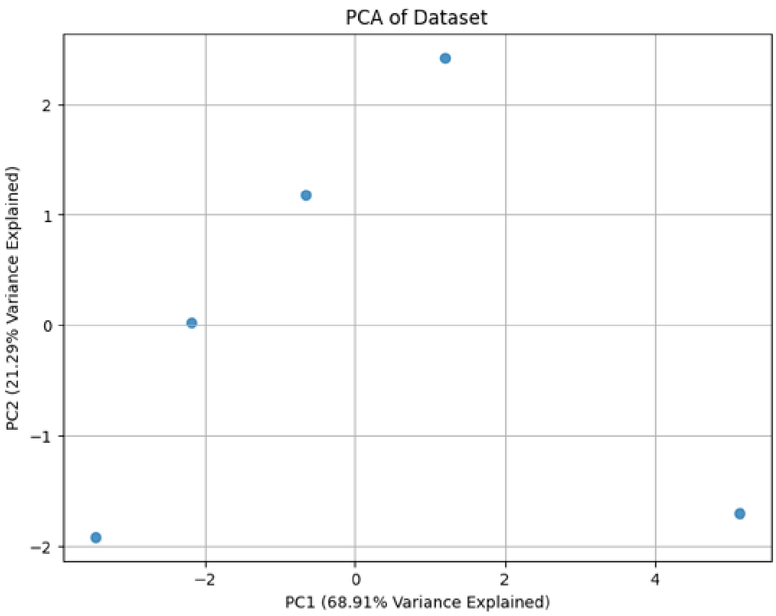
In our study, principal component analysis (PCA) was applied to investigate the relationships and underlying patterns in the data collected from various vermicompost (VC) dosing levels and their impact on plant growth parameters and soil properties (Figure 3). This multivariate statistical technique was utilized to reduce the dimensionality of the dataset, comprising measurements such as plant height, leaf length, fresh and dry weight of plants, total phenolic content (TPC), microbial biomass carbon (MBC-C), dehydrogenase activity (DHA), basal respiration (CO_2-C), pH, electrical conductivity (EC), organic matter (OM) content, total N, and P content.

According to the results, PCA deciphers the patterns in how VC affects plant growth and soil properties, identifying two principal components that explain 90.20% of the data variance. The first principal component (PC1) explained 68.91% of the variance, highlighting its correlation with key growth parameters and nutrient dynamics, showcasing VC's significant impact on nutrient enhancement in plants and soil. The second principal component (PC2), accounting for 21.29% of the variance, focused on more specific soil chemical properties and microbial interactions, underscoring subtle effects on soil health.

The PCA scatter plot illustrated the distribution and relationship of different VC dosing groups, revealing how similar or unique their impacts are, which helps in identifying optimal VC dosages. This analysis proves invaluable in simplifying complex multivariate data, allowing for a concentrated examination of essential factors affecting plant and soil health. It guides further research and practical applications by improving understanding of VC's broad and nuanced impacts, thus enhancing agricultural outcomes. PC1 primarily indicates that VC boosts plant growth through nutrient availability, while PC2 suggests VC's secondary effects modify soil chemistry and microbial activity, crucial for nutrient cycling. This streamlined approach helps in optimizing VC dosing, enhancing plant productivity, soil health, agricultural sustainability, and crop quality.

The Pearson correlation coefficient analysis in our study aimed to determine the relationships between various plant growth parameters, soil properties, and biochemical indicators (Table 6).

The outcomes of Pearson correlation coefficient analysis highlight significant relationships between plant growth parameters, soil properties, and vermicompost dosages, providing valuable insights for enhancing agricultural practices and sustainability. The analysis reveals strong positive correlations between plant height, leaf length, fresh and dry weights, indicating that plants with greater height and leaf length generally exhibit higher biomass. This correlation extends to the total phenolic content (TPC), which correlates positively with plant biomass, CO_2-C, microbial biomass carbon (MBC-C), and total nitrogen (Total N, %), suggesting that healthier, more robust plants tend to accumulate more phenolics, beneficial for plant defense mechanisms. Moreover, soil biochemical properties such as CO_2-C and MBC-C, indicators of microbial activity, show positive correlations with each other and dehydrogenase activity (DHA), indicating interconnected microbial processes. These markers, however, negatively correlate with soil pH, implying that lower pH levels could enhance microbial and enzyme activities, beneficial for basal respiration and microbial biomass.



(array([0.68909194, 0.2129486])

	PC1	PC2
0	5.117979	-1.700923
1	1.190113	2.424325
2	-0.666132	1.177648
3	-2.180160	0.018320
4	-3.461801	-1.919370

Figure 3. Principal component analysis (PCA) of the dataset showing the distribution of different vermicompost dosages and their impact on basil.

Table 6. Correlation matrix showing the relationships among various plant growth parameters, soil properties, and biochemical indicators for basil

	PH	LL	FW	DW	TPC	CO_2-C	MBC-C	DHA	pH	EC	OM	Total N	P	K
PH			☑	☑				☑						
LL			☑		✓			✓						
FW	☑	☑		☑				☑						
DW	☑		☑					☑						
TPC		✓				✓	✓			✓	☑	✓	☑	✓
CO_2-C					✓		☑	☑	⊗	☑	☑	☑	☑	☑
MBC-C					✓	☑		☑	⊗	☑	☑	☑	☑	☑
DHA	☑	✓	☑	☑		☑	☑		⊗	☑	☑	☑	☑	☑
pH						⊗	⊗	⊗		⊗	⊗	⊗	⊗	⊗
EC					✓	☑	☑	☑	⊗		☑	☑	☑	☑
OM					☑	☑	☑	☑	⊗	☑		☑	☑	☑
Total N					✓	☑	☑	☑	⊗	☑	☑		☑	☑
P					☑	☑	☑	☑	⊗	☑	☑	☑		☑
K					✓	☑	☑	☑	⊗	☑	☑	☑	☑	

✓= positive correlation ($P<0.05$), ☑= strong positive correlation ($P<0.01$), ⊗= strong negative correlation ($P<0.01$), *n*= 15, PH= plant height, LL= leaf length, FW= fresh weight, DW= dry weight.

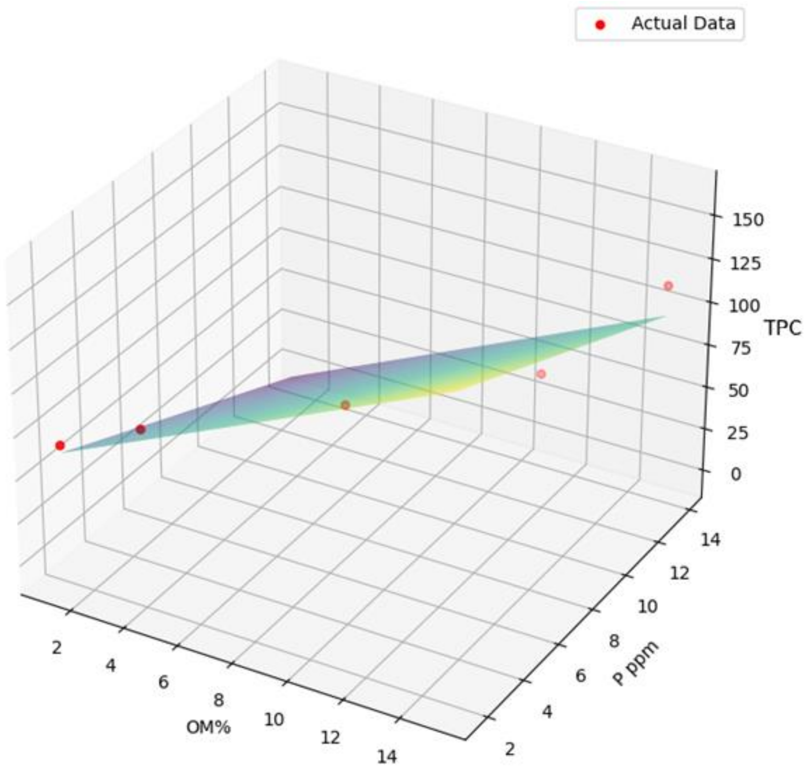


Figure 4. 3D plot of the regression model illustrating the relationship between soil organic matter, (OM) content (%), phosphorus (P) content (ppm), and total phenolic content (TPC) in basil plants.

The analysis also demonstrates strong negative correlations between soil pH and microbial activities, underscoring the critical role of pH in enhancing microbial efficiency and nutrient cycling. Optimally managing soil pH can significantly improve nutrient availability and uptake by plants. In addition, strong positive correlations between organic matter content and key soil nutrients like total N content and phosphorus (P) highlight the importance of organic matter as a nutrient reservoir, improving soil structure and fertility. The relationships between TPC and nutrients such as total N and phosphorus suggest that nutrient management influences phenolic content, important for plant health and disease resistance. Balanced fertilization strategies can enhance microbial activity, supporting overall soil health and plant growth.

Multiple regression analysis was also carried out to explore the impact of soil properties, specifically organic matter (OM) and phosphorus (P) contents, on the total phenolic content (TPC) of basil plants because of the result as a strong correlation according to Pearson correlation analysis. We utilized multiple regression analysis to model the relationship between these variables, providing a quantitative assessment of how changes in soil composition influence TPC. The model was constructed using data gathered from various vermicompost treatments applied to the soil, which were expected to modify the soil's OM (%) and P content (ppm) (Figure 4).

The derived multiple regression equation given in Equation 1:

$$y = 7.09356 \times OM - 5.53497 \times P + 63.99723 \quad (1)$$

where $\hat{y}$ represents the predicted TPC. The model indicates that each unit increase in OM%, holding P constant, is associated with an increase of 7.09356 in TPC. Conversely, each unit increase in P, with OM% held constant, is associated with a decrease of 5.53497 in TPC. Our research results emphasize the need for careful management of P levels in soil to avoid inhibiting phenolic production, particularly in phosphorus-rich environments. This finding is consistent with the observations of Malusà et al. (2006), who reported that phosphate deficiency increased soluble phenolic content in bean roots, suggesting that high P levels have the opposite effect. Similarly, Asami et al. (2003) highlighted the importance of managing soil phosphorus to prevent a reduction in phenolic synthesis. Further supporting our findings, Phares et al. (2020) observed that higher P availability for plant uptake was associated with reduced total flavonoid content, which includes phenolic compounds. This suggests a disruption in phenolic synthesis due to elevated soil pH resulting from increased P levels. Zargoosh et al. (2019) also noted a correlation between soil P availability and total phenol content, indicating that increased soil phosphorus could play a key role in reducing phenol levels in specific environmental contexts. Additionally, Pang et al. (2022) found that phosphorus addition reduced lignin and vanillyl-type phenols, indicative of disrupted phenolic synthesis in plants. Zhang et al. (2023) further reported that high soil phosphorus inhibited the accumulation of polyphenols in tea plants, aligning with our conclusions about the negative impacts of excessive P on phenolic content in plants. This study also highlights the complex interactions influenced by agricultural practices and environmental conditions. High P levels have been consistently shown to correlate negatively with phenolic content in various plants, suggesting that phosphorus might suppress the synthesis of defense-related secondary metabolites.

4. Conclusion

Further research is essential to understand the intricate relationships between different soil nutrients and secondary metabolite production in plants. This includes exploring interactions between phosphorus and other micronutrients, as well as the effects of varying soil pH and microbial activity on phenolic synthesis. By leveraging these insights, agricultural practitioners can refine their fertilization strategies to improve crop yield and quality, focusing on the beneficial phenolic compounds that contribute to the health benefits of many plants and herbs like basil. This analysis underscores the complexity of plant-soil interactions and highlights the importance of understanding these relationships for optimizing agricultural inputs, improving crop quality, and enhancing environmental sustainability. Future studies will be pivotal in deepening our understanding of these dynamics, leading to more informed and sustainable agricultural practices.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	F.Ş.H.T.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1501228

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 451-458 / September 2024

DEVELOPMENT OF CLUSTER TOMATO VARIETIES RESISTANT/TOLERANT TO TOMATO YELLOW LEAF CURL VIRUS (*TYLCV*) AND *Fusarium oxysporum* f.sp. *Radicis-lycopersici* (*Forl*) THROUGH MOLECULAR MARKER-BASED PLANT BREEDING

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Abstract: The global spread of viral and fungal diseases has led to a decline in tomato production as farmers are forced to abandon their crops. To combat these diseases, researchers have developed techniques using molecular-assisted selection to identify plant varieties that are resistant to these diseases. This study focused on cultivating cluster tomato varieties that are resistant or tolerant to *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*Forl*) and *Tomato Yellow Leaf Curl Virus* (*TYLCV*) using molecular DNA markers. The breeding program involved isolating genomic DNA from 69 cluster tomato varieties and then using PCR with *C2-25* and *Ty3P6-25* primers to identify which varieties were resistant or tolerant to *Forl* and *TYLCV*, respectively. Out of the 66 cluster tomato varieties, 20 were resistant or tolerant (*RR*) to *Forl*, 37 were heterozygous resistant or tolerant (*Rr*), and 9 were susceptible (*rr*). Among the 3 cluster tomato varieties, some were resistant or tolerant (designated as *RR*) to *TYLCV*, while others were heterozygous resistant or tolerant (*Rr*), and some were susceptible (*rr*) to the disease. This indicates that DNA molecular markers can reliably determine the presence of resistance or tolerance to *Forl* and *TYLCV* in cluster tomatoes. Molecular markers can efficiently screen thousands of tomato plants in a shorter time period, leading to the selection of more high-quality, resistant or tolerant varieties.

Keywords: *Forl*, *TYLCV*, Resistance, Cluster tomato, Molecular marker, Plant breeding

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Received: June 14, 2024
Accepted: July 13, 2024
Published: September 15, 2024

Cite as: Basım H, Kandil O. 2024. Development of cluster tomato varieties resistant/tolerant to *Tomato Yellow Leaf Curl Virus* (*TYLCV*) and *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*Forl*) through molecular marker-based plant breeding. BSJ Agri, 7(5): 451-458.

1. Introduction

Vegetables are an essential part of human nutrition, and one such vegetable that is often underrated is the tomato (*Solanum lycopersicum* L.). Tomatoes are rich in cancer-fighting compounds and are widely traded agricultural products. Their extensive use in the food industry, particularly in canned goods and sauces, has established them as one of the primary tropical vegetables in global agricultural production (Toor et al., 2006; Gull and Nayik, 2020). The tomato is a diploid plant with 24 chromosomes and belongs to the Solanum genus and the Solanaceae family of the Solanoideae subfamily (Davies et al., 1981). The Solanum genus contains not only cultivated species like *S. lycopersicum*, but also wild species such as *S. pimpinellifolium*, *S. glandulosum*, and *S. cheeseman* (Kil et al., 2016). The tomato, originally from Central and South America, was first cultivated on the coast of Peru (Hedrick, 1919). It is widely cultivated in approximately 144 countries, making it one of the most valuable vegetables (Hassan, 2020). Global tomato production reached 187 million tons in 2020. Türkiye ranked third with 13.204 million tons, following China and India (FAO, 2022).

There are different varieties of tomatoes that grow as single fruits or clusters. The market value, production quantities, and demand for tomato fruits are determined based on their visual characteristics and quality. These fruits are then presented to the market for consumption and export. Cluster tomatoes generally have a greater market advantage than single harvested tomatoes due to their fresh appearance and visual appeal. Harvesting tomatoes in clusters reduces labor costs and time (Beno-Moualem et al., 2004). Tomatoes are an important part of the global economy, but they are susceptible to various diseases caused by pathogens and insects. These diseases can lead to a significant decrease in crop yield, nutrient content, shelf life, and fruit quality, and can even kill the plant. Fungi and viruses are examples of the types of pathogens that can infect tomato plants.

The major viral pathogens that can negatively impact tomato production include: *Tomato Yellow Leaf Curl Virus* (*TYLCV*), *Tomato Brown Rugose Fruit Virus* (*TBRFV*)

(Salem et al., 2016; Luria et al., 2017), *Tomato Mosaic Virus* (*ToMV*)*, Tomato Ring Spot Virus* (*ToRSV*)*, Potato Y Virus* (*PYV*)*, and Tomato Spotted Wilt Virus (TSWV)* (Wani et al., 2010). In addition, *Fusarium oxysporum* is known to be one of the most important fungal pathogens affecting tomato plants (Hassan, 2020). Two specific strains of *Fusarium oxysporum* can infect tomatoes. The first one, called *F. oxysporum* f.sp. *radicis-lycopersici* (*Forl*), causes *Fusarium* crown and root rot, while the second one, called *F. oxysporum* f.sp. *lycopersici* (*Fol*), causes vascular wilt (Armstrong and Armstrong, 1981). *Forl* is a pathogenic fungus found in the soil around various plant species worldwide and is known to cause *Fusarium* crown and root rot in tomatoes and other crops, making it an economically significant problem. The pathogen was first discovered in South Florida, USA (Sonoda, 1976) and was later detected in Turkey for the first time in 2004 (Can et al., 2004). Symptoms of crown and root rot caused by *Forl* include yellowing and wilting of plants, as well as severe root rot. These symptoms can worsen under certain conditions, such as low temperatures (10-20 °C), moist soil, saline water, and low pH levels, ultimately resulting in rapid spread and increased impact on the plant (Hassan, 2020).

Given the lack of an effective fungicide to combat *Fusarium* wilt disease in tomato cultivation, as well as the inefficiency of soil solarization, the most viable solution appears to be the use of tomato plants resistant to *Forl* (Szczechura et al., 2013). This resistance is due to a single dominant gene, *Frl*, on the 9th chromosome of the tomato (Fazio et al., 1999; Truong et al., 2011). *Fusarium oxysporum* has become a significant soil-borne pathogen affecting tomato production. It has led to substantial losses in tomato cultivation in various countries, including the United States, Mexico, Israel, and specific regions of Turkey (Geng et al., 2012). *Tomato Yellow Leaf Curl Virus* (*TYLCV*) is a pathogen belonging to the Begomovirus genus of the Geminiviridae family. It has a single-stranded circular DNA genome of around 2.8 kb. *TYLCV* is responsible for significant losses in the tomato economy (Abhary, 2007; Hull, 2009). Earliest evidence of *TYLCV* was found in Israel during 1939-1940. It was associated with outbreaks of the whitefly (*Bemisia tabaci*) and observed to be a harmful disease agent affecting tomato cultivation in Jordan during the 1960s (Cohen and Nitzany, 1966). It was named *Tomato Yellow Leaf Curl Virus* (*TYLCV*) (Cohen and Harpaz, 1964). The presence of *Tomato Yellow Leaf Curl Virus* (*TYLCV*) was first reported in areas where tomatoes were grown in Türkiye (Yılmaz, 1978).

Tomato crops can suffer significant economic losses due to the *TYLCV* disease, resulting in yield losses of up to 100% depending on the stage of infection (Moriones and Navas-Castillo, 2000). *TYLCV* is a disease that poses a significant threat to countries with economically important tomato production, such as China, India, the United States, and Türkiye. The infection caused by this disease has spread to the many regions worldwide, including tropical, subtropical, and temperate regions, and affects a wide range of hosts, including tomatoes. The severity of the infection in tomato plant populations is directly related to the level of the vector for *Tomato yellow leaf curl virus* (*TYLCV*) known as *Bemisia tabaci* (Ghanim et al., 1998). To prevent losses caused by the *TYLCV* disease, it is important to take measures to stop the virus from spreading in areas where tomatoes are grown (Czosnek and Laterrot, 1997; Czosnek and Ghanim, 2011). One suggested method is to use tomato plant varieties that are resistant to pests and diseases (Moriones et al., 2007). Genetic studies have revealed that the resistance of tomatoes to *TYLCV* is primarily controlled by multiple genes. The development of resistant tomato varieties relies mainly on Ty-3 (Zamir et al., 1994; Hanson et al., 2000; Ji et al., 2007; Ji et al., 2009; Anbinder et al., 2009).

The use of molecular markers and genetic mapping techniques has sped up the selection of high-quality, disease-resistant tomato varieties in breeding programs. This allows for the screening of thousands of plants in a shorter time. This selection process was first introduced in tomato plants (Tanksley, 1983; Tanksley et al., 1992). Molecular markers are tools that help identify gene loci resistant to certain diseases in an organism. This information aids in selecting parents for breeding programs. Two types of molecular markers have been developed: hybridization-based and PCR (Polymerase Chain Reaction)-based. PCR-based methods include Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD), Single Nucleotide Polymorphism (SNP), and Amplified Fragment Length Polymorphism (AFLP). These techniques are used to develop and detect disease-resistant tomato varieties (Yang et al., 2014; Hanson et al., 2016). Sequence Characterized Amplified Region (SCAR) and Cleaved Amplified Polymorphic Sequence (CAPS) are important PCR-based molecular techniques used for the selection of tomatoes resistant to *TYLCV* and *Fusarium* (Nevame et al., 2018).

The aim of this study is to develop tomato varieties that are resistant or tolerant to *TYLCV* and *Forl* diseases using molecular marker-assisted selection techniques developed with the advancement of modern biotechnology in our breeding program.

2. Materials and Methods

In this study, samples from the young leaves of 69 cluster tomato varieties grown in a greenhouse were placed into sterile 1.5 mL Eppendorf tubes. Genomic DNA was then extracted from the samples using the CTAB method (Doyle and Doyle, 1990). The concentration of the genomic DNA samples was measured using a Thermo ND-1000 spectrophotometer, which showed a concentration of 100 ng/mL. Finally, the samples were stored at +4°C for further use.

To determine the resistance or tolerance of cluster tomato varieties (69) to *TYLCV* and *Forl*, PCR (Polymerase Chain Reaction) was performed using gene-specific primers *C2-25* and *Ty3P6*-25, respectively (Table

1). The PCR reactions for *Forl* and *TYLCV* consisted of 1.2 µL DNA (100 ng/µL), 1.25 µL 10X Dream *Taq* Buffer (containing 20 mM MgCl2), 1 µL dNTP (2.5 mM), 0.25 µL *Taq* polymerase (5U), 0.25 µLforward and reverse primers (10 mM). The final volume was adjusted to 12 µL with ddH_2O.

For the *Forl* test, the PCR cycling parameters were as follows: initial denaturation at 94 °C for 1 minute, followed by denaturation at 94 °C for 25 seconds, annealing at 55 °C for 35 seconds, extension at 72 °C for 1 minute and 30 seconds, for a total of 35 cycles. This was followed by a final extension at 72 °C for an additional 5 minutes.

Similarly, for the *TYLCV* test, the PCR cycling parameters were as follows: initial denaturation at 94 °C for 3 minutes, denaturation at 94 °C for 30 seconds, annealing at 53 °C for 1 minute, extension at 72 °C for 1 minute for a total of 35 cycles. This was followed by a final extension at 72 °C for an additional 10 minutes.

PCR products for *Forl* were digested using *Xap*I enzyme. For each sample, a reaction was set up with 10.5 µL of PCR product, 0.66 µL of 10X buffer, 0.66 µL of *Xap*I enzyme (500 U), and the final volume was adjusted to 20 µL with ddH_2O. The samples were incubated overnight at 34 °C. The PCR products *(Ty3P6*-259) and cleavage products (*C2-25*) were loaded onto a 1.5% agarose gel prepared in 0.5X TAE (Tris-acetate-EDTA) buffer containing ethidium bromide (0.5 mg/mL). The gel was run at 100 volts for 150 minutes. The PCR and cleavage results were visualized using an ultraviolet (UV) light imaging system (Vilber Lourmat, France) and recorded for further analysis.

Table 1. Molecular markers and primers used

Gene	Marker	Primer Sequence 5'…………..3'	Reference
Ty	*Ty3 P6*-25	F:GGTAGTGGAAATGATGCTGCTC R:GCTCTGCCTATTGTCCCATATATAACC	Jensen et al., 2007
frl	*C2-25*	F:ATG GGC GCT GCA TGT TTC GTG R:ACACCTTTG TTGAAAGCCATC CC	Staniaszek et al., 2014

3. Results

Based on the results of PCR tests using the *C2-25* and *Ty3P6-25* primers to determine the genotype of 69 cluster tomato varieties, it was found that 20 varieties were homozygous resistant/tolerant (*RR*) to *Forl*, 37 varieties were heterozygous resistant/tolerant (*Rr*), and 9 varieties were susceptible (*rr*) to the disease. Similarly, for *TYLCV*, 3 varieties were homozygous resistant/tolerant (*RR*), 27 were heterozygous resistant/tolerant (*Rr*), and 39 were susceptible (*rr*). No varieties were found to be simultaneously resistant or tolerant to both diseases. It is worth noting that some samples did not show any band formation. The genotypic analysis was provided in Table 2.

Analysis was conducted to determine the genotypes using C2-25 primers for *F. oxysporum* subsp. *radicis-lycopersici*. Samples with the homozygous resistance (*RR*) genotype showed a 700 bp band, while samples with the heterozygous resistance (*Rr*) genotype displayed bands at 700 and 1000 bp (Figure 1). Similarly, samples with the recessive genotype (*rr*) exhibited a single band at 1000 bp. Additionally, genotypic analysis using *Ty3P6-25* primers for Tomato yellow leaf curl virus (*TYLCV*) revealed that samples with the homozygous resistance (*RR*) genotype produced a single band at 630 bp. Meanwhile, samples with the heterozygous resistance (*Rr*) genotype showed bands at 630 and 320 bp, and samples with the recessive genotype (*rr*) displayed a single band at 320 bp (Figure 2).

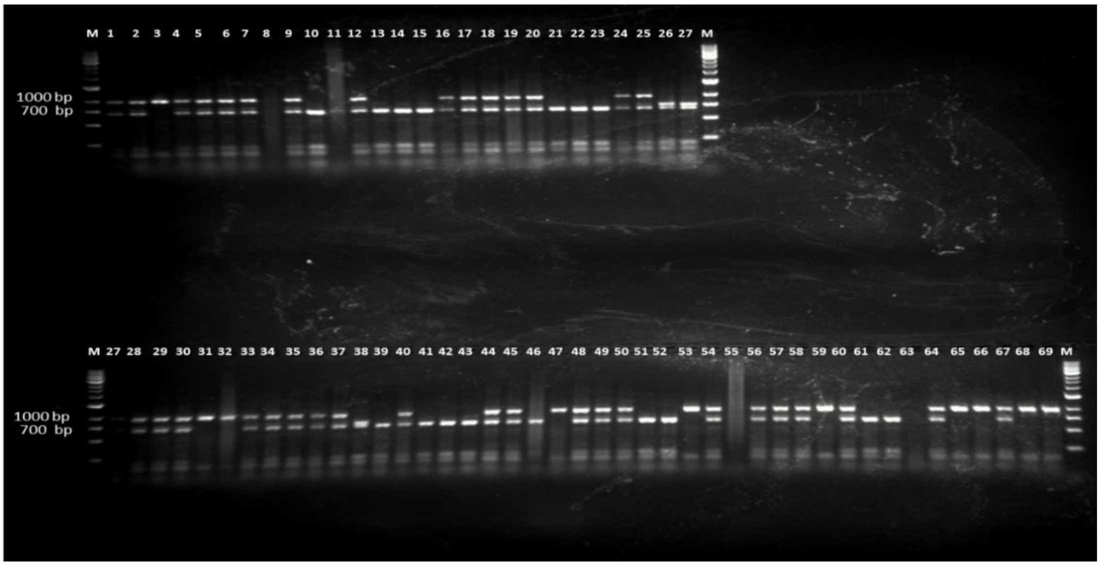

Figure 1. PCR of Cluster Tomato Varieties with *C2-25* primer (M = Marker, 1 kb; 1-69, Cluster Tomato Varieties).

Table 2. Molecular marker-based analyses of cluster tomato varieties (1-69) analyzed by PCR

Test No	*Ty3P6*-25	*C2-25*	Test No	*Ty3P6*-25	*C2-25*
1	*rr*	*Rr*	*36*	*RR*	*Rr*
2	*rr*	*Rr*	*37*	*Rr*	*Rr*
3	*rr*	*rr*	*38*	*rr*	*Rr*
4	*rr*	*Rr*	*39*	*rr*	*RR*
5	*rr*	*Rr*	*40*	*rr*	*RR*
6	*Rr*	*Rr*	*41*	*Rr*	*Rr*
7	*Rr*	*Rr*	*42*	*rr*	*RR*
8	*Rr*	-	*43*	*rr*	*RR*
9	*Rr*	*Rr*	44	*rr*	*RR*
10	*Rr*	*RR*	45	*Rr*	*Rr*
11	*rr*	*RR*	46	*Rr*	*Rr*
12	*rr*	*Rr*	47	*Rr*	*RR*
13	*rr*	*RR*	48	*rr*	*rr*
14	*Rr*	*RR*	49	*rr*	*Rr*
15	*rr*	*RR*	50	*rr*	*Rr*
16	*rr*	*Rr*	51	*rr*	*Rr*
17	*rr*	*Rr*	52	*rr*	*RR*
18	*Rr*	*Rr*	53	*rr*	*RR*
19	*Rr*	*Rr*	54	*Rr*	*rr*
20	*rr*	*Rr*	55	*rr*	*Rr*
21	*rr*	*RR*	56	*Rr*	-
22	*Rr*	*RR*	57	*rr*	*Rr*
23	*Rr*	*RR*	58	*rr*	*Rr*
24	*Rr*	*Rr*	59	*Rr*	*Rr*
25	*Rr*	*Rr*	60	*Rr*	*rr*
26	*rr*	*RR*	61	*rr*	*Rr*
27	*Rr*	*RR*	62	*rr*	*RR*
28	*Rr*	*Rr*	63	*rr*	*RR*
29	*Rr*	*Rr*	64	*rr*	-
30	*rr*	*Rr*	65	*Rr*	*Rr*
31	*rr*	*Rr*	66	*rr*	*rr*
32	*Rr*	*rr*	67	*rr*	*rr*
33	*RR*	*rr*	68	*rr*	*Rr*
34	*RR*	*Rr*	69	*rr*	*rr*
35	*Rr*	*Rr*			

RR= homozygous resistant/tolerant, *Rr*= heterozygous resistant/tolerant, *rr*= susceptible, -= not detected.

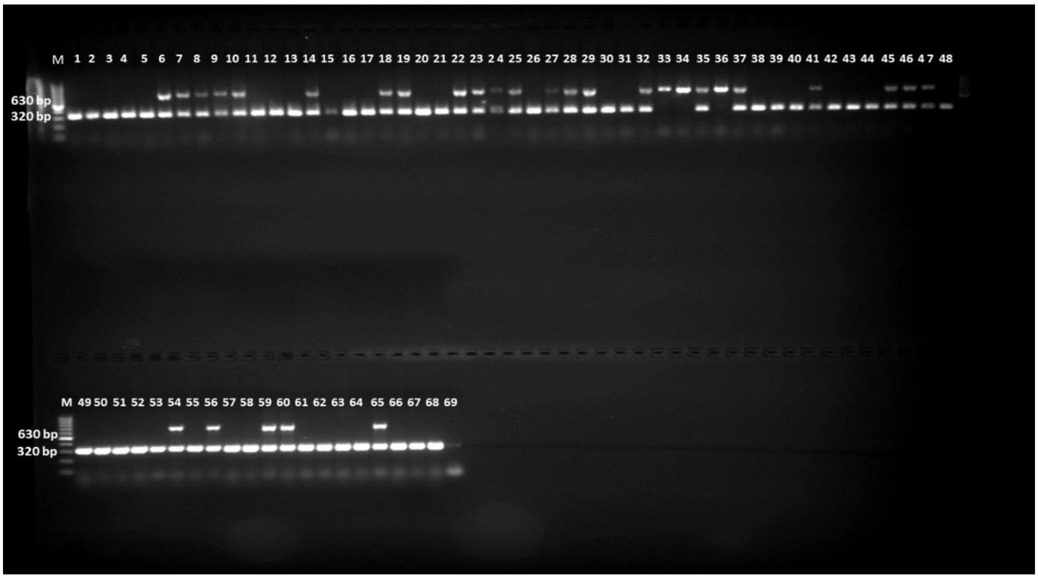

Figure 2. PCR for clustert varieties with *Ty3P6*-25 primer (Marker = Marker 100 bp; 1-69, Cluster Tomato Varieties).

4. Discussion

The tomato (*Solanum lycopersicum* L.) is a popular and nutritious component of the human diet. It contains various vitamins and phenolic compounds that are believed to be effective against many types of cancer. Furthermore, it is one of the most extensively produced and traded agricultural commodities worldwide. However, the widespread occurrence of fungal and viral diseases has forced many farmers to give up on tomato farming altogether. In response to these challenges, modern breeding techniques have been developed to combat these diseases. Traditional breeding methods have primarily focused on selecting plants based only on their physical traits rather than their genetic sensitivity to disease. However, this approach is complicated by the influence of environmental factors and specific farming practices on plant traits, making field screening a complex and time-consuming process (Hanssen et al., 2010; Junker et al., 2015). The most effective way to manage diseases is by selecting resistant varieties. Not only is this method affordable and straightforward, but it's also environmentally safe (Hanssen et al., 2010).

Molecular-assisted selection can accelerate the process of selecting resistant plants. However, not all molecular markers known are suitable for tomato breeding programs. Therefore, further research is necessary to identify and develop allele-specific molecular markers that can improve the use of the molecular-assisted selection method in tomato breeding programs (Foolad and Panthee, 2012).

Marker-Assisted Selection (MAS), has significantly increased the speed and effectiveness of developing more resistant varieties through phenotypic selection (Grube et al., 2000).

"The spread of *TYLCV* presents a significant threat to tomato yield and production. Producers are looking for solutions, and studies suggest that using virus-resistant plant species offers advantages. Modern MAS techniques can greatly facilitate the selection and cultivation process of resistant varieties, reducing the time and effort required (Grube et al., 2000).

Studies have shown that domesticated tomatoes are susceptible to the *Tomato Yellow Leaf Curl Virus* (*TYLCV*). However, certain wild tomato species such as *S. arcanum, S. cheesmaniae, S. chilense, S. galapagense, S. chmielewskii, S. corneliomulleri, S. habrochaites, S. neorickii, S. peruvianum, S. pimpinellifolium,* and *S. pennellii* exhibit symptoms when infected with the virus. This has sparked further investigation into the resistance of these species. Previous studies have identified different gene regions that provide resistance to *TYLCV* (*Ty-1, Ty-2, Ty-3, Ty-4, Ty-5, Ty-6*), with *Ty-1, Ty-2, Ty-3*, and *Ty-4* being dominant, and *Ty-5* being recessive (Zamir et al., 1994; Hanson et al., 2000; Ji et al., 2007; Ji et al., 2009; Anbinder et al., 2009). Both the *Ty-1* and *Ty-2* genes have shown high resistance to the *Tomato Yellow Leaf Curl Virus* (*TYLCV*) and have been widely utilized by breeders. In later studies, it was discovered that *Ty-2* and *Ty-3* genes have no impact on begomoviruses, and their resistance has been overcome by specific strains of *TYLCV* (Ji et al., 2007). The *Ty-2* and *Ty-3* genes may not be as effective as the Ty-3 and Ty-3a genes in providing resistance against *TYLCV*. Resistance to *TYLCV* is dependent on the *Ty-3* and *Ty-3a* genes (Ji et al., 2007; Hanson et al., 2016; Nevame et al., 2018). The *Ty-3* gene, which has two allelic genes obtained from the *S. chilense*, were named *Ty-3* and *Ty-3a. Ty-3* was derived from *LA2779* and *Ty-3a* from *LA1932*. Co-dominant markers were developed for both genes, and it was found that the *P6-25* marker yielded results for both *Ty-3* and *Ty-3a*. While the Ty-3 gene provides broader resistance, the *Ty-3a* gene is preferred due to its association with fewer undesirable traits, while still retaining the resistance gene (Ji et al., 2007).

Specific markers known as *Ty3P6-25* markers were created to identify the *Ty3* gene region. PCR analysis using *Ty3P6-25* revealed that samples with a homozygous (*RR*) resistance genotype produced a single band of 630 bp. On the other hand, samples with a heterozygous (*Rr*) resistance genotype showed two bands at 630 bp and 320 bp. Additionally, samples with a homozygous recessive (*rr*) genotype exhibited a single band of 320 bp (Jensen et al., 2007). Tomato varieties that are resistant or tolerant against the *Tomato Yellow Leaf Curl Virus* (*TYLCV*) and have both homozygous and heterozygous traits were developed using the *Ty-3* gene-specific *Ty3P6-25* primer. Currently, there is no effective fungicide to combat Fusarium wilt (*Forl*) disease in tomato cultivation. Though soil solarization is inadequate, the use of *Forl*-resistant tomato plants has been considered the most viable method to combat the disease (Szczechura et al., 2013).

The first mapping study concerning *Forl* in tomatoes identified a *Forl*-resistant gene region in *Solanum peruvianum*, a wild species of tomato, known as *Forl* (Laterrot and Moretti, 1991; Fazio et al., 1999). The gene (*frl*) provides genetic resistance to tomato and is controlled by a single dominant gene on the 9th chromosome (Laterrot and Moretti, 1991).

A study was conducted to identify markers associated with *Frl* and explore the connection between the *Forl*-resistant *Frl* locus in tomato and the *Tm-2* locus, which confers resistance to multiple strains of *Tobacco mosaic virus* (*TMV*). A cross was made between the 'Motelle' breeding line and 'IRB-301-31', and fifteen to sixty seedlings from the F3 generation were tested for resistance to *Fusarium* and the *TMV* "0" strain. The results showed a strong linkage between *Frl* and *Tm-2* (Vakalounakis et al., 1997).

The gene-specific marker *C2-25* for the *frl* gene region was created by Staniaszek et al. (2014). In PCR analysis using this marker, samples with homozygous resistant (*RR*) genotype displayed a 700 bp band, while samples with heterozygous resistant (*Rr*) genotype showed two bands at 700 and 1000 bp. Susceptible varieties (*rr*) produced a single 1000 bp band. This study developed homozygous and heterozygous resistant or tolerant cluster tomato varieties among the selected cluster tomato varieties using the *C2-25* marker specific to the *Frl* gene region.

5. Conclusion

Molecular markers have been developed against two economically important diseases caused by *Forl* and *TYLCV*. These molecular markers can be used successfully to create resistant/tolerant cluster tomato varieties against these diseases. The use of molecular markers in plant breeding programs has been proven to be fast, easy, and advantageous in numerous studies. However, it is important to note that genotypes developed through molecular marker-assisted selection may produce different results in real-world conditions due to the complex nature of pathogens, the emergence of new variants, and variability in virulence. A genotype that is resistant in one region may exhibit susceptibility in another region. Therefore, it is necessary to confirm the reliability of the marker and the true resistance of genotypes to the disease after inoculation with region-specific pathogens.

After completing this study, we will conduct further research to confirm the resistance or tolerance of cluster tomato varieties obtained by conducting pathogenicity tests using pathogens from different geographical regions. Additionally, we will separately measure the susceptibility of *Forl* and *TYLCV* resistant or tolerant varieties to different biotic markers. This will enable us to develop resistance to different biotic agents within the same cluster tomato variety.

This method will enable testing a larger variety of materials and reduce the duration of breeding programs, ultimately increasing the success rate. By using modern breeding methods, individuals with desired traits can be hybridized among heterozygous individuals selected from the two disease resistances. This will result in cluster tomato varieties that are resistant or tolerant to both diseases simultaneously.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.B.	O.K.
C	70	30
D	70	30
S	100	
DCP	60	40
DAI	70	30
L	100	
W	100	
CR	50	50
SR	50	50
PM	30	70
FA		100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

The authors give thanks to İstanbul Tarım A.Ş. for funding this search.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1511604

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 459-465 / September 2024

DETERMINATION OF CHANGES IN PHYSICAL AND TECHNOLOGICAL CHARACTERISTICS OF SOME POTATO (*Solanum tuberosum* L.) VARIETIES GROWN IN KONYA UNDER LONG STORAGE CONDITIONS

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Abstract: In this study conducted in 2022 year under the ecological conditions of Konya, five registered potato varieties (Innovator, Russet Burbank, Metro, Brooke, Lady Olympia) were cultivated, and certain physical and technological characteristics were examined before and after a six-month long storage period at conditions of 4-6 °C and 90-98% humidity. These characteristics, including dry matter content, chips yield, French fries yield, and the color values of chips after frying (L*, a*, b*) were assessed both before and after storage, and weight losses at the end of storage were also recorded. At the end of the storage period, there were variations in the physical and technological characteristics of the tubers. According to the overall average of the potato varieties, by the end of storage compared to pre-storage, the dry matter content of potato tubers increased by 2.72%, chip yield by 0.48%, French fries yield by 5.09%, and the a* value by 55.37%. On the other hand, the L* value decreased by 8.39%, the b* value by 28.17%, and the weight loss during storage showed a decrease of 4.61%. In terms of industrial type, based on dry matter content, Brooke and Innovator varieties had the highest values. Excluding the Melody variety, all other varieties showed high yields in chips and French fries production. The variety with the least weight loss detected was the Innovator.

Keywords: Physical characteristics, Potato, Storage, Technological characteristics, Variety

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Received: July 06, 2024
Accepted: July 26, 2024
Published: September 15, 2024

Cite as: Çöl Keskin N. 2024. Determination of changes in physical and technological characteristics of some potato (*Solanum tuberosum* L.) varieties grown in Konya under long storage conditions. BSJ Agri, 7(5): 459-465.

1. Introduction

Potatoes, even after being harvested, continue to exhibit metabolic activities, making them living organisms. This characteristic elevates the significance of proper storage conditions to preserve their quality and extend shelf life. During storage, potato tubers undergo quality changes negatively influenced by moisture loss, decay, and physiological deteriorations. The alteration in physical, technological, and quality characteristics depends on the duration of storage, the temperature and humidity level, the presence of air, and gas components of the storage location. The desired outcomes post-storage are minimized weight loss, reduced changes in tuber quality, and the least amount of observed diseases in the tubers (Eltawil et al., 2006). To minimize storage losses, delay physiological aging, and achieve positive results in chip quality at the end of storage, pre-harvest applications of natural and synthetic sprout growth inhibitors have been conducted in many studies, yielding successful outcomes (Baydar et al., 2009; Şanlı and Karadoğan, 2013; Ok and Şanlı, 2021). On the other hand, factors such as storage types, storage temperature, the differences in storage structures, storage duration, and understanding how pre-harvest conditions affect tuber quality and its relationship with tuber dormancy are among the factors that directly influence the changes in the tuber before and after storage (Öztürk, 2010; Kibar, 2012; Yurtlu et al., 2012; Öztürk et al., 2016; Alamar et al., 2017; Şanlı et al., 2019). The purpose of storage is crucial in determining the storage temperature. Generally, after the harvested tubers are placed in storage, the storage temperature is gradually reduced by 1 °C every 1-2 days. Additionally, it is documented in the literature that tubers intended for seed use should be kept at 2-4 °C to prevent dormancy for a longer period, those intended for culinary use should be kept at 3-5 °C to preserve cooking properties, and industrial tubers should be kept at 7-10 °C to maintain the increase in reducing sugars at a certain level (Karadoğan and Şanlı, 2019). Depending on storage conditions, potato tubers lose water through transpiration, which will manifest as weight loss and shriveling of the tubers. Additionally, metabolic processes such as respiration in potato tubers require energy, utilizing a portion of the dry matter, particularly starch, resulting in a reduction in tuber weight due to dry matter loss. Storage diseases and pests can further exacerbate these losses. Consequently, there will be changes in the chemical composition of the tubers during the storage period (Wustman and Struik, 2007). Differences in pre-harvest care practices, such as the amount of water provided and nitrogen fertilization, also lead to changes in quality parameters such as the protein content of the tuber (Çalışkan and Akkamış, 2023). On the other hand, climate changes are also expected to adversely affect the storage of potatoes, tuber sprouting, and storage diseases (Zhang et al., 2024). After long-term storage, the loss of apical dominance occurs, and the resulting bud sprouting and branching directly affect the number of stems in the plant during the growing season. Since the number of main stems determines the tuber size distribution, it is crucial to bring the seed potatoes to the growing season with minimal storage weight loss (Eshel, 2015). After the end of dormancy, the cumulative temperature during storage directly affects physiological aging. Additionally, light conditions and genotypic characteristics are among the factors that influence physiological aging (Struik, 2007).

In potato breeding and cultivation, determining post-storage quality and physical changes is as important as yield. This study aims to identify the physical and technological changes of some registered potato varieties under long-term storage conditions (6 months).

2. Materials and Methods

The experiment was conducted at the Prof. Dr. Abdülkadir Akçin trial field of the Faculty of Agriculture, Selçuk University, during the 2022 growing season (May-September). Before planting, soil samples were taken from various points in the trial field at a depth of 0-30 cm. According to the soil analysis results, the texture class was identified as loamy, with an organic matter content of 1.37%, inorganic nitrogen at 8.6 mg kg^{-1}, phosphorus at 5.0 mg kg^{-1}, potassium at 48 mg kg^{-1}, and a pH of 7.81. According to climate data obtained from the Konya Meteorology Regional Directorate, the average temperature and relative humidity values during the study period in 2022 were found to be close to the long-term averages (1980-2021). The average temperature for the months of May to September was recorded as 20.6 °C over the long term, while it was 21 °C in 2022. The average relative humidity values were 46.3% over the long term and 45.1% in 2022. The total precipitation in June was significantly higher in the long-term average (26.1 mm) compared to 2022 (10.4 mm), whereas the total precipitation amounts for the other months were found to be similar.

The experiment was conducted using a "Randomized Complete Block Design" and the storage data were analyzed using the same design with factorial. Five registered potato varieties (Innovator, Russet Burbank, Metro, Brooke, and Lady Olympia) were used as materials in the study. The characteristics of the varieties used in the research are provided in Table 1. In the experimental field, tubers were hand-planted into rows marked with markers, with each plot measuring 70x30 cm. Each plot consisted of 4 rows, and the plot length was set to 5 meters. The experiment was set up with 3 replications. Tubers harvested from each plot were placed into 5 kg mesh potato bags and stored in a storage facility with a temperature of 4-6 °C and humidity adjusted to 90-98%. In addition to the 5 kg of tubers placed in mesh potato bags from each plot, additional randomly selected tubers were taken their pre-storage technological properties were measured. At the end of the storage period (September-March, "6 months"), the technological properties of randomly selected tubers from each mesh potato bags were determined as post-storage properties. Additionally, at the end of the storage period, the weights of the tubers in the mesh potato bags were measured, and the weight loss percentage was calculated by comparing it to the pre-storage weight (Öztürk et al., 2016). The technological properties calculated both pre and post storage included dry matter content (%) (Kacar, 1994), chips yield (%) (Anonymous, 2001), French fries yield (%) (Şenol, 1973), and post-frying color values measured using a Minolta Chromo Meter CR 200b device, with lightness (L*), red/greenness (a*), and yellow/blueness (b*) values recorded respectively (Torrico et al., 2019).

The data obtained as a result of the study were subjected to analysis of variance using JMP 11 software (JMP Version 11, SAS Institute Inc. Cary, NC, 1989–2021) and the differences between the averages were grouped by “LSD Multiple Comparison Test” with the computer statistic program MSTAT-C (Michigan State University, v. 2.10).

Table 1. Characteristics of the varieties used in the experiment

Varieties	Maturity Group	Usage Area
Brooke	Mid-Late	Table and Industrial
Innovator	Mid-Late	Chips and French Fries
Lady Olympia	Mid	Chips and Industrial
Metro	Mid-Early	Table and Industrial
Russet Burbank	Late	French Fries

3. Results

3.1. Dry Matter Content (%)

Before storage, the dry matter content of the varieties showed that the highest value was found in the Brooke variety (22.46%), followed by Russet Burbank (22.24%), Innovator (21.51%), Lady Olympia (21.17%), and Metro (19.26%). The changes in dry matter content post-storage varied among the varieties. The overall average increased from 21.33% to 21.91%. The varieties that showed a decrease in dry matter content after storage were Brooke (1.47% decrease) and Russet Burbank (6.97% decrease). It was determined that the dry matter content of the other varieties increased after storage (Table 2).

3.2. Chip Yield (%)

Pre- and post-storage chip yields of varieties were found to be higher in varieties bred for chipping and French fry production, while the table variety Metro exhibited the lowest values (37.18%-31.49%). Excluding the Metro variety, all other types were categorized in group (a), and numerical comparisons revealed the highest yield of 46.46% with the Lady Olympia, followed by 45.63% with Brooke, 44.79% with Innovator, and 43.03% with Russet Burbank variety. Post-storage chip yield values paralleled the increase in dry matter content, showing a 0.48% rise, thus recording an experimental average of 43.63%. At the end of storage, chip yields of Innovator, Lady Olympia, and Russet Burbank varieties showed increases, whereas Brooke and Metro varieties displayed decreases (Table 2).

3.3. French Fry Yield (%)

The changes in French fry yields from post-harvest to post-storage were parallel to the chip yield values. The lowest French fry yield was observed in the Metro variety at 33.40%. Post-storage, this value increased to 38.15%. The Russet Burbank and Innovator varieties exhibited increases from 44.22% to 44.57% and from 42.37% to 43.00%, respectively.

Table 2. Mean values and statistical groupings of dry matter content and chips yield of cultivars

Cultivars \ Traits	Pre-storage	Post-storage	Pre-storage	Post-storage
	Dry matter content (%)		Chips yield (%)	
Brooke	22.46^{a}	22.13^{b}	45.63^{a}	44.17^{a}
Innovator	21.51^{bc}	23.61^{a}	44.79^{a}	48.55^{a}
Lady Olimpia	21.17^{c}	22.81^{ab}	46.46^{a}	48.83^{a}
Metro	19.26^{d}	20.30^{c}	37.18^{b}	31.49^{b}
Russet Burbank	22.24^{ab}	20.69^{c}	43.03^{a}	45.14^{a}
Mean	21.33	21.91	43.42	43.63^{a}
LSD(%)	0.27**	1.31**	4.60**	4.75**

a,b= The differences between the means shown with the same letters and the same column in the same group are insignificant (*: P<0.05, **: P<0.01, NS: non-significant).

Table 3. Mean values and statistical groupings of French-fry yield and storage weight loss of cultivars

Cultivars \ Traits	Pre-storage	Post-storage	Storage weight loss (%)
	French-Fry Yield (%)		
Brooke	45.53^{a}	42.60^{b}	5.34
Innovator	42.37^{a}	43.00^{b}	3.52
Lady Olimpia	41.85^{a}	49.58^{a}	5.47
Metro	33.40^{b}	38.15^{c}	3.87
Russet Burbank	44.22^{a}	44.57^{b}	4.88
Mean	41.47	43.58	4.61
LSD(%)	7.00*	3.68**	NS

a,b= The differences between the means shown with the same letters and the same column in the same group are insignificant (*: P<0.05, **: P<0.01, NS: non-significant).

Table 4. Mean values and statistical groupings of a and b of cultivars

Traits \ Cultivars	Pre-storage	Post-storage	Pre-storage	Post-storage	Pre-storage	Post-storage
	Chips of L* value		Chips of a* value		Chips of b* value	
Brooke	41.76	41.76	-4.18a	-1.82	21.43	14.07
Innovator	45.65	45.65	-3.23a	-1.10	20.29	16.55
Lady Olimpia	38.30	38.30	-2.28ab	-1.68	19.70	15.35
Metro	39.18	39.18	-1.51bc	-0.46	18.59	13.85
Russet Burbank	35.38	35.38	-0.91c	-0.33	15.83	9.06
Mean	40.05	40.05	-2.42	-1.08	19.17	13.77
LSD	NS	NS	1.72*	NS	NS	NS

A decrease in French fry yields was noted in other varieties post-storage. The trial average showed a proportional increase from 41.47% to 43.58%, amounting to a 5.09% rise (Table 3).

3.4. Storage Weight Loss (%)

The weight loss rates of the varieties before and after storage did not exhibit statistical significance. However, when comparing the numerical data of weight losses among the varieties, the highest weight loss was recorded in the Lady Olympia variety at 5.47%, followed by 5.34% in Brooke, 4.88% in Russet Burbank, 3.87% in Metro, and 3.52% in Innovator (Table 3).

3.5. *L Value of Chips**

The L* values of chips before and after storage did not show statistical significance after frying. Numerically compared, the highest L* value was measured in the Innovator variety both pre-storage (45.65) and post-storage (39.92), while the lowest value was recorded in the Russet Burbank variety before storage (35.38) and in the Metro variety after storage (32.26). A decrease in the trial average from 40.05 to 36.69 was observed (Table 4).

3.6. a* Value of Chips

The pre-storage a* values of the varieties were found to be statistically significant at the 5% level. The highest values were observed in the Russet Burbank (-0.91) and Metro (-1.51) varieties, while the lowest value was recorded for the Brooke variety (-4.18). No statistically significant differences were found among the a* values of the varieties after storage. Numerically, as before storage, the highest value was again determined in the Russet Burbank variety (-0.33), followed by the Metro variety (-0.46). According to trial averages, the a* value increased from -2.42 before storage to -1.08 after storage (Table 4).

3.7. b* Value of Chips

According to trial averages, the b* values were 19.17 before storage and decreased to 13.77 after storage. No statistical differences were identified between the two periods, and a decrease was observed in all varieties post-storage. Numerically, the highest pre-storage b* value was 21.43 in the Brooke variety, while post-storage, the highest was 16.55 in the Innovator variety. The lowest b* value was recorded in the Russet Burbank variety, with 15.83 before storage and 9.06 after storage (Table 4).

4. Discussion

4.1. Dry Matter Content

As reported by Pinhero et al. (2009), the dry matter content varies according to cultivation conditions and genotype. During storage, tubers undergo respiration, reducing starch to sugar within the tuber, which indirectly leads to a decrease in dry matter. Additionally, the physiological age of the tuber, maturity group, storage temperature, and duration directly affect the dry matter content (Mazza, 1983a; 1983b). In a study conducted over two years, the dry matter content of tubers showed variability among varieties, with a trial average decrease of 1.46%. By variety, Marfona showed a decrease of 4.32% in dry matter content after storage, Toscana 3.91%, Binella 2.43%, Granola 9.96%, and Natascha 6.64%, while Banba and Slaney exhibited increases of 4.24% and 5.72%, respectively (Ozturk and Polat, 2016). In a research conducted by Baijal and Van Vliet (1966), a reduction in the dry matter contents of tubers was observed by the end of storage. The dry matter content consists of 70% starch, and fluctuations in dry matter content also impact the starch level. A study designed to investigate changes in tubers over various storage durations explained that the reduction in starch content at the end of storage was due to the decomposition of starch necessary for the respiration of the tubers. Additionally, it was noted that as the storage duration increased, the development of shoots in the tubers led to greater moisture loss from these shoots, potentially causing a proportional increase in dry matter contents (Özcan et al., 2019). The study observed variability in dry matter contents among different varieties; decreases were noted in Brooke and Russet Burbank varieties, while increases were detected in other varieties. The findings of the researchers aligned with the dry matter values recorded in the study, demonstrating that the lowest dry matter content found in the Metro variety, a culinary type, was consistent with its characteristics.

4.2. Chip Yield

The chemical composition of a tuber determines whether a potato is suitable for industrial use. Industrial-type potato varieties are desired for their high dry matter content, high chip yield, and low oil absorption during frying. Specific gravity directly affects chip yield, with varieties having a higher specific gravity typically showing higher yields. As specific gravity correlates positively with dry matter content, varieties with high dry matter levels are expected to also have high chip yields. Additionally, it is preferred that chips do not darken excessively during frying. Besides these criteria, the physiological age of the tuber is also significant (Sowokinos, 1978; Lulai and Orr, 1979; 1980). Research findings have varied by varieties. A study conducted by Karadoğan (1994) under Erzurum conditions reported that chip yields for 15 potato varieties ranged between 27.56% and 48.21%, with a positive correlation noted between chip yields and dry matter contents. Kara (1996) found an increase in chip yields corresponding to a decrease in tuber weight loss during storage. A study initiated by Kita (2002) reported that chip texture underwent various changes during storage, linked to alterations in the water content within the chip texture. Another study conducted under storage conditions found that post-storage chip yields ranged between -8.40% and 3.85%. Some varieties showed an increase in chip yield values, while others showed a decrease (Ozturk and Polat; 2016). The variations in chip yield values in this study are consistent with existing literature.

4.3. French Fry Yield

In a study conducted by Özcan et al. (2019), it was found that as storage duration increased, the yields of French fries decreased. The highest French fry yield was observed in the Russet Burbank variety, with a pre-storage value of 35.4% decreasing to 30.9% post-storage. The researchers have explained this by relating it to the dry matter content, noting that an increase in dry matter content proportionally enhances French fry yields. It has also been stated that a reduction in moisture loss in tubers increases their specific gravity and indirectly their dry matter content, which in turn improves French fry yields over the course of storage according to their specific gravity and dry matter content (Şenol, 1970; Şanlı, 2012). The findings related to French fry values in the study are consistent with the researchers' observations.

4.4. Storage Weight Loss

There is a close relationship between the dormancy periods of potato tubers and post-storage weight losses. It has been observed that breaking dormancy leads to an increase in weight losses after storage (Özcan et al., 2019). In this field, the findings of some researchers have varied. Accordingly, weight changes identified as storage weight losses, such as those reported by Ozturk and Polat (2016) (2.03%), were found to be above the range of data obtained in this study, while those reported by Okur (2008) (7.2-9.5%) and Özcan et al. (2019) (5.78-13.49%) were closer to the lower end of the observed range. The differences in weight losses among genotypes are influenced by factors such as respiration, transpiration, and sprout formation (Kolbe and Stephan-Beckmann, 1997).

4.5. Color Values of Chips after Frying (L*, a*, b*)

One of the most significant challenges in the potato chip industry is maintaining the color of chips and ensuring consumer appeal. The key factors affecting chip color include variety, storage conditions, storage duration, maturity of the variety, specific gravity and, indirectly, the dry matter content of the variety, as well as cultivation practices (Hill, 1974). When potato tubers are stored at 9-10 °C, tubers exhibit high reducing sugar content, known as low-temperature sweetening (LTS). Tubers stored at this temperature result in chips and French fries that appear dark brown during frying (Maillard browning reaction), which is undesirable to consumers. To prevent this, storage temperatures should be lowered. Storing tubers at lower temperatures offers several advantages: reduced respiration rates leading to lower storage weight losses, natural control of sprouting, control of bacterial and fungal pathogens, avoidance of chemical sprouting inhibitors, and benefits to human health and environmental protection (Pinhero et al., 2012).

Studies on various accessions of the *Solanum* genus have concluded that flesh color is related to carotenoids. The primary carotenoids are zeaxanthin, lutein, violaxanthin, and β-carotene (Burgos et al., 2009). Carotenoids vary with flesh color, with lutein being predominant in yellow-fleshed varieties and zeaxanthin in orange-fleshed varieties (Sulli et al., 2017). Studies have shown a significant relationship between dry matter content, reducing sugar and sucrose levels, and chip color in tubers, directly affecting chip color (cited from Mazza, 1983a;b in Pinhero et al., 2009). Except for the a* value before storage, L* and b* values were not statistically significant in post-frying chip color values, and no dark color appearances were observed during the studies. The frying colors were found to have the brightness and yellowness desired by consumers.

5. Conclusion

Long-term storage has been observed to cause changes in the physical and technological aspects of potato tubers. These changes vary depending on the characteristics of the variety. The Innovator variety exhibited the lowest numerical weight loss, indicating its high resistance to storage conditions. The varieties Brooke, Innovator, Lady Olympia, and Russet Burbank were found to have desirable values for industrial use. Dry matter content is the most critical industrial criterion and can vary according to variety, storage duration and conditions, and the differences in cultural practices during cultivation. Values of this study are significant for increasing researches on storage, one of the most crucial aspects of potato breeding, and for comparing the potential of breeding materials with standard varieties. Further studies should incorporate different storage types and conditions and include additional varieties to enhance the quality and scope of the research.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	N.Ç.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1508023

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 466-470 / September 2024

DETERMINATION OF FISH CONSUMPTION HABITS IN DİYARBAKIR PROVINCE

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Abstract: This study was conducted to determine the fish consumption habits in Diyarbakır province and the reasons that influence these habits. A questionnaire survey was carried out on a total of 3310 individuals, consisting of 1910 males and 1400 females, in the center and districts of Diyarbakır. According to the results, it was found that 16% of the respondents consumed fish and the most preferred fish were anchovy (30.81%) and carp (25.98%). When buying fish, 40.04% of people said that they preferred it to be cheap and 20.63% said that they preferred it to be tasty. The study concluded that the majority of fish consumption occurs during the winter season. The primary reasons for not consuming fish, as indicated by the respondents, were the high price (42.38%) and a lack of purchasing power (38.42%). Looking at the monthly consumption of individuals, 49.78% consume less than 1kg of fish. As a result of the study, it was found that fish consumption in Diyarbakır province is far below the national and world average. In addition to socio-economic reasons, this situation is thought to be due to the fact that fish cannot be consumed in all seasons because the city is far from the sea coast. In addition, the rate of aquaculture and consumption of aquaculture products in Turkey is quite low. In this context, the consumption of aquaculture products should be increased.

Keywords: Fish consumption, Diyarbakir, Consumption habits, Socio-Demographic factors

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Received: July 01, 2024
Accepted: August 03, 2024
Published: September 15, 2024
Cite as: Özcan F, Aydin MK. 2024. Determination of fish consumption habits in Diyarbakır province. BSJ Agri, 7(5): 466-470.

1. Introduction

The fisheries and aquaculture production sector has become one of the food production sectors that has attracted attention in the 21st century due to the need for protein-rich food, especially with the growing population in recent years. As indicated in the FAO (2019) report, aquaculture products represent 17% of the global consumption of animal protein. According to the report of the Food and Agriculture Organization of the United Nations (FAO, 2022), the sector that has shown the most development among food sectors in the last 10 years is the aquaculture sector. In Turkey, as in the world, the fisheries sector is growing every year. Our country, which has three distinct marine ecosystems and a coastline spanning 8,333 km, boasts a significant inland and marine fisheries production capacity, supported by an extensive network of dams, lakes, and rivers (Arslan and Yıldız 2021). The production, which has been based on hunting for many years, has turned to aquaculture in recent years with the increase of aquaculture with the advancement of technology. Although hunting has been less developed than aquaculture in Turkey and in the world in recent years, it has maintained its importance in aquaculture production. When the distribution of sea fish caught by species was examined, anchovy was the fish caught in the largest quantity with 151.598 tons. Anchovy was followed by sprat with 28.041 tons and horse mackerel with 19.590 tons. In 2021, 335 thousand 644 tons of aquaculture production took place in seas and 136.042 tons in inland waters. The most important fish species cultivated in inland waters were trout with 135,732 tonnes, sea bass with 155.151 tons and sea bream with 133.476 tons (TUİK, 2022). As the world's population grows, the agricultural products needed to provide an adequate and balanced diet are decreasing. Water resources are one of the most important factors in nutrition. Animal foods are the main source of protein in the human diet. Among animal foods, the protein and nutritional value of fish is high. Omega-3 fatty acids are mainly found in fish (Kris-Etherton et al., 2002). People in developed countries pay attention to nutrition and choose foods that are best for their health. Seafood is a rich source of polyunsaturated fatty acids, which are beneficial for human nutrition. These acids positively impact human metabolism and physiological functions, and are essential for maintaining a healthy lifestyle (OKA, 2014).

In Türkiye, the income level of the consumer, the price of aquaculture products, consumer preferences, consumer habits and the social and economic structure of the region are the factors that influence the demand and consumption of aquaculture products. The fact that the annual per capita consumption of fish is very low in

Eastern Anatolia, Southeastern Anatolia and Central Anatolia, while it is quite high in the Black Sea and other coastal regions, indicates that the amount of aquaculture products consumed in Türkiye varies by region. For example, the per capita consumption of fish in the Black Sea region is around 25 kg, while this value is calculated to be less than 1 kg in Eastern and Southeastern Anatolia (Karakaya and Kırıcı 2016). Studies on fish consumption in Turkey have mostly focused on determining the structure of fish consumption (Karakaya and Kırıcı 2016; Terin et al., 2016; Şen and Şahin 2017). Considering both the benefits of fish for healthy nutrition and its production potential and added value in Turkey, it is necessary to conduct research to determine the factors affecting fish consumption in Turkey and develop necessary strategies. Survey is the most popular and systematic method of data collection when used under appropriate conditions. This study was conducted to determine the structure of fish consumption and the purchasing tendencies of consumers in the city center of Diyarbakır province. In this way, it aims to determine the place of fish meat in the dietary structure of consumers living in Diyarbakır province.

2. Materials and Methods

The study was conducted to determine the fish consumption habits and quantity in Diyarbakır city center and districts. In order to determine the fish consumption habits of the people living in this city and the reasons for them, the questionnaire consisting of 20 questions was administered to a total of 3310 participants, including 1400 women and 1910 men, who were randomly selected, face to face and in the form of question and answer. At the same time, questions were asked about the type of meat, type of fish, frequency of consumption, amount consumed, reasons for preference and non-preference, and the way the products were prepared. Since it is not possible to survey all individuals in the provinces and districts in terms of time and financial means, the equal probability simple random sampling method was applied and the sampling size (equation 1) was obtained using the following equation when the number of population units is over 10,000 (Yazıcıoğlu and Erdoğan 2014).

$$n=P\times Q\times Z\alpha 2/d2 \qquad (1)$$

n: Sample size,
P: probability of the event occurring,
Q(1-P): Probability that the event does not occur,
$Z\alpha 2$: confidence coefficient (this number is taken to be 1.96 for a 5% margin of error),
d: Sampling error accepted according to the frequency of the event.
The data obtained were analyzed and interpreted in MS-Excel and the results were compared with the results of similar studies.

3. Results

3.1. Demographic Structure

It is observed that 42.30% of the consumers are male and 57.70% are female. In terms of age distribution, 39.87% of the age group is between 21 and 30 years old, while 21.75% of the age group is between 31 and 40 years old. The number of family members is as follows: 28.24% of respondents have four family members, 23.65% have five, 9.06% have three, 8.76% have seven, 7.85% have eight, and 7.55% have six. The respondents were found to have the following levels of education: 53.17% had completed university studies, 25.67% had completed primary school, and 20.24% had completed secondary school. The occupational groups are as follows: 32.77% are employed in some capacity, 26.58% are retired, and 22.35% are civil servants.

3.2. Fish Consumption Habits

In terms of fish consumption, 42.90% of respondents consume chicken, 40% red meat, 16.01% fish and 1.08% meat. The general opinion on fish prices is that 88.15% of the respondents think that fish prices are expensive. 10.27% of the respondents stated that the prices are normal; 0.96% stated that they have no information about fish prices (Figure 1).

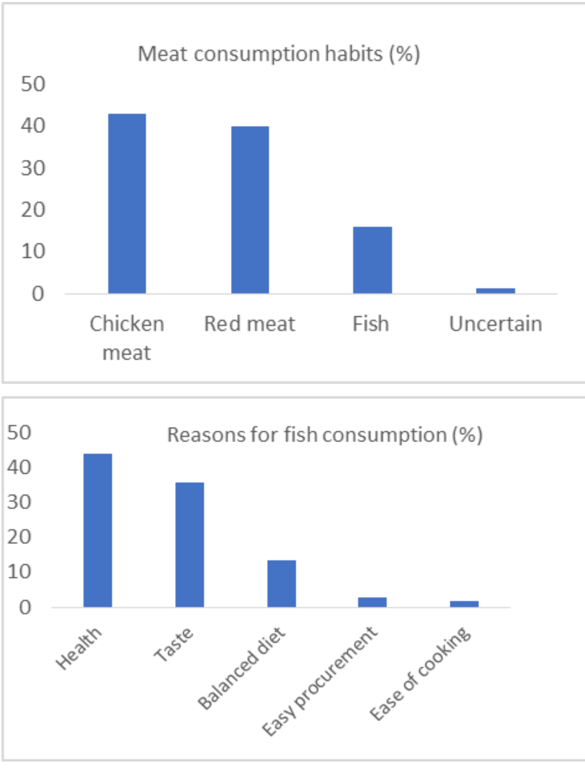
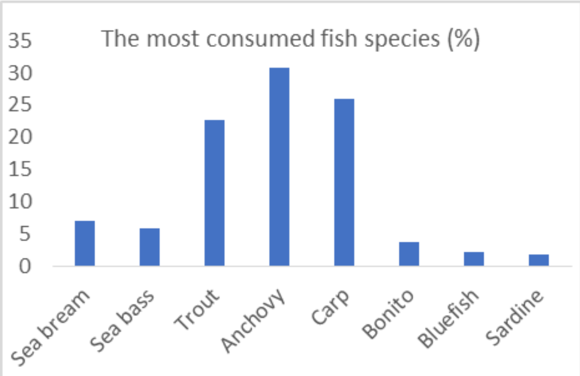


Figure 1. Individuals' meat consumption preferences and reasons for fish choice.

If we look at the frequency of fish consumption, 47.43% of the participants consume fish once a month; 38.67% once a year; 12.53% once every fifteen days; 0.84% do not consume fish; 0.51% consume fish once a week. The reasons for preferring fish are: 43.98% health; 35.64% taste; 13.29% balanced diet; 2.71% easy to obtain; 1.81% easy to cook. According to the respondents, 48.03% of the people who took part in the survey get their fish from the market, 24.16% from the fish market, 14.44% from hawkers, and 11.78% from the fish market. When buying fish, 46.04% of the participants look for factors such as economy, 29.63% for taste, and 22.26% for less bones. If we look at the most consumed fish, 30.81% say anchovy, 25.98% carp, 22.65% trout, 6.94% sea bream, 5.74% sea bass, 3.62% bonito, 2.11% bluefish, and 1.78% sardine (Figure 2).



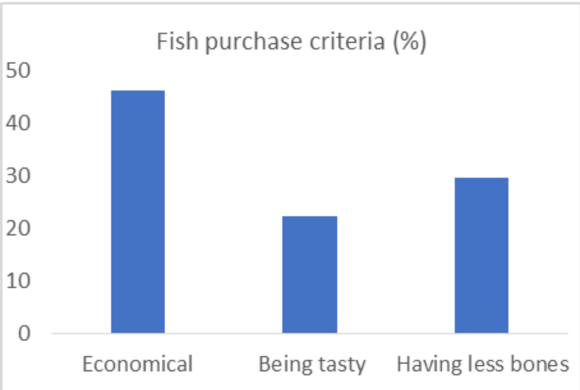


Figure 2. Preferred species of fish and the reasons for this preference.

As a result of the questionnaire, 40.78% of the people consumed less than 1 kg; 24.47% consumed between 1 and 3 kg; 14.19% consumed between 4 and 6 kg; 9.36% consumed more than 10 kg; 8.45% consumed between 6 and 10 kg. 58.61% of the participants stated that they consumed fish mainly in the winter season, 14.35% in the autumn, 11.93% in the summer and 8.45% in the spring. If we look at the way people consume fish, 90.42% eat it fresh, 3.65% eat it canned, 3.53% eat it salted and 1.26% eat it pickled. When it comes to cooking fish, 63.83% of people prefer frying, 33.98% grilling and 1.20% steaming. The reasons for not consuming fish were identified by 42.38% as being expensive; 38.42% as not having the purchasing power; 11.90% as not being tasty; 4.74% as not being available where they live; 1.75% as household size.

4. Discussion

The macro and micro minerals present in fish are of significant importance for maintaining bone health, dental hygiene, dermal integrity, and cellular protection. Furthermore, they are essential for maintaining healthy heart rhythm, blood pressure, fluid balance, muscle function, reproductive system function, and gut flora (Varlık et al., 2004). Cevher (2018) conducted a study in Konya Province and found that 53% of the participants were university graduates, 26% were high school graduates and 11% were middle school graduates. In a study conducted in Antalya province, 34.72% of the participants were university graduates, 31.72% were primary school graduates, 23.08% were high school graduates and 10.48% were middle school graduates (Arslan and İzci 2016). When analyzing the educational status of the participants in Tunceli province, 55.5% of them have university education and 24.4% of them are high school graduates (Yüksel et al., 2011). In this study, 53.17% of the respondents were university graduates, 25.67% were primary school graduates and 20.24% were secondary school graduates. The occupational groups are 32.77% workers, 26.58% pensioners and 22.35% civil servants. In the study conducted by Kırıcı et al. (2018) in the city center of Siirt province, it was found that 40.6% of people consumed white meat, 31.4% consumed red meat and 22.5% consumed fish. Soylu (2018) reported in his study conducted in Kayseri that red meat was the most consumed, followed by chicken meat and then fish. Yüksel et al. (2011) found that red meat (40%), chicken meat (38%) and fish meat (22%) were the most consumed types of meat by people living in Tunceli. Olgunoğlu et al. (2014) conducted a study to determine fish meat consumption habits in Adıyaman and found that the most consumed meat products were chicken meat (56%), red meat (38%) and fish meat (5%). Arslan and İzci (2016) found that the meat consumption rate was 46.96% for chicken meat, 36.12% for red meat and 16.92% for fish meat, respectively. In this study, 42.90% of the respondents consume chicken meat, 40% red meat and 16.01% fish meat. Reasons such as the high nutritional value or the healthiness of fish meat were found to be first in the preference for fish consumption with 72.3% in Tekirdağ province by Abdikoğlu et al. (2015), 29% in Ankara and Çanakkale provinces by Bayraktar et al. (2019), 51.2% in Erzurum, 67.9% in Bayburt and 67.3% in Erzincan by Doğan (2019). In the studies conducted by Kırıcı et al. (2018) in Siirt province and Karakaya and Kırıcı (2016) in Bingöl, the deliciousness factor ranked first in fish consumption preference with 57.6% and 60.1%, respectively. In general, it can be said that fish meat consumption is higher in places closer to water resources, while red meat and chicken meat are consumed more than fish meat in places far from water resources. It can be said that this situation is caused by the eating culture, the transport or the way of getting the fish to the region and the prices. Cevher (2018) conducted a study in Konya and found that 57% of the participants consumed 1-3 kg and 32% consumed 4-6 kg of fish per month. Çelik (2014) found in his study in Manisa that 33% consumed 1-2 kg, 24% consumed 2-4 kg, 20% consumed 1 kg and

23% consumed more than 4 kg of fish, and the per capita consumption of aquaculture products was 7.7 kg/year. In the study conducted in Ankara and Çanakkale provinces, it was found that 81% of participants preferred to consume fish once a week or once a month (Bayraktar et al., 2019). Çiçek et al. (2014) found that 28% of consumers in Elazığ province consumed fish meat once every fifteen days, 25% once a week, 23% once a month, 15% several times a year, 4% two to three times a week, and 5% did not consume fish at all. In a study conducted in Siirt province, the frequency of fish consumption was found to be once a month, with 32.5% of respondents indicating this as their preferred frequency (Kırıcı et al., 2018). In a study conducted by Terin et al. (2016) in Van, the frequency of fish consumption every fifteen days was identified as the most prevalent, with a rate of 30.6%. In their study conducted in Mersin, Şen and Şahin (2017) reported that 43% of individuals consumed fish once a week and 42% consumed fish once a month. In this study, 47.43% of the participants consumed fish once a month, 38.67% consumed fish once a year, 12.53% consumed fish once every fifteen days, 0.84% did not consume fish, and 0.51% consumed fish once a week. Bayraktar et al. (2019) reported that the most consumed fish type was anchovy with 59%, and the highest fish consumption was in winter (37%). In a study conducted in Süleymanpaşa district of Tekirdağ province, it was found that the most consumed marine fish was anchovy with 25.66%, the most consumed freshwater fish was trout with 46.78%, and people consumed fish mostly in winter (34.78%) (Abdikoğlu et al., 2015). In this study, the most consumed fish were anchovy (30.81%), carp (25.98%), trout (22.65%), sea bream (6.94%), sea bass (5.74%), bonito (3.62%), bluefish (2.11%), and sardine (1.78%). In many previous studies carried out throughout Turkey, it has been observed that individuals consume anchovies the most. In other studies, Çolakoğlu et al. (2006) stated that people living in Çanakkale province buy fish from fish markets and fish markets. Temel (2014) stated that 80% of people living in Rize province buy fish from fish markets. Balık et al. (2013) found that the people living in Aybastı and Fatsa districts of Ordu prefer to buy fish from peddlers and fish markets, while Aydın and Karadurmuş (2013) found that the people living in Trabzon and Giresun provinces generally (50.81%) procure fishery products from fish stalls. Erdal and Esengün 2008 found that families living in Tokat prefer certain fish sellers (85%) when buying fish. In this study, 48.03% of the participants said that they bought fish from the market, 24.16% from the fish market, and 14.44% from travelling vendors, 11.78% from the fish market and 0.60% from the fish market. In a study conducted in Adıyaman, 41% of the participants fried the fish in oil, 35% cooked it in the oven and 23% cooked it on the grill (Olgunoğlu et al., 2014). Cevher 2018, in his study in Konya, found that 60% of the participants preferred frying and 20% preferred grilling. In this study, 63.83% of individuals preferred frying, 33.98% preferred grilling and 1.20% preferred steaming. In a study conducted in Antalya province, it was found that 80% of the individuals consumed fresh seafood products (Arslan and İzci, 2016).

In this study, the consumption pattern of fish among individuals was examined and it was found that 90.42% consumed fresh fish, 3.65% consumed canned fish, 3.53% consumed salted fish and 1.26% consumed salted fish. A review of the data from the surveys conducted in our country reveals that a significant proportion of the population consumes fresh fish. In contrast, the consumption of processed fish products remains relatively low. The reasons for this can be attributed to the fact that both aquaculture and fishing supply the market with fresh fish, which is readily available at all times. Consequently, the consumption of processed fish products is not a habit that is widely practiced. In a study conducted in Tokat province, the average annual per capita fish consumption was 13 kg/year (Erdal and Esengün 2008), 4.1 kg/year in Tunceli province (Yüksel et al., 2011), 3.6 kg/year in Elazığ province (Çiçek et al., 2014), 21.5 kg/year in Hatay province (Demirtaş et al., 2014), 20.07 kg/year in Rize province (Temel 2014), 14.69 kg/year in Tekirdağ province (Abdikoğlu et al., 2015), 7.7 kg/year in Manisa province (Dereli et al., 2016), 16.8 kg/year in Van province (Terin et al., 2016). In this study, as a result of the survey, monthly fish consumption was determined as follows: 40.78% of individuals consumed less than 1 kg; 24.47% between 1 and 3 kg; 14.19% between 4 and 6 kg; 9.36% more than 10 kg; 8.45% between 6 and 10 kg.

5. Conclusion

The consumption rate of aquaculture products in the province is well below the world and national averages. A review of the research findings reveals that the majority of consumers perceive fish as a nutritious and healthy food. Nevertheless, a considerable number of respondents indicated that fish prices are elevated. It is very important to analyses the market demand very well and to meet the demand in time. It is important that fish is available on the market at the desired time, especially in the winter season, and that it is fresh. Promotional and production activities should be emphasized to encourage the consumption of aquaculture products, which are healthy food, in the province with a high youth population. Product promotion, especially of processed products, cold chain transport of fish, widespread use of hygienic fish markets and activities to promote fish consumption in schools will increase fish consumption.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	F.Ö.	K.A
C	100	
D	100	
S	80	20
DCP	80	20
DAI	80	20
L	80	20
W	80	20
CR	80	20
SR	80	20
PM	80	20
FA	80	20

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there is no animal or human research. Written and informed consent forms were obtained from participants for the study.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1500833

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 471-476 / September 2024

DETERMINATION OF THE RELATIONSHIP OF HARMONIZATION RATIO AND SOME YIELD CHARACTERISTICS WITH GRAIN YIELD IN BARLEY

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Abstract: In this study, it was aimed to calculate the harmonization ratio before and after the flowering period (GFP_{tt}/VP_{tt}) in barley depending on thermal times, to determine the vegetative period (VP) and grain filling period (GFP) values for higher grain yield (GY) capacity and the possibility of using these qualities as adaptation and selection criteria and to determine the relationship between HR and GY calculated according to phenological periods depending on thermal times under Kahramanmaraş conditions. The experiments were carried out between 2014 and 2016 for 2 successive years with 3 replicates according to the randomized completed block design with 9 genotypes. Phenological traits such as VP, GFP, GY and days to maturity (DM) were measured in relation to thermal times. According to the results, genotypes were found to be significantly different in terms of harmonization ratio in barley. Considering the two-year averages, the highest grain yields were determined as Samyeli (534.3 kg da^{-1}) and Şahin-91 (532.8 kg da^{-1}). The lowest grain yields were determined as Kendal (404.9 kg da^{-1}) and Sur-93 (416.9 kg da^{-1}). The highest harmonization ratios were determined as Samyeli (0.753) and Kendal (0.672), the lowest harmonization ratios were determined as Şahin-91 (0.486) and Athena*Yabani (0.558). Although there was a general relationship between grain yield and harmonization ratio, some genotypes had values outside this trend.

Keywords: Harmonization ratio, Vegetative period, Grain filling period, Yield, Barley

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Received: April 02, 2024
Accepted: July 30, 2024
Published: September 15, 2024

Cite as: Tanrıkulu A. 2024. Determination of the relationship of harmonization ratio and some yield characteristics with grain yield in barley. BSJ Agri, 7(5): 471-476.

1. Introduction

Barley, which was previously used directly in human nutrition, is now cultivated and bred for two main purposes in the world and in our country: as a feed for animal nutrition and for malt-beer production in the industry (Kılınç et al., 1992).

In Türkiye, there is 3 million hectares of cultivation and 8.3 million tons of production in 2020 (TUIK, 2021a) and the most barley production are Konya, Ankara, Afyonkarahisar, Aksaray, respectively (TUIK, 2021b). In 2022, barley cultivation area was 3.2 million ha, production was 8.5 million tons and yield was 266 kg da^{-1} (Anonymous, 2022).

With a production of 157 million tons and a cultivation area of 51 million hectares, barley ranks 4th among cereals in the world after wheat, maize and rice, with a share of 3.5% in agricultural areas, 17.4% in cool climate cereals and 7% in total cereal areas (FAO, 2020).

About half of the increase in yield per unit area was due to genetic improvement and the other half to improvements in breeding technique in all grains. Genetic improvement has been made possible by the improvement of agronomic characters that increase yield potential or tolerance to stresses. Increases in yield potential have also led to changes in phenological periods. Determining the effects of phenological periods on yield will allow the development of new high-yielding varieties. In addition, knowing how phenological periods affect yield will help to make more realistic yield predictions. For the first time, the effects of VP (vegetative period), GFP (grain filling period) and DM (days to maturity) on grain yield were evaluated together for durum wheat cultivars GFP_{tt} /VP_{tt} and it was revealed that the harmonization ratio (HR) obtained by proportioning them was significantly related to grain yield (Akkaya et al., 2006).

The relationship between growing-degree-days (GDD) and the time of peak tassel emergence in maize varieties, GDD index values were calculated by taking max. and min. temperature values for each day of the period from sowing to peak tassel emergence, using the formula (equation 1)

$$GDD = \text{Daily max. temperature} + \text{Daily min. temperature}/2\text{-}10 \quad (1)$$

The peak tassel emergence time shortened with the increase in air temperatures, and that the delay in sowing time and the earliness characteristics of the varieties also played a role in this decrease. The fact that the GDD value started to decrease from the beginning of July revealed that the planting time should not be delayed further (Choelho and Dale, 1980). It was reported that barley varieties with short heading time had long heading-maturity duration, varieties with high grain yields had high harvest indexes, varieties with low grain yields had low harvest indexes, and varieties with high yields were generally two-rowed in terms of thousand grain and hectoliter weight (Kırtok and Genç, 1979), (Choelho and Dale, 1980), the time of peak tassel emergence in maize is also related to temperature totals and the method used to determine this is GDD (Kiniry and Keener, 1982), in a study investigating the direct effects of temperature on developmental stages in soybean, thermal time provides a better determination of dry matter accumulation (Mayers et al., 1991) aimed to observe the development rate of barley, bread wheat, durum wheat, triticale and oat cereal varieties grown in 5 different locations with rainfed cereals, especially with flowering, and barley varieties matured earlier, barley tillered more, flowered earlier, had higher grain yield than other cereals, matured as barley, bread wheat, triticale, durum wheat and oat, respectively, and barley reached physiological maturity 10 days earlier than other species (Lopez and Richards, 1994) was determined.

The thermal time from sowing to harvest was reported to be 1808 (± 23) GDD at 3 main temperatures (9 - 29 - 39^{o}C) used in the maturity estimation of peanut (Bell and Wright, 1998), while leaf formation at different sowing times in triticale, except when the photoperiod was below 11 hours, (above 0^{o}C) (Naylor and Su, 1998), the number of fully developed leaves on the main stem is directly related to thermal time from sowing onwards in the development of photoperiod sensitive sorghum in Mali (Vaksmann et al., 1998).

In this study, it was aimed to evaluate barley genotypes in terms of phenological periods (VP, GFP, DM) in barley genotypes grown in Kahramanmaraş conditions, to examine their relationships with GY and to calculate the harmonization ratios evaluating GFP_{tt} /VP_{tt} = HR_{tt} together with phenological traits and to determine the traits that can be used as selection criteria for this region.

2. Materials and Methods

In this research, genotypes belonging to barley species (Kendal, Samyeli, Promesa*Yabani, Eralam*Yabani, Şahin-91, Efes-98, Sur-93, Athena*Yabani, Altıkat) were used in Kahramanmaraş conditions in the growing periods of 2014 - 2015 and 2015 - 2016 with 3 replications according to the randomised completed block design.

When the 2-year growing period was analyzed, the lowest temperature was realized in January with 1.6^{o}C in 2015-2016 period and the highest temperature was realized in June with 33.7^{o}C in the same period (Anonymous, 2016). The Mediterranean climate is effective in the region and the temperature difference between seasons is high. Winters are generally warm and rainy, and summers are hot and dry (Figure 1 and Figure 2). In the soil of the experiment, phosphorus and potassium from macro plant nutrients were moderately sufficient, calcium was too much, magnesium was too much, micronutrients except manganese were low or insufficient, organic matter coverage was low, lime coverage was high, and it was slightly alkaline (Anonymous, 2015).

Sowing was carried out on December 5, 2014 in the first year and on December 28, 2015 in the second year with a 6-row plot seeder with a spacing of 20 cm on plots of 8.30 meters in length and 400 grains per/m^2 in barley. Sowing depth was 3 - 4 cm and plot size was 1.2 m x 8.3 m = 9.96 m^2 in both years.

Fertilization was completed with 20-20-0 (compound) commercial fertilizer at 7 kg da^{-1} of pure N and 7 kg of pure P_2O_5 as base fertilizer at sowing in both years, and with 33% Ammonium Nitrate (NH_4NO_3) at 7 kg da^{-1} of pure N at the end of tillering in the second part and 35 kg da^{-1} in the second part.

In the rain-fed study, weeds were controlled manually and narrow and broad-leaved weeds were controlled with selective herbicide (2.4 - D Amine) in the spring season.

Near the harvest time, 1.15 meters from the beginning and end of each plots and 1 row from the edges of the plots were removed as edge effect, and the remaining part (0.8 m x 6 m = 4.8 m^2) was left. In addition, during the ripening period of the plants, the plots were mowed with a sickle for 1 meter each, the mowed plants were dried for 2-3 days and then weighed and biomass yields were determined. Then the parcels were threshed with a threshing machine.

HR (Harmonization ratio) values were calculated with the formula $HR_{tt} = GFP_{tt} / VP_{tt}$ (Harmonization ratio $_{thermal\ times}$= Grain Filling Period $_{thermal\ times}$ / Vegetative Period $_{thermal\ times}$) depending on thermal times. Days to maturity (DM), grain filling period (GFP), vegetative period (VP) were evaluated by Gebeyehou et al. (1982) and Lopez Castaneda and Richards (1994). Celsius scale was used with a base 0^{o}C for thermal time calculations for VP, DM, and GFP. The analysis of variance of the data belonging to the mentioned characters was performed using SAS package program (SAS, 1999) and Duncan multiple comparison test was used to compare the means.

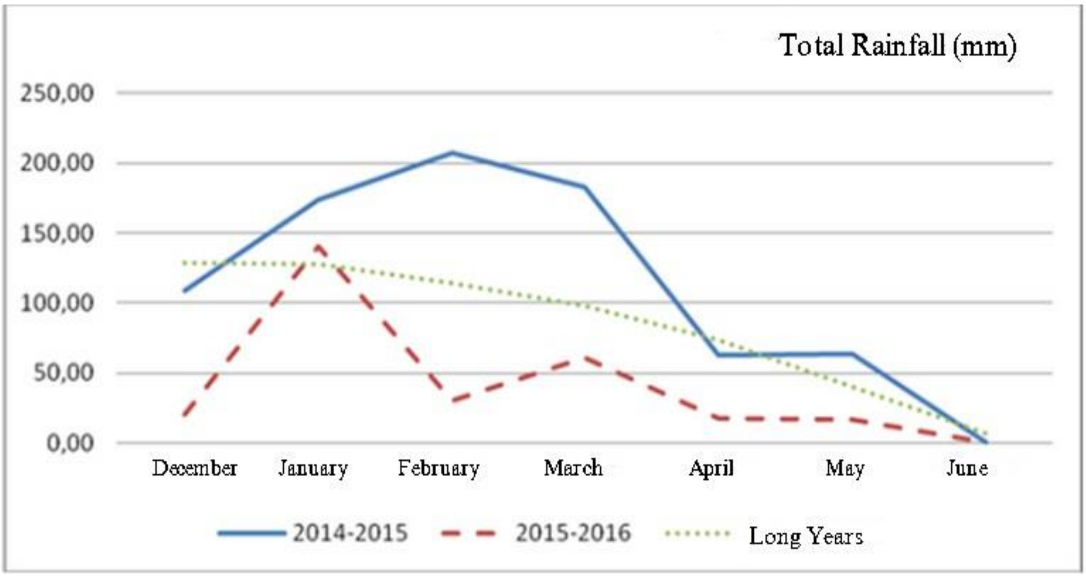

Figure 1. Graph of total rainfall (mm) data for the experimental years and long years (1960 - 2015).

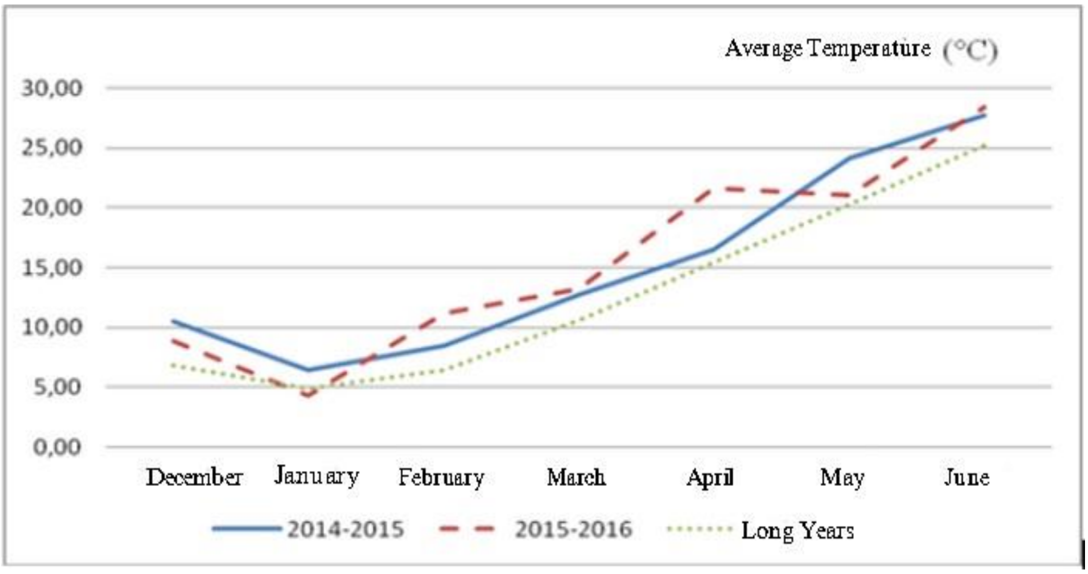

Figure 2. Graph of average temperature (°C) data for the trial years and long years (1960 - 2015).

3. Results and Discussion

As a result of the evaluation of the genotypes in terms of the traits examined by considering the two-year averages; they were found to be significantly different in terms of heading duration, vegetative period, grain filling period, days to maturity, harmonization ratio, grain yield, protein content, 1000-grain weight and hectoliter weight (Table 1, Table 2 and Table 3).

According to the results of correlation analysis, there is no statistically significant difference between grain yield and harmonization ratio in barley (Table 4). The reason for this is thought to be problems such as grain shedding, bird damage, not all varieties are registered and they have different genetic structure.

In terms of vegetative period values, the highest value was obtained from Şahin-91 genotype with 132.5 days, while the lowest value was obtained from Samyeli genotype with 114.1 days (Table 1). In another study, vegetative period values were 67.3 and 64.6 days and grain filling period values were 38.6 and 37.0 days, respectively (Öztürk et al., 2001). In terms of grain filling period values, the highest value was obtained from Samyeli genotype with 41.1 days, while the lowest value was obtained from Şahin-91 genotype with 31.0 days. (Table 1). In terms of days to maturity values, the highest value was obtained from Şahin-91 genotype with 163.5 days, while the lowest value was obtained from Samyeli genotype with 155.3 days (Table 1).

Table 1. Phenological characteristics of the genotypes in the years of the study

Genotypes	Vegetative Period			Grain Filling Period			Days to Maturity		
	14-15	15-16	Average	14-15	15-16	Average	14-15	15-16	Average
Kendal	130.3^{f}	108.3^{c}	119.3^{d}	41.0^{ab}	34.3^{abc}	37.6^{b}	171.3^{bc}	142.6^{de}	157.0^{de}
Samyeli	124.0^{g}	104.3^{d}	114.1^{e}	45.0^{a}	37.3^{a}	41.1^{a}	169.0^{c}	141.6^{e}	155.3^{e}
Promesa*Yabani	135.6^{d}	114.6^{b}	125.1^{b}	37.3^{bc}	32.3^{bc}	34.8^{cd}	173.0^{bc}	147.0^{b}	160.0^{bc}
Eralam*Yabani	138.0^{c}	114.3^{b}	126.1^{b}	37.0^{bc}	32.0^{bc}	34.5^{cd}	175.0^{ab}	146.3^{bc}	160.6^{b}
Şahin-91	146.0^{a}	119.0^{a}	132.5^{a}	31.6^{d}	30.3^{c}	31.0^{e}	177.6^{a}	149.3^{a}	163.5^{a}
Efes-98	139.6^{b}	113.3^{b}	126.5^{b}	34.6^{cd}	33.0^{bc}	33.8^{d}	174.3^{ab}	146.3^{bc}	160.3^{bc}
Sur-93	138.0^{c}	112.6^{b}	125.3^{b}	35.3^{cd}	34.0^{abc}	34.6^{cd}	173.3^{abc}	146.6^{bc}	160.0^{bc}
Athena*Yabani	137.3^{c}	113.3^{b}	125.3^{b}	36.3^{bc}	31.3^{bc}	33.8^{d}	173.6^{ab}	144.6^{bcd}	159.1^{bcd}
Altıkat	132.6^{e}	109.0^{c}	120.8^{c}	39.3^{bc}	35.3^{ab}	37.3^{bc}	172.0^{bc}	144.3^{cd}	158.1^{cd}
Averages	135.7^{a}	112.1^{b}	123.9	37.5^{a}	33.3^{b}	35.4	173.2^{a}	145.4^{b}	159.3

14= 2014, 15= 2015, 16= 2016

Table 2. Traits of the genotypes in the years of the study

Genotypes	Harmonization Ratio			Grain Yield			Heading Duration		
	14-15	15-16	Average	14-15	15-16	Average	14-15	15-16	Average
Kendal	0.692^{ab}	0.652^{abc}	0.672^{b}	140.5^{c}	669.4^{ab}	404.9^{c}	133.0^{e}	110.6^{c}	121.8^{e}
Samyeli	0.764^{a}	0.741^{a}	0.753^{a}	340.0^{ab}	728.7^{a}	534.3^{a}	127.3^{f}	106.3^{d}	116.8^{f}
Promesa*Yabani	0.604^{cd}	0.530^{de}	0.567^{c}	308.8^{b}	587.9^{bc}	448.4^{bc}	141.3^{c}	117.0^{b}	129.1^{bc}
Eralam*Yabani	0.596^{cd}	0.533^{de}	0.564^{c}	334.9^{ab}	558.3^{c}	446.6^{bc}	143.6^{b}	116.0^{b}	129.8^{b}
Şahin-91	0.509^{e}	0.463^{e}	0.486^{d}	415.6^{a}	650.0^{abc}	532.8^{a}	148.6^{a}	120.6^{a}	134.6^{a}
Efes-98	0.563^{de}	0.560^{cd}	0.562^{c}	325.7^{ab}	612.9^{bc}	469.3^{abc}	143.6^{b}	116.3^{b}	130.0^{b}
Sur-93	0.568^{de}	0.588^{bcd}	0.578^{c}	275.4^{b}	558.3^{c}	416.9^{c}	142.0^{c}	106.3^{b}	128.3^{c}
Athena*Yabani	0.583^{cde}	0.534^{de}	0.558^{c}	355.5^{ab}	662.0^{ab}	508.8^{ab}	142.3^{bc}	114.6^{b}	128.8^{bc}
Altıkat	0.650^{bc}	0.660^{ab}	0.655^{b}	366.8^{ab}	579.6^{bc}	473.2^{abc}	136.0^{d}	111.3^{c}	123.6^{d}
Averages	0.614^{a}	0.584^{b}	0.599	318.1^{a}	623.0^{b}	470.6	139.7^{a}	114.2^{b}	127.0

14= 2014, 15= 2015, 16= 2016

Table 3. Quality characteristics of the genotypes in the years of the study

Genotypes	Protein Content in Grain			1000-Grain Weight			Hektoliter Weight		
	14-15	15-16	Average	14-15	15-16	Average	14-15	15-16	Average
Kendal	13.7^{c}	12.1^{ab}	12.9^{ab}	34.1^{e}	30.1^{f}	32.1^{f}	55.4^{a}	58.9^{c}	57.1^{b}
Samyeli	13.7^{c}	13.6^{a}	13.6^{a}	45.9^{ab}	50.4^{ab}	48.1^{ab}	56.9^{a}	68.9^{a}	62.9^{a}
Promesa*Yabani	13.4^{d}	12.5^{a}	13.0^{ab}	46.3^{ab}	47.0^{bcd}	46.6^{bc}	55.4^{a}	64.2^{abc}	59.8^{ab}
Eralam*Yabani	14.1^{b}	12.4^{a}	13.3^{ab}	43.7^{bc}	45.9^{cde}	44.8^{cd}	56.0^{a}	63.5^{abc}	59.7^{ab}
Şahin-91	12.7^{e}	12.4^{a}	12.6^{b}	44.1^{abc}	48.3^{bc}	46.2^{bc}	55.7^{a}	65.5^{ab}	60.6^{ab}
Efes-98	13.8^{c}	12.7^{a}	13.2^{ab}	41.9^{c}	43.9^{de}	42.9^{de}	53.0^{a}	60.9^{bc}	56.9^{b}
Sur-93	14.8^{a}	12.4^{a}	13.6^{a}	47.0^{a}	52.4^{a}	49.7^{a}	54.8^{a}	68.9^{a}	61.8^{a}
Athena*Yabani	13.8^{c}	12.1^{ab}	12.9^{ab}	39.0^{d}	42.5^{e}	40.8^{e}	56.9^{a}	62.9^{abc}	59.9^{ab}
Altıkat	12.1^{f}	10.5^{b}	11.3^{c}	33.8^{e}	31.1^{f}	32.5^{f}	53.5^{a}	49.5^{d}	51.5^{c}
Averages	13.6^{a}	12.3^{b}	12.9	41.8^{a}	43.5^{b}	42.6	55.3^{a}	62.5^{b}	58.9

14= 2014, 15= 2015, 16= 2016

Table 4. Correlation coefficients of investigated characteristics of the genotypes as an averages of two years of the study

Traits	PC	HD	DM	HR	VP	GFP	1000-GW	HW	GY
PC	1.000								
HD	0.513**	1.000							
DM	0.536**	0.963**	1.000						
HR	0.092	-0.174	0.073	1.000					
VP	0.498**	0.997**	0.959**	-0.195	1.000				
GFP	0.294*	0.198	0.448**	0.878**	0.178	1.000			
1000-GW	0.256	-0.032	-0.082	-0.255	-0.630	-0.189	1.000		
HW	-0.433**	-0.863**	-0.923**	-0.203	-0.855**	-0.509**	0.352**	1.000	
GY	-0.535**	-0.800**	-0.867**	-0.145	-0.797**	-0.499**	0.178	0.862**	1.000

*Significant at 5 % level, **Significant at 1 % level. PC= protein content per grain, HD= heading duration, DM= days to maturity, HR= harmonization ratio, VP= vegetative period, GFP= grain filling period, 1000-GW= 1000-grain weight, HW= hectoliter weight, GY= grain yield.

The harmonization ratios varied between 0.486 - 0.753, the highest harmonization ratios were observed in Samyeli (0.753) and Kendal (0.672) genotypes, while the lowest harmonization ratios were observed in Şahin-91 (0.486) and Athena*Yabani (0.558) genotypes, respectively (Table 2). In terms of grain yield values, the highest value was obtained from Samyeli genotype with 534.3 kg da^{-1} and the lowest value was obtained from Kendal genotype with 404.9 kg da^{-1} (Table 2). The reason why the grain yield of Kendal variety was very low in the first year of the experiment was due to bird damage to the plots of this variety in the first year. In another study, grain yield of different barley genotypes varied between 158- 458 kg da^{-1} under barren conditions and between 2.82-6.34 kg da^{-1} under irrigated conditions (Sönmez and Yüksel, 2019). When the average of two years was analyzed, the highest heading duration value was obtained from Şahin-91 genotype with 134.6 days and the lowest value was obtained from Samyeli genotype with 116.8 days (Table 2). In the studies conducted in Bafra plain, it was determined that the heading duration of the varieties used in the studies ranged between 126.0-133.5 days (Sirat and Sezer, 2017a) and the heading duration ranged between 160-170 days in Samsun (Sirat and Sezer, 2017b).

In terms of 1000-grain weight values, the highest value was obtained from Sur-93 genotype with 49.7 g and the lowest value was obtained from Kendal genotype with 32.1 g. In this study, considering the fact that the sowing was made later in the second year compared to the first year and higher 1000-grain weight was obtained in the second year, it is seen that it is in harmony with the studies of researchers such as Kandemir (2004) and Öztürk et al. (2007) who stated that high 1000-grain weight was obtained in late sowing (Table 3). In terms of hectoliter weight values, the highest value was obtained from Samyeli genotype with 72.4 kg, while the lowest value was obtained from Altıkat genotype with 61.1 kg (Table 3). These results are also in accordance with the researches indicating that hectoliter weight was 73.5 kg in the first year and 79.1 kg in the second year (Bleidere et al., 2013) and 1000-grain weight was between 24-47 g (Balouchi et al., 2005). In terms of protein content values in grain, the highest value was obtained from Samyeli genotype with 13.6% and the lowest value was obtained from Altıkat genotype with 11.3% (Table 3). Gündüzalp (1992) reported that the protein content of barley was between 8-15% and Kenar and Şehirali (2001) reported that two-row varieties had higher protein content than six-row varieties. Considering that Kendal and Altıkat varieties used in our research were six-rowed and the other genotypes were two-rowed, it is seen that these results coincide with our research results. In previous studies conducted with barley genotypes, protein content was between 6.35 and 23.4% (Wang et al., 2011).

4. Conclusion

According to the results obtained from the research; Samyeli and Şahin-91 varieties can be recommended for high grain yield under regional terms. In addition, it was determined that hectoliter weight, 1000-grain weight, days to maturity, vegetative period, heading duration, grain filling period, harmonization ratio and protein content traits can be used as selection criteria for high grain yield in the regional conditions. It is thought that all these differences between the traits and genotypes are due to the different environmental and ecological conditions of the two years, the use of different genotypes, genetic differences between the genotypes and the different adaptation ability of the genotypes. It can also be said that the genotypes responded differently to ecological circumstances.

Considering this research, it may be recommended to carry out studies with different locations, plants and genotypes in order to move forward in this field in terms of agricultural production activities. Definition of the characteristics of the genotypes is also important for future breeding studies.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	F.Ö.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1463877

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 477-485 / September 2024

ASSESSMENT OF GERMINATION AND SEEDLING DEVELOPMENT FACTORS OF SOYBEAN CULTIVARS IN DIFFERENT SALINITY LEVELS

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Abstract: Salinity poses a significant abiotic stress factor that exerts detrimental effects on plant growth during germination and early seedling stages. The global prevalence of high salt concentration has transformed salinity into a serious problem, impacting vast expanses of land worldwide. This experiment aims to examine the effects of various concentrations of sodium chloride (NaCl), including 200 mM, 150 mM, 100 mM, and 50 mM, on the seed development at early stage and germination of different cultivars of soybean to determine the variety with the highest value of tolerance, while exploring the underlying mechanisms responsible for salt tolerance in these plants. The parameters considered for measurement included relative injury rate, mean germination time, germination percentage, water uptake percentage, seedling height reduction, seedling biomass, and salt tolerance. Among these parameters, seedling height was highly affected with up to 72.58% reduction in 200 mM, followed by fresh weight and water uptake percentage. The parameters with minimum changes from 0 mM to 200 mM were mean germination time and relative injury rate. By assessing these parameters, a comprehensive understanding of the effects of salinity on soybean genotypes can be obtained. In conclusion, the study suggests that seedling traits are a reliable way to identify genotypes with increased tolerance to salinity stress by farmers according to the salinity situation in their soils.

Keywords: NaCl, Soybean, Germination, Salinity, Salt stress

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Received: April 02, 2024
Accepted: July 30, 2024
Published: September 15, 2024

Cite as: Üstün R, Amjid M. 2024. Assessment of germination and seedling development factors of soybean cultivars in different salinity levels. BSJ Agri, 7(5): 477-485.

1. Introduction

Soybean (*Glycine max*) is one of the most economically significant crops worldwide, contributing extensively to food and oil production. It is widely cultivated for edible vegetable oil production and serves as a high-protein feed for livestock. Its chemical composition includes approximately 40-50% protein, 20-30% oil, and 26-30% carbohydrates (Linh et al., 2021). The majority of cultivated soybeans are used as animal feed, particularly in the USA. Soybean oil is used as raw material for biodiesel production and finds applications in industries such as bakery, food, textile, medicine, and chemicals (Hill et al., 2006).

Soybean cultivation faces numerous challenges, including abiotic stresses such as salinity, which can significantly impact germination, seedling establishment, and overall plant growth (Han and Lee 2005; El Sabagh et al., 2015). Salinity, resulting from high levels of soluble salts in soil or irrigation water, poses a major threat to the agricultural productivity of irrigated land worldwide (Zhang et al., 2017). Understanding the responses of soybean cultivars to varying salinity levels is imperative for devising strategies to mitigate its adverse effects and sustain crop yields (Kumar 2017). The ability of plants to tolerate salinity is a dynamic characteristic that undergoes constant changes and demonstrates variations within the same species. This variability is primarily influenced by the stage of growth that the plants are in (Hosseini et al., 2002). It has been observed that non-halophytes, such as white clover, wheat, and rice, tend to be more susceptible to salinity stress during the germination and the seedling stage (Hampson and Simpson, 1990; Rogers et al., 1995; Hosseini et al., 2002). However, it is essential to acknowledge that the sensitivity observed during the early seedling growth stage may not necessarily mirror the subsequent sensitivity of the mature plant to salinity. Remarkably, several species of plants demonstrate an enhanced ability to tolerate higher levels of salinity as they reach maturity, surpassing their sensitivity during the early growth of seedlings. This phenomenon highlights the dynamic nature of salt tolerance and underscores the importance of considering the growth stage when assessing a plant's ability to withstand salinity stress (Dehnavi et al., 2020).

The prevailing belief suggests that the detrimental effects of NaCl on plant tissues primarily arise from the absorption and accumulation of Na^+ ions, rather than Cl^- (Hosseini et al., 2002). Many studies were carried out on common beans, soybeans, castor beans, corn, and wheat have provided evidence indicating the absence of a notable correlation between salt sensitivity and the concentration of Cl^- ions in plant tissues (Jeschke and Wolf, 1988a and 1988b; Kinraide, 1999; Hosseini et al., 2002). However, it is important to recognize that, in addition to Na^+, other cation concentrations present in plant tissues may also exert a noteworthy influence under saline conditions. Several authors have presented compelling evidence suggesting that the presence of Ca^{2+} and K^+ ions can provide a protective effect against the toxic impacts of Na^+ ions (Huang and Rozelle, 1995; Volkmar et al., 1998; Tester and Davenport 2003; Lindberg and Premkumar 2023). These findings underscore the complexity of ion interactions within plant tissues and emphasize the role of multiple cations in mitigating the harmful effects of Na^+ accumulation in saline environments.

The tolerance of plants to salinity is a trait that displays variability within the same species across different growth stages. Specifically, non-halophytic plants like white clover (Rogers et al., 1995), wheat (Hampson and Simpson, 1990), and rice (Pearson et al., 1966) exhibit higher sensitivity during the seedling stage compared to the germination stage. It is important to emphasize that the sensitivity observed during early seedling growth does not necessarily reflect the subsequent sensitivity of the mature plant to salinity. Interestingly, many plant species demonstrate a substantial increase in salt tolerance as they reach maturity, surpassing their sensitivity during the early seedling growth phase (Allen et al., 1986; Rogers and Noble, 1991).

The impact of salinity on plant growth can be attributed to two distinct mechanisms: osmotic effects and toxic effects, as highlighted in previous studies (Redmann, 1974; Hampson and Simpson, 1990). It is important to note that osmotic and toxic effects are not mutually exclusive, as evidenced by varying responses observed when comparing isosmotic solutions of NaCl and polyethylene glycol (PEG) during germination and growth. These differences indicate that the plant's response to salinity cannot be solely attributed to osmotic effects alone. For example, in PEG solutions, soybean germination is hindered at higher osmotic potentials but can recover when the seeds are transferred to deionized water. Conversely, in high concentrations of NaCl, germination inhibition seems to be a consequence of toxic effects, as recovery is not observed when the seeds are transferred to water (Hosseini et al, 2000). This highlights the complex interplay between osmotic and toxic effects in the context of salinity's impact on plant germination and growth.

2. Materials and Method

2.1. Plant Material and Seed Sterilization

In this experiment, Turbo, Lider, Agroyal, Arısoy, A3966 and Antsoy in total six soybean cultivars were used as plant material. The study was conducted at the Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, and Türkiye. Petri dishes, Whatman filter paper, and chemicals including NaOCl and NaCl used in this experiment were available in the molecular lab of the same department.

The seed sterilization procedure was carried out by Hassan's described method (Hassan et al., 2009). A total of 75 soybean seeds from each genotype, carefully chosen for their uniform size, were subjected to the sterilization process. Initially, they were thoroughly rinsed twice with a 2% solution of NaOCl to ensure proper disinfection. Subsequently, seeds were washed with deionized water for two minutes, effectively eliminating any residual chemicals from the seed surface.

2.2. Salt Solution Preparation and Design of the Experiment

To prepare the solution, the desired amount of distilled water was filled in four different flasks. The calculated weight of NaCl salt was measured with precise balance for each flask accordingly to prepare 50mM, 100mM, 150mM, and 200mM solution respectively (Table 1). Salt was added to distilled water contained in each flask and mixed on an electric stirrer for 15 minutes. The distilled water was used for control treatment.

Table 1. Preparation of salt solutions

Treatments	Grams of NaCl	Distilled Water
50 mM	0.584g	200 ml
100 mM	1.1688g	200 ml
150 mM	1.7532g	200 ml
200 mM	2.3376g	200 ml

The study was conducted using a completely randomized design (CRD) with five treatments, including the control treatment, and three replicates per treatment. Petri dishes with 9cm diameter were sterilized in the autoclave to prevent any possible fungal growth inside the treated petri dish. Two layers of Whatman filter paper were placed in each petri and 10 ml of the solution was added to filter paper layers in Petri dishes with three replications of each treatment and 5 ml same concentrate solution was 5 days later. The treatments, T0, T1, T2, T3 and T4 correspond to: 0; 50 mM, 100 mM, 150 mM and 200 mM NaCl respectively. Five seeds were placed in each petri dish with uniform size at a uniform distance. The seeds were then placed in a plant growth room with a temperature range of 25 to 27.1ºC to trigger germination. To prevent any loss of water from the experiment with evaporation, petri dishes were wrapped with parafilm and placed in the dark.

2.3. Assessment of Germination and Seedling Growth

During each cycle of the experiment, daily counts were made of germinated seeds over 10 days. Germination was defined as root protrusion beyond the pericarp by at least 2 mm. At the end of the experiment, the soybean seedlings were preserved to record the data measurements including fresh weight, dry weight, and seedling length. These parameters allowed for an assessment of seedling growth and development, providing insights into their physiological, phenotypical, and biomass characteristics under experimental conditions.

2.3.1. Water uptake

After evaluating the seedling fresh weight and the seedling dry weight, the water uptake was determined using the given formula (equation 1).

Water Uptake %age = [(Fresh weight – dry weight)/Fresh weight] × 100 (1)

2.3.2. Germination time

The number of germinated seeds was counted daily from each replication and determined the mean value. The overall mean germination time was calculated by using the formula given below (equation 2).

Mean time of germination = $\sum Dn / \sum n$ (2)

Here, 'n' represents the count of seeds that have germinated on day 'D,' where 'D' denotes the number of days since the germination process started.

2.3.3. Germination percentage

Germination data was collected for 10 days daily and determined the germination time after 10 days. The given formula was used to determine the germination time (equation 3).

Germination Percentage = (Germinated seed count / total sown seed count) × 100 (3)

2.3.4. Relative injury

The calculation of the relative injury rate followed the formula established by Tsegay and Gebreslassie (2014). The calculation included subtracting the percentage of germination in salt-treated seeds from the percentage of germination in the control, followed by dividing the resulting value by the percentage of germination in the control (equation 4).

Rate of Relative Injury = [%age of germination in control – %age of germination in salt treatments] / %age of germination in control (4)

2.3.5. Reduction in seedling height

The reduction of the germinated plant height is obtained by subtracting the percentage of shoot length under salt stress conditions from the percentage of shoot length under normal conditions, relative to the percentage of shoot length under normal conditions. This reduction is calculated using the following equation 5.

Shoot height reduction = [(Controlled shoot height – salt-treated shoot height) / Controlled shoot height] x 100 (5)

2.3.6. Biomass of seedling

To ensure standardization of mass, the biomass of the seedlings was determined by weighing them using an analytical balance. Before weighing, the seedlings were placed in an oven at 55ºC temperature for 48 hours.

2.3.7. Salinity tolerance rate

The salt tolerance rate was carried out with the help of the prescribed standard formula provided hereafter (equation 6).

Salinity Tolerance Rate = (Salt treated seedling dry weight / Control seedling weight) x 100 (6)

3. Results and Discussion

The effect of different concentrations of NaCl on germination and early growth of soybean seedlings was analyzed in this study. The results revealed that there was no significant difference in germination percentage between seeds treated with 0 mM (control) and those treated with 50 mM NaCl. However, the germination percentage notably declined with increasing salinity levels. Under salt stress, the germination percentages decreased in the “Turbo” variety from 100% in the control to 86.67% at 200 mM NaCl. It decreased from 90% in the “Lider” variety at 0 mM, to 73.33%. In the “Agroyal” variety, from 86.67% (control) to 66.67% (similar to A3966). This percentage decreased from 93.33% in the controlled “AntSoy” to 70% and from 100% to 66.67% in “Arisoy”, with significant differences observed between all varieties (Table 2). The “Turbo” variety exhibited the highest cumulative germination percentage of 94.67%, while the A3966 genotype showed the lowest cumulative germination percentage of 77.33% (Table 3). Cumulative analysis across all varieties indicated a significant reduction in germination percentage from 92.78% (0 mM) to 71.67% (200 mM) for all treatments (Table 4).

In some previous studies, it has been stated that although salt stresses up to 50 ppm do not negatively affect seed germination, or plant growth, increasing levels of salinity stress have negative effects on these parameters (Shanko et al., 2017; Açıkbaş et al., 2023). In this respect, the results of this study are like those previously reported. These results suggest that the germination stage in the soybean is moderately salt-tolerant. However, high levels of salinity can still inhibit seed germination. This is likely due to the toxic effects of Na^+ and Cl^- ions, which can disrupt the water balance and metabolism of the seeds.

Table 2. Variance analysis of Soybean varieties under applied salinity levels

G	T	GP	SH (cm)	HR (%)	RIR (%)	MGD	FW (g)	B (g)	WUP (%)	ST (%)
Turbo	T0	100.000±0.000a	3.710±0.031a	100.000±0.000a	0.000±0.000b	2.550±0.040d	0.608±0.004a	0.210±0.002a	65.397±0.006a	100.000±0.000a
	T1	100.000±0.000a	3.510±0.059a	94.600±0.010a	0.000±0.000b	2.673±0.043d	0.568±0.001b	0.209±0.001a	63.285±0.002ab	99.212±0.004ab
	T2	93.333±0.033ab	2.500±0.012b	67.396±007b	6.667±0.033ab	3.030±0.035c	0.527±0.001c	0.205±0.002ab	61.261±0.003bc	97.632±0.012abc
	T3	93.333±0.033ab	1.533±0.133c	41.297±0.034c	6.667±0.033ab	3.273±0.014c	0.501±0.011d	0.200±0.001bc	59.842±0.009cd	95.110±0.013bc
	T4	86.667±0.033b	1.250±0.029c	33.695±0.008c	13.363±0.033a	3.463±0.019a	0.467±0.001e	0.197±0.001c	57.917±0.001d	93.520±0.011c
		0.0220*	<.0001*	<.0001*	0.0220*	<.0001*	<.0001*	0.0005*	<.0001*	0.0029*
Lider	T0	90.000±0.000a	3.800±0.023a	100.000±0.000a	0.000±0.000b	2.283±0.009d	0.733±0.002a	0.223±0.001a	69.637±0.001a	100.000±0.000a
	T1	90.000±0.000a	3.640±0.031a	95.806±0.014a	0.000±0.000b	2.410±0.010d	0.658±0.004b	0.218±0.001ab	66.714±0.003b	98.355±0.004ab
	T2	83.333±0.033ab	2.500±0.031b	65.787±0.006b	7.407±0.037ab	2.620±0.025c	0.548±0.002c	0.218±0.001ab	60.450±0.001c	97.461±0.005ab
	T3	76.667±0.033b	1.800±0.115c	47.335±0.028c	14.813±0.037a	2.783±0.060b	0.513±0.001d	0.210±0.004b	60.312±0.003cd	94.323±0.018bc
	T4	73.333±0.033b	1.233±0.120d	32.450±0.031d	18.517±0.037a	2.990±0.006a	0.484±0.001e	0.193±0.002c	58.799±0.006d	86.672±0.005
		0.0027*	<.0001*	<.0001*	0.0027*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
A	T0	86.667±0.067a	3.567±0.067a	100.000±0.000a	0.000±0.000b	2.730±0.091c	0.373±0.001a	0.112±0.007a	0.717±0.011a	100.000±0.000a
	T1	80.000±0.058a	3.000±0.115b	84.000±0.036b	7.500±0.038ab	3.027±0.054b	0.327±0.001b	0.095±0.003ab	0.700±0.006ab	85.843±0.062ab
	T2	80.000±0.058a	2.567±0.067c	72.046±0.028bc	7.500±0.038ab	3.130±0.015b	0.287±0.002c	0.087±0.002bc	0.688±0.011ab	78.132±0.039b
	T3	76.667±0.033a	2.300±0.115c	64.633±0.043c	10.833±0.058ab	3.150±0.029	0.255±0.001d	0.086±0.002bc	0.662±0.005bc	77.547±0.038b
	T4	66.667±0.033a	1.200±0.058d	33.719±0.022d	22.500±0.052a	3.400±0.021a	0.207±0.002e	0.077±0.002c	0.626±0.007c	69.533±0.041a
		0.1705	<.0001*	<.0001*	0.0418*	<.0001*	<.0001*	0.0007*	0.0002*	0.0040*
A3966	T0	86.667±0.033a	2.800±0.058a	100.000±0.000a	0.000±0.000b	3.167±0.089b	0.417±0.002a	0.107±0.001a	74.260±0.001a	100.000±0.000a
	T1	83.333±0.033a	2.100±0.100b	75.193±0.051b	3.703±0.037b	3.217±0.044b	0.310±0.001b	0.105±0.001ab	67.271±0.002b	97.834±0.013ab
	T2	76.667±0.033ab	1.367±0.120c	48.726±0.036c	11.573±0.005ab	3.553±0.024a	0.291±0.005c	0.101±0.002bc	66.708±0.002b	94.413±0.014bc
	T3	73.333±0.033ab	1.133±0.067cd	40.424±0.018cd	15.276±0.035ab	3.723±0.023a	0.282±0.001cd	0.099±0.000c	65.169±0.003c	91.615±0.000c
	T4	66.667±0.033ab	0.900±0.058d	32.129±0.018d	22.683±0.061a	3.770±0.025a	0.275±0.002d	0.090±0.001d	64.565±0.005c	83.850±0.003d
		0.0118*	<.0001*	<.0001*	0.0073*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
AntSoy	T0	93.333±0.067a	3.400±0.058a	100.000±0.000a	0.000±0.000b	2.623±0.039d	0.355±0.002a	0.103±0.001a	70.982±0.004a	100.000±0.000a
	T1	86.667±0.033ab	2.500±0.115b	73.631±0.042b	6.667±0.033ab	2.767±0.089d	0.301±0.001b	0.097±0.001b	68.488±0.002ab	94.202±0.014ab
	T2	76.667±0.033ab	1.700±0.100c	50.116±0.038c	17.500±0.025ab	3.150±0.029c	0.275±0.002c	0.090±0.002c	68.134±0.005b	87.084±0.022bc
	T3	73.333±0.033b	1.500±0.115cd	44.203±0.039cd	20.833±0.051a	3.400±0.021b	0.27±0.002c	0.085±0.001c	67.186±0.005b	82.219±0.013c
	T4	70.000±0.000b	1.133±0.033d	33.382±0.015d	24.167±0.058a	3.643±0.043a	0.178±0.001d	0.075±0.002d	57.859±0.010c	72.858±0.022d
		0.0096*	<.0001*	<.0001*	0.0069*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Arisoy	T0	100.000±0.000a	3.800±0.058a	100.000±0.000a	0.000±0.000b	2.730±0.091d	0.402±0.002a	0.133±0.002a	69.747±0.002a	100.000±0.000a
	T1	93.333±0.033ab	3.300±0.115b	86.975±0.044b	6.667±0.033cd	3.017±0.044cd	0.36±0.001b	0.122±0.002b	66.972±0.005b	91.741±0.011b
	T2	83.333±0.033bc	2.367±0.033c	62.332±0.018c	16.667±0.033bc	3.217±0.044bc	0.357±0.001b	0.108±0.002c	66.469±0.002b	81.226±0.011c
	T3	73.333±0.033cd	1.600±0.058d	42.102±0.013d	26.667±0.033ab	3.440±0.029ab	0.285±0.001c	0.096±0.001d	65.879±0.004b	72.194±0.009d
	T4	66.667±0.033d	0.900±0.058e	23.719±0.018e	33.333±0.033a	3.630±0.085a	0.215±0.003d	0.088±0.001e	59.106±0.010c	66.194±0.014e
		<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*

A= agroyal, G= genotype, T= treatment, GP= germination percentage, SH= seedling height, HR= height reduction, RIR= relative injury rate, MGD= mean germination days, FW= fresh weight, B= biomass, WUP= water uptake percentage, ST= salt tolerance.

Table 3. Pair-wise multiple comparisons of means of all of the 6 Soybean varieties for studied traits

	Turbo	Lider	Agroyal	A3966	AntSoy	Arisoy
GP (%)	94.667±0.020	82.667±0.020	78.000±0.050	77.333±0.033	80.000±0.033	83.000±0.027
SH (cm)	2.501±0.052	2.595±0.064	2.527±0.084	1.667±0.080	2.047±0.084	2.393±0.064
HR (%)	67.391±0.012	68.280±0.016	70.910±0.026	59.287±0.024	60.270±0.027	63.030±0.018
RIR (%)	5.333±0.019	8.15±0.022	9.667±0.037	10.646±0.027	13.828±0.033	16.667±0.027
MGD	3.00±0.030	2.620±0.022	3.086±0.042	3.488±0.041	3.120±0.044	3.213±0.058
FW (g)	0.534±0.004	0.587±0.002	0.290±0.001	0.315±0.002	0.276±0.002	0.324±0.002
B (g)	0.204±0.002	0.212±0.002	0.092±0.003	0.100±0.001	0.090±0.001	0.109±0.001
WUP	61.540±0.004	63.182±0.003	67.866±0.008	67.587±0.003	66.530±0.005	65.629±0.004
ST	97.091±0.008	95.364±0.006	82.21±0.036	93.535±0.006	87.266±0.014	82.297±0.009

GP= germination percentage, HR= height reduction, RIR= relative injury rate, MGD= mean germination days, FW= fresh weight, B= biomass, WUP= water uptake percentage, ST= salt tolerance.

Table 4. Pair-wise multiple comparison of means of all the 5 treatments for studied traits

T	GP	SH (cm)	HR (%)	RIR (%)	MGD	FW (g)	B (g)	WUP (%)	ST (%)
0mM	92.778±0.019a	3.513±0.086a	100.000±0.000a	0.000±0.000a	2.681±0.068a	0.481±0.034a	0.148±0.012a	70.294±0.007a	100.000±0.000a
50mM	88.889±0.020ab	3.008±0.137b	85.062±0.024b	4.089±0.012b	2.852±0.067ab	0.421±0.034ab	0.141±0.013a	67.124±0.005b	94.531±0.015ab
100mM	82.222±0.019bc	2.167±0.115c	61.067±0.023	11.219±0.015a	3.117±0,068bc	0.381±0.028ab	0.135±0.013a	65.306±0.008bc	89.325±0.020bc
150mM	77.778±0.021cd	1.644±0.093d	46.667±0.023	15.849±0.021b	3.295±0.071cd	0.351±0.027b	0.129±0.013a	64.0870.007c	85.501±0.022cd
200mM	71.667±0.020d	1.103±0.042	31.516±0.011	22.423±0.022b	3.483±0.063d	0.304±0.030b	0.120±0.013a	60.138±0.007d	78.771±0.025d
Sig.	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0015*	0.5862	<.0001*	<.0001*

T= treatment, GP= germination percentage, SH= seedling height, HR= height reduction, RIR= relative injury rate, MGD= mean germination days, FW= fresh weight, B= biomass, WUP= water uptake percentage, ST= salt tolerance.

The seed germination process was meticulously monitored at regular intervals of 12 hours, providing insights into the germination initiation time, which was observed at the 36-hour mark after seed placement. Statistical analysis of observed data shows variation in mean germination time. A gradual increase in mean germination time was recorded with the increase in salinity level. We found that using a 200 mM solution made soybean seeds take longer to sprout. In the Turbo variety, it went from 61.2 hours to 83.1 hours. For Lider, it increased from 54.8 hours to 71.8 hours.

Agroyal showed the same effect, going from 65.5 hours to 81.6 hours. A3966 seeds took more time to germinate as well, moving from 76.0 hours to 89.4 hours. The AntSoy seeds went from 62.95 hours to 87.43 hours. Arisoy seeds also took longer, going from 65.52 hours to 87.12 hours when we used the 200 mM solution (Table 2). Variations among the varieties have been recorded in cumulative analysis, but there is no significance level between used genotypes. Lider showed the shortest mean germination time with 62.88 hours and A3966 showed the highest value of mean germination time with 83.71 hours (Table 3). In the treatment-wise cumulative statistical analysis, there was no significant difference between the control group and the group treated with 50 mM salt solution, which showed a moderate salt tolerance at the seed germination stage of soybean. However, there was a significant change with higher doses of salinity (Table 4). These findings reveal the varying responses of soybean genotypes to salinity stress during germination, offering valuable insights for future studies on stress resilience and crop improvement strategies. Previous soybean studies showed that salinity stress has been consistently documented to negatively impact various parameters, including seed germination, plant height, shoot dry weight (Essa, 2002), seedling fresh weight (Farhoudi and Tafti, 2011), germination percentage (Neves et al., 2005; Ahmadvand et al., 2012; Ndifon, 2013), as well as plant height and root length (Ahmed et al., 2023). Notably, higher salt concentrations have been demonstrated to reduce shoot dry weight significantly (Le et al., 2021).

The analysis demonstrates that the percentage of water uptake of soybean decreased inversely with increasing NaCl salt concentration. The water uptake percentage reduced significantly ($p<0.05$) at 200 mM NaCl, but there was no significant difference between the water uptake percentage of seeds treated with 50 mM NaCl solution and the control group. For all soybean varieties studied, statistical analysis shows a reduction in water absorption when treated with 200 mM saline compared to the control. The reduction ranging from 65.39 to 57.92% for the "Turbo" variety, from 69.64 to 58.80% for "Lider", from 71.71 to 62.67% for "Agroyal", from 74, 26 to 64.56% for "A3966", from 70.98 to 57.86% for "AntSoy" and from 69.75 to 59.11% for "Arisoy" (Table 2).

Cumulative statistical analysis showed that there is slight difference without significance level between different used genotypes. The highest cumulative water absorption 67.87% recorded in "Agroyal", and the lowest water absorption 61.54% has been recorded in the "Turbo" variety (Table 3). Cumulative treatment analysis showed a reduction in water uptake percentage from 70.29% at 0 mM to 60.14% at 200 mM salinity level (Table 4).

It's noteworthy to acknowledge that seed health and size play crucial roles in influencing the water absorption percentage during the germination and early seedling development stage. These findings emphasize the intricate dynamics of salt-induced water uptake reduction in soybeans, explain the potential impacts of seed characteristics on water absorption capacity, and offer valuable insights for future research in stress resilience and crop improvement strategies.

The decrease in water uptake percentage with increasing salinity is likely due to the dehydration of the cell cytoplasm caused by the high concentration of salt in the soil. Water absorption by the seed is also affected by the seed coat nature, which may become more impermeable to water as the salinity level increases. Higher salinity levels cause a reduction in water absorption during cell division and cell differentiation, which eventually leads to increased osmotic pressure. The high osmotic pressure of the soil solution inhibits water uptake in the seedlings, which in turn reduces the water uptake percentage of the seedlings.

Seedling height reduction is a common observation in crop plants grown in saline environments. This is due to the toxic effects of high salt concentrations, which can cause plant cells to dehydrate and shrink. In this study, we investigated the effects of different NaCl concentrations on seedling height reduction in different soybean varieties. We found that seedling height reduction increased significantly with increasing salinity, but the level of height reduction was different in every variety.

The statistical analysis showed a reduction of 66.31% in the height of the seedlings obtained at 200 mM saline solution compared to the control (0 mM) in the "Turbo" variety, a reduction of 67.55% in the "Lider", a reduction of 66.28% in "Agroyal", a reduction of 67.87% in the "A3966", a reduction of 66.62% of the mutant variety "Arisoy", and a maximum reduction of 76 .28% was recorded in the "Antsoy" variety (Table 2).

The cumulative height of all treatments recorded 67.39% in Turbo variety compared to the control group, 68.28% in Lider, 70.91% in Agroyal, 59.29% in A3966, 60.27% in AntSoy, and 63.03% has been recorded in the Arisoy (Table 3). Among these varieties, Agroyal showed the highest cumulative height and A3966 showed the lowest cumulative seedling height. Treatment wise cumulative analysis of all varieties showed that seedling height reduced from 100 percent at control to 31.52 percent at 200 mM salinity level (Table 4).

These findings shed light on the varied responses of soybean varieties to salinity stress, providing crucial data for advancing our understanding of salt-induced growth inhibition. The differential impact of salinity on seedling height among various genotypes warrants further investigation and offers valuable insights for devising strategies to enhance stress resilience in soybean cultivation. These results suggest that soybean is moderately salt-tolerant. However, high levels of salinity can still significantly reduce seedling height. This is likely due to the decrease in cell division and the cell elongation that occurs under salt stress. The reduction in seedling height can have several negative consequences for plant growth. For example, it can lead to slower leaf appearance and leaf size, which can reduce photosynthesis and overall plant productivity.

The greatest seedling biomass was achieved with seeds treated under 0 mM salinity in the control group, whereas the lowest values were observed with seeds subjected to 200 mM salinity. A reduction of 6.19% recorded in Turbo, 13.45% in Lider, 31.25% reduction in Agroyal, 15.89% reduction in A3966, 27.18% reduction in Antsoy, and 27.18% reduction in Arisoy variety from 0 mM to 200 mM salinity solution with a significant difference (Table 2). These results showed the highest reduction in the biomass occurred in the Agroyal variety while the lowest biomass reduction was recorded in the Turbo variety (Table 3). Treatment-wise collective results of all the varieties didn't show a significant difference. Cumulative analysis showed that Lider variety had the highest biomass value (0.212g) and AntSoy had the lowest value (0.090g). No significant difference (p value 0.5862) was observed in seedling biomass among the different salt treatments while analyzing all the varieties cumulatively (Table 4).

Variations in the fresh weight of all varieties with changes in salinity level were more significant than the significance level in the biomass. The highest fresh weight value was recorded in the controls of all the varieties while the lowest fresh weight value was recorded in the seedlings treated with 200 mM salinity level. From the control group (0 mM) to the highest salinity level (200 mM) a reduction of 23.19% in "Turbo"; 33.97% in "Lider"; 44.50% in "Agroyal"; 34.05% in "A3966"; 49.86% in "AntSoy"; and 46.52% in "Arisoy" (Table 2).

Cumulatively, "Lider" showed the highest fresh weight value while the mutant "Arisoy" showed the lowest one (Table 3). Treatment wise the fresh weight of all genotypes cumulatively reduced from 100 percent at 0 mM treatment to 36.80%, at treatment applied with 200 mM solution (Table 4).

The biggest reason behind the relative injury is ion toxicity and osmotic stress caused by salinity. Relative injury rate increased with the increase in salinity level but with very low significance compared to other parameters. There was no relative injury in 0 mM and seedlings treated with 50 mM salinity solution in the

Turbo and Lider varieties. Any variety did not show relative injury in the seedlings of the control group (0 mM) seedlings.

The relative damage increased significantly, recording a maximum of 13.36% in the “Turbo” variety, subjected to 200 mM salinity; 18.52% in “Lider”; 22.50% in “Agroyal”; 22.68% in “A3966”; 24.17% in “AntSoy” and 33.33% in “Arisoy” (Table 2).

Cumulative treatment analysis of all varieties showed a 22.42% increase in relative damage rate under salinity conditions (200mM), compared to 0% in controls.

The maximum cumulative relative injury rate was recorded in “Arisoy” and the minimum was recorded in the “Turbo” variety (Table 3). Treatment-wise cumulative analysis of all varieties showed an increase of 22.42% relative injury rate at 200 mM solution from 0 percent at 0 mM saline solution (Table 4).

The precise mechanisms underlying the impact of salt on germination remain incompletely understood. Excessive salinity negatively impacts plant growth in several ways. The higher salinity creates an osmotic imbalance, making it harder for plants to take up water. It leads to osmotic stress, restricting water uptake and cell expansion. A severe imbalance of ions including K^+, Na^+, K^+/Na^+, Ca^{2+}, and Cl^- causes several physiological changes in the cell (Tunçtürk et al, 2011). Sodium and chloride ions accumulate in plant tissues, disrupting physiological processes and nutrient uptake. Nonetheless, it has been proposed that osmotic (Kingsbury and Epstein, 1986; Kumar and Sharma, 1990) and/or toxic effects may contribute to salt-induced injury (Essa, 2002). Furthermore, soluble salts can induce osmotic stress, leading to specific ion toxicity and ionic imbalances, potentially culminating in plant mortality (Munns, 2002).

Salt tolerance is often investigated by applying the NaCl solution to the medium of plant growth to create salinity. In this study, the levels of tolerance of soybean seedlings to salt stress were assessed using various salt solutions. The results demonstrated that soybean seeds exhibited tolerance across all salinity levels, with an inverse linear relationship between salt tolerance and increasing salt concentrations. Statistical analysis showed a significant difference between the treatments among all varieties. These results show that the minimum drop in tolerance level 6.5% was recorded in the “Turbo” variety, and the maximum drop in the tolerance level 33.81% was recorded in “Arisoy” (Table 2). Cumulatively Turbo showed the highest level of salt tolerance with 97.09%, and “Agroyal” showed the lowest value (82.21%) of relative tolerance (Table 3). Treatment-wise cumulative analysis showed a significant reduction from a 100% tolerance level in the control group to 78.77% in the 200 mM saline group (Table 4).

The current study reveals that soybean's salt tolerance decreases with higher salinity levels, but also confirms its capability to germinate under saline conditions. However, it is important to note that salt tolerance screening during the germination period may have limited implications for assessing crop salt tolerance since many germination studies are conducted in laboratory settings using Petri dishes with varying salinity solutions. It has been observed that in some plants, as the salinity rate increases, the plants' sensitivity to salt increases during plants germination stages.

4. Conclusion

The osmotic pressure resulting from salt stress adversely affects soybean's germination time and early growth of seedlings. Elevated levels of salinity result in a decrease in the percentage of water absorption, seed vitality, and an extension of the average germination time. Throughout this experiment, soybean seeds developed tolerance under lower salinity levels and experienced germination delays under high salinity. Germination percentage and index decreased with increasing salinity. Furthermore, variations in response to elevated salinity were evident in both seedling biomass and the relative injury rate. Increased NaCl concentrations had a noticeable impact on seedling height, contributing to a significant enhancement in salt tolerance for soybeans as salinity levels rose. Nevertheless, the early seedling growth phase revealed only modest salt tolerance. Interestingly, it was observed that increasing salinity levels did not yield significant changes in biomass.

It is crucial to raise awareness among people about the importance of cultivating tolerant varieties of soybean according to their soil salinity conditions. The selection of soybean varieties should be based on the specific environmental conditions of the area. It is essential to adopt recommended soybean varieties known for their tolerance levels and standard traits, while pairwise interaction results in a final yield.

This study provides valuable insights into the germination and seedling development factors of soybean varieties under different salinity levels. The observed variations in tolerance among varieties underscore the importance of genetic diversity in breeding programs aimed at developing salt-tolerant soybean varieties

Among the tested varieties, “Turbo” exhibited the strongest salt tolerance with a higher performance in all tested parameters comparing the others. However, although a high variation was observed in the tested parameters in terms of response to different salt concentrations, the variety “Arısoy” performed a higher susceptibility towards the highest salt concentration of 200 mM. When the average of all genotypes was evaluated, it was observed that while germination was 100% in the control groups, it decreased to 77% in the highest salt concentration solution.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	R.U.	M.A.
C	50	50
D	50	50
S	100	0
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	100	0
PM	100	0
FA	100	0

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1432970

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 486-496 / September 2024

EFFECTS OF DIFFERENT SOWING FREQUENCIES AND NITROGEN DOSES ON THE YIELD COMPONENTS OF SILAGE SORGHUM X SUDAN GRASS HYBRID (*Sorghum bicolor* X *Sorghum sudanense* Mtapf.) IN THE IĞDIR BASIN

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Abstract: The study was conducted in 2021 to evaluate the yield and yield parameters of sorghum for silage (Sorghum bicolor x Sorghum sudanense Mtapf., Hayday hybrid) under different nitrogen application rates (0, 6, 12 and 18 kg/da nitrogen application, respectively) and different row distances (5 cm, 10 cm, 15 cm and 20 cm). The experiment was established at Igdir University Agricultural Application and Research Centre in Igdir ecological conditions according to split-plot experimental design with three replications. The data obtained from the experiment were as follows: plant height 190.9 cm and 257.3 cm; herbage yield 1756.1 kg/da and dry herbage yield 3228.5 kg/da; dry herbage yield 522.2 kg/da and 925.2 kg/da; stem ratio 69.1% to 74.8%; leaf ratio 15.3% to 74.8%; 15.3% to 20.2%; crude protein content ranged from 6.4% to 8.1%; NDF content ranged from 58.5% to 63.1%; ADF content ranged from 31.7% to 34.5%. The results showed that N application rates increased plant height, fresh herbage yield and dry matter, while decreasing the NDF ratio of the plants. In the study, row spacing did not affect dry matter, stem and leaf ratio, NDF and ADF of sorghum plants. It was concluded that 28.570 plants/da sowing and 6 kg N/da application could be applied in terms of fresh and dry matter yield under Igdir ecological conditions.

Keywords: Silage sorghum, Nitrogen doses, Green herbage yield, Yield, Quality

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Received: February 06, 2024
Accepted: August 15, 2024
Published: September 15, 2024

Cite as: Akdeniz H, Keskin B, Eren B. 2024. Effects of different sowing frequencies and nitrogen doses on the yield components of silage sorghum x sudan grass hybrid (*Sorghum bicolor* x *Sorghum sudanense* Mtapf.) in the Iğdır basin. BSJ Agri, 7(5): 486-496.

1. Introduction

Climate change, which poses a threat to agricultural production and access to clean food in many parts of the world, and food problems caused by intensive population growth have emerged as one of the most important challenges faced by mankind in the modern age. Agricultural areas are the most affected areas due to environmental problems (Arici and Avci, 2022). It is one of the main problems of agricultural production even in countries that have advanced in agriculture. Forage crops play an important role in the agricultural economy of developing countries by providing the cheapest feed source for livestock. Although our country has a significant potential in terms of livestock, the return obtained from each animal is quite negligible (Keskin et al., 2018). Insufficiency of quality roughage resources is one of the most important factors contributing to the inability to achieve the required amount of animal production. Our meadows and pastures, which are the most important food source for animals, are experiencing a decrease in production as a result of both intensive and irregular grazing (Özkan and Şahin Demirbağ, 2016).

Considering the current need for roughage in Turkey, sorghum is a suitable substitute for maize in animal feed. It shows stability in dry periods, high temperature tolerance and drought resistance. It also regrows rapidly after harvest. Especially sorghum for silage surpasses maize as an alternative due to its improved resistance to pests and diseases (Keskin et al., 2005). Sorghum (*Sorghum bicolor* L.) is an important plant with a wide range of ecological adaptations due to its xerophytic properties. It is widely cultivated as fodder and forage by subsistence farmers in both wetland and non-wetland regions of Turkey. Its feed is fed to almost all classes of animals and can be used as hay or silage. However, the quality of sorghum forage is poor due to low protein content and the presence of hydrocyanic acid (Salman and Budak, 2015). The performance of dairy animals depends on the availability of quality feed in sufficient quantity and continuously. Therefore, the critical limitation on profitable animal production in developing countries is the shortage of quality feed (Sarwar et al., 2002). Among the many options to overcome the shortage of roughage, the best one is the introduction of high-yielding crop varieties (Akbay et al., 2023).

Sorghum is one of the most important multifunctional crops grown all over the world for both grain and feed purposes. The genus Sorghum, which belongs to the grass family known as Poaceae, is home to over 25 different flowering plant species (Salman and Budak, 2015). There are several different names for sorghum such as orshallu, milo, durra and Asian millet. Vitamin B1, niacin, 10% protein, 3.4% fat, carbohydrate and trace amounts of iron are some of the nutrients that can be found in sorghum. It is one of the five most widely cultivated crops in the world and has a number of potential economically important applications. These applications include food (grain), feed (grain and biomass), fuel (ethanol production), fibre (paper), fermentation (ethanol production) and fertiliser (using organic by-products). Most species are nitrogen-efficient, heat- and drought-tolerant, and are particularly important in arid regions where grain is a staple food for the poor and rural people (Kimutai et al., 2023). These characteristics make cereals particularly important in agricultural environments. It is considered one of the most important cereal crops in the world, in addition to wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*) and barley (Hordeum vulgare). In addition to maize and pearl millet (*Pennisetum glaucum* L.), it accounts for a large proportion of the dryland cereal crop grown in semi-arid tropical regions.

Sorghum (Sorghum bicolor (L.) Moench = S. vulgare Pers) is categorised under four main groups as grain sorghums, sugar sorghums, forage sorghums and broom sorghums according to their cultivation purposes (Açıkgöz, 2001). Sorghum varieties with thin stalks, tall stems, tillering, high grass yield and herbage yield are grown for fodder production (Balabanlı and Türk, 2005; Parlak and Özaslan Parlak, 2006). Sorghum species have a great potential in the utilisation of dry areas with sufficient rainfall and as an alternative to maize and other cultivated crops in irrigated agricultural areas in seasons when water is limited (Tiryaki, 2005; Temel et al., 2017). Sorghum is a warm season plant of tropical and subtropical origin and is used in African countries for bread making, as raw material for alcoholic beverages, in syrup and starch production due to its rich sugar content, as grain and green, biofuel, broom and animal feed (Kazungu et al., 2023). Sorghum, a C_4 crop, is characterised by its high and high quality grass yield as well as its ability to thrive in a variety of agro-ecosystems. Feed, food and industrial use are the main reasons for its cultivation. According to House (1985), sorghum gives twice as many roots as maize. Sorghum is more efficient than maize and other crops in utilizing water and plant nutrients (N, P, and K) (Kimbrough, 2002; Bean et al., 2002). As a result, it was able to produce a greater amount of biomass.

This study aimed to evaluate the potential of sorghum for high quality biomass production in the Igdir Aras basin and similar regions. There is a dire need to develop such varieties with higher yield potential to meet the increasing demand for feed for livestock (Chohan et al., 2006). Therefore, this study was designed to find the most suitable sorghum variety capable of producing large quantities of biomass in terms of feed yield and quality.

2. Materials and Methods

The investigation was conducted to determine the effects of different green herbage yield and nitrogen doses on the Sorghum x Sudan grass hybrid variety Hayday under the conditions of Iğdır Ecology in n 2021 at the Agricultural Application and Research Center of Iğdır University. The experiment Split Plot Desing was established in three replications according to four different nitrogen doses (0, 6, 12, 18 kg/da) and four sowing frequencies (5 cm, 10 cm, 15 cm, and 20 cm = 28.570, 14.285, 8.570 and 7,142 plants/da) were applied. The average lime ratio of the research area trial soil was 6.53, pH 7.98, electrical conductivity 1.8 dS m^{-1}, potassium 0.3 t ha^{-1}, phosphorus 0.008 t ha^{-1}, and organic matter was very low 1.6%, soil structure was clay loam. On July 5, 2021, when the total parcel area reached 17.5 m2 and the planting soil temperature reached 18-20 °C, with a distance of 70 cm between rows and five rows in each parcel, sowing was applied to the rows at a depth of approximately 4-5 cm in each row using hand markers. Irrigations were irrigated by sprinkler method on July 14, 2021, and July 20, 2021, when the soil moisture meter consumed 50% of the field capacity. Furrows were irrigated the rows with a fertilizer machine, and the first dose of nitrogen was given by sowing. Irrigation was carried out on August 16, 2021, and the last irrigation was on August 26, 2021. All of the triple super phosphate and half of the nitrogen were given equally to all plots, 6 kg/da at the sowing time. Half of the nitrogen and all of the phosphorus were applied; the rest of the nitrogen was given when the plant reached 50 cm height. After leaving 50 cm of edge rows at the beginning of each plot, it was cut with a sickle based on 4 m x 3 rows = 8.4 m^2 area, 3 rows in the middle. In the research, the plant height of 10 plants randomly selected from each plot, the part from the soil surface to the tip of the plant from 5-6 cm was measured, and then the leaf ratio, stem ratio, and panicle ratios were found by cutting. The green herbage yield was cut in the middle 3 rows and immediately weighed; the dry matter ratio (%) and the yield of 2 or 3 plants taken from each plot were immediately weighed as a fresh weight and then dried in the laboratory at 70 ^{o}C for 48 hours, and the dry matter ratio was found. Dry matter was calculated on the green grass yield using the ratio of hay. The crude protein ratio was determined using Kacar (1984), NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), and Van Soest et al. (1991).

3. Results and Discussion

Under the conditions of Iğdır Ecology, together with the agricultural characteristics of different row distances and nitrogen doses on Sorghum x Sudan grass-Hayday variety, crude protein ratio, NDF, and ADF ratios are presented in Table 1.

3.1. Traits of Plants Examined in the Study

3.1.1. Plant height (cm)

Green grass yield, nitrogen doses and the interaction of these two factors were found to be significant in Sorghum x Sudan grass-Hayday cultivar (Figure 1). Plant height varied between 190.9 cm and 257.3 cm (Table 1). Among the different sowing frequency and nitrogen doses, the highest plant height was 257.3 cm obtained from the plants with 20 cm sowing frequency and 12 nitrogen doses, while the lowest plant height was 190.9 cm obtained from the plants with 5 cm sowing frequency and 0 (zero) nitrogen dose. In terms of nitrogen applications, plant height increased with increasing nitrogen applications (Table 1).

Table 1. Some plant and chemical properties of the sorghum sudan grass hybrid

Row distances	Nitrogen doses	Plant height, cm	Green herbage, kg/da	Dry matter ratio, %	Dry matter yield, kg/da	Stem ratio, %	Leaf ratio, %	Panicle ratio, %	Crude protein ratio, %	NDF, %	ADF, %
	0	190.9	1837.7	28.4	522.2	69.7	16.8	13.3	6.4	60.7	32.1
5	6	251.6	3228.5	28.7	925.2	72.3	18.6	9.1	7.5	61.8	33.2
	12	236.1	2439.1	28.9	706.3	72.9	17.4	9.7	7.5	60.9	33.6
	18	220.7	3071.9	27.7	851.7	74.3	16.8	8.9	8.1	59.2	31.7
	0	234.4	2363.2	28.9	680.9	72.4	16.3	11.4	7.1	60.5	32.3
10	6	228.2	2449.4	30.0	735.5	70.1	18.3	11.6	6.9	63.1	34.5
	12	253.5	2804.5	28.9	804.3	73.9	15.3	10.8	6.9	61.0	33.4
	18	249.6	2684.6	28.0	750.1	71.4	16.7	11.9	7.3	60.7	33.7
	0	250.1	2404.7	29.8	715.8	74.8	15.3	9.8	8.0	62.4	33.1
15	6	247.5	2765.6	28.1	776.6	72.6	16.5	10.8	6.9	60.6	32.5
	12	242.0	2765.5	29.6	818.6	72.6	17.0	10.4	7.2	60.9	33.5
	18	245.2	2198.4	27.7	608.7	71.3	16.9	11.7	8.1	60.6	33.7
	0	223.5	1756.1	30.0	526.7	73.4	17.3	9.4	6.8	60.2	32.1
20	6	224.5	2386.1	28.0	669.3	69.1	20.2	10.7	7.6	63.6	33.9
	12	257.3	2623.9	27.2	714.6	73.2	16.3	10.4	7.8	60.6	33.5
	18	247.5	2976.2	27.9	832.7	74.2	15.7	10.7	7.4	58.5	33.2

Frequency means

Row distances	Plant height	Green herbage, kg/da	Dry matter ratio	Dry matter yield	Stem ratio	Leaf ratio	Panicle ratio	Crude protein ratio	NDF	ADF
5 cm	218.5 ^{c}	2644.3 ^{a}	28.4	751.3 ^{a}	72.3	17.4	10.2	7.4 ^{a}	60.6	32.7
10 cm	234.7 ^{b}	2575.4 ^{ab}	28.8	742.7 ^{a}	71.9	16.6	11.4	7.0 ^{b}	61.3	33.4
15 cm	239.9 ^{a}	2533.5 ^{b}	28.7	729.9 ^{a}	72.8	16.4	10.7	7.5 ^{a}	61.1	33.2
20 cm	231.2 ^{b}	2435.6 ^{c}	28.2	685.8 ^{b}	72.4	17.3	10.3	7.4 ^{a}	60.7	33.2
Nitrogen										
0	224.7 ^{c}	2090.5 ^{b}	29.2 ^{a}	611.4 ^{b}	72.6 ^{a}	16.4 ^{b}	10.9	7.0 ^{c}	60.9 ^{b}	32.3 ^{b}
6 kg	237.9 ^{b}	2707.3 ^{a}	28.7 ^{a}	776.6 ^{a}	71.0 ^{b}	18.3 ^{a}	10.5	7.2 ^{bc}	62.3 ^{a}	33.5 ^{a}
12 kg	247.2 ^{a}	2658.2 ^{a}	28.6 ^{a}	760.9 ^{a}	73.1 ^{a}	16.5 ^{b}	10.3	7.3 ^{b}	60.8 ^{b}	33.6 ^{a}
18 kg	240.7 ^{a}	2732.7 ^{a}	27.8 ^{b}	760.8 ^{a}	72.8 ^{a}	16.5 ^{b}	10.8	7.8 ^{a}	59.7 ^{c}	33.1 ^{a}

a,b= different letter within column shows the statistical diference (P<0.05).

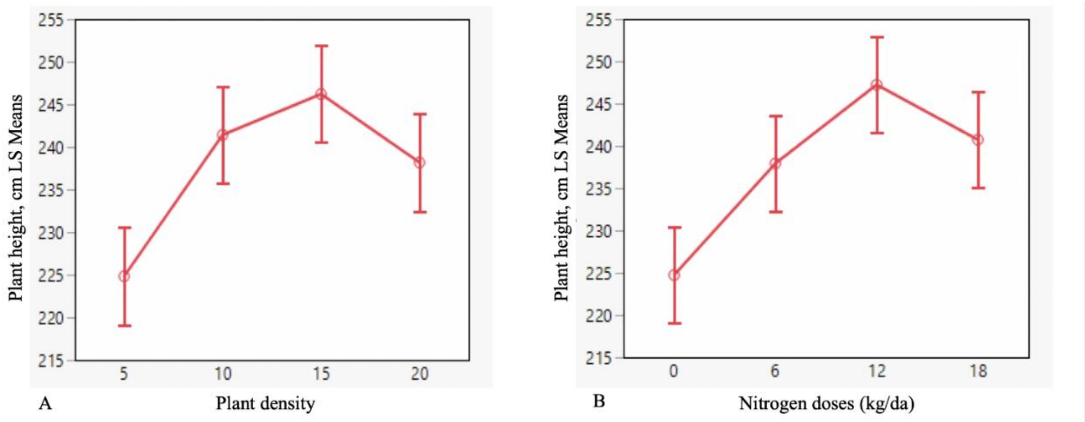

Figure 1. A- Plant height and density, B- Plant height and Nitrogen dose.

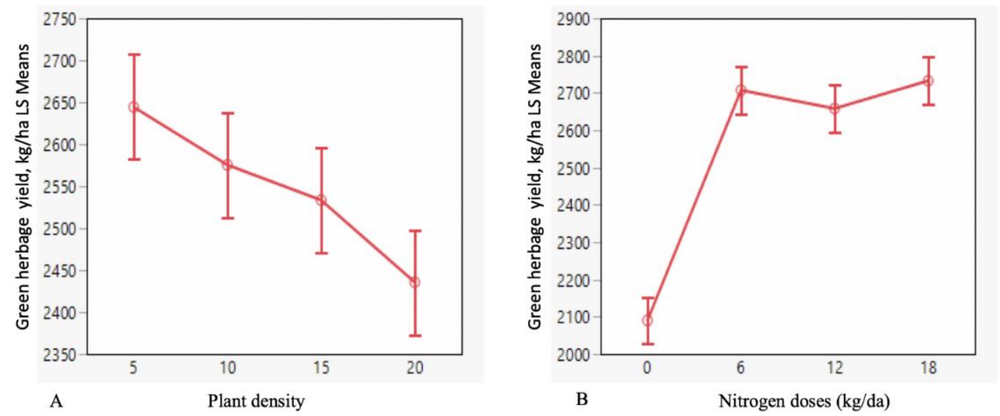

Figure 2. A- Green herbage yield and density, B- Green herbage yield and Nitrogen dosage.

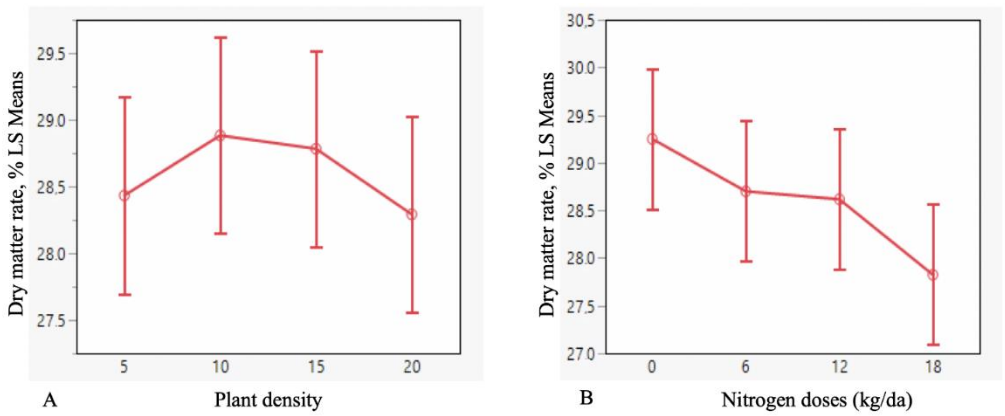

Figure 3. A-Dry grass ratio and density, B-Dry grass ratio and Nitrogen dosage.

When the plant height data of sorghum plants were evaluated in terms of sowing frequency, the plant height increased as the row spacing increased, but showed a decrease at 20 cm row spacing (Figure 1). However, due to the increase in N doses, plant height increased up to 12 N doses per hectare and showed a decreasing trend after the next N dose of 18 kg/ha (Figure 2). In the study, plant height varied between 190.9 cm and 257.3 cm, and Karadağ et al. (2014) found plant height as 183.9-224.2 cm, which is very close to the results of the study. At the same time, Gonulal, (2020) reported the average plant height as 269.0 cm in a study using hayday variety. Some researchers reported that different row spacings (30-40-50 cm) in different regions did not affect plant height of sorghum silage varieties (Yılmaz et al., 2003; Güler et al. 2003). It is similar to the results obtained in the study conducted by Ghazal and Al-Juheishy (2024) with plant height values (158.58-187.60 cm). Additionally, Ozkan et al. (2023) reported that the average plant height was 210.37 cm.

As the distance between rows increases, it may cause an increase in dry matter rather than plant height due to the plant's tendency to tiller more. As can be understood in Figure 1, both the green herbage yield rate and the increase in nitrogen doses up to a certain amount and the decrease after that show the importance of determining the most suitable planting density and nitrogen applications in cultivated plants.

3.1.2. Green herbage yield (kg/da)

According to the variance analysis of green herbage yield, both green Herbage yield and nitrogen doses and the interactions of these two factors were found to be statistically significant ($P<0.05$). In this study, green herbage yield of sorghum, which is an important feed source in arid and semi-arid regions, varied between 1756.1 kg/ha and 3228.5 kg/ha (Table 1).

When Table 1 is analysed, the highest green herbage yield with 3228.5 kg/ha was obtained from 5 cm row spacing and 6 nitrogen doses. The lowest green herbage yield was obtained from 20 cm row height and 0 (zero) nitrogen dose application with 1756.1 kg/ha. Table 1 shows that the increase in row height showed a decrease in green herbage yield. However, nitrogen increase caused an increase in green herb yield in the above-row applications (Figure 2-A). According to the fertiliser doses, less green herbage yieldwas obtained from the control plots without nitrogen. There was no statistically significant difference between 6, 12 and 18 kg N fertiliser doses (Table 1 and Figure 2-B). Green herbage yield of sorghum plants, in which nitrogen doses and different sowing frequencies were examined, varied between 1756.1 kg/ha and 3228.5 kg/ha. Some researchers found these values lower than the values of Karadağ et al. (2014), green herbage yield 2128.2-4764.3 kg/da, Keskin et al. (2018), green herbage yield 3482.0-8337.6 kg/da.

3.1.3. Dry matter ratio (%)

In this study, green grass yield and nitrogen doses were discussed; Nitrogen doses have a partial effect on dry matter (Figure 3-A) and their interactions are insignificant (P>0.05). The dry matter ratio varied between the lowest 27.2% and the highest 30%. Despite the increase in nitrogen doses, a decreasing trend in the hay ratio of Sorghum was determined (Table 1 and Figure 3-B). The results of the study by Keskin et al. (2018) showed similar results with hay ratio values of 32.0-38.0%. Ozkan et al. (2023) reported the average dry matter ratio as 32.53% in their study.

3.1.4. Dry matter yield (kg/da)

In terms of variance analysis on dry matter yield, as in green grass yield, both in-row and nitrogen doses and their interactions were found to be statistically significant on dry matter yield (P<0.05). According to the data obtained, dry matter yield varies between 522.2 kg/da and 925.2 kg/da. Keskin et al. (2018) reported the hay yield between 1141.2 -2658.1 kg/da in their study. The data obtained from Keskin et al. (2018) was observed to be lower than the results.

In parallel with the increasing green herbage yield, Dry matter yield has also increased, and the lowest Dry matter yield was obtained from the plant plots planted at least frequently, 20 cm over rows (Table 1 and Figure 4-A). The green herbage yield alone shows that Sorghum can be planted in rows of 5 to 15 cm. Dry matter yield is not statistically significant between applications given 6, 12 and 18 kg / N per hectare (Table 1), Dry matter yield from 6 kg N / da to 18 kg N / da is almost constant with a very decrease if the plant is dry If it will be considered as grass, it shows that 6 N application per decare will be sufficient (Figure 4-B).

While the Dry matter yield values of this experiment are between 522.2-925.2 kg/da, dry matter yield 1433.7-3422.3 kg da; Karadağ et al. (2014), dry matter yields 935.0-1924.0 kg/da; Yazıcı (2005) found that Dry matter yield is lower than the yields of 977-2055 kg/da. Güler et al. (2003), in their studies conducted under Diyarbakır conditions, emphasized that in order to increase the Dry matter yield per unit area in silage sorghum cultivation, the gaps between rows should be narrowed in relation to green grass yield per unit area. On the other hand, Kaplan et al. (2019), in sweet Sorghum, the greater the distance between the rows, the lower the feed yield.

Increasing green herbage yield in sorghum cultivation affects feed yield and quality (Zhao et al., 2022). Beyaert and Roy (2005) reported that the maximum feed yield was obtained at 125 kg N/ha. Some studies have reported that increasing the amount of nitrogen above the threshold does not benefit the dry matter yield of forage sorghum (Marsalis et al., 2010).Some researchers reported that with increasing row spacing, the total biomass yield decreased, and the highest yield was obtained in the first year (May et al. 2015).

3.1.5. Stem ratio (%)

The stem ratio of Sorghum varied between 69.1% and 74.8% (Table 1). From nitrogen applications, the sap ratio value of 6 kg/da N dose was lower than control and other applications with a very small difference. However, neither the densities nor the effects of nitrogen on the stem ratio were seen clearly and obviously in these applications (Figures 5-A, 5-B). The stem ratios of the sorghum plant were similar to the stem values (69.7% and 73.2%) of a study conducted by Yilmaz (2000) under Van conditions. Additionally, Keskin et al. (2018) reported the stem ratio as 71.7-78.0% in their study. The data obtained are consistent with other literature data. Some researchers have shown that the effect of green herbage yield and nitrogen applications on grain and Dry matter yield is important in grain sorghum (Musemwa and Musara, 2020).

3.1.6. Leaf ratio (%)

The effect of nitrogen dose on leaf ratio was found to be significant (P<0.05). The leaf ratio in forage plants is one of the important factors affecting the quality and efficiency of the produced feed, and in this experiment, the leaf ratio varied between 15.3% and 20.2% (Table 2).

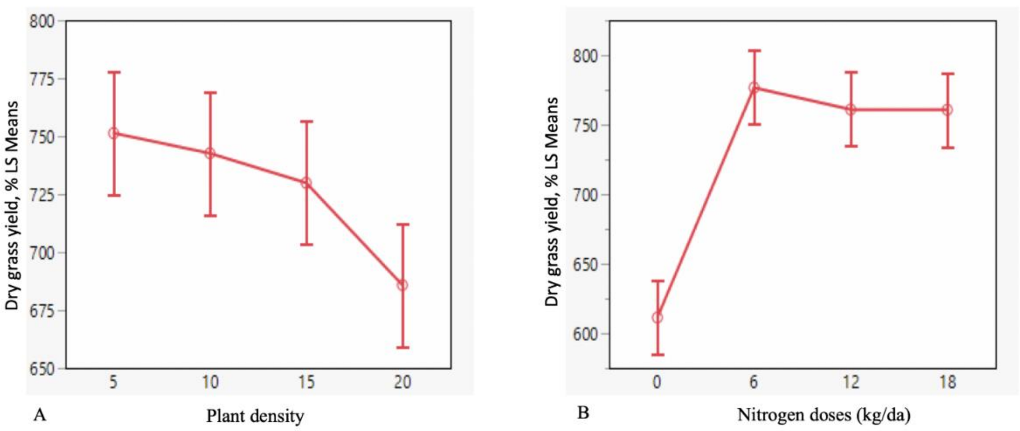

Figure 4. A-Dry grass yield and density, B- Dry grass yield and Nitrogen dose.

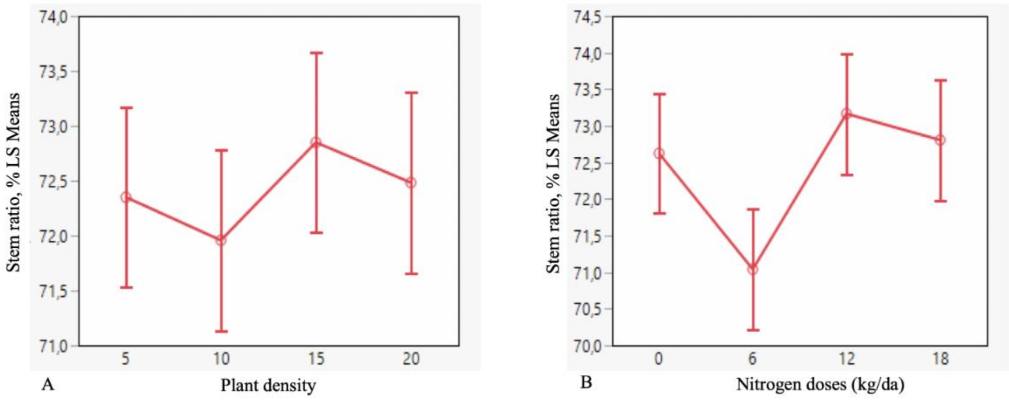

Figure 5. A-Stalk ratio and Frequency, B-Stalk ratio and Nitrogen dosage.

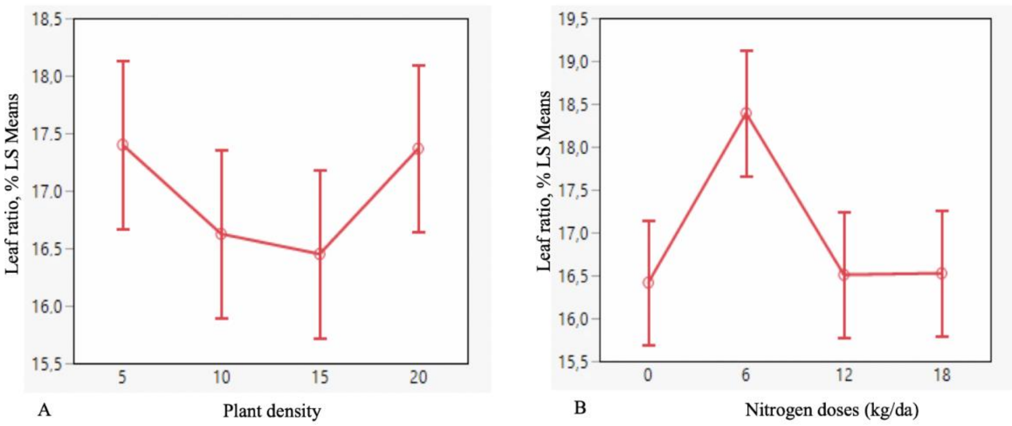

Figure 6. A-Leaf ratio and Frequency, B-Leaf ratio and Nitrogen dose.

The green herbage yield decreased after 5 cm in the study and showed a fluctuating trend (Figure 6-A), but there was no significant difference between applications. Similarly, the leaf ratio of the plots applied at 6 kg N / da was found to be higher and significant compared to other applications (Figure 6-B) but not statistically significant (P>0.05).

A slight difference was observed between leaf ratios depending on nitrogen doses. In a similar study, it was observed that different nitrogen doses (0, 5, 10, 15 and 20 kg/da) did not affect the number of leaves of the plant in Bursa sugar sorghum. Keskin et al. (2018) reported that the leaf ratio varied between 15.7% and 20.0%. Also Kaplan et al. (2019) reported in their study that the leaf ratio was between 16.81 - 20.97%. Nitrogen fertilization positively affected yield and yield components, leaf area index, growth rate, and characteristics such as leaves, stems and clusters (Szabó et al., 2022).

3.1.7. Panicle ratio (%)

The factors and their interaction on the panicle ratio of the plant were not statistically significant (P>0.05). However, as the green grass yield increased from 5 cm to 10 cm, the bunch ratio increased slightly and followed an unstable trend (Figure 7). It is estimated that at 12 k/da N dose, the statistically insignificant decrease in panicle ratio is large. Kaplan et al. (2019) reported that the average cluster rate in their study with different nitrogen applications was between 68.88 - 71.37%. Some researchers have reported that N rates significantly affect the total weight of the panicle, with the optimum occurring at 9 kg/ha N (Xorse Kugbe, 2019). Others found that green grass yield and nitrogen levels were not important on the number of seeds per cluster, but they achieved green grass and hay yield with the application of 240 kg N/ha (Shahrajabian et al., 2011).

3.1.8. Crude protein ratio (%)

It was observed that different row distances and nitrogen doses applied to the sorghum plant were very important on the crude protein yield rate of the plant (P<0.05). The nitrogen content of sorghum varies between 6.4% and 8.1%. Although there was not a large variation between the nitrogen rate of the plant and the nitrogen application in terms of density, the nitrogen rate in the 10 cm density application was slightly lower than in other applications; In terms of nitrogen, the crude protein rate of the plant to which 18 doses of nitrogen was applied per decare was higher at 7.8% compared to other applications, and the rate of the plant without nitrogen was the lowest at 7.0%. The density effect on crude protein differed little (Figure 8-A); However, no significant differences were found between nitrogen treatments; the crude protein ratio increased in parallel with the dose increase (Figure 8-B).

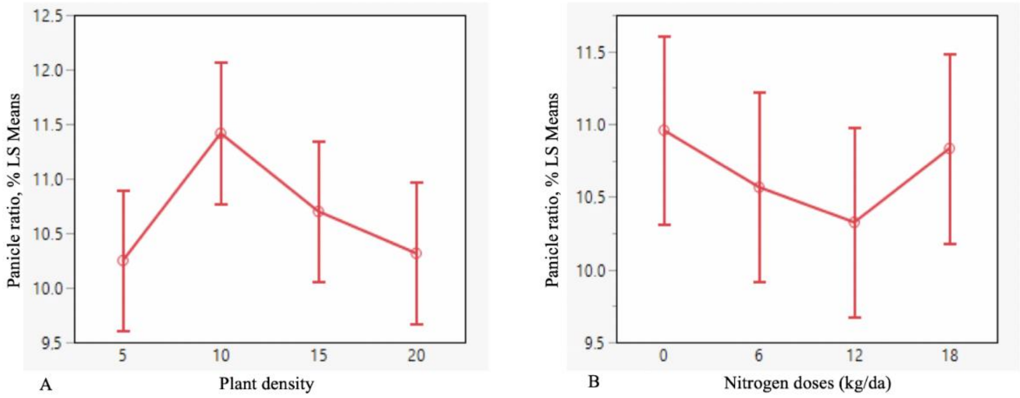

Figure 7. A-Bunch ratio and Frequency, B-Bunch and Nitrogen dosage.

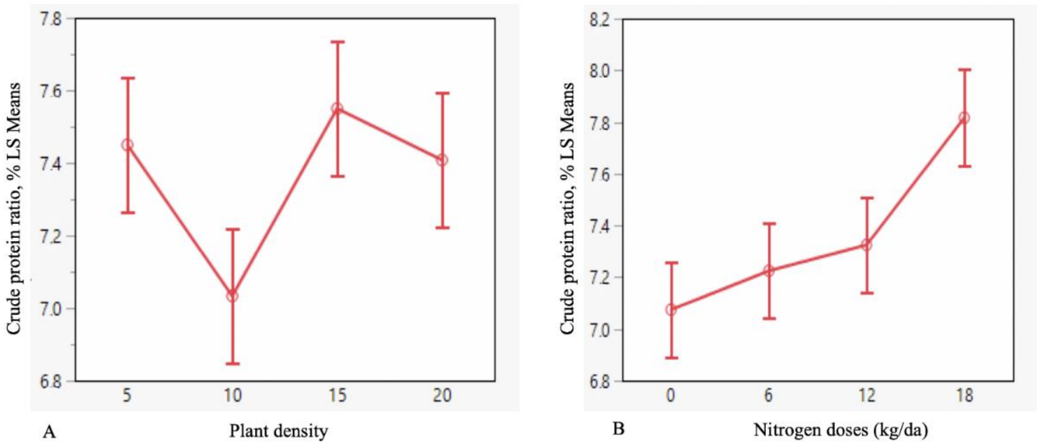

Figure 8. A-Crude protein ratio and Frequency, B-Crude protein content and Nitrogen dosage.

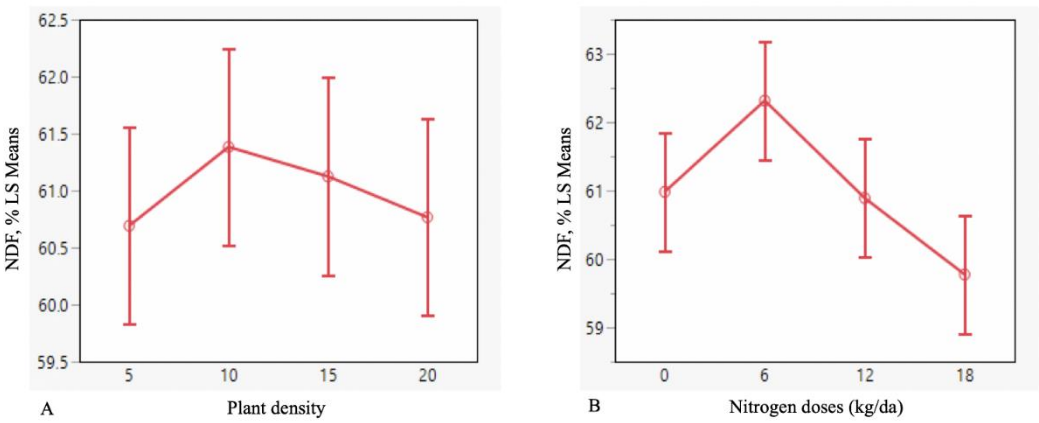

Figure 9. A-NDF ratio and Frequency, B-NDF ratio and Nitrogen dose.

In this research, the crude protein content of sorghum varied between 6.4% and 8.1%. Kaplan et al. (2019) reported in their study with different nitrogen applications that crude protein ratios varied between 4.88 - 6.70%. Similar results are seen when compared to the literature. In our study, it was determined that there was an increase in the crude protein ratio with increasing nitrogen content.

3.1.9. NDF ratio (%)

The NDF ratio of the plant varies between 58.5% and 63.6% depending on density and nitrogen averages. Only the applied nitrogen doses had an effect on the NDF ratio of the plant; the NDF ratio of the plants in the plot where 6 kg nitrogen was given per decare was highest with 62.3%; The lowest one was taken from the parcel with the highest nitrogen (Table 1 and Figure 9-A). Although the density was insignificant, NDF started to decrease in the plant after 10 cm; similarly, if the control plot is not taken into account, the NDF content of the plant decreased after 6 kg N/da. Similar results were reported by Kaplan et al. (2019) in their study, the NDF ratio is similar to the values of 57.20 - 61.33%.

3.1.10. ADF ratio (%)

The interaction of density and nitrogen dose was not statistically significant, except for nitrogen doses in sorghum (P>0.05). The ADF rate of sorghum varies between 31.7% and 34.5%. However, nitrogen doses, although significant, may have appeared more pronounced between treatments. It was observed that the ADF rate in the control plot where only fertilizer was not applied was lower than in nitrogen applications (Table 1). Nitrogen increased the ADF rate from 32.7% at 5 cm row spacing to 33.4% at 10 cm density (Figure 10), the ADF rate of nitrogen applications is shown in Figure 10. The ADF rate obtained in the study was compared to that of Kaplan et al. (2019) found that the ADF rate was lower than 34.80 - 38.83%.

Structural carbohydrates found in roughage are divided into two groups: NDF (cellulose, hemicellulose and lignin) and ADF (cellulose, hemicellulose) (Tekce and Gül, 2014). The NDF rate of the sorghum plant used in the experiment varies between 58.5-63.1% and the ADF rate varies between 31.7-34.5%. Karadağ et al. (2014), NDF rates are 61.23-63.00% and ADF rates are 39.14-40.92%. Additionally, Kaplan et al. (2019), NDF rate, 57.20 - 61.33%, ADF rate, 34.80 - 38.83%. This study, Karadağ et al. (2014), Kaplan et al. (2019) is supported by the results.

Atalay (2019) examined the effects of different nitrogen applications on yield and some quality characteristics of sorghum x sudangrass (*Sorghum bicolor* (L.) Moench x *Sorghum sudanense* (Piper) Stapf) hybrid varieties, and the increased nitrogen content, excluding the leaf ratio in the plant. The total green and hay yield and crude protein ratios are investigated; on the other hand, the crude fiber, ADF, and NDF ratios have decreased with the increase in the amount of pure nitrogen applied per decade. It is seen that it is very in line with the results of this study (Atalay, 2019). In a similar study, Olak and Tan (2016) reported that different nitrogen doses significantly affected other parameters, except for nitrogen doses in Gin Millet (*Panicum italicum* L.) ADF and NDF ratios in millet.

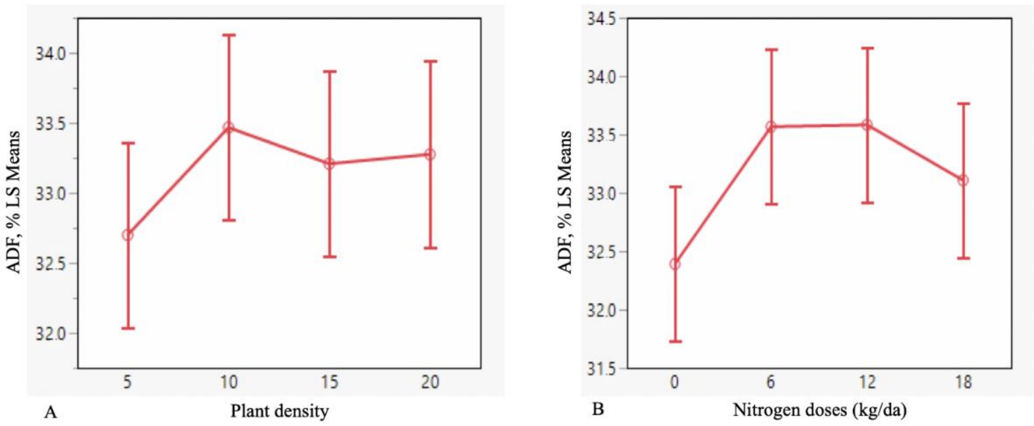

Figure 10. A- ADF ratio and Frequency, B- ADF ratio and Nitrogen dose.

Table 2. Correlations between some agronomic and quality traits of sorghum plant

	Plant height, (cm)	Green herbage yield, (kg/da)	Hay ratio, (%)	Hay yield, (kg/da)	Leaf ratio, (%)	Stem ratio, (%)	Panicle ratio, (%)	Crude protein ratio, (%)	PDF ratio, (%)	ADF ratio, (%)
Plant height, cm	1	0.510**	-0.064	0.482**	-0.332*	0.483**	-0.308*	0.209	0.142	0.418**
Green herbage yield, kg/da		1	-0.179	0.957**	-0.129	0.341*	-0.299*	0.356*	-0.120	0.088
Hay ratio,%			1	0.102	0.105	-0.045	-0.039	-0.186	0.050	-0.069
Hay yield, kg/da				1	-0.099	0.324*	-0.303*	0.306*	-0.112	0.057
Leaf ratio,%					1	-0.726**	-0.152	-0.064	0.178	0.125
Sap ratio,%						1	-0.518**	0.300*	-0.133	-0.086
Panicle ratio,%							1	-0.261	-0.083	-0.103
Crude protein ratio,%								1	-0.057	0.051
PDF ratio,%									1	0.581**
ADF ratio,%										1

Türk and Alagöz (2019) support the results of this study, with the results that nitrogen applications on a different plant, bee grass, increase the hay yield and crude protein ratio while decreasing the ADF and NDF ratios.

3.1.11. Correlation between investigated properties of the Sorghum

The correlation between plant height, green herbage yield, hay rate, hay yield, leaf rate, stem ratios, and crude protein, NDF, and ADF ratios are shown in Table 2. Plant height and green herbage yield, hay yield, and ADF ratio are very significantly and positively related, and the leaf ratio has been found to be significantly but negatively related to the panicle ratio ($P<0.05$). The positive and strongest relationship between green herbage and hay yield was r=0.957. However, a strong negative relationship between leaf ratio and stem ratio r=0.726 was observed. In a study conducted with grain sorghum, they stated that the plant given 45 and 90 kg N per decare caused an increase of 13% and 48% in yield, respectively, compared to the control. There was a strong relationship between the number of seeds and the total biomass and grain yield (Mahama et al., 2014). The fact that this study is a single year was insufficient to reveal the relationships between the investigated features.

4. Conclusion

Climate change and rapid population growth have exacerbated global food production problems, particularly affecting agricultural areas. Despite Turkey's significant potential in livestock production, returns per animal remain low, mainly due to inadequate quality forage resources and declining meadow and pasture production. Sorghum as an alternative to maize considering Turkey's roughage needs, sorghum is an excellent maize substitute for animal feed. Sorghum is tolerant to high temperatures and drought and regrows rapidly after harvest. Due to its xerophytic properties, adaptable sorghum (*Sorghum bicolor* L.) is widely grown as forage and roughage. The availability of quality feed is critical for dairy animal performance and shortages of quality feed limit profitable livestock production in developing countries. Introduction of high yielding crop varieties is vital to overcome this shortage.

This study aimed to assess the potential of sorghum for high quality biomass production in the Igdir Aras basin. Development of high yielding varieties is necessary to meet the increasing demand for animal feed (Chohan et al., 2006). This study focussed on the agronomic characteristics of different row spacing and nitrogen doses in Sorghum x Sudan grass-Hayday cultivar, including crude protein, NDF and ADF ratios. According to the data obtained as a result of the study, plant height varied between 190.9 cm and 257.3 cm with the highest at 20 cm sowing frequency and 12 nitrogen doses and the lowest at 5 cm sowing frequency without nitrogen dose. Increasing nitrogen applications are associated with increasing plant height (Karadağ et al., 2014; Gonulal, 2020; Özkan et al., 2023; Ghazal and Al-Juheishy, 2024). Green grass yield varied between 1756.1 kg/ha and 3228.5 kg/ha. The highest yield was obtained at 5 cm row spacing and 6 nitrogen dose and the lowest yield was obtained at 20 cm row height without nitrogen. Increasing nitrogen doses were associated with higher yield (Karadağ et al., 2014; Keskin et al., 2018). Dry matter ratio varied between 27.2 and 30%. Increasing nitrogen doses showed a decreasing trend in dry matter ratio (Keskin et al., 2018; Özkan et al., 2023). Increasing green grass yield is associated with increasing dry matter yield. Dry matter yield varied between 522.2 kg/ha and 925.2 kg/ha. Optimum sowing frequency and nitrogen applications are very important for yield (Keskin et al., 2018).

Stem ratio varied between 69.1% and 74.8%. Nitrogen applications did not significantly affect the stem ratio. Leaf ratio varied between 15.3% and 20.2% and nitrogen doses positively affected leaf ratio and quality (Yılmaz, 2000; Keskin et al., 2018; Kaplan et al., 2019). Crude protein ratio varied between 6.4 and 8.1%. It can be said that increasing nitrogen doses positively affect the crude protein content (Kaplan et al., 2019). NDF ratio varied between 58.5% and 63.6% and ADF ratio varied between 31.7% and 34.5%. Nitrogen doses significantly affected these rates, and increasing nitrogen was associated with higher NDF and ADF rates (Tekce and Gül, 2014; Kaplan et al., 2019).

Sorghum, a resilient and versatile crop, has significant potential to improve animal feed quality and yield in arid and semi-arid regions. To maximise the agronomic benefits of sorghum, it is very important to optimise sowing frequency and nitrogen applications. In this study, it was concluded that sorghum for silage (Sorghum bicolor x Sorghum sudanense Mtapf.) Hayday can be sown at a sowing rate of 28.570/piece per decare and 6 kg nitrogen fertiliser per decare may be sufficient.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.A.	B.K.	B.E.
C	25	25	50
D	50	25	25
S	40	30	30
DCP	40	30	30
DAI	50	25	25
L	40	30	30
W	40	30	30
CR	50	25	25
SR	40	30	30
PM	40	30	30
FA	50	25	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

This study was supported by Iğdır University BAP (2017-FBE-L09).

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1466167

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 497-504 / September 2024

CONSUMPTION ESTIMATES OF FOOD CALORIES IN PORT HARCOURT HOUSEHOLDS, SOUTH-SOUTH NIGERIA: LA/AIDS APPROACH

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Abstract: This research analyzed the households in Port Harcourt city identified the demand patterns through survey and evaluated the effect of price, income and other factors on demand for food calories. Broadly this paper estimated the demand for calories consumed households by utilizing data sets that include household consumption amounts, food commodity prices and expenditures. The paper employs the Linear Approximate Almost Ideal Demand System (LA/AIDS) to estimates expenditure elasticities for the aggregate commodities: rice, yam, garri, and fufu, providing an insight into differences in calories consumption levels and patterns across households. Results of the survey shows that one year increase in the education of household head decreases the share of fufu by 0.378 per cent while that of rice and yam increases by 0.067 and 0.711 percentage respectively. Again, male headed households spend more on fufu (0.590) compared to their female headed counterparts, while it is 0.590 and -0.365 percent smaller for garri and yam. The coefficient of the variables for household size equivalence is negatively significant for garri (-0.897) and fufu (-0.976). Results of tests of homogeneity shows that homogeneity condition in the estimated demand system holds only for fufu, yam and rice. In this connection, it is recommended that greater capacity must be built for multi-disciplinary research and development. Such research and development efforts will undoubtedly reveal a host of calorie food-based products and alternative processing technologies that will enhance household consumption of the products.

Keywords: Urban, Households, Elasticities, Consumption, Gender, LA/LAIDS

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Received: April 07, 2024
Accepted: August 22, 2024
Published: September 15, 2024

Cite as: Edaba MIE, Aroyehun AR, Onyenma GC, Edaba FC. 2024. Consumption estimates of food calories in Port Harcourt households, South-South Nigeria: LA/AIDS approach. BSJ Agri, 7(5): 493-504.

1. Introduction

Changes in the demand and availability of household food in Nigeria are primarily caused by shifts in income growth, urbanization, and patterns of food consumption. Due to the high rate of population growth in the nation, which averages 5 million new births annually at a rate of 2.4%, food needs are rising at a pace higher than 3.5% while food supply is increasing at a rate of over 2.1% (FAO, 2020). Evidence of this tendency has also been observed in China, Kenya, Nepal, and India (Gido et al., 2017; Korir et al., 2018; Zhao et al., 2022). Despite improvements in ensuring adequate supply of food in the last ten years, a sizable percentage of impoverished households still exist in developing nations, such as Nigeria. In order to prevent hunger and malnutrition, the majority of people must consume enough food to meet the minimum daily energy requirement of 2260 kcal per capita, as suggested by the Food and Agriculture Organization FAO (2017). The analysis from FAOSTAT indicates that per capita supply of calories has remained almost negatively skewed in Nigeria and has recently fallen in many homes due to economic recession, unemployment and inflation (Oladimeji et al., 2018). While the number of hungry individuals fell from almost one billion to less than 800 million, throughout this period, the average percentage per capital daily calorie consumption (kcal per capita per day) in developing countries climbed from 2151 in 1976 to 2850 in 2015 and estimated to reach 2980 by 2030 (FAO, 2003). However, this is below the 2500 kcal for men and 2000 kcal for women recommended by FAO and National Health Service NHS (2023) as the standard minimum daily requirement. The estimated 800 million undernourished people are chronically poor and will not be able to achieve food security unless dramatic steps are implemented to boost the incomes of the underprivileged while raising agricultural productivity and reducing reliance on food imports.

According to estimates, 795 million people worldwide lack access to food that is both sufficient and of the correct quality, and one in three of them are severely malnourished (Obalola et al., 2021). According to estimates, almost 40% of Nigerians are undernourished (Ashagidigbi et al., 2012). Most households' productivity is negatively impacted and their capacity to make the best use of food is hindered due to several types of deprivation of basic necessities. Three main variables influence the demand for food: population expansion, urbanization, and changes in consumption habits that lead to an increase in the number of people and an improvement in infrastructure (Pieters et al., 2013).

The need for calories to satisfy our daily productive schedules, particularly in the areas of health, labour, and food security, is the basis for demand estimates of household calorie intake (Aromolaran, 2004). Most definitions of enough food place more focus on the number of calories required for an active, healthy existence than on the necessity of merely surviving (Subramalan and Deaton, 1996). Food consumption has been demonstrated to have a high empirical correlation with productivity and human health from biblical views. Nuani et al. (2022) have noted that an individual is at risk for health problems and therefore unproductive if their lifetime calorie consumption is below a certain threshold.

The Federal Government of Nigerian has established food policies and programmes, such as the Agricultural Transformation Agenda, the Agricultural Credit Guarantee, and the Microcredit Scheme for the production of root and tuber crops. These programmes were aimed at promoting the sustainable development of the country's agricultural sector in a bid to expedite import substitution while placing special emphasis on reducing food importation. Since the majority of demand estimates now in use are derived from time series data based on average consumer behaviour rather than the conduct of the homes of interest, they may not be sufficient for assessing the effects of these programmes. Inferentially, determining demand linkages from household surveys becomes imperative and critical for determining the actual situation of urban calories consumers.

The response of households to changes in socioeconomic features in low-income nations is a topic of much dispute; yet, the empirical evidence seems contradictory and layered. For example, estimates of household demand elasticities for food calories by Strauss (1984), Kormawa et al. (2002), and Nuani et al. (2022) are relatively high, supporting the idea that calorie consumption increases with economic expansion. However, estimates of elasticity's that are close to zero have been obtained by Musyoka et al. (2014) and Rozi et al. (2021), indicating that a rise in income will not likely result in a significant improvement in food consumption. They contend that, even among the most impoverished, households tend to substitute taste preferences, accessibility, and nutrient-free options as wealth grows, creating a flat income distribution.

Despite all of these findings, it is evident from the literature on demand analysis that relatively few researches have really looked at the factors influencing calorie intake in sub-Saharan Africa. This stands in contrast to the numerous empirical studies on the consumption of calories by households in South America, Europe, and Asia. Therefore, by closing the gaps in the empirical literature regarding food consumption trends in Nigeria, this paper adds to the present body of literature. The study adopts a unique data on households' characteristics in Port Harcourt, South-Southern Nigeria. The principal objective of this study is therefore aimed at investigating the relationship that exist between consumption expenditure and prices of garri, rice, yam and fufu (a major cassava product in Nigeria) in the study area.

The theory of calories is of paramount importance to this study. Baba et al. (1999) assert that calories are the primary factor in weight gain or loss and that "a calorie is a calorie" regardless of the source (for instance carbohydrates, fat, protein among others). A calorie is a calorie is a principle that is supported by most nutritionists, including Gillian McKeityh and Jack Groppell. According to this school of thinking, "calories in, calories out" determines how much weight is lost or gained. In other words, regardless of the source of the calories, weight loss will occur if an individual burns more calories than they consume. Conversely, weight gain will occur if someone consumes more calories than they burn each day. This idea is based on the observation that carbs and protein both contain around 4 calories per gram, and it doesn't matter where these calories come from. They base this on the many calories consumed daily; weight reduction is the outcome, and vice versa; if one consumes an additional x number of calories daily, weight gain results (Piatti et al., 1994). As a result, sufficient nourishment influences growth and efficiency, and healthy feeding promotes the body's natural development.

Another relevant theory is the Brink's unified theory of nutrition. A person's weight growth or loss is determined by their macronutrient ratio, not by their total calorie intake. According to studies based on this theory, people who follow a higher-protein, lower-carb diet lose roughly the same amount of weight as those who follow a higher-carb, lower-protein diet. This simple statement helps people understand the differences that some people experience when they consume the same number of calories but have very different ratios of fat, carbohydrate, and protein. However, according to Layman et al. (2005), the group following the higher protein diet lost less lean body mass (muscle) and more real fat. The study also shows that a higher protein diet may actually result in less weight loss than a lower protein, higher carbohydrate diet when the same number of calories are consumed but different macronutrient intakes are made. The lower carbohydrate, higher protein diets actually result in more fat reduction. As one might anticipate, exercise generally has a greater impact.

2. Materials and Methods

The data collection procedure and scope of data for the present study came from a sample of urban household dwellers in the city of Port Harcourt, in Nigeria. This is stratification into strata at this first stage and then random selection. Therefore, 2-stage selection was adopted based on the kinds of homes and residential houses that is low-, medium-, and high-income households they occupied. Two hundred and forty (240) respondent households, drawn at random from each of the three income categories, make up the sample. Data on socioeconomic characteristics, expenditure share of the commodity consumed and products (garri, fufu, yam and rice) were collected from the houses using a well-designed questionnaire. Household heads who responded were asked to recall and state how much they spent, how much they consumed of each food item under consideration, how much they spent in naira, and how much food was physically consumed in the household over the course of the previous seven days.

The survey items included household consumption quantities, income, prices and total expenditure data on food commodities as well as demographic characteristics of each sampled household. In particular, data on age, sex, marital status, educational levels, and household size equivalence etc. were gathered. Other information collected in the surrey included major occupation of respondents and constraints limiting consumers in the area.

2.1. Model Specification and Estimation

In this study, we used an approximation of the Almost Ideal demand system, proposed by Deaton and Manlbaurer (1980) and employed in several food consumption studies such as Deaton and Muellbauer (1986), Edgerton (1997), Tsegai and Kormawa (2009), Bett et al. (2012), and Nuani et al. (2022) and it's applied to a system of three products namely garri, fufu, yam and rice. These three products are key food staples in Port Harcourt household diets. In this model, quantity demand is represented by the budget share of each commodity while price and income are expressed in logarithms. Household consumption expenditure were used in this analysis as a proxy for income because data on expenditures are generally more reliable than income data as questions of income are sensitive and is expected that households underestimate their income (Gibson,1995; Tsegai and Kormawa, 2009). To ensure a more accurate estimation we first used a multistage budgeting strategy, where the household first distributes its expenditure in terms of its total expenditure over broad categories of household commodities, such as food and non-food goods, in order to assure a more accurate estimation. After then, it divides the corresponding spending percentages across the several subcategories of the preceding broad categories (Bett et al., 2012).

Due to the model's practical qualities and adaptability, demand analysis has made extensive use of it. First, the predicted budget shares also add up to 0.1 if the system of equations is complete, meaning that the actual budget share sums up to 1.0. This is referred to as adding up. Once more, imposing or testing for symmetry in the cross-price items is not too difficult. Thirdly, the demand equation and its derivation from a well-behaved utility function are consistent with economic theory. In our analysis, the model aggregates the following variables: educational attainment, gender of the household head, married status of the household head, and family size (equivalence). To incorporate these demographic variables, the LA/AIDS (linear approximate almost ideal demand system) was specified as given in Equation 1:

$$W_it = \alpha_i t \ P_ie + P_is + P_im + P_iq + \beta \log \left(\frac{Xt}{Pt^{ж}}\right) + \sum_j Y_{ij} \log P_jt \quad (1)$$

where;

W_it = Expenditure share of the thy commodity at period t

α_i= Interceptive of the expenditure in the absence of price, income and other demographics

P_ie = Number of years spent in school

P_is = Gender of household head (male 1, otherwise = 0)

P_im = Marital status of household head (married=1, otherwise = 0)

P_iq = Household size equivalence derived as Es and given as in Equation 2:

$$Es = (A + ac)\ \hat{}\ b \quad (2)$$

where;

Es = Household equivalence scale which divides household expenditure to obtain per capita expenditure

A = Number of adults in the individual household

C = Number of children in the individual household

b = Household economics of scale constant (0.80)

a = Child adult equivalence constant (0.54)

b = Expenditure coefficient (slope)

Xt = Total expenditure on all commodities under the study period

P_{jt} = Price of the jth commodity during period, t

$P_t^{ж}$ = Price index which makes the system linear (approximated using Stone's price index) and it's defined as given in Equation 3:

$$P^{ж}_t = \sum WjwgPjt \quad (3)$$

where;

Wj = Expenditure share of the jth commodity

Yij = Coefficient of the row sums of prices and expenditure matrix (Homogeneity testing).

The equation applied to each of the three food commodities. The three equations can be estimated independently under two conditions.

a) If we use the same explanatory variable in each equation or
b) If we do not wish to impose any cross-equation on the system of equations (Gibson, 1995; Abdulai and

Aubert, 2004).

In this study the homogeneity condition is tested, the adding-up condition is imposed by the model and so it's not testable (Deaton and Muellbaurer, 1986). In the context of the LA/AIDS, testing or imposing the symmetry restriction is valid only when the theoretical price index, which is non-linear is used, and not when an approximation index (Stone's price index) is used. Since the theoretical price index is not used in this study, symmetry restrictions are neither tested nor imposed.

The idea behind the concept of household equivalency is that disposable income or expenditure needs to be adjusted in order to compare the dynamics, characteristics, and disparities of households. This is done by determining which age group each member of the household belongs to. This is especially important if we want to accurately assess the factors that affect the welfare and living standards of various households or anticipate behavioural patterns that are common to them (Easton, 2001). Converting the incomes to a per capita basis could be a straightforward adjustment, but that would neglect the impact on household economics of scale and the various characteristics of the population. According to USCB (2021) it is not true that two can live as cheaply as one but two living together are likely to spend less than if they live separately in order to attain the same standard of living.

3. Results and Discussion

The first LA/AIDS regression results as presented in Table 1 indicate that, all things being equal, a one-year increase in the household head's education lowers the share of fufu by 0.378 percent, while the shares of rice and yam increase by 0.067 and 0.711 percentages, respectively. Also, households headed by men spend 0.590 percent more on fufu than households headed by women; similarly, for garri and yam, the expenditures are 0.590 and -0.365 percent lower, respectively. Given that many of the female home heads are widows, it is possible that these discrepancies reflect shifts in the ages of the household heads rather than gender differences in taste.

Table 1 further shows that household size (equivalence) has well-determined effect on garri and fufu expenditures. The coefficient of the variable for household size equivalence is negatively significant for garri (-0.897) and fufu (-0.976) indicating that consumption of calories declines with increasing household size. This outcome is in line with the economic laws established by Engels and Bennett. According to Engels' law, the percentage of a budget allocated to food expenditures tends to decrease as income increases. Bennett's law, which asserts that consumers reallocate their food budget away from starchy staples like yam, rice garri, and maize and towards more expensive sources of calories like fruits, vegetables, and animal items, is a comparable pattern. Nuani et al. (2022) discovered that households with higher income levels have greater purchasing power, which allows them to vary their consumption habits to include wheat goods, fruits, meat products, and beverages. The finding also agrees with Rono et al. (2017), who found negative own-price elasticities for calories such as cassava and yam as expected *a priori*. On the contrary, Manyong et al. (2007) found a positive own price elasticities for cassava flour and *fufu*, confirming the prevalence of the existence of close substitutes.

The domestic demand for non-food items and services appears to be growing faster than the need for food as the economy expands and income levels rise. As a result, demand for fruits, vegetables, and animal products is increasing more quickly than that of grains and tubers. Given that Port Harcourt is Nigeria's third-biggest industrial and commercial hub after Lagos and Abuja, higher income levels are anticipated in the city relative to the nation's average per capita. According to Subramanian and Deaton (1996), even when comparing families with just adults, it is not expected that members of the household will choose to consume the same amount of calories given twice as many resources. On the other hand, economies of scale or shared public goods could free up resources to allow for higher food and calorie consumption. In addition, a home in an urban region seems to have fewer calories accessible than a household in a rural area.

Table 1. Summary of parameter estimates

Products	A	R^2	Pie	Pis	Pim	Piq
Garri (1)	-1.251	0.281	0.237	-0.374	-2.058	-0.897
	(-3.344)^k		(2.010)	(-0.431)^k	(-2.764)^k	(-3.331)^k
Fufu (2)	-3.181	0.362	-0.378	0.590	-5.233	-0.976
	(-1.442)		(-2.568)^k	(1.077)	(-1.233)	(-6.047)^k
Yam (3)	-1.061	0.413	0.711	-0.365	0.88	-0.152
	(-2.547)^k		(4.744)^k	(-0.371)	(1.071)	(-0.364)
Rice (4)	-3.053	0.6110	0.067	1.613	0.088	0.188
	(-2.389)^k		(-2.021)^k	(2.034)^m	(0.931)	(8.011)^k

The numbers in parenthesis are t-values while the dependent variables are presented as budget share; ^{k} significant at 1 percent level; ^{l} significant at 5 percent level; ^{m} significant at 10 percent level; α_i= interceptive of the expenditure in the absence of price, income and other demographic; P_ie = number of years spent in school; P_is= sex of household head (male 1, otherwise = 0); P_im = marital status of household head (married=1, otherwise = 0); P_iq = household size equivalence.

Table 2. Tests of homogeneity

Product	P	Yi1	Yi2	Yi3	Yi4	∑yij
Garri	1.621	-5.664	1.391	5.622	-1.026	3.401
	(15.156)[k]	(-2.068)[m]	(0.458)	(-1.614)[m]	(-2.149)[k]	(1.751)[m]
Yam	1.104	3.723	-0.511	2.052	0.688	3.384
	(16.733)[k]	(1.642)	(-0.138)	0.438	(0.133)	(0.147)
Fufu	6.369	7.233	5.394	5.277	1.241	-1.589
	(1.061)[k]	(6.514)[k]	(7.203)[k]	(5.536)	(5.312)	(1.238)
Rice	2.142	-3.747	-0.301	-0.490	-0.2352	(-5.722)
	(2.489)[k]	(-1.469)	(-0.841)	(-0.186)	(0.591)	(6.355)

The number in parenthesis are t-values while the dependent variables are presented as budget share; [k] significant at 1 percent level; [l] significant at 5 percent level; [m] significant at 10 percent level; Yij = coefficient of the row sums of prices and expenditure matrix (Homogeneity testing); $P_t^{ж}$ = Stone's price index.

Table 3. Expenditure estimates

Food	Expenditure			R^2
Categories	Coefficients (β)	t-stats	Elasticities	Values
Rice	2.134	(22.389)[k]	0.4963	0.5452
Yam	1.896	(16.632)[k]	0.6998	0.3483
Garri	1.469	(15.125)[k]	0.8011	0.2891
Fufu	0.543	(10.711)[k]	0.6252	0.3216

Results of tests of homogeneity are revealed and explained in Table 2. This table has the column headed ∑Yij, the row sums of the unconstrained matrix, which explains the absolute effect on each value share of a 1 percent rise in all prices and total share of household expenditure. The condition for homogeneity requires a zero value (or insignificant). Thus, increase in prices and expenditure will increase expenditure on garri. From the result, test of homogeneity condition in the estimated demand system holds only for fufu, yam and rice. It is assumed that some consumers, especially those who live in cities tend to perceive their wealth and income in nominal naira or dollar terms rather than realizing their actual value, adjusted for inflation. This suggests that money illusion may exist among the consumers. These findings emphasize the need for more superior data to support demand theory. According to research by Deaton and Muellbaurer (1980), Tsegai and Kormawa (2009), Almas et al. (2019), and Obalola et al. (2021) numerous food staples do not meet the homogeneity requirements.

Table 2 further indicates that some of the coefficients on the prices of the calories under consideration were not jointly statistically significant. Several factors such as spatial variation in the sample collection of the 240 respondents as well as wider variation in taste and preferences may be responsible for these visible disparities.

As shown in Table 3, the explanatory power of the independent variables was moderately weak, except in the case of rice. Based on the values of the R2, the equation explains 55% of the variation in rice budget shares, while on the contrary, equations of the other three commodities (yam, garri and fufu) explains 35%, 29% and 33% of the variation. This observed weakness on the values of the R^2 is consistent with some findings in literature (Addo, 2016; Zhao et al., 2022). On the other hand, the expenditure coefficient is statistically significant in all the five equations. Because the budget share (or share allocation) is the dependent variable, a positive and statistically significant expenditure coefficient implies that the share increases with total expenditure. Thus, when income increases, households will consume more of garri (0.801), yam (0.701), fufu (0.6252), and rice (0.4963). The implications of these findings are simple: when income increase by 1 percent, garri expenditure consequently increases by 0.801. There is also a positively increasing pattern that follows the share of yam, fufu and rice in descending order. This observed pattern implies that the expenditure elasticity of rice is more inelastic than the other commodities considered. This therefore means that rice is an income inelastic food and it therefore appears to be gaining grounds as a very essential food alternative in Port Harcourt households. This shows that rice is fast becoming a choice food staple and a necessity in Port Harcourt households. Few factors are perceived to be responsible for this finding; First, the increase in economic importance of rice as a major source of metabolic energy. Second, the increase in the welfare of households due to the growing livelihood options and inclining economic activities in the city, as well as the gradual increase in real income of household heads.

4. Conclusion

In Nigeria, particularly in Port Harcourt, the demand for food commodities, especially calories, has increased significantly during the past few years. This development is the result of a number of factors, including population growth, improved income levels, technological advancements, and improved production resource requirements. This also includes the trade promotion policies of the federal government, and their exceptional quality of providing excellent sources of dietary energy. The subsector requires improved vertical coordination to increase the need for calories even further. Without better price signals, the subsector is unable to meet the population's demand for the kinds of items.

This paper employs both parametric and non-parametric estimation techniques to investigate the relationship between household calorie products consumption and per capita household expenditure and other socio demographic variables in Port Harcourt households. The result of the survey confirms that Port Harcourt households spend a significant share of their budgets on garri, yam, rice and fufu. In addition, one year increase in the education of household head decreases the share of fufu by 0.378 per cent while that of rice and yam increases by 0.067 and 0.711 percentage respectively, other thing being equal. In addition, households headed by men spend 0.590 percent more on fufu than households headed by women; similarly, for garri and yam, the expenditures are 0.374 and -0.365 percent lower, respectively. Overall, the findings imply that while development methods aimed at boosting economic growth might enhance diet quality, they might not be adequate to increase nutritional intake. The results of this study also imply that methodological issues, contextual features, and demographic differences are primarily to blame for Nigeria's variation in food calories and expenditure elasticity. The research, therefore, recommends that:

i. A problem facing Nigeria's food consumption subsector is a lack of budget which affect there expenditure patterns of calorie demand. Due to a lack of understanding of these factor, resources were inefficiently allocated to projects and investments that produced returns that were below optional. It is advised that more infrastructure be created to support multidisciplinary research and development in this regard. Undoubtedly, research and development initiatives of this kind will uncover a plethora of food goods low in calories and alternative processing methods that will increase the products' use in homes.

ii. The prospects in the utilization of food products lies in convincing the end users of the safety of the products and possible use in diversified forms, demonstrating and harnessing high quality products from their original farm gate produce. This will enhance the quality, and identify future areas of utilization.

iii. A variety of institutional and technical approaches could be used to guarantee better food access through agricultural transformation initiatives. These strategies will help to feed the growing population, lessen hunger, and enhance the lives of the poorest people, as well as to prevent or lessen the degradation of the planet's finite resources. Therefore, it is imperative to adjust to the socioeconomic and local conditions. Doing so might enhance planning and creation of strong connections to intelligent incentive packages that are sustainable and could boost agricultural production, food supply, and demand.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.E.	A.A.	G.O.	F.E.
C	30	20	20	30
D	35	25	20	20
S	30	30	20	20
DCP	30	30	20	20
DAI	30	30	20	20
L	30	30	20	20
W	35	30	15	20
CR	30	30	20	20
SR	35	35	10	20
PM	35	35	10	20
FA	30	30	20	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

This study obtained approval from the Ethics Committee of the University of Port Harcourt. The study participants were also informed about the purpose of the study and responded to the questionnaires anonymously, and they were allowed to skip any item they did not wish to answer.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1517294

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 505-514 / September 2024

INFLUENCE OF DIFFERENT ORGANIC FERTILIZERS ON GROWTH, YIELD, QUALITY, AND ELEMENT CONTENT IN LETTUCE (*Lactuca Sativa* var. *Longifolia*)

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Abstract: This study was performed in order to determine the effects of pigeon manure, chicken manure, and vermicompost on lettuce's (*Lactuca sativa* var. *longifolia*) yield, vegetative growth, element content, and quality characteristics. The study was carried out in greenhouse. In the study, 0% (control), 0.50% pigeon manure, 0.50% chicken manure, and 0.50% vermicompost were applied via drip irrigation. The treatments began 20 days after the seedling planting, at 10-day intervals and four times. The experiment was set up with four replicates as per the randomized blocks experimental design. As per the research findings, the chicken manure significantly increased the lettuce's average head weight and total yield compared to the control and two other treatments. Even if the pigeon manure and vermicompost increased the yield parameters similar to chicken manure, they were statistically in the same group as the control. The pigeon manure again significantly increased the chlorophyll amount, root's fresh and dry weight, leaf's K and Zn content, leaf color's L value, and fruit juice's pH content compared to control. On the other hand, vermicompost generated the highest results for the leaf's Ca and Pb content. The Pb content in the lettuce leaves increased in all the organic fertilizer treatments compared to control, and the highest Pb content was obtained by the vermicompost, chicken manure, and pigeon manure, respectively. The treatments' effect on the leaf's Ni content was found to be insignificant. But the vermicompost increased the leaf's Ni content compared to the control. The treatments' effect on leaf color's a* and b* values, brix degree, head diameter, head height, leaf relative water content, and Na, Mg, Cu and Mn contents was again found to be insignificant. In conclusion, it is recommended to apply 0.50% chicken manure in greenhouse lettuce cultivation to obtain large heads and high yield. To achieve bright-colored plants, high chlorophyll content, high root fresh and dry weight, and high potassium and zinc content, 0.50% pigeon manure is suggested. However, 0.50% vermicompost is not recommended for greenhouse lettuce cultivation due to its potential to increase the Pb and Ni heavy metal content in the leaves.

Keywords: Organic fertilizer, Pigeon manure, Chlorophyll, Heavy metals, Calcium

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Received: July 16, 2024
Accepted: August 24, 2024
Published: September 15, 2024

Cite as: Nas Y. 2024. Influence of different organic fertilizers on growth, yield, quality, and element content in lettuce (*Lactuca sativa* var. *longifolia*). BSJ Agri, 7(5): 505-514.

1. Introduction

Lettuce (*Lactuca sativa* var. *longifolia)* is a winter vegetable falling within the *Asteraceae* genus with economic significance. It is grown throughout the year, both in the field and greenhouse. It is generally consumed fresh. According to the production data from FAO, 27 million tons of lettuces were produced on an area of 1.2 million hectares in 2022. The Republic of Türkiye is in the position of being the 8th country with the highest lettuce production in the world.

In recent years, the usage of chemical fertilizers and pesticides in agriculture has increased. For this reason, the biological diversity and soil structure of agricultural areas are deteriorating (Chaudhary et al., 2022). Soil health and crop yield are closely related to the microorganisms living in the soil (Harman et al., 2021). In healthy soil, the activity of microorganisms is high, and thus, the soil's organic matter content also becomes high. Accordingly, the agricultural activities performed for the prevention and increase of organic matter in the soil become crucial.

In terms of sustainable agriculture, biofertilizers and organic fertilizers are used as alternatives to chemical fertilizers for the prevention of soil and environmental health. The use of biofertilizers and organic fertilizers improves the microbial content of the soil and increases the dissociation of organic matter, thus increasing accessibility of plants to dissolved nutrients (Wang et al., 2019; Chaudhary et al., 2021).

Vermicompost and chicken manure are organic fertilizers widely used in agriculture as alternatives to chemical fertilizers. Similarly, pigeon manure has recently emerged as another organic fertilizer alternative in agriculture. Numerous studies have investigated the effects of chicken manure and vermicompost on plant growth, yield, and quality. Likewise, in recent years, there has been growing research on the effects of pigeon manure on various plant species. But it was observed that the studies conducted regarding pigeon manure were less compared to the ones performed regarding chicken manure and vermicompost. In addition, the studies conducted regarding the effects of pigeon manure, chicken manure, and vermicompost on vegetables and their usage doses are very limited. In the previous studies, the reaction of lettuce against different organic fertilizers was investigated (Abd-Elmoniem et al., 2000; Hernández et al., 2010; Islam et al., 2012; Özkan and Müftüoğlu, 2016; Durak et al., 2017; Ullah et al., 2017; Pizarro et al., 2019; Üçok et al., 2019; Tarakçıoğlu and Özenç, 2022; Karademir and Kibar, 2022; Korkmaz and Akıncı, 2022; Altuntaş et al., 2022). But most of the studies conducted are relevant to vermicompost. No study was conducted in which the effects of pigeon manure, chicken manure, and vermicompost on lettuce were examined together. In this context, the determination of the effects of pigeon manure, chicken manure, and vermicompost on lettuce's plant development, yield, and quality characteristics will contribute to future studies.

The aim of this study was to search for the effects of pigeon manure, chicken manure, and vermicompost on lettuce's vegetative growth, yield, element content, and quality characteristics. The study has the characteristic of being the initial study in which the effects of pigeon manure, chicken manure, and vermicompost on lettuce, grown under the ecological conditions of the GAP region, were investigated together.

2. Materials and Methods

2.1. Plant Materials and Study Site

The study was carried out at the research and application greenhouse (37°20'22.2"N, 41°53'55.7"E) of Şırnak University's Faculty of Agriculture (İdil, Şırnak, Republic of Türkiye). In the experiment, the Cospirina F1 lettuce cultivar from Syngenta Company was used as plant material. The Cospirina cultivar is suitable for production in the greenhouse and field. It has a heavy head structure with its strong leaves. Moreover, its tolerance for leaf tip blight is high (Anonymous, 2023a). In the study, pigeon manure (PM), chicken manure (CM) *(*Anonymous, 2023b), and vermicompost (VM) (Anonymous, 2023c) were used (Table 1).

Table 1. Some chemical properties of biofertilizers

Pigeon manure		Chicken manure		Vermicompost	
Content	Amount	Content	W/W%	Content	W/W%
Fe	0.12 ppm	Organic matter	10%	Organic matter	25%
Cu	0.025 ppm	Total (Humic+Fulvic) Acid	6.3%	Total (Humic+Fulvic) Acid	8%
Pb	0.44 ppm	Total Nitrogen	1.5%	Total Nitrogen	2%
SO_4^{2-}	19 ppm	Organic Nitrogen	1%	Organic Carbon	10%
Zn	2.81 ppm	pH	5-7	pH	5.5-7.5
K	221.5 ppm	Max. EC	4 dS/m	Max. EC	12 dS/m
NO_3^-	0.9 ppm	-	-	Water-Soluble Potassium Oxide	3.5%
NH_4	1.206 ppm	-	-	-	-
pH	7.19	-	-	-	-
EC	3.84 dS/m	-	-	-	-

2.2. Experimental Design

The seedlings were transplanted into the greenhouse on November 14, 2023. The treatments began 20 days after the seedling planting. In the study, 0% (control), 0.50% pigeon manure, 0.50% chicken manure, and 0.50% vermicompost were applied via drip irrigation. Fertilizer doses were determined based on previous studies to ensure they would not harm the plants. The treatments were performed at 10-day intervals and four times. The study was formed of 16 parcels, with 4 treatments and 4 replicates as per the randomized blocks experimental design. A total of 160 seedlings were used, with 10 seedlings per replicate.

Seedling plantings were performed at 30 cm by 40 cm intervals. Plant nutrition, plant disease management, and pesticide management actualized at the experiment area were performed in the direction of the suggestions of Vural et al. (2000). The harvest was conducted on January 27, 2024.

2.3. Yield and Quality Measurement

2.3.1. Total yield (kg/da)

The total yield was calculated as a result of the weighing of all the heads collected from the parcels.

2.3.2. Average head weight (g)

It was calculated as a result of the weighing of the head weights of 5 plants that were determined prior to each replicate and that reached harvest maturity.

2.3.3. Head diameter (cm)

In each replicate, the head diameter of 5 plants was measured by a digital caliper.

2.3.4. Head height (cm)

In each replicate, the head height of 5 lettuces was measured with the aid of a digital caliper.

2.3.5. Chlorophyll analysis

In lettuce plants, the chlorophyll rate was determined by a Minolta brand chlorophyll meter (Konica Minolta SPAD-502 Plus) from about the 5th leaf from the plant's top.

2.3.6. Determination of the fresh and dry weight ratio in the roots and leaves

In the harvest period, roots and leaves were sampled from each treatment, and fresh and dry weights were measured (Şahin et al., 2022).

2.3.7. Leaf color

In randomly selected 5 plants from each replicate, the color of the leaves was measured by a Minolta CR-300 colorimeter as L, a*, and b*. In this system, the colors are determined as a point in a three-dimensional spherical space. L indicates the color's lightness or darkness; positive a indicates the red color, and negative a indicates the green color; positive b indicates the yellow color, and negative b indicates the blue color (McGuire, 1992).

2.3.8. Determination of the leaves' relative water content

By the end of the harvest, the fresh weights of the leaf samples were measured. Then, the leaf samples were kept in pure water for 4 hours, and by the end of the referred period, their turgor weights were determined. After drying the leaf samples, whose weights were determined, by keeping them in an 80ºC drying oven for 48 hours, their dry weights were determined in g. The leaves' relative water contents (%) were calculated by the following formula (equation 1) using the obtained dry and fresh weights (Sanchez et al., 2004).

$$TuW: (FW-DW)/(TuW-DW)x100 \quad (1)$$

which is;

FW: fresh weight, DW: dry weight, TuW: turgor weight.

2.3.9. Mineral element analyses

Mineral element analyses were performed on the ICP-MS device. About 500 mg was weighted from the serum samples and transferred to the teflon cups of the microwave oven. Concentrated 10 ml of Merck nitric acid at 65% was added to each sample. For the blank, 10 ml of nitric acid at 65% was added to an empty teflon cup. Teflon cups were placed in the microwave disintegrator oven. The maximum temperature was increased to 210°C within 25 minutes, and they were kept at that temperature for 15 minutes. They were kept in a closed system for 40 minutes in total, and the dissolution operation was actualized. Following the decrease of the microwave oven's temperature to ambient conditions, the solution in the teflon cups was well washed along with the teflon cups with pure water and taken into 50 ml volumetric flasks. Following a suitable dilution of the solutions, their readings under the determined conditions were made on the ICP-MS device.

The ICP-MS calibration solutions were diluted with 1% Suprapur nitric acid – ultrapure water as per the commercially available multiple element standards, and the calibration graph was formed by preparing the concentrations specified in Table 2. In the study conducted, for the element analyses of the samples, a quartz nebulizer, a cyclonic spray chamber, and an ICP-MS NexION® 2000 C (PerkinElmer®Inc., USA) device with an integrated autosampler were used. Using the 18.2 MΩ ultrapure water obtained from the Sartorius™ Wall Mounted Arium Pro Ultrapure Water System device, and the irrigation solution containing 1% Suprapur nitric acid – ultrapure water was prepared in the concentrations specified in Table 2. For the control of element analyses, the ^{89}Y internal standard of 25 ppb concentration was used (Table 2).

By means of the peristaltic pump, the samples were sent to the cyclonic spray chamber by the argon gas flow. In order to prevent the attempts, high rates of helium gas as well as argon gas were used. In the analyses, ICP-MS software version 2.2 was used for checking the device and for the adjustment, data collection, and data analysis. All the operating conditions of the ICP-MS device are shown in Table 3.

2.4. Statistical Analysis

The data obtained by the end of the experiment was subjected to variance analysis by the JMP 8 (SAS Institute Inc., Cary, NC, USA) software pack, and the differences among the averages were calculated as per the Tukey's test.

3. Results

Organic fertilizer treatments increased the average head weight and total yield (Table 4). The pigeon manure, chicken manure, and vermicompost treatments increased the average head weight at a rate of 27.63%, 65.25%, and 19.23%, respectively. The highest average head weight (238.51 g) was obtained by the chicken manure treatment. Similarly, all the treatments increased the total yield compared to the control. The chicken manure constituted the highest total yield (2.868 kg/da). As shown in Table 4, following the chicken manure, the pigeon manure and vermicompost treatments constituted the highest total yield, respectively (Table 4). In terms of head diameter and head height, we couldn't determine a significant effect of the treatments ($P<0.05$; Table 5). In addition to this, organic fertilizer treatments increased both the head diameter and head height compared to the control (Table 5).

Table 2. Calibration standards

Analytes	Std1 (ppb)	Std2 (ppb)	Std3 (ppb)	Std4 (ppb)	Std5 (ppb)	Std6 (ppb)	Internal standard
Cu, Mn, Ni, Pb, Zn	0.5	1	5	25	50	100	^{89}Y

Table 3. Operating conditions of the ICP-MS analysis

Parameter	Description / Value
Nebulizer	MEINHARD® plus Glass Type C
Spray Chamber	Glass cyclonic (baffled), 4°C
One-Piece Torch	w/2.5mm Quartz Injector
Injector	2.0 mm i.d.
Nebulizer Flow	Optimized for < 2% oxides
RF Power	1600 W
Cones	Ni
Replicates	3.0
Dwell time	50 ms
Aerosol Dilution	Set to 2.5x
Sample Delivery Rate	350 µL/min
Rinse time	45 seconds
Nebulizer gas flow rate	0.93 L/min
Deflector voltage	−12 V
Analog stage voltage	−1750 V
Pulse stage voltage	1100 V
Discriminator threshold	26
Sample Tubing (Orange-Yellow)	Flared PVC Pump Tubes 0.51mm/0.89mm
Internal Standard Tubing (Orange-Red)	Flared PVC Pump Tubes 0.19mm/0.91mm
Peristaltic pump rate	35 rpm
Alternating current (AC) rod offset	−4.0

Table 4. The effect of the treatments on the yield values

Treatment	Average Head Weight (g)	Total yield (kg/da)
Control	231.46±7.29 b	1.735,98±54.65 b
PM	295.43±14.97 b	2.215,73±112.25 b
CM	382.51±18.91 a	2.868,86±141.84 a
VM	275.98±10.74 b	2.069,90±80.55 b
P	0.0004*	0.0004*

*The averages indicated with the same letters on the same column are statistically different from each other as per the Tukey's test (P<0.05).

Table 5. The effect of the treatments on the head diameter and head height

Treatment	Head Diameter (cm)	Head Height (cm)
Control	12.91±0.60	29.41±0.96
PM	18.25±1.31	31.33±0.62
CM	17.41±0.70	31.91±1.03
VM	15.75±1.63	32.27±0.40
P	0.0639^{ns}	0.1659^{ns}

ns= not significant, *The averages indicated with the same letters on the same column are statistically different from each other as per the Tukey's test (P<0.05).

Based on the results, while the effect of organic fertilizer treatments on the plant color's a* and b* values was found to be insignificant, their effect on the L value was found to be significant (Table 6). While the highest L value was obtained by the pigeon manure treatment, the lowest L value was determined by the control treatment. The L color value increased in all the organic fertilizer treatments compared to the control (Table 6). In the CIE color system, L represents the lightness, +a represents the red, -a represents the green, +b represents the yellow, and -b represents the blue. While the differences among the leaf color's a* values were found to be statistically insignificant, organic fertilizer treatments ensured the formation of darker green leaves. When the pigeon manure was compared to the control and other treatments, it ensured the formation of darker green leaves (Table 6).

The effect of the treatments on the water-soluble dry matter amount was found to be insignificant ($P<0.05$; Figure 1). Organic fertilizer treatments didn't positively affect the water-soluble dry matter amount (Figure 1).

The effect of organic fertilizer treatments on the pH content of fruit juice was found to be statistically significant at the level of $P \leq 0.05$ (Figure 2). While the highest fruit juice pH content was obtained by the pigeon manure treatment, the lowest fruit juice pH content was determined by the control treatment. The chicken manure, vermicompost, and control treatments statistically fell within the same group (Figure 2).

Significant differences were determined in the effect of organic fertilizer treatments on the chlorophyll content ($P<0.05$; Figure 3). The pigeon manure increased the chicken manure content at a rate of 13.15% compared to the control. Similarly, while chicken manure increased the leaf chlorophyll content at a rate of 4.14%, vermicompost increased it at a rate of 3.67% (Figure 3).

The leaf relative water content was not significantly affected by the organic fertilizer treatments (Figure 4). In terms of root fresh weight and root dry weight values, the effect of organic fertilizer treatments was found to be significant ($P<0.05$; Table 7). The highest root fresh weight and root dry weight were determined by the pigeon manure treatment. As shown in Table 7, the pigeon manure increased the root fresh and dry weights by about two times. The chicken manure and vermicompost increased the root fresh and dry weights compared to the control even if not as much as the pigeon manure (Table 7).

In terms of leaf fresh weight and leaf dry weight values, we couldn't find a significant effect of organic fertilizer treatments ($P<0.05$; Table 8). While the effect of the treatments on the leaf's sodium (Na) and magnesium (Mg) content was not found to be significant, their effect on the leaf's calcium (Ca) and potassium (K) content was found to be significant ($P<0.05$; Table 9). The vermicompost treatment increased the leaf's Ca content. But the pigeon manure and chicken manure treatments decreased the leaf's Ca content. On the other hand, the leaf's K content showed an increase with the organic fertilizer treatments. The pigeon manure treatment constituted the leaf's highest K content. The chicken manure treatment and vermicompost treatments followed the pigeon manure treatment, respectively (Table 9).

Table 6. The effect of the treatments on the plant color

Treatment	L	a*	b*
Control	37.03±1.15 b	-30.61±3.51	42.60±2.11
PM	45.75±1.53 a	-39.58±2.12	46.35±0.84
CM	39.72±1.24 b	-33.23±1.85	43.00±0.63
VM	41.99±1.09 ab	-37.17±2.46	43.71±1.65
P	0.0061*	0.1442ns	0.4053ns

ns= not significant, *The averages indicated with the same letters on the same column are statistically different from each other as per the Tukey's test (P<0.05).

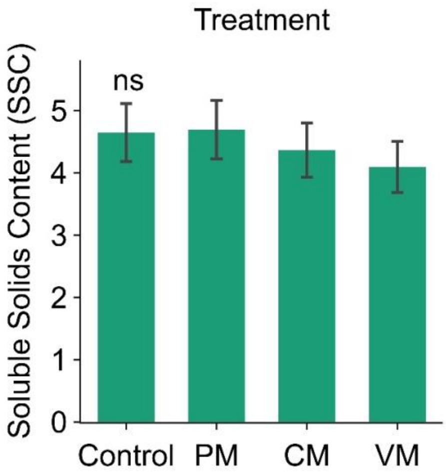

Figure 1. The analysis of soluble solids content in tomato fruit. Vertical bars are used to display all data, which are the means of 4 replicates (+SE). Different letters above the error bars indicate significant differences among treatments at the 5% level.

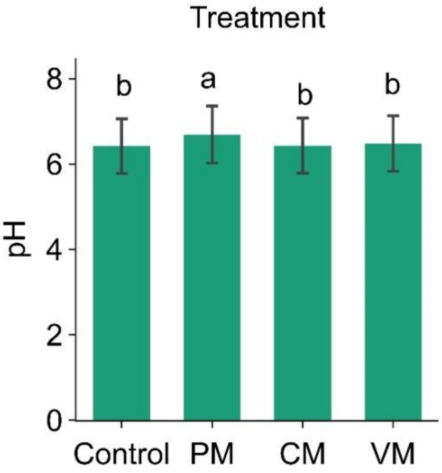

Figure 2. The effect of organic manure on pH content in tomato fruit. Vertical bars are used to display all data, which are the means of 4 replicates (+SE). Different letters above the error bars indicate significant differences among treatments at the 5% level.

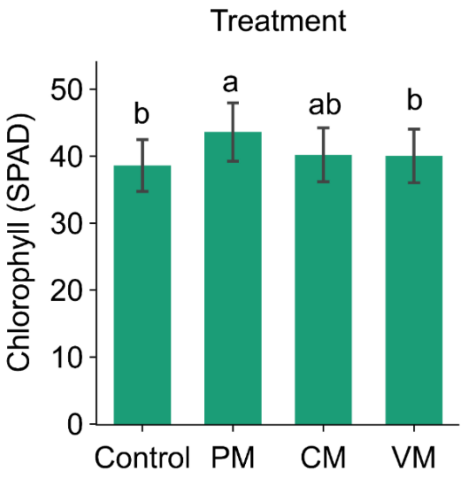

Figure 3. The effect of organic manure on chlorophyll content. Vertical bars are used to display all data, which are the means of 4 replicates (+SE). Different letters above the error bars indicate significant differences among treatments at the 5% level.

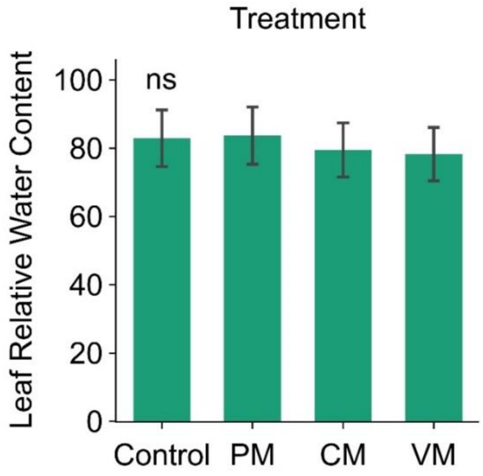

Figure 4. The effect of organic manure on leaf relative water content. Vertical bars are used to display all data, which are the means of 4 replicates (+SE). Different letters above the error bars indicate significant differences among treatments at the 5% level.

Table 7. The effect of the treatments on the root fresh and root dry weights

Treatment	Root Fresh Weight (g)	Root Dry Weight (g)
Control	15.74±0.89 b	3.08±0.33 b
PM	28.90±2.06 a	6.00±0.47 a
CM	22.58±2.02 ab	4.03±0.50 ab
VM	16.13±1.56 b	3.17±0.37 b
P	0.0011*	0.0041*

*The averages indicated with the same letters on the same column are statistically different from each other as per the Tukey's test (P<0.05).

Table 8. The effect of the treatments on the leaf fresh and leaf dry weights

Treatment	Leaf Fresh Weight (g)	Leaf Dry Weight (g)
Control	5.07±0.45	0.58±0.03
PM	8.78±0.87	0.69±0.05
CM	5.98±0.90	0.61±0.07
VM	5.98±1.06	0.61±0.09
P	0.1030^{ns}	0.6447^{ns}

ns= not significant.

Table 9. The effect of the treatments on the leaf's Ca, Na, K, and Mg content

Treatment	Ca (ppm)	Na (ppm)	K (ppm)	Mg (ppm)
Control	7.459,00±490.75 ab	1.002,93±203.34	6.605,66±224.67 b	925.06±37.63
PM	5.833,33±313.27 ab	796.60±76.77	11.744,33±562.92 a	886.56±12.43
CM	5.643,33±484.61 b	725.66±49.39	7.747.00±119.72 ab	859.90±39.35
VM	7.657,00±261.86 a	671.53±38.89	7.276.00±53.98 ab	947.16±5.08
P	0.0213*	0.2204^{ns}	0.0390*	0.3120^{ns}

ns= not significant, *The averages indicated with the same letters on the same column are statistically different from each other as per the Tukey's test (P<0.05).

While the effect of the organic fertilizer treatments on the leaf's copper (Cu) and manganese (Mn) content was found to be insignificant, their effect on the leaf's zinc (Zn) and iron (Fe) content was found to be significant (P<0.05; Table 10). It was observed that the pigeon manure increased the leaf's Zn content. But the chicken manure and vermicompost decreased the leaf's Zn content compared to the control. Similarly, the leaf's Fe content decreased with the organic fertilizer treatments. The pigeon manure decreased the leaf's Fe content at a rate of 48.78% compared to the control (Table 10).

The effect of organic fertilizers on the leaf's nickel (Ni) content was found to be insignificant (P<0.05; Table 11). But their effect on the leaf's lead (Pb) content was found to be statistically significant at a level of P≤0.05. The Pb content of the lettuce leaves increased with the organic fertilizer treatments. The highest Pb content was obtained by the vermicompost treatment. The chicken manure and pigeon manure ranked second and third, respectively. In addition, the pigeon manure, chicken manure, and control statistically fell within the same group (Table 11).

Table 10. The effect of the treatments on the leaf's micro-element content

Treatment	Cu (ppb)	Mn (ppb)	Zn (ppb)	Fe (ppb)
Control	399.17±21.14	3.212,47±147.15	2.358,80±209.65 ab	439.53±23.17 a
PM	396.47±25.59	3.508,19±119.60	3.001,31±219.22 a	295.41±4.28 c
CM	324.83±2.89	2.907,59±54.74	1.828,58±217.83 bc	331.07±35.22 bc
VM	326.21±42.91	2.616,53±335.00	1.126,67±101.42 c	418.56±14.17 ab
P	0.1918^{ns}	0.1161^{ns}	0.0050*	0.0036*

ns= not significant, *The averages indicated with the same letters on the same column are statistically different from each other as per the Tukey's test (P<0.05).

Table 11. The effect of the treatments on the leaf's heavy metal content

Treatment	Ni (ppb)	Pb (ppb)
Control	67.79±4.38	7.15±0.72 b
PM	61.22±5.51	10.83±1.49 b
CM	57.84±12.10	11.41±0.62 b
VM	72.15±1.19	19.66±1.71 a
P	0.3803^{ns}	0.0023*

ns= not significant, *The averages indicated with the same letters on the same column are statistically different from each other as per the Tukey's test (P<0.05).

4. Discussion

The study revealed that the pigeon manure, chicken manure, and vermicompost treatments ensured improvements in the lettuce's yield, vegetative growth, some element contents, and quality characteristics.

4.1. Effect of Pigeon Manure, Chicken Manure, and Vermicompost on the Yield and Quality of Lettuce

Biofertilizers and organic fertilizers increase the crop yield, soil productivity, and nutrient cycle (Singh et al., 2020; Haroun et al., 2023; Wei et al., 2024). The lettuce yield and quality results obtained from the study revealed that the pigeon manure, chicken manure, and vermicompost treatments have significant potential for increasing the yield and some quality parameters (average head weight, yield per decare, fruit juice pH, leaf color's L value, root fresh and dry weights). But they didn't significantly increase the water-soluble dry matter and leaf color's a* and b* values. The increase in average head weight and total yield may be attributed to the role of the organic fertilizers used in root development, the functioning of photosynthesis, and the increased effectiveness of many physiological processes in the plant (Chaudhary et al., 2022). Hence, in our study, root fresh and dry weights (Table 7) and leaf's chlorophyll content (Figure 3) increased with all three organic fertilizer treatments. Özkan and Müftüoğlu (2016) reported that vermicompost in different doses increased the lettuce's yield. Hernández et al. (2010) suggested the treatment of 60 t ha^{-1} chicken manure in order to obtain a higher yield in lettuce cultivation. Similarly, Pizarro et al. (2019) specified that the yield increased in lettuce cultivation with the treatment of 4 kg m^{-2} chicken manure. In another study, Islam et al. (2012) specified that, instead of commercial fertilizer, the use of cow manure in lettuce cultivation constituted maximum leaf number, root length, and yield. In the literature, it was determined by the studies conducted that the organic fertilizer treatments had different results on the quality, similar to their effect on the yield of lettuce. Karademir and Kibar (2022) specified that, with the vermicompost treatment of different doses applied to lettuce, the leaf color's L and a* values were not affected, but the leaf color's b* value decreased. In another study conducted regarding vermicompost (Korkmaz and Akıncı, 2022), it was determined that the lettuce's L value decreased but its a* and b* values increased. In another study, the vermicompost increased the lettuce's L color value compared to chemical fertilizer and chicken manure (Üçok et al., 2019). These results show parallelism with the findings obtained by us.

4.2. Effect of Pigeon Manure, Chicken Manure, and Vermicompost on the Vegetative Growth of Lettuce

The results revealed that pigeon manure, chicken manure, and vermicompost didn't positively affect some of the growth and development parameters in lettuce (head diameter, head height, leaf relative water content, leaf fresh weight, and leaf dry weight). But the effect of all three manures on the leaf chlorophyll content, root fresh weight, and root dry weight was positive. The reason behind that may be due to the vegetation period of the lettuce plant. Hence, when compared with other plant species, the lettuce plant has a shorter vegetation period. Moreover, organic fertilizers are taken in by the plant more slowly compared to chemical fertilizers. This status arises from the slowness of the dissolution of organic fertilizers in the soil. For this reason, the plants' exploitation of organic fertilizers takes a long time. Üçok et al. (2019), in a study in which they studied the effects of organic fertilizers (vermicompost, chicken manure) and chemical fertilizers on the lettuce plant, reported that the chemical fertilizers constituted better results by themselves in terms of yield and some growth parameters compared to organic fertilizers. Similarly, Abd-Elmoniem et al. (2000) specified that, in lettuce plants grown for two seasons in aquaculture, inorganic fertilization generally ensured the highest growth compared to organic fertilization (pigeon manure and chicken manure). These results tally with the information reported in the literature.

The leaf's chlorophyll content showed an increase with the organic fertilizer treatments. Ullah et al. (2017), in a study in which they examined the effects of farm manure and chicken manure on local iceberg cultivar lettuce and Chinese cultivar lettuce, specified that the chicken manure treatment ensured more leaf and total chlorophyll content in Chinese cultivar lettuce. Similarly, Çukurcalıoğlu et al. (2023) specified that the chicken manure increased the leaf's chlorophyll content in bean plants. The researchers attributed this status to the richness of chicken manure in terms of nutrients, such as nitrogen and phosphorus, in the first place. Again, similarly, Hosseinzadeh et al. (2016) specified that the vermicompost treatment positively affected the chlorophyll content in chickpea cultivation. In other studies conducted by other researchers, it was determined that the organic fertilizers positively affected the leaf's chlorophyll content (Keçe et al., 2024; Ye et al., 2022; Toor et al., 2024; Khalid et al., 2017).

With the organic fertilizer treatments, root fresh and dry weights increased. Similar to the results of this study, Tarakçıoğlu and Özenç (2022) specified that the lettuce's root fresh and dry weights increased with the vermicompost treatments. In another study carried out by Kashem et al. (2015), it was reported that the vermicompost treatments increased the root dry weight of tomatoes. Similarly, Özkan et al. (2016) reported that the root fresh weight of spinach increased with increasing vermicompost treatments. These findings tally with the results obtained by the study.

4.3. Effect of Pigeon Manure, Chicken Manure, and Vermicompost on the Mineral Content of Lettuce

When organic fertilizers are added to the soil, they mineralize the compounds in the soil and make it more favorable for agriculture (Wei et al., 2024). In addition to this, increasing the organic matter in the soil (Altuntaş et al., 2022) ensures the secretion of plant hormones that are useful for the plants and the biodegradation of organic matter (Khan et al., 2023). Our results indicated that the organic fertilizer treatments caused significant increases in lettuce's macro, micro, and heavy metal contents. Ca and Pb content reached the highest level in vermicompost, and K and Zn content reached the highest level in pigeon manure. The chicken manure increased the leaf's K and Pb content compared to the control. This status indicates that all three organic fertilizers have the macro- and micro-nutrient contents required for vegetative growth and development. Hence, Karademir and Kibar (2022) specified that the vermicompost treatments significantly increased the nitrogen, phosphorus, potassium, calcium, magnesium, sodium, copper, iron, and zinc content in curly lettuce. Similarly, Hernández et al. (2010) specified that the highest Mg, Fe, Ca, Cu, and Mn content in lettuce was obtained by vermicompost. In another study, through vermicompost treatments on lettuce, the P, Mg, Mn, Fe, Cu, and Zn content increased (Durak et al., 2017). Similar results were also obtained in other studies (Üçok et al., 2019). These results show parallelism with the findings obtained by us.

5. Conclusion

In this study, the usage potential of pigeon manure, chicken manure, and vermicompost in lettuce cultivation was examined. To summarize, chicken manure significantly increased the average head weight and total yield. The highest yield was obtained with chicken manure. However, pigeon manure resulted in the best values for leaf color L, fruit juice pH, chlorophyll content, root fresh and dry weight, and leaf K and Zn content. On the other hand, vermicompost resulted in the highest values for leaf Ca and Pb content. Vermicompost increased the heavy metal (Ni and Pb) content in the leaves. In conclusion, for high yield in greenhouse lettuce cultivation, 0.50% chicken manure is recommended. However, for improving fruit quality, 0.50% pigeon manure is recommended. Vermicompost generally had a positive effect on lettuce yield and quality. However, due to its potential to increase the heavy metal (Ni and Pb) content in the leaves, the application rate of vermicompost in greenhouse lettuce production should be carefully considered.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	Y.N.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

We thank the Abdullah TUTUŞ for his assistance in collecting this data.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1523858

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 515-523 / September 2024

WATER FOOTPRINT ASSESSMENT OF AGRICULTURAL PRODUCTION IN BILECIK PROVINCE

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Abstract: The freshwater sources are under serious pressure both in terms of quality characteristics due to pollution and in terms of quantity due to the increase in parameters such as temperature and evaporation under the influence of global warming. To ensure sustainable use of these resources, it is necessary to employ high-efficiency pressurized irrigation systems and cultivate plant species that are resilient to various stress factors and highly productive. In determining the water usage characteristics of plants, rapid atmospheric effects brought by climate change, plant water and temperature stress, soil moisture should be monitored, and water production indicators should be determined. In the water-intensive agricultural sector, monitoring the water footprint has become one of the important indicators in terms of ensuring water-food-energy sustainability, efficient use and fair sharing of water resources. This study aims to determine the water footprint of agricultural production in Bilecik province and its districts located in the transitional zone. Accordingly, values of crop and livestock production throughout the province and using a volume-based approach, the water footprint of crop production is estimated at 0.6 billion cubic meters (BCM), while the water footprint of livestock production is 0.5 BCM, resulting in a total agricultural water footprint of 1.1 BCM. In crop production, green water footprint constitutes 33%, blue water footprint 59%, and grey water footprint 8% of the total water footprint. The data obtained will form the basis for developing strategies in sustainable water and food management, aligned with climate change scenarios, to achieve sectoral supply-demand balance.

Keywords: Agriculture, Water usage, Effectiveness, Sustainability, Water management

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Received: April 02, 2024
Accepted: July 30, 2024
Published: September 15, 2024

Cite as: Gültaş HT. 2024. Water footprint assessment of agricultural production in Bilecik province. BSJ Agri, 7(5): 515-523.

1. Introduction

The importance of sustainable, fair, and integrated management of water resources is increasing day by day. It is clear that current usage shows signs of inadequacy due to the increasing industrial use of existing water resources and unforeseen increases in population. Ensuring food supply and secure transfer of water to the future are essential for sustainable living. According to studies conducted by the IPCC and other international organizations, extreme events occurring in meteorological parameters such as temperature and precipitation, due to global climate change, particularly adversely affect the Mediterranean Basin water resources (IPCC, 2023).

Water is used in domestic (drinking), industrial, and agricultural sectors. Globally, agricultural usage accounts for approximately 69%, industrial usage for 19%, and domestic usage for around 12% of total water usage. In the agricultural sector, which consumes the largest portion of water, the primary goal is to maximize the benefits derived from each unit of water. Evaluating and preserving surface and groundwater sources used in agricultural irrigation in terms of quantity and quality are essential. Therefore, it is important to develop scientific data-based models to strengthen sustainable water management strategies and enhance their implementation with robust indicators (DSİ, 2022; Ahi and Çakmak, 2023).

In the assessment and sustainability of resource efficiency and management, life cycle analyses such as ecological footprint, water footprint, and carbon footprint have become important indicators. Footprints represent the efficiency level of resources in meeting society's needs and are often expressed in volumetric terms. The total volume of water used by an individual, sector, and/or country in production processes is defined as water footprint, encompassing the total water resources consumed during the production of domestically produced and imported goods. Water footprint is categorized into three types: blue, green, and grey water footprint. Blue water footprint represents the total volume of surface and groundwater used in producing a product or service. Green water footprint refers to the total amount of rainwater used in the production of a commodity. Grey water footprint, an indicator of water pollution, represents the volume of freshwater used for removing or reducing pollution load based on current water quality standards (Hoekstra, 2003).

In assessing global water and carbon cycles, life cycle assessment (LCA) takes into account spatial variability and reveals the environmental, social, and economic impacts of where production occurs. Life cycle assessment (LCA) is the most widely used method to evaluate the environmental impacts of agricultural products and facilitate the transition to more sustainable production and consumption models (Notarnicola et al., 2017). This approach encompasses various environmental impact categories, including ecological footprint and water footprint. The most significant difference between ecological water footprint and water footprint lies in the fact that EF estimates reflect only consumption differences based on global average efficiencies, while WF estimates reflect both production and consumption based on actual efficiencies. However, both analyses share the common capability of being conducted for specific organizations, activities, and products across all spatial scales (Hoekstra, 2009). Thus, the concept of water footprint measures the supply and use of all humanity's freshwater resources (blue, green, and grey), identifying geographical distinctions between production and consumption regions (Hoekstra and Mekonnen, 2012).

The use of water footprint and carbon footprint indicators in crop production contributes to shaping production policies. In Italy, water consumption was evaluated using wheat production data from different regions between 2011 and 2015. The green water footprint was highest in Umbria at 6525 m^3/ha and lowest in Sardinia at 3125 m^3/ha. It was noted that the blue water footprint ranged from 42 m^3/ha to 88 m^3/ha (Casolani et al., 2016). Yousefi et al. (2017) collected data in Iran during the summer of 2012 using a random sampling method to calculate the water footprint, carbon footprint, and energy requirements of sunflower production. They reported a green water footprint of 0.63 m^3/kg (18%), a blue water footprint of 2.78 m^3/kg (82%), resulting in a total water footprint of 3.41 m^3/kg. Agricultural water footprint appears high in Turkey, consistent with global trends. According to a report prepared by the General Directorate of Water Management for the Büyük Menderes Basin, the total water footprint is 13.70 billion m^3 (SYGM, 2023), and in another thesis (Erdem, 2021), the agricultural water footprints of the Seyhan, Ceyhan, and Asi Basins were calculated as 3.53 billion m^3, 6.58 billion m^3, and 2.51 billion m^3, respectively. The agricultural water footprint calculated in the Konya Closed Basin irrigation networks was 1.36 billion m^3 (Çakmak and Torun, 2023).

This study will examine and evaluate agricultural water usage in Bilecik province from a different perspective, discussing the current situation. The water footprint approach will analyze water resource consumption at the regional level and provide guidance for future projections. It will highlight successful practices and identify necessary actions if deficiencies are found, thereby providing valuable insights to scientists and decision-makers. It will also contain valuable information for ensuring sustainability in water resource management and food supply.

2. Materials and Methods

2.1. Materials

The study was conducted using data related to agricultural and animal production as well as water resources of Bilecik province. Bilecik is located in the southeast of the Marmara Region, between 39° and 40° 31' north latitudes and 29° 43' and 30° 41' east longitudes. The altitude of the province ranges from 200 to 500 meters above sea level. Mountains cover a significant portion, approximately 32%, of the province's geography, whereas usable plains constitute only 7%. In terms of climate, Bilecik Province has a transitional climate, exhibiting characteristics of both the Marmara and Central Anatolian climates. According to log term meteorological data, the average precipitation is 453.9 mm, and the average temperature is 12.5 °C (MGM, 2023).

The main surface water source within the boundaries of Bilecik province is the Sakarya River, which traverses 80 km and has a flow rate of 100 m^3/s. The river water is used for irrigation and energy production purposes. In second place is the Karasu Stream, covering a distance of 65 km with a flow rate of 3.6 m^3/s. The only natural lake within the boundaries of Bilecik province is Lake Çerkeşli. The total surface area of natural lake surfaces is 4,790 hectares, with 3 dams totaling 5,716 hectares of net irrigation area used for irrigation purposes, and 5 reservoirs totaling 1,410 hectares of net irrigation area. The total water source volume in Bilecik province is 374.7 hm^3/year, with surface water potential of 320 hm^3/year and groundwater potential of 54.7 hm^3/year. The total area of the province is 430,200 hectares, with 140,743 hectares classified as agricultural land, 32,200 hectares as pasture land, 205,825 hectares as forest land, and 51,432 hectares as other types of land. In Bilecik province, irrigated agriculture is practiced on an area of 20,298 hectares (Anonymous, 2017). The areas where irrigated agriculture is concentrated include particularly the districts of Osmaneli, Gölpazarı, Söğüt, and the central district.

The agricultural and animal production data for the year 2022 used for analysis belong to Bilecik Province, sourced from the report of the Provincial Directorate of Agriculture and Forestry of Bilecik Governorship (Anonymous, 2023), while some data related to water footprint indicators were obtained from tables published by Mekonnen and Hoekstra (2011; 2012).

2.2. Methods

The study focused on calculating the volume-based blue, green, and grey water footprints described by Hoekstra et al. (2011). The blue water footprint (WF_{blue}) indicates the portion of consumed groundwater or surface water. The agricultural water footprint has been determined by calculating the total green, blue, and grey water requirements of crops grown in the region. The Green Water Footprint (WF_{green}) is considered as the total volume of rainwater used in the production of a product, while the grey water footprint (WF_{grey}) is calculated as the total volume of water needed to neutralize pollutants (Hoekstra et al., 2011; Ercin ve Hoekstra, 2012).

The method developed by Chapagain and Hoekstra (2004) for determining the water footprint of crop production has been used. In crop production, the water footprint largely depends on the water consumption of the plants. The distribution of the 2022 crop pattern by product groups in the research area is shown in Figure 1.

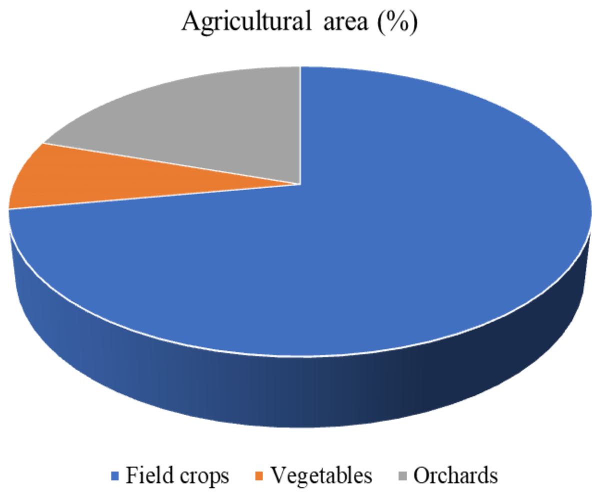

Figure 1. Crop cultivation rates in Bilecik province.

Plant water consumption consists of two main components: rainfall and irrigation water. In the research area, water footprint values in m^3/year and m^3/ton have been calculated using the water footprint method developed by Chapagain et al. (2006). The necessary meteorological data for the calculations were obtained from the General Directorate of Meteorology (MGM, 2023). To determine the water footprint of crop production, plant water consumption and effective rainfall were first calculated using the TAGEM-SUET (tagemsuet.tarimorman.gov.tr) application, resulting in the green and blue water needs. The Penman-Monteith method was used for plant water consumption and the USDA-SCS method for effective rainfall in the application. Plant water consumption (ET, m^3/ha) is calculated as the sum of the blue and green water needs (Chapagain and Hoekstra, 2004).

The water footprint components of crop water consumption (m^3/ha) are values dependent on the green and blue water needs of the crop during its growing season (crop water consumption ET, mm). Green crop water consumption is the amount of crop water consumption covered by effective rainfall. When effective rainfall (Peff) is equal to or greater than plant water consumption, green crop water consumption is equal to crop water consumption in equation 1. When crop water consumption exceeds effective rainfall, green crop water consumption is equal to Pe in equation 2 (Lovarelli et al., 2016).

$$ET \leq Peff \text{ then } ET_{green} = ET \quad (1)$$
$$ET > Peff \text{ then } ET_{green} = Peff \quad (2)$$

In the equations, ET_{green} represents the amount of crop water consumption covered by rainfall (mm); Pe denotes effective rainfall (mm), and ET refers to total crop water consumption (seasonal evapotranspiration, mm).

The difference between crop water consumption and effective rainfall is expressed as blue crop water consumption or net irrigation water requirement. When crop water consumption is equal to and/or greater than effective rainfall, blue crop water consumption (dn, $ET_{blue-theoretical}$) is equal to the difference between crop water consumption and effective rainfall and is calculated using equation 3. When effective rainfall exceeds crop water consumption, there is no need for irrigation, so blue crop water consumption equals zero in equation 4 (Lovarelli et al., 2016).

$$ET \geq Peff \text{ then } ET_{blue} = ET - Peff \quad (3)$$
$$ET < Peff \text{ then } ET_{blue} = 0 \quad (4)$$

Blue crop water consumption (d_n, $ET_{blue-theoretical}$) theoretically represents the amount of irrigation water needed by the crop. This amount includes the water losses that occur as the irrigation water delivery from the water source to the crop. Therefore, blue crop water consumption has been divided by the irrigation efficiency (E) to calculate the total theoretical irrigation water requirement using equation 5 (Hoekstra et al., 2012).

Crop water use (CWU, m^3/ha) represents the total evapotranspiration amount (ET) during the crop growing season (lgp) and is determined by equation 6.

$$ET_{blue-theoretical} = ET_{blue}/E \quad (5)$$
$$CWU_{green/blue} = 10 \times \sum_{d=1}^{lgp} ET_{green/blue} \quad (6)$$

The water footprint of crops is obtained from the sum of green, blue, and grey water footprint components throughout the crop growth process by equation 7. Green and blue water footprints (m^3/ton) are calculated by dividing crop water use (m^3/ha) by crop yield (ton/ha) using equations 8 and 9. The green, blue, and total water footprint values during the growing season were calculated using equations 10, 11 and 12, based on the total volume of water used for crop production (Hoekstra et al., 2011). Grey water footprint for crop production has been calculated using the average water footprint per ton of commodity per country, weighted based on origin (WF* in m^3/ton) values described in Mekonnen and Hoekstra (2011).

$$WF_{proc} = WF_{proc-green} + WF_{proc-blue} + WF_{proc-grey} \quad (7)$$
$$WF_{proc-green} = \frac{CWU_{green}}{Y} \quad (8)$$
$$WF_{proc-blue} = \frac{CWU_{blue}}{Y} \quad (9)$$
$$WF_{proc-green}\ (m^3) = WF_{proc-green}\ (m^3/ton) \times Production\ (ton/year) \quad (10)$$

$WF_{proc\text{-}blue}\ (m^3) = WF_{proc\text{-}blue}\ (m^3/ton)\ x\ Production\ (ton/year)$ (11)

$WF_{grey}\ (m^3) = WF_{proc\text{-}grey}\ (m^3/ton)\ x\ Production\ (ton/year)$ (12)

The water footprint of livestock includes the total amount of water used directly or indirectly in the production of beef, dairy, and other products from cattle, sheep, and poultry raised in the region. In animal production, the blue water footprint per animal is obtained by multiplying the number of livestock (HSi,j) by average water footprint at end of life time (HSUi,j, m^3/animal) reported by Mekonen and Hoekstra (2012) using equation 13.

$Mavi\ SA_{hayvancılık} = \sum HSi,\ \times HSUi,j$ (13)

The blue, green, and grey water footprints of animal products such as meat, milk, and eggs were obtained by multiplying the water footprint values per ton described by Mekonen and Hoekstra (2012) with the total production quantities in Bilecik province.

3. Results

The total values for the water footprint of crop production, animal husbandry, and overall agricultural production covering Bilecik provincial center and districts for the year 2022 are detailed in Tables 1 and 2. The water footprint of plant production is 0.608 billion m^3, the water footprint of animal husbandry is 0.516 billion m^3, and the total agricultural water footprint is calculated as 1.12 billion m^3. The share of the plant production water footprint within the agricultural production water footprint is found to be higher at 54% compared to the animal production water footprint share of 46%. The total water footprint of plant production consists of 33% green water footprint, 59% blue water footprint, and 8% grey water footprint (Figure 2).

The distribution of the total water footprint of plant production across different plant product groups and crops within the province is illustrated in Figure 3. According to the graph, cereals have the largest total water footprint with 390.16 million m^3 (65%) in the province, followed by fruits with 152.84 million m^3 (25%), vegetables with 56.72 million m^3 (9%), and greenhouse production with 8.10 million m^3 (1%). The total water potential for the province has been reported as 374.7 million m^3 by the State Hydraulic Works (DSİ) in 2022. Even excluding the green water footprint in plant production, the total of blue and grey water footprints has been calculated as 410.3 million m^3.

In animal production, the water footprint calculated based on water needs per animal totals 394.65 million m^3, while the water footprint of animal products as milk, egg, chicken meat and beef is calculated as 121.11 million m^3, with the highest share being 77% attributed to the water footprint based on live animal inventory. The total water footprint in animal production is 515.76 million m^3. Within the total water footprint of animal product production, the share of green water footprint is 87%, the share of blue water footprint is 6%, and the share of grey water footprint is 7% (Figure 4).

Table 1. The green, blue and grey water footprint along process of growing crops

Crop Category	Crop variety	Cultivated area (ha)	Crop Production (ton/year)	$WF_{proc\text{-}green}$ (m^3/ton)	$WF_{proc\text{-}blue}$ (m^3/ton)	WF_{green} (m^3)	WF_{blue} (m^3)	WF_{grey} (m^3)	WF_{proc} (10^6 m^3)
Field Crops	Wheat	30520.00	70391.00	1011.10	1489.77	71172640.00	104866720.00	13515072.00	189.55
	Barley	7365.80	15074.00	1139.51	1620.34	17177045.60	24424992.80	346702.00	41.95
	Rye	511.80	1518.00	786.24	1677.68	1193517.60	2546716.80	220110.00	3.96
	Oats	1980.00	12114.00	381.16	702.17	4617360.00	8506080.00	1756530.00	14.88
	Dry Beans	224.60	470.00	863.04	2651.24	405627.60	1246080.80	117030.00	1.77
	Chickpea	665.60	875.00	1373.80	3231.39	1202073.60	2827468.80	335125.00	4.36
	Sunflower (Oil)	8454.50	15324.00	996.40	4583.66	15268827.00	70239986.00	2160684.00	87.67
	Sunflower	1512.40	3074.00	888.55	4087.51	2731394.40	12565019.20	433434.00	15.73
	Maize (Silage)	582.50	30719.00	34.25	113.92	1051995.00	3499660.00	5037916.00	9.59
	Sainfoin	319.20	4526.00	294.87	-	1334575.20	-	497860.00	1.83
	Clover	1921.80	40123.00	200.26	214.49	8035045.80	8605820.40	1203690.00	17.84
	Hops	182.00	1047.00	726.78	229.11	760942.00	239876.00	15705.00	1.02
	Total	54240.20	195255.00	8695.96	20601.28	124951043.80	239568420.80	25639858.00	390.16
Vegetables	Pumpkin	148.50	4126.00	72.63	303.19	299673.00	1250964.00	169166.00	1.72
	Kidney bean	423.50	4810.00	177.68	461.71	854623.00	2220834.00	1197690.00	4.27
	Peas	101.50	698.00	293.45	675.31	204827.00	471366.00	316892.00	0.99
	Pepper	375.00	10975.00	68.95	274.85	756750.00	3016500.00	3643700.00	7.42
	Tomato	1560.20	108635.00	28.98	113.23	3148483.60	12300616.80	2281335.00	17.73
	Beans (Fresh)	344.70	3499.00	198.80	530.40	695604.60	1855864.80	1588546.00	4.14
	Spinach	147.80	1800.00	165.70	15.11	298260.40	27195.20	73800.00	0.40
	Watermelon	1045.60	68560.00	30.78	62.89	2110020.80	4312054.40	5827600.00	12.25
	Melon	536.70	13529.00	80.05	273.88	1083060.60	3705376.80	1149965.00	5.94
	Eggplant	53.10	1790.00	59.86	191.75	107155.80	343238.40	73390.00	0.52
	Onion (Fresh)	148.90	4013.00	74.88	59.52	300480.20	238835.60	140455.00	0.68
	Onion (Dry)	63.20	1236.00	103.19	391.88	127537.60	484364.80	43260.00	0.66
	Total	4948.70	223671.00	1354.95	3353.73	9986476.60	30227210.80	16505799.00	56.72
Greenhouse production	Tomato (Table)	214.40	18791.00	0.00	155.40	0.00	2920128.00	394611.00	3.31
	Cucumber (Table)	222.60	17034.00	0.00	123.88	0.00	2110248.00	357714.00	2.47
	Lettuce	468.30	14059.00	0.00	111.92	0.00	1573488.00	576419.00	2.15
	Green Onion	19.60	1149.00	0.00	109.51	0.00	125832.00	40215.00	0.17
	Total	924.90	51033.00	0.00	500.72	0.00	6729696.00	1368959.00	8.10
Orchards	Cherry	2189.30	14274.00	641.27	597.86	9153463.30	8533891.40	1213290.00	18.90
	Peach	2201.50	36651.00	251.14	354.27	9204471.50	12984447.00	3115335.00	25.30
	Olive	1751.10	3341.00	2191.36	460.18	7321349.10	1537465.80	283985.00	9.14
	Sour Cherry	567.60	1015.00	2338.06	2202.18	2373135.60	2235208.80	86275.00	4.69
	Grape	1046.60	6530.00	670.11	464.48	4375834.60	3033046.80	555050.00	7.96
	Walnut	5664.30	2166.00	10933.72	21020.15	23682438.30	45529643.40	184110.00	69.40
	Quince	474.60	6591.00	301.06	431.90	1984302.60	2846650.80	560235.00	5.39
	Apple	394.80	2816.00	586.17	577.34	1650658.80	1625786.40	239360.00	3.52
	Plum	320.30	1803.00	742.75	1104.62	1339174.30	1991625.40	153255.00	3.48
	Pomegranate	280.50	7104.00	165.09	331.59	1172770.50	2355639.00	603840.00	4.13
	Pear	84.90	606.00	585.75	837.51	354966.90	507532.20	51510.00	0.91
	Total	14975.50	82897.00	19406.49	28382.08	62612565.50	83180937.00	7046245.00	152.84
Total Water Footprint of the process of growing crops, WFproc, million m^3									607.81

Table 2. Annual water footprint of animal category and some selected food products

Water footprint of animal type				Water footprint of animal products				
Animal category	Number of animal head	WF_{mean} (m^3/animal)	WF_{total} (10^6 m^3)	Product	WF_{green} (m^3)	WF_{blue} (m^3)	WF_{grey} (m^3)	WF_{total} (10^6 m^3)
Cattle	35461	1889	66.99	Milk	18986000	1892000	1584000	22.46
Buffalo	49	20558	1.00	Eggs	5412096	509472	895752	6.82
Sheep	122098	141	17.22	Chicken meat	30884040	2726856	4068504	37.68
Goat	40887	76	3.10	Beef	50636382	1932150	1584363	54.15
Broiler	17757	6	0.11					
Egg poultry	6515539	47	306.23					
Total			394.65		105918518	7060478	8132619	121.11

WF_{mean}= Average water footprint at end of life time by Mekonnen and Hoekstra (2012).

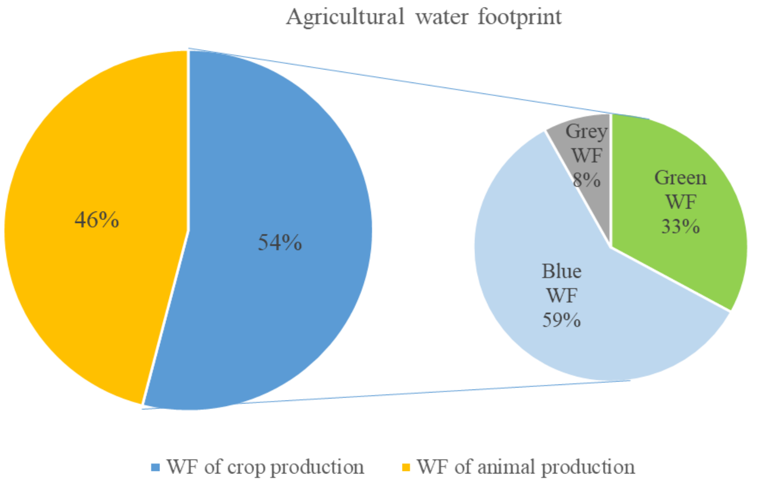

Figure 2. Percentages of agricultural water footprint.

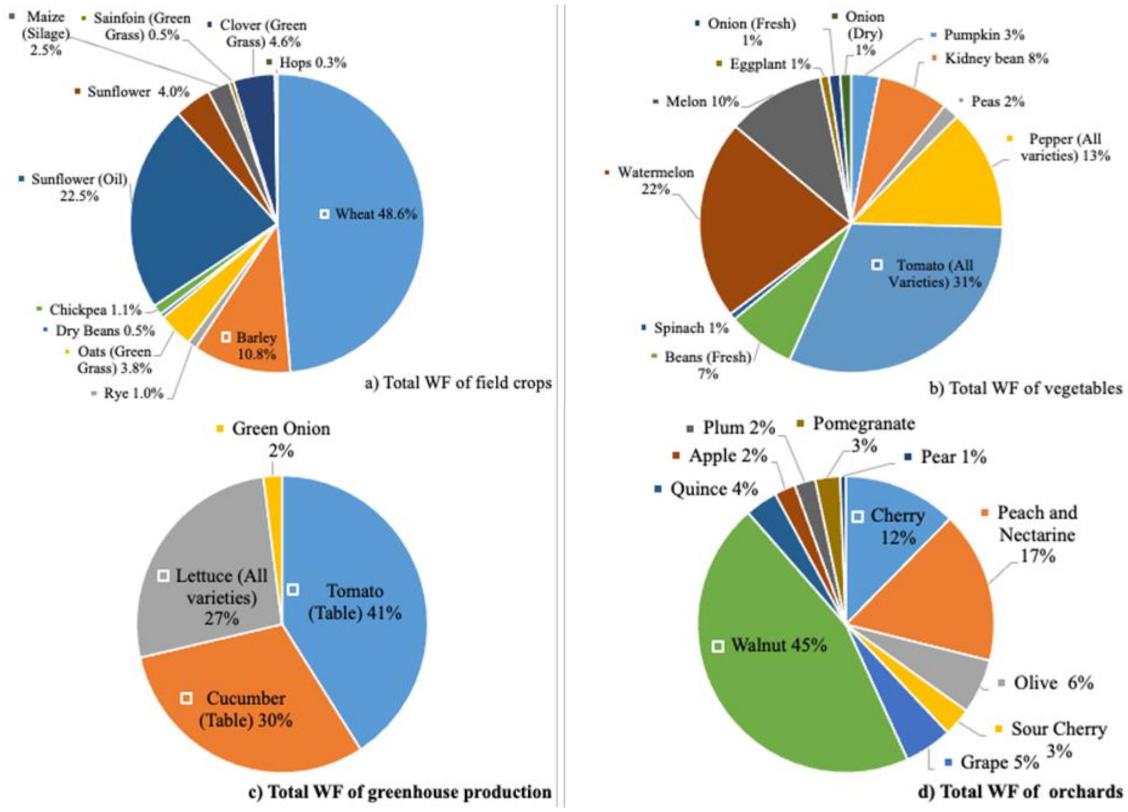

Figure 3. The distribution of the total water footprint of plant production across different plant product groups and crops.

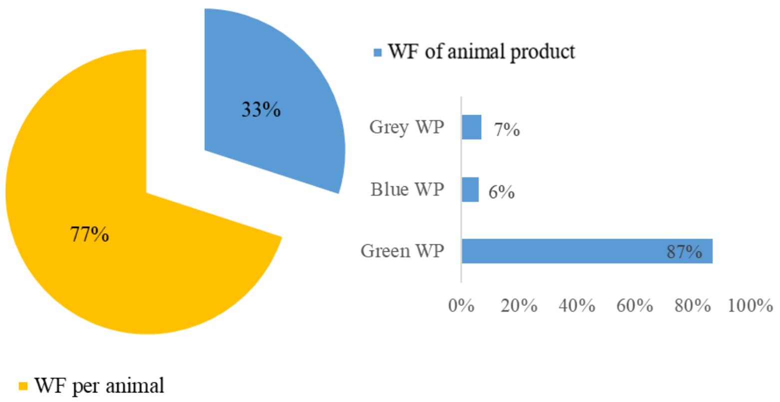

Figure 4. Water footprint of animal production

4. Discussion

According to similar studies found in the literature, agricultural production has the largest share among the components of the water footprint. On a global average, agricultural production accounts for 70% of direct water use and 90% of indirect water use. In Turkey, agricultural production accounts for 74% of direct water use, and this percentage can even reach up to 86% in arid regions with a continental climate (Mekonnen and Hoekstra, 2011; Ekinci, 2015; Batan, 2021).

In addition to the intensity of water use for agricultural purposes, rainfall anomalies are increasing in the so-called gateway regions where the study was conducted. In addition to the classical methods of studying how water is used, the use of techniques such as water footprinting, which can distinguish between more uses and assess the impacts on the ecosystem, has increased especially in the last decade. In particular, it is seen that many studies have been carried out on this subject with the need for detailed studies on the agricultural sector, which is the main user of water in our country and in the world (Ababaei and Etedali, 2017; Novoa et al., 2019; Hossain et al.2021; Yang et al., 2020; Cai et al., 2022). In the study, the total values of animal and crop water footprint were obtained as ZZ and FF%, respectively. This situation is similar to the official institution statistics where water use is explained and reveals the reliability of the results of the study (Anonymous 2023).

In watersheds or special production zones, the water footprint method can be used robustly and reliably to assess the impacts of crop and livestock production on water resources. This method explains well the reactions of crops and livestock production to water (Novoa et al., 2019; Yang et al., 2020). Specifically, field crops, vegetables, greenhouse cultivation and fruit cultivation groups were examined in the study and total water footprint values of 390.16, 56.72, 8.10 and 152.84 million m^3 were obtained respectively. It is seen that the dominant values belong to field crops and fruit cultivation. This situation is similar to the production statistics and other study results. In the study conducted by Novoa et al. 2019, the agricultural water footprint was obtained as 18,221 m^3. In the study where the water footprints of the main river basins in Europe were calculated, the river basins with the highest values were Thames, Scheldt, Rhine and Po and the agricultural water footprint values were announced as 130,363 m^3 km^{-2}, 200,524 m^3 km^{-2}, 109,720 m^3 km^{-2} and 219,630 m^3 km^{-2}, respectively (Vanham and Bidoglio, 2014). In the study conducted by Cai et al., 2022, the agricultural water footprint in China was analyzed between 2000-2017 and the average was announced as 5.039 x 109 m^3/year. In the study conducted by Çakmak and Torun (2023), in Konya closed basin in our country, agricultural water footprint was evaluated for irrigation networks. The agricultural water footprint was calculated as 1.09 million m^3/ha in Konya Closed Basin. In the study conducted by Muratoğlu (2020) in order to evaluate the agricultural water footprint and usage of Diyarbakır Province, the average agricultural water footprint value was calculated as 3.43 billion m^3/year. Another study was conducted by Erdem (2021) for the water footprint assessment of Seyhan, Ceyhan and Asi Basins. The water footprint values in these basins were calculated as 3.53, 6.58 and 2.51 billion m^3, respectively. When the studies and the data obtained are examined, it is seen that the water footprint data varies according to the plants grown in the relevant region, plant planting rates, agricultural techniques, irrigation methods, and is also significantly affected by dry and normal rainfall conditions. The fact that it depends on many natural and artificial parameters can be considered as a positive factor in reflecting natural conditions.

The concept of water footprint includes sectoral data on general usage, as well as specific green, blue and grey water footprint components. Thanks to these components, it reflects the usage characteristics of water resources more accurately and reliably. In the study, green, blue and grey footprint values for plant cultivation are 197.5, 359.7 and 50.5 million m^3, respectively. Data on animal products are calculated as 105.9, 7.06 and 8.1 million m^3, respectively. Across the country, the total water footprint of crop production ranges between 2.13 and 114.79 billion m^3, while the total water footprint of animal production ranges between 0.43 and 9.98 billion m^3 (Muratoğlu, 2020; Erdem, 2021; Ahi and Çakmak, 2023; Çakmak ve Torun, 2023). It is consistent with many results obtained under similar conditions in the international literature conducted by Lovarelli et al. (2016), Ababaei and Etedali (2017), Novoa et al. (2019), Yang et al. (2020), Hossain et al. (2021), and Cai et al. (2022).

5. Conclusion

In the study, after discussing classical concepts and methods for assessing water resources, the concept of the water footprint, one of the techniques considered today, was used as a basis, and the use of water resources in agricultural production in Bilecik Province was examined. Bilecik Province and the region's freshwater resources face challenges such as pollution and increased consumption due to factors like irregularities and reductions in precipitation, improper use of irrigation and cultivation techniques, irregular use of natural resources, poor land planning, negative impacts of industrial development on the ecosystem, and intensive migration due to its location at the intersection of transportation axes. The total water footprint of agricultural production obtained from the study (1.1 billion m^3) aligns with the water use statistics at the provincial level, clearly demonstrating the increase in the use of water for animal and plant cultivation in agricultural production. Bilecik Province is considered one of the areas predicted to be most affected by global climate change, along with a significant part of the Mediterranean and Aegean regions of Turkey. Therefore, due to meteorological parameters such as increased temperature-evaporation and precipitation anomalies, providing quality water in the desired amounts and times, especially for agricultural production, will become more challenging. In the future, the creation of water-intensive units such as organized industrial zones in the city, parallel population growth due to industrial development, and the transition of existing agricultural production from dry farming to irrigated agriculture highlight the importance of planning the province's future with a focus on water resources and natural resources.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	H.T.G.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

The author thanks to Dr. Buğra Erdem, the Director of Crop Production at the Bilecik Provincial Directorate of Agriculture and Forestry, for providing the data.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1495158

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 524-533 / September 2024

EVALUATION OF PROXIMATE, PHYTOCHEMICALS, ANTIOXIDANT CAPACITY, ENZYMATIC INHIBITION, AND ANTI-INFLAMMATORY PROPERTIES OF AVOCADO SEED MEAL AS POTENTIAL FEED ADDITIVE

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Abstract: This study examined the proximate composition, phytochemical profile, antioxidant capacity, enzymatic inhibition, and anti-inflammatory properties of avocado seed meal. The proximate analysis revealed that avocado seed meal is rich in protein (17.32%), fat (15.33%), and carbohydrates (48.73%), with moderate levels of moisture (10.26%), ash (2.39%), and crude fiber (5.97%). Phytochemical analysis indicated high contents of phenols (111.56 mg/g), saponins (96.94 mg/g) and flavonoids (66.66 mg/g), but lower levels of alkaloids (14.60 mg/g), tannins (1.17 mg/g), and steroids (0.89 mg/g). The antioxidant properties assessed showed significant DPPH (50.05%) and FRAP (63.04%) free radical scavenging activities, though lower inhibition of lipid peroxidation (34.88%) and vitamin C content (0.03 mg/g). Enzymatic inhibition assays demonstrated alpha-lipase, alpha-glucosidase, and alpha-amylase inhibition rates of 31.46%, 27.56%, and 50.88%, respectively. Anti-inflammatory properties were also notable, with 24.02% albumin denaturation inhibition and 37.99% antiprotease activity. These findings suggest that avocado seed meal holds considerable potential for nutritional and therapeutic applications as natural feed supplement for livestock and humans.

Keywords: Antioxidant, Plant-based additives, Phytochemicals, Proximate, Protein denaturation, Seed meal

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Received: June 04, 2024
Accepted: August 26, 2024
Published: September 15, 2024

Cite as: Atiba OI, Falowo AB, Oloruntola OD, Olarotimi OJ, Ayankoso M. 2024. Evaluation of proximate, phytochemicals, antioxidant capacity, enzymatic inhibition, and anti-inflammatory properties of avocado seed meal as potential feed additive. BSJ Agri, 7(5): 524-533.

1. Introduction

In recent years, interest in the use of feed ingredients or additives endowed with bioactive properties has increased significantly, especially within the system of intensive livestock production, due to occurrence of antibiotic residues in animal products, which are injurious to consumer health (Falowo et al., 2014; Hotea et al., 2023). Part of these feed ingredients/additives could be obtained from processed fruit and vegetable wastes, which are typically discarded after processing, packing, distribution and consumption (FAO, 2013; Ikusika et al., 2024). .On yearly basis, substantial quantities of fruit and vegetable wastes are generated and disposed of in landfills or water bodies, presenting potential health and environmental hazards if not appropriately channeled into productive products (FAO, 2013; Ikusika et al., 2024). These wastes represent abundant resources on a global scale. However, fruit wastes, encompassing seeds, peels, pulp, pomace, and their extracts, can be processed and harnessed as natural feed ingredients or additives to promote growth and health in animal production, because they possessed a plethora of bioactive compounds that offers antioxidant, antimicrobial, immune-protective and other beneficial secondary metabolites (Achilonu et al., 2018). In addition, they are rich sources of dietary fiber, protein, vitamins C and E, carotenoids, minerals, and other micronutrients, offering potential as alternative to synthetic growth hormones and antibiotics within the livestock industry (Achilonu et al., 2018; Ikusika et al., 2024). Recent studies have indicated that dietary supplementation with fruit seed meals can enhance growth performance and improve nutrient digestibility and meat quality in livestock (Akure et al., 2021; Haruna et al., 2021). Also the dietary inclusion of fruit seed meals containing phytochemicals have been reported to lower blood glucose and cholesterol content and enhance liver glycogen storage in animals (Uchenna et al., 2017). Utilization of fruit seed meals as feed additives in animal diets is believed to be natural, less toxic, residue-free, and safer than synthetic ones (Manuelian, et al., 2021).

One of the fruit seeds that can be explored as natural feed additives is avocado fruit seeds. Avocado (*Persea americana*) is a dicotyledonous tropical plant belonging to the *Lauraceae* family (Barbosa-Martín et al., 2016; Bangar et al., 2022). Renowned for its sensory attributes and nutritional benefits, avocado is widely cultivated and highly sought after internationally, often regarded as the world's healthiest fruit for human consumption (Mahawan et al., 2015; Bangar et al., 2022; Siol and Sadowska, 2023). Apart from its use as food, the avocado fruit is traditionally used for several medicinal purposes including hypotensive, hypoglycemic, anti-viral and anti-diarrheal and cardiovascular diseases (Vinha et al., 2020). The processing of avocado fruits into products such as avocado oil and paste often generate a sizeable amount of waste products, including peels and seeds which are always discarded as waste (Bangar et al., 2022). Each fruit contains a single large seed, accounting for approximately 13–18% of the fruit's weight (Barbosa-Martín et al., 2016; Siol and Sadowska, 2023). These seeds are rich in nutrients and possess various medicinal properties, making them potential candidates as valuable feed additives in animal diets. This is primarily due to their substantial reserves of inherent phytochemicals such as alkanols, terpenoid glycosides, furan ring-containing derivatives, flavonoids and coumarin (Uchenna et al., 2017; Setyawan et al., 2021). Emerging research indicates that the phytochemicals in seed meals may offer health benefits, including antioxidant and antimicrobial effects (Siol and Sadowska, 2023). Understanding these compounds could pave the way for new health supplements and functional foods, while reevaluating them could enhance the commercial use of these resources in food and feed industries. It is known that different varieties of seed meals including avocados produce different content of nutrients and bioactive compounds (Setyawan et al., 2021). Therefore, this study aims to evaluate the proximate composition, phytochemical profile, and potential anti-diabetic, anti-inflammatory, and antioxidant properties of avocado seed meal.

2. Materials and Methods

2.1. Collection and Processing of Avocado Seed Meal

Fresh avocado fruits were purchased from a local market in Ondo State, Nigeria. The seeds were extracted from the fruits, cleaned, sliced into small cubes, and air-dried in an open shade for 14 days. After drying, the seeds were ground into a meal and stored at 4 °C until analysis. All chemicals used for the analyses were of analytical reagent grade and were purchased from Sigma-Aldrich.

2.2. Determination of Proximate Composition of Avocado Seed Meal

The proximate composition (moisture, fat, protein, ash, crude fiber) of the seed meal was determined according to the procedures described by the Association of Official Analytical Chemists (AOAC, 2010). The carbohydrate content of the seed meal was calculated by the difference method (AOAC, 2010), subtracting the sum (g/100g dry matter) of crude protein, crude fat, ash, and fiber from 100g.

2.3. Determination of Phytochemical Composition of Avocado Seed Meal

2.3.1. Tannin content

Approximately 0.2g of each finely ground sample was weighed into a 50mL sample bottle. Then, 10mL of 70% aqueous acetone was added, and the bottle was properly sealed. The bottle was placed in an ice bath shaker and shaken for 2 hours at 30 °C. Each solution was then centrifuged, and the supernatant was stored on ice. From each solution, 0.2mL was pipetted into a test tube, and 0.8mL of distilled water was added. Standard tannic acid solutions were prepared from a 0.5mg/mL stock solution, and the volume was adjusted to 1mL with distilled water. Next, 0.5mL of Folin-Ciocalteu reagent was added to both the sample and the standard, followed by 2.5mL of 20% Na_2CO_3. The solution was then vortexed and allowed to incubate for 40 minutes at room temperature. Its absorbance was read at 725nm against a reagent blank. The tannin concentration was determined using a standard tannic acid curve (Makker and Goodchild, 1996).

2.3.2. Saponins content

Saponin was quantified using the vanillin and concentrated sulfuric acid colorimetric method described by He et al. (2014). A 0.1mL sample was combined with 0.5mL of 50% ethanol, 4.0mL of 77% sulfuric acid (w/w), and 0.5mL of freshly prepared 8% vanillin solution (w/v). The mixture was allowed to reach ambient temperature before being heated in a water bath at 60 °C for 15 minutes. The absorbance was measured at 545nm using a UV/Vis spectrophotometer. A tea saponin calibration curve was used to quantify the total saponin content in the seed sample, which was expressed as mg tea saponin equivalent per gram dry weight (TSE/g DW).

2.3.3. Alkaloids content

The alkaloid content of the seed sample was determined using the gravimetric technique described by Adeniyi et al. (2009). Five grams of the sample were dispersed in 50mL of 10% acetic acid in ethanol. After stirring, the mixture was left to stand for approximately 240 minutes before being filtered. The filtrate was then reduced to a smaller volume on a heated plate. Concentrated ammonium hydroxide was added dropwise to precipitate the alkaloids. The precipitate was collected on filter paper and rinsed with a 1% ammonium hydroxide solution. It was then oven-dried at 60 °C for 30 minutes, transferred to desiccators, and weighed repeatedly until a constant weight was achieved. The weight of the alkaloids was calculated as a percentage of the total sample weight.

2.3.4. Steroids content

The steroid content of the seed sample was determined using the method described by Odeyemi et al. (2023). One gram (1.0g) of the powdered sample was mixed with 100mL of distilled water in a conical flask. The mixture was filtered, and the filtrate was eluted with 0.1N ammonium hydroxide solution. Two milliliters (2mL) of the eluent were transferred to a test tube and mixed with

2mL of chloroform. Subsequently, 3mL of ice-cold acetic anhydride was added to the mixture. A standard sterol solution (200mg/dL) was prepared and treated as a blank. The absorbance of the standard and the test sample was measured using a spectrophotometer set to 420nm, zeroed with the blank.

2.3.5. Vitamin C

The vitamin C content of the seed sample was determined using the method described by Benderitter et al. (1998). A 500 µL extract mixture (300 µL extract diluted with 100 µL of 13.3% trichloroacetic acid and water) was prepared, to which 75 µL of DNPH solution (containing 2g dinitrophenyl hydrazine, 270mg copper sulfate ($CuSO_4.5H_2O$), and 230mg thiourea in 100mL of 5mL/L H_2SO_4) was added. The reaction mixture was incubated at 37 °C for 3 hours. After incubation, 0.5mL of 65% H2SO4 (v/v) was added to the mixture, and the absorbance was measured at 520nm using a UV spectrophotometer. The vitamin C content of the seed sample was then quantified using ascorbic acid as a reference standard.

2.3.6. Phenol content

The total phenol content of the seed aqueous extract was determined using the method described by Singleton et al. (1999). Briefly, 0.2mL of the extract was mixed with 2.5mL of 10% Folin-Ciocalteu reagent and 2mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45 °C for 40 minutes, and the absorbance was measured at 700nm using a spectrophotometer. The phenol content was expressed as mg gallic acid equivalent.

2.3.7. Flavonoid content

The total flavonoid content of the seed aqueous extract was determined using a colorimetric assay described by Bao et al. (2005). Briefly, 0.2mL of the extract was added to 0.3mL of 5% $NaNO_3$ at zero time. After 5 minutes, 0.6mL of 10% AlCl3 was added. Six minutes later, 2mL of 1M NaOH was added to the mixture, followed by 2.1mL of distilled water. The absorbance was read at 510nm against a reagent blank. The flavonoid content was expressed as mg rutin equivalent.

2.4. Determination of Anti-oxidant Properties of Avocado Seed Meal

2.4.1. 2,2-Diphenyl-2-picrylhydrazyl radical scavenging capacity

The free radical scavenging ability of the seed aqueous extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) was estimated according to the method described by Gyamfi et al. (1999). One milliliter of the extract was mixed with 1mL of a 0.4 mM methanolic solution of DPPH, and the mixture was left in the dark for 30 minutes before measuring the absorbance at 516 nm

2.4.2. Lipid peroxidation inhibition

The lipid peroxidation inhibition of the seed aqueous extract was determined using a method previously described by Bajpai et al. (2015). In the presence or absence of seed extract (50-250 g/mL) or a control substance, a reaction mixture containing 1 mM $FeCl_3$, 50 µL of bovine brain phospholipids (5 mg/L), and 1 mM ascorbic acid in 20 mM phosphate buffer was incubated at 37 °C for 60 minutes. Malondialdehyde (MDA), a byproduct of lipid peroxidation caused by hydroxyl radicals, was measured using the 2-thiobarbituric acid (TBA) reaction. The percentage of inhibitory activity was calculated using the equation 1:

$$\% \text{ inhibition} = [(AC - AT)/(AC)] \times 100 \quad (1)$$

where, AC= absorbance of control, AT= absorbance of test

2.4.3. Ferric reducing antioxidant power (FRAP)

The reducing property of the seed aqueous extracts was assessed by their ability to reduce ferric chloride ($FeCl_3$) solution, following the method described by Oyaizu (1986). A 2.5 mL aliquot of the extract was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 minutes, followed by the addition of 2.5 mL of 10% trichloroacetic acid. After centrifugation at 2000 x g for 10 minutes, 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer, and the ferric reducing antioxidant property was subsequently calculated, with ascorbic acid used as the standard.

2.5. Determination of Antidiabetic Properties of Avocado Seed Meal

2.5.1. Alpha-amylase inhibitory activity

The α-amylase inhibition study was conducted using the 3,5-dinitrosalicylic acid (DNSA) method, as outlined by Wickramaratne et al. (2016). Seed extract concentrations ranging from 10 to 1000 g/mL were prepared by treating the extract with at least 10% dimethylsulfoxide and diluting it in buffer solution (0.006 M NaCl, 0.02 M Na_2HPO_4/NaH_2PO_4, pH 6.9). Two hundred microliters of extract and 200 µL of α-amylase solution were mixed and incubated at 30 °C for 10 minutes. Subsequently, 200 µL of starch solution (1% in water, w/v) was added to each tube, and the mixture was further incubated for 3 minutes. The reaction was stopped by adding 200 µL of DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL 2 M NaOH and 20 mL 96 mM 3,5-dinitrosalicylic acid solution) to a water bath at 85-90 °C, followed by boiling for 10 minutes. After cooling to room temperature, the mixture was diluted with 5 mL distilled water and analyzed using a UV-Visible spectrophotometer at 540nm. A blank with 100% enzyme activity was created by substituting 200 µL of buffer for the plant extract, while a blank reaction without the enzyme solution was generated using the plant extract at each concentration. As a positive control, acarbose (100–200 µg/mL) was used, and the reaction was conducted similarly to the plant extract reaction. The inhibitory activity of α-amylase was calculated as a percentage of inhibition using the equation provided. IC50 values were determined by plotting the percentage of α-amylase inhibition versus the extract concentration (Equation 2):

$$\% \alpha \text{ - amylase inhibition} = 100 \times (A\%C - (AS/A\%C) \quad (2)$$

where, A%C= absorbance 100% control, AS= absorbance sample

2.5.2. Alpha-glucosidase inhibitory activity

An assay for assessing the glucosidase inhibitory activity of the seed was adapted from Dej-adisai and Pitakbut (2015). The glucosidase enzyme converts the substrate, p-nitrophenyl-D-glucopyranoside (pNPG), into the yellow product, p-nitrophenol (pNP), which is used to analyze the glucosidase reaction. Fifty microliters of a 10 mM phosphate buffer solution (pH 7), containing 0.2 mg/mL sodium azide and 2 mg/mL bovine serum albumin, were added to a well plate. One unit/mL of Saccharomyces cerevisiae α-glucosidase and 50 µL of an 8 mg/mL sample solution were added to the phosphate buffer solution (Type I, lyophilized powder, Sigma, EC 3.2.1.20). The solvent control was a 5% DMSO solution, and the positive control was 8 mg/mL of acarbose in each well. The mixtures were incubated at 37 °C for 2 minutes. Fifty microliters of 4 mM pNPG were then added to each well, and the mixture was further incubated for 5 minutes under the same conditions. The release of pNP was measured every 30 seconds for 5 minutes using a microplate reader at 405 nm. The velocity (V) was calculated using the following linear relationship equation (3) between absorbance and time:

$$\text{Velocity} = \Delta\text{Absorbance at 405 nm} / \Delta\text{Time} \quad (3)$$

The initial highest velocity of each sample's reaction was recorded, and the percentage of inhibition was calculated using the equation (4) provided:

$$\% \text{ Inhibition} = ((\text{V control} - \text{V sample}) / (V\ control))\ X\ 100 \quad (4)$$

2.5.3. Lipase inhibition activity

The lipase inhibitory activity of the seed samples was determined following the method described by Ambigaipalan et al. (2017), with modifications as mentioned by Fathi et al. (2021). In brief, lipase enzyme (5 mg) was dissolved in 1 M Tris-HCl (pH 8.5). One hundred microliters of each tested sample (concentrations ranging from 0.2 to 1 mg/mL) were added to an equal volume of lipase solution and 4 mL of Tris-HCl buffer (1 M, pH 8.5). The mixtures were then incubated at 37 °C for 25 minutes, and the enzymatic reactions were initiated by adding 100 µL of the substrate (5 mM palmitate in dimethyl sulfoxide (DMSO): ethanol (at 1:1 w/v)) to the reaction mixtures, followed by a second incubation period (37 °C, 25 minutes). The absorbance of the samples was measured in a microplate reader (BioTek, Winooski, Vermont, US) at 412 nm. Lipase inhibitory activity was determined using the following equation 5:

$$\text{Lipase inhibitory (\%)} = ((As - Asb)/(Ac - Acb)) \times 100 \quad (5)$$

where As, Asb, Ac, and Acb represent the absorbance of the sample, sample blank, control, and control blank, respectively. The blank and control samples were generated by following the aforementioned experimental steps without adding the enzyme or without adding the inhibitor and enzyme, respectively.

2.6. Determination of Anti-inflammatory Properties of Avocado Seed M

2.6.1. Albumin denaturation inhibition

The assay was conducted following the protocol outlined by Osman et al. (2016). Ibuprofen and diclofenac, two positive standards, were prepared at a concentration of 0.1% each (1.0 mg/mL), alongside the seed extracts. Each reaction vessel contained 1000 µL of the test extract, 1400 µL of phosphate-buffered saline, and 200 µL of egg albumin. Distilled water was used as a negative control in place of the extracts. The mixtures were incubated at 37 °C for 15 minutes and then heated for 5 minutes at 70 °C. After cooling, their absorbances at 660 nm were measured. The protein denaturation inhibition percentage was calculated using the following formula given in Equation 6:

$$\% \text{ DI} = (1 - \text{ARTS}/\text{ARTS } (-\text{ve control})) * 100\% \quad (6)$$

where, DI= denaturation inhibition, ARTS= absorbance reading of the test sample

2.6.2. Antiproteinase activity

The test was conducted following the procedure outlined by Rajesh et al. (2019). The reaction mixture (2 mL) consisted of 1 mL of 20 mM Tris-HCl buffer (pH 7.4), 0.06 mg of trypsin, and 1 mL of the test sample with varying concentrations (100–500 µg/mL). The mixture was heated at 37 °C for five minutes. Subsequently, 1 mL of 0.8% (w/v) casein was added to the mixture, and it was further incubated at 37 °C for 20 minutes. To terminate the reaction, 2 mL of 70% perchloric acid was added to the mixture. After centrifugation, the absorbance of the supernatant was measured at 210 nm using buffer as a blank. The experiment was conducted in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated using the following formula given in Equation 7:

$$\% \text{ inhibition} = (\text{Abs control-Abs sample})*100/\text{Abs control.} \quad (7)$$

2.7. Statistical Analysis

Data obtained on each experiment were carried out three times and the results were analyzed using descriptive statistic of SPSS version 2021 to compute the mean. The means were used to construct bar graphs in Excel.

3. Results

The result of proximate composition of avocado seed meal is presented in Figure 1. The result revealed that avocado seed meal possessed high protein (17.32%), fat (15.33%) and carbohydrate (48.73%) content and moderate moisture (10.26%), ash (2.39%) and crude fibre (5.97%) content. As presented in Figure 2, the result of the phytochemical analysis showed that avocado seed meal contained high phenol (111.56mg/g), saponins

(96.94mg/g) and cardiac glycosides (56.78mg/g) contents and low flavonoid (66.66mg/g), alkaloid (14.60mg/g), tannin (1.17mg/g) and steroid (0.89mg/g) contents. The antioxidant properties of avocado seed meal is presented in Figure 3. The result showed that avocado seed meal had strong DPPH (50.05%) and FRAP (63.04%) free radical scavenging ability and low percent inhibition of lipid peroxidation (34.88%) and vitamin C content (0.03mg/g). The percentage of lipase inhibition, alpha-glucosidase and alpha-amylase inhibition activities in the avocado seed meal were 31.46, 27.56 and 50.88%, respectively (Figure 4). The result of the anti-inflammatory properties showed that avocado seed meals had a 24.02 percent albumin denaturation inhibition and a 37.99 percent antiprotease activity, respectively (Figure 5).

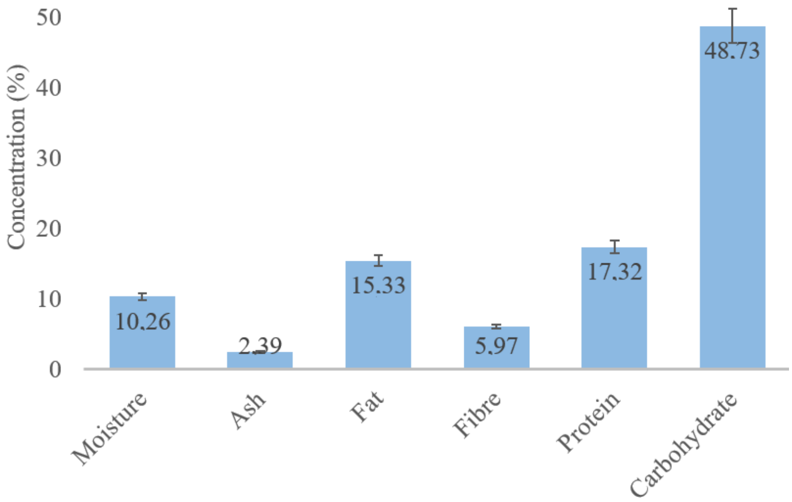

Figure 1. Proximate composition of avocado seed meal.

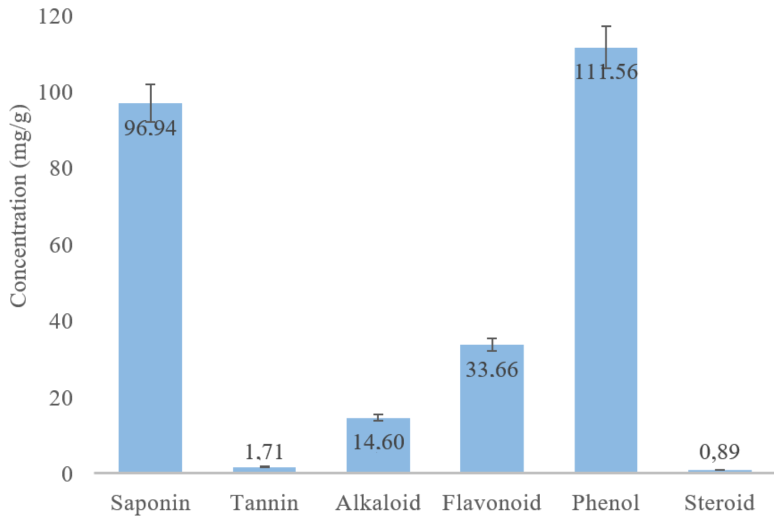

Figure 2. Phytochemical composition of avocado seed meal.

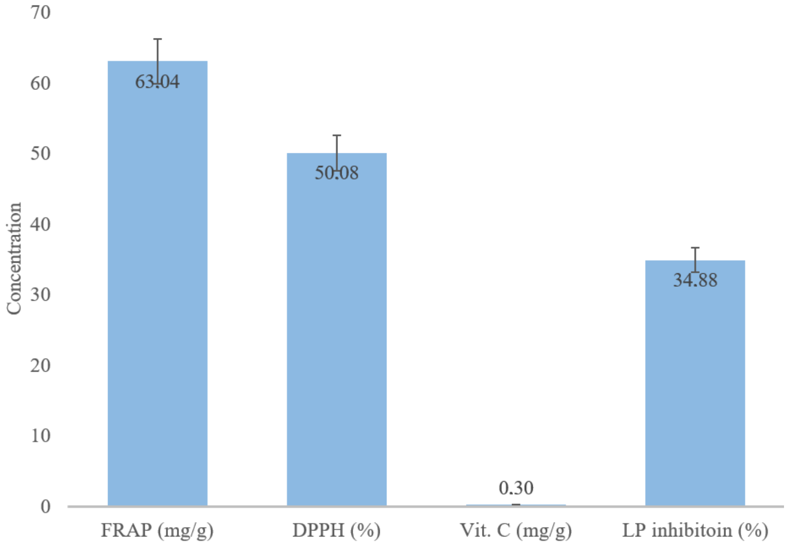

Figure 3. Anti-oxidant properties of avocado seed meal: LP= lipid peroxidation, DPPH= 2,2-diphenyl-1-picrylhydrazyl, FRAP= Ferric reducing antioxidant power, Vit. C= Vitamin C.

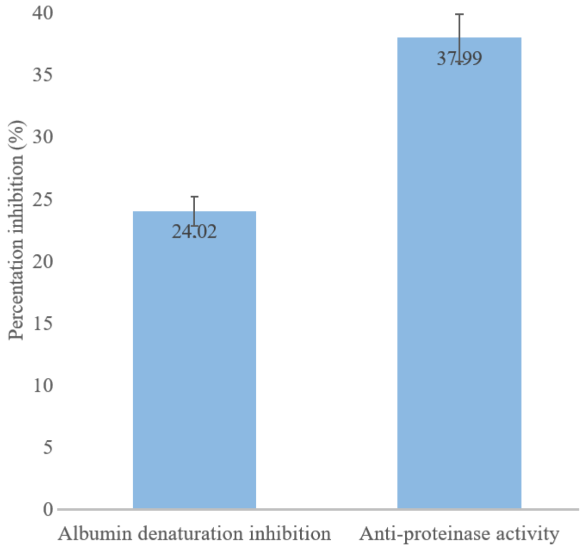

Figure 4. Anti-inflammatory properties of avocado seed meal

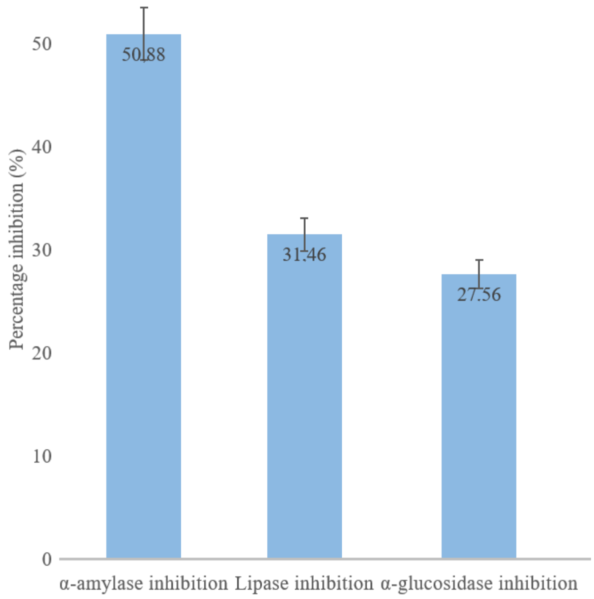

Figure 5. Antidiabetic properties of avocado seed meal.

4. Discussion

Evaluation of the proximate composition of food and feedstuffs is crucial for determining their safety, suitability, and nutritional integrity before consumption or incorporation as ingredients in animal diets (Oloruntola and Ayodele, 2022). In this study, the analysis of the proximate composition of avocado seed meal revealed significant nutritional attributes, positioning it as a promising food supplement or feed ingredient. The high protein content observed in avocado seed meal suggests its potential as an alternative protein source when utilized as feed ingredient to reduce the cost of using conventional protein in diet. Proteins are vital complex molecules that play pivotal roles in growth and development, cell signaling, enzyme regulation, and serving as biocatalysts in animals (Bangar et al., 2022).

The recorded level of fat content in avocado seed meal indicates its capacity to provide additional energy to the diet, thereby promoting efficient nutrient utilization and feed conversion efficiency. Studies have shown that the inclusion of feed ingredients rich in fat content can elevate dietary energy levels, leading to improved feed efficiency, growth rates, and enhanced quality of animal products (Hao et al., 2020; Omidi et al., 2020). Similarly, the substantial carbohydrate content found in avocado seed meal suggests it could be used as energy source in animal diets, particularly when combined with other carbohydrate or energy sources. Carbohydrates serve as supplementary energy sources for animals, supporting metabolic functions and aiding in maintaining body temperature (Navarro et al., 2019). The observed low crude fiber content in avocado seed meal indicates that the seed meal could easily be digested and absorbed in the gastrointestinal tract when included as a feed ingredient or additive in animal diets, especially for monogastric animals. Fiber is crucial in the diet of farm animals, as it acts as a diluent to enhance proper bowel movement (Odoemelam and Ahamefule, 2006; Udo et al., 2018). However, feed with higher crude fiber content has been reported to remain longer in the stomach, widening the interior wall of the colon, facilitating waste movement, and preventing constipation (Kolu et al., 2021). The low ash content of avocado seed meal implies that the seeds may not be a rich source of minerals, necessitating supplementary diets when included in animal diets. The ash content of feed samples is typically used to determine the organic content, from which the mineral content can be derived (Bello et al., 2008; Kolu et al., 2021). The values of moisture content obtained for avocado seed meal in this study fall within the range (5-12%) previously reported for other seed meals (Ogunbode and Raji, 2024). Moisture content in feed is indicative of its water activity, with higher moisture content samples having a higher tendency for spoilage of food materials (Famuwagun and Taiwo, 2023). The moisture content levels observed in avocado seed meal in this study suggest a prolonged shelf life when included in animal diets. In overall, the proximate composition values (protein, fat, crude fiber, carbohydrate, and moisture) obtained for avocado seed meal in this study align with the range of values reported by Bangar et al. (2022) and Nyakang et al. (2023) for avocado seed meal.

Phytochemical screening of plant materials, including seeds, is crucial to determine their potential and suitability as natural feed additives before inclusion in animal diets (Oloruntola et al., 2024). This is because the presence of specific phytochemical contents can elucidate the product's action or reaction when used in biological systems. The amount of phenol, flavonoid, and alkaloid contents obtained in avocado seed meal revealed that the seed contained rich antioxidants and could be utilized as a potential natural food and feed supplement/additive. The presence of phenol and flavonoid content in avocado seed has been associated with its high antioxidant and antimicrobial bioactivity (Rodriguez-Carpena et al., 2004; Achilonu et al., 2018). Phenol assists in the prevention of several chronic diseases that arise through the activity of free radicals, while flavonoid and alkaloids help combat various resistant bacterial strains (Achilonu et al., 2018; Kurek, 2019). The application of feed additives rich in antioxidant content, such as phenol and flavonoid, has been reported to increase serum and muscle antioxidants, improve the growth, nutrient digestibility and absorption, and meat quality of livestock (Falowo, 2023; Oloruntola et al., 2024).

The presence of saponins and tannins shows that inclusion of avocado seed meal in animal diets can boost the immune system and reduce the level of cholesterol content in animals. Saponins, steroids, and tannins are important secondary metabolites that play significant roles as immunostimulants and hypocholesterolaemic properties in animals (Das et al., 2012; Oloruntola and Ayodele, 2022). Specifically, saponins are used to reduce serum cholesterol levels, enhance feed efficiency and body weight gain of animals, increase the permeability of intestinal mucosal cells in vitro, inhibit active mucosal transport, and facilitate uptake of substances that are usually not absorbed (Oloruntola et al., 2021). Tannins are also used to control bloat, pathogenic bacteria load, and intestinal parasites in animals. Steroids are used to increase feed intake, induce hypocholesterolemia, and increase muscle yield in animals (Petit et al., 1995; Skoupa et al., 2022). The results of the antioxidant content (phenol, flavonoid, saponin, tannins, steroids, and alkaloid) of avocado seed meal obtained in this study are inconsistent with values reported by Setyawan et al. (2021). This variation may be due to factors such as variety, season of harvest, geographical origin and location, processing, and methods of extraction (Kupnik et al., 2023).

The analysis of ferric reduction activity potential (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity is utilized to determine the antioxidant potentials of plant materials including seed meal. It was evident that extracts of the avocado seed meals displayed good antioxidant activity in both the DPPH and FRAP assays. This could be attributed to the high contents of inherent phenolic compounds in the seed meal. Numerous studies have shown that the antioxidant activities of plant materials are mainly due to the concentration of inherent phenolic compounds (Falowo et al., 2014; Oloruntola et al., 2021; Falowo, 2022). The level of lipid peroxidation inhibition recorded in this study is a direct confirmation that when avocado seed meal is utilized as feed additives/ingredient, it could inhibit the production of oxidation due to its high inherent phenolic content and antioxidant activities as shown earlier. Lipid peroxidation is a complex chemical process that occurs when free radicals attack lipids to alter the physicochemical properties of membrane lipid bilayers and cause severe cellular dysfunction in animals (Ayala et al., 2014). The low amount of vitamin C recorded in this study shows that the use of avocado seed meals in the diet may require additional supplementation for vitamin C. Vitamin C is a natural antioxidant that helps to scavenge free radicals and strengthen the body's immunity against infections (Robert et al., 2003).

In addition to the inherent phytochemicals and antioxidant activities, avocado seed meal can act as good inhibitors of enzymes. Inhibition of digestive enzymes such as α-amylase, α-lipase, and α-glucosidase has been reported as one therapeutic strategy for the treatment and management of chronic health conditions such as diabetes and obesity (Oluwagunwa et al., 2021; Ogundipe et al., 2022). The results of the α-amylase, α-lipase, and α-glucosidase inhibition activities recorded in this study suggest that avocado seed meal could be used to decrease the digestion of carbohydrates and postprandial hyperglycemia, particularly in diabetic and obese patients (Zhang et al., 2015; Poovitha and Parani, 2016). The antidiabetic activity of avocado seed has been linked to its polyphenolic content, antioxidant activity, and polyunsaturated fatty acids content (Razola-Díaz et al., 2023). This result is similar to the antidiabetic activities of medicinal herbs and fruits recently reported by Oloruntola et al., 2024, and Rybak and Wojdyło, 2023, respectively. The percentage albumin denaturation and anti-proteinase activity of avocado seed meal revealed its anti-inflammatory properties, which also makes it eligible for use as natural food supplements to combat inflammation in body. This result is in line with other studies that have revealed the utilization of plants and their parts as anti-inflammatory agents, protecting the body against denaturation of tissue proteins and proteinases causing tissue damage development during inflammatory reactions (Iqbal et al., 2019; Truong et al., 2019; Mustafa et al., 2023).

5. Conclusion

This study have showed that avocado seed meal could be utilized as potential source of dietary protein, lipid and energy for livestock. Its phytochemicals, anti-diabetic, anti-inflammatory, and antioxidant properties also showed it could be utilized as supplements to improve the health of humans and livestock.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.I.A.	A.F.	O.D.O.	O.J.O.	M.A.
C	20	40	20	10	10
D	30	50	20		
S		50	50		
DCP	50	20	10	10	10
DAI		50	40	10	
L	10	40	20	10	20
W	20	20	20	20	20
CR	20	20	20	20	20
SR	20	20	20	20	20
PM	20	20	20	20	20
FA	20	20	20	20	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1437292

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 534-541 / September 2024

EVALUATION OF LIVESTOCK ENTERPRISES IN TERMS OF FARMER SATISFACTION AND CURRENT SITUATION

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Abstract: In addition to meeting the basic needs and nutritional needs of people, the agricultural sector is of vital importance for countries due to many factors such as its place in employment, the supply of raw materials to the industrial sector and the use of the products produced as inputs, and its share in national income. The continuity of the agricultural sector depends on the satisfaction of agricultural enterprises and the strong structure between them and their current situation. Many factors such as demographic characteristics of agricultural enterprises, labor force utilization, presence of young population, capital structure and profitability, production planning and use of technology, agricultural policies and supports are included in this situation. In this study, the current situation and satisfaction of livestock enterprises were evaluated. Within the scope of the study, there are data on the socio-economic status of the enterprises, their opinions and expectations regarding agricultural supports and agricultural policies, their organizational status and credit use savings, the current status of the enterprises and their breeding preferences, the opinions and demands of the owners. In this study, the current situation and satisfaction of livestock enterprises are evaluated. The research was carried out in agricultural enterprises engaged in cattle and sheep breeding in Seben district of Bolu province. Face-to-face questionnaire application was used to collect the data, and SPSS package program was used to analyze the data obtained. As a result of the study, it was concluded that 74.4% of the enterprises were not satisfied with the situation and future of their agricultural enterprises, and producers wanted their children to turn to different sectors and not to continue in the agricultural sector. In addition, producers stated that agricultural supports are important for the continuity of production and sustainability of enterprises (62.8%), and that they take agricultural supports into consideration in their production planning. In addition, the high technology and expenditure costs required for production activities, aging of enterprises and increasing labor shortage, problems in land planning and future planning of enterprises, and insufficient credit and agricultural supports also come to the fore in the research.

Keywords: Sustainability in agricultural enterprises, Satisfaction in agricultural enterprises, Livestock enterprises, Agricultural economics

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Received: February 14, 2024
Accepted: August 27, 2024
Published: September 15, 2024
Cite as: Çetin F, Olhan E. 2024. Evaluation of livestock enterprises in terms of farmer satisfaction and current situation. BSJ Agri, 7(5): 534-541.

1. Introduction

In addition to the basic characteristics of the agricultural sector, the increase in input costs, the aging of the labor force, the negativities and fluctuations in the markets, as well as many negativities such as the pandemic process caused by the Covid-19 outbreak and global climate crises pose problems in terms of the satisfaction, planning and continuity of agricultural enterprises. The protection and support of the agricultural sector within the agricultural law includes the development of agricultural production activities in accordance with the demands, ensuring and improving the continuity of biological and natural resources, increasing yield and quality, strengthening food safety and security in a sustainable manner, and developing agricultural organizations. In addition, the law includes agricultural policy objectives that aim to increase the welfare level of the agricultural sector by ensuring rural development, eliminating problems in agricultural markets and strengthening markets (Anonymous, 2006). In this way, the future of the enterprises and their satisfaction with their current situation are tried to be guaranteed.

Regardless of the level of development of countries, the agricultural sector is of vital importance. Therefore, ensuring the continuity of the agricultural sector varies in direct proportion to the current situation of agricultural enterprises and the satisfaction of the enterprises. In terms of enterprises, many factors such as demographic characteristics, labor force and population status, agricultural policies, production activity models and planning, technology used, land availability significantly affect the current structure of the enterprises and the satisfaction of the owners with their enterprises and thus their future thoughts.

In terms of agricultural enterprises, it is possible for them to maintain, increase and improve their earnings over the long term with the effective and efficient use of their assets. Although the existing goals of enterprises are to achieve maximum profit, all components of sustainability should be taken into account in planning the future and continuity of enterprises. Although the good financial and economic performance of enterprises is an indicator of the continuity of their activities in the short term, it does not guarantee the existence and sustainability of enterprises in the long term. Therefore, the degree of satisfaction with the situation of businesses, their expectations and their current situation are becoming more important (Doane and MacGillivray, 2001; Ertan, 2018).

In our country, the livestock sector has an important place in terms of high potential and economic values. Factors such as the use and evaluation of some plant products in production activities, increasing labor productivity and business efficiency, ensuring high profitability, using natural and economic resources and reducing risk factors etc. provide positive effects for enterprises. In addition, when the ecological and natural resources and ecological conditions of our country are evaluated, it has very favorable conditions for ovine and bovine breeding. This situation contributes to the success criteria of the enterprises. It will help to increase the benefit to be obtained from the existing conditions with planned production models suitable for the conditions of the region, to improve the current situation of the enterprises and to meet their expectations (Vural and Fidan, 2007; Turan et al., 2017).

In addition to production and consumption, the policies implemented in our country have become one of the important factors affecting businesses and markets. When we look at it, the fact that red meat production and consumption have a large area is valuable for the national economy. However, in recent years, meat imports have been resorted to due to the inability of red meat production to meet meat consumption. This issue negatively affects the degree of satisfaction of agricultural enterprises, their current situation and the future of the sector. In its solution, the importance of the protection of enterprises and the necessity of structural agricultural policies come to the fore (Saygın and Demirbaş, 2017).

One of the indicators that reveal the level of development of countries is the parameter of healthy and adequate nutrition. In order to have adequate and balanced nutrition, 60% of the daily protein consumption should be plant-based and 40% should be animal-based. Contrary to the fact that developed countries meet the criteria for balanced nutrition, the desired levels of animal-derived protein consumption have not been reached in our country and developing countries (Terin et al., 2017). Animal protein intake has become a priority issue in balanced and adequate nutrition in developed and developing countries. Food and nutrition problems are increasing due to the unbalanced growth of the world population and many problems. In addition to feeding people, the red meat sector also helps to reveal the development levels of countries with socio-economic variables. Therefore, the elimination of nutritional problems and the continuation of production activities are possible by improving and developing the current situation of the enterprises and ensuring the satisfaction of the enterprises in terms of the sector. Otherwise, the continuity of agricultural enterprises that form the basis of the food chain will be negatively affected.

The aim of this research is to reveal the current situation of livestock enterprises and their degree of satisfaction with their own enterprises. In this context, issues such as socioeconomic status and labor force, production methods, existing assets, agricultural supports and opinions of the enterprises are included.

2. Materials and Methods

2.1. Materials

Seben district was chosen as the research area because it has a voice in the region in terms of animal husbandry activities, it is the main sector preferred by enterprises and access to reliable information is convenient. The main material of the study consists of data obtained from face-to-face interviews with animal breeding enterprises in Seben district of Bolu province. In addition, secondary data obtained from theses prepared on this subject, Turkish and foreign publications, books and internet resources were also utilized.

2.2. Methods

In the research, a face-to-face survey was conducted with the producers to obtain the data. In determining the enterprises (121 enterprises with 5 or more cattle breeding and small ruminant breeding), complete census method was used. Within the scope of the questionnaire studies, in addition to the socio-economic and demographic characteristics of the producers, their current assets (animals, tools, machinery, land), production activities and their thoughts on their satisfaction status are included. In this study, it was interpreted by tabulating it with frequency and percentage distribution. The current labor force utilization in the enterprise was evaluated through the Male Work Unit (MWU) (Erkuş and Demirci, 1996). In addition, in order to determine the number of animals in the enterprises and to make comparisons, the common unit of large animal unit (LAU) was used (Erkuş et al., 1995).

2.3. Statistical Analysis

SPSS package program was used in the analysis and evaluation of the data obtained through the questionnaire form regarding the social structure and economic activities of the enterprises examined.

3. Results and Discussion

3.1. Socioeconomic and Demographic Characteristics of Enterprises

When the age distribution of household members in the study was evaluated, 1.2% (4 people) was found among the 0-6 age group; 5.4% (18 people) among the 7-14 age group; and 79.8% among the 15-64 age group (226 people). In addition, individuals in the 65 and over age group constitute 14.3% (48 people) (Table 1).

Table 1. Age group distribution of enterprises

Age Group	Person	%
0-6	4	1.2
7-14	18	5.4
15-64	226	79.8
65-+	48	14.3
Total	296	100

When the distribution of the population by age and gender in the investigated enterprises is examined; it is seen that the ratio of boys and girls between the ages of 0-6 is equal to each other. Among the individuals between the ages of 7-14, males account for 22.2% and females account for the remaining 77.8%. It was concluded that 57.9% of the individuals between the ages of 15-64 were male and 42.1% were female. In individuals over 65 years of age, 45.8% are male and 54.2% are female.

Table 2. Age group distribution of enterprises

Education Status	Person
Primary School	178
Middle School	32
High School	80
License	32
Associate Degree	14
Total	336
Agricultural Education	72

When the educational status of the household members participating in the study is analyzed (Table 2), it is seen that primary school graduates have the largest share (178 people). This is followed by individuals with high school (24-80%), undergraduate (10-32%), secondary school (9%) and associate degree (4-14%). 59.5% of the owner farmers also participated in one or more of the agricultural trainings organized in the region (72 people). The training includes trainings on different production activities such as herd management, young farmer, agricultural insurance, pruning and vaccination, dairy processing and development, animal nutrition and ration preparation, beekeeping, etc. In addition, the number of household members in the enterprise should be at least 2 and at most 8 individuals. While the average household size of the enterprises is 2.8, the average age is 45.33. The average age of the business owners is 59.5. According to similar studies, the average age is above the results (Aksoy and Yavuz, 2012; Kara and Kızıloğlu, 2012; Demir et al., 2015; Özsayın and Everest, 2019; Lianou and Fthenakis, 2021). It was concluded that there were eighteen male individuals in the 22-30 age group who had completed their basic education and were engaged in production activities with their families and that these individuals participated in one or more of the agricultural trainings.

Table 3. Distribution of enterprises by occupational status (%)

Profession Group	%
Farmer	36
Housewife	26.8
Student	17.3
Worker	4.8
Retired	6.8
Tradesmen	1.5
Other	6.8
Total	100

When the occupational status of the households within the scope of the survey is analyzed (Table 3), it is concluded that farmers (36%) have the largest share. It was determined that 26.8% of the family members were housewives, 17.3% were students, 4.8% were workers in public-private institutions or individual fields, 6.8% were retired, 1.5% were tradesmen, and the remaining 6.8% were included in other occupational groups. In addition, considering the marital status of the individuals in the enterprises, it was revealed that 36.3% were married and 76.7% were single. It was also concluded that 25.6% of the business owners are retired and continue production.

3.2. Characteristics of Enterprises

The total land area of the analyzed enterprises was 7960.6 da. The average land width per enterprise was found to be 62.1 da. When evaluated in terms of enterprises, it was determined that the smallest enterprise had 14.3 da and the largest enterprise had 281.2 da of land. In addition, the average number of pieces of land is 6.2. Considering the number of parcels owned by the enterprises, it was seen that the number of parcels consisted of at least one and at most seventeen parcels. When their land ownership status is evaluated, there are three enterprises that are engaged in animal production activities but not in crop production. When the land saving status of the enterprises was analyzed, it was determined that 64.9% of them owned land and 35.1% of them rented or rented land to a partner (Table 4).

When the land assets of the enterprises are evaluated according to their qualities, 801.1 decares of the land is irrigated and 7159.5 decares is dry land. Considering the production pattern of the enterprises, it was concluded that the majority of the producers (75%) produce wheat. Oat, alfalfa, barley, vetch, fruit (apple, cherry, walnut, peach etc.), vineyard and vegetable production are among the production preferences of the enterprises respectively. In addition, 25.7% of the producers prefer to utilize at least a part of their land as fallow. On average; 35.9 da of barley-wheat land, 2.4 da of alfalfa land, 8.3 da of oat land, 2.8 da of fresh vegetable-fruit and vineyard land are used for different productions. In addition, 5.4 da of fallow land, 4.3 da of silage corn-sunflower land and 3 da of other crops.

In total, 2203 bovine animals, 18655 ovine animals, 1425 poultry and 3 passenger animals were found in the total number of the enterprises examined. When the average animal assets of the holdings are examined, it is seen that there are 63.6 sheep, 33.06 goats/sheep, 29.23 lambs, 8.78 goats, 8.02 kids in ovine breeding. In cattle breeding, there are on average 6.8 cows, 2.53 heifers, 2.41 calves, 4.13 calves and 1.33 heifers per enterprise. The average of the holdings in terms of large animal units was calculated as 30 units (LAU) (Table 5). This result is above similar studies on average LAU (Aktürk et al., 2005; Özalp and Sayın, 2018; Karadaş, 2018). The average animal capital of the holdings was 435,245 TL. Large animal unit varies between a minimum of 9.08 < and an average of 30 < and a maximum of 108.31.

Table 4. Land asset and saving status of enterprises

Total Land Area (da)	7960,6		
Enterprises size (da)	The Smallest	Average	The biggest
	14.3	62.1	281.2
Enterprises Land Width (da)	The Smallest	Average	The biggest
	1	6.2	17
Savings Status (da)	Property Land		Rent - Sharecropper
	5166.8		2793.8

Table 5. Animal assets of enterprises

Animal type	Mean/ Number	LAU
Cow	6.8	6.8
Calf	2.41	1.20
Cowling	4.13	0.83
Bullock	1.33	0.93
Heifer	2.53	1.77
Goat	33.06	3.3
Sheep	63.6	12.72
Yearling lamb	8.78	0.06
Lamb	29.23	1.46
Kid	8.02	0.4
Heder	2.84	0.34
Billy- goat	1.04	0.12
Layer chicken	8.53	0.03
Turkey	2.13	0.008
Goose	0.48	0.002
Donkey	0.02	0.027
Beehive	1.95	-
Total		30

The building assets of the enterprises were found to be 490,750 TL. Of the building assets, 39.7% were housing assets, 22.4% were barns, 16.3% were corrals and 21.6% were other assets. In production activities, tractors and equipment are widely preferred in the region. It has been observed that the equipment of some enterprises have completed their economic life, but they continue to be used and take part in production activities. When the agricultural tools and machinery owned by the enterprises are examined, the average number of tractors is 1.09; the average number of tractor- trailer is 1.13; the average number of plows is 0.92; the average number of seeder is 0.47; the average number of sweep is 0.63; the average number of motopumps is 0.14; the average number of disc harrows is 0.33; the average number of harrows is 0.66; the average number of rollers is 0.31; the average number of cobra machines is 0.16; the average number of irrigation equipment is 0.94; the average number of hoeing machines is 0.12; the average number of meadow mowers is 0.65; the average number of balers is 0.14; the average number of combine harvesters is 0.04; the average number of feed mixer is 0.14; the average number of milking machines is 1.02. The average equipment asset value was calculated as 350,670 TL (Table 6).

Table 6. The tools and machinery (equipment) owned by the enterprises

Agricultural Equipment Type	Average per Enterprise (piece)	Equipment Value (TL)
Tractor	1.09	169 250
Tractor- Trailer	1.13	25 340
Plow	0.92	19 800
Seeder	0.47	16 340
Sweep	0.63	12 550
Motopomp	0.14	2 120
Disc harrow	0.33	7 610
Harrow	0.66	12 900
Roller	0.31	4 410
Cobra machine	0.16	2 400
İrrigation equipment	0.94	13 920
Hoeing machine	0.12	5 160
Meadow mower	0.65	11 750
Baler	0.14	21 000
Combine harvester	0.04	14 000
Feed mixer	0.14	3 960
Milking machine	1.02	8 160
Total		350 670

3.3. Views of enterprises on animal production

In the study, the opinions of the producers on why they do animal husbandry activities and their preferences were included (Table 7). When these opinions are evaluated, the main reasons for preferring animal husbandry activities are that it is widespread in the region and there is currently no other employment area. Of the enterprises participating in the study, 15.7% stated that the sector was profitable, 9.9% stated that they preferred it because it was their ancestors' and fathers' profession, 0.8% stated other reasons for preference, and 1.7% stated that they thought of leaving animal husbandry activities for different reasons. 46.3% of the people surveyed stated that they would like to work in different fields other than the agricultural sector. On the other hand, 53.7% of the enterprises stated that they did not have the idea of working in a field other than the agricultural sector due to factors such as health, age, education, etc.

Table 7. Opinions of the enterprises about aquaculture

Preference of Livestock Activities	n	%
I find it profitable	19	15.7
Widespread in the region	49	40.5
Lack of diverse employment opportunities.	38	31.4
Ancestor, father's profession	12	9.9
Thinking of quitting	2	1.7
Other	1	0.8
Total	121	100

Considering the evaluation of the enterprises in the study according to the care type of their animals, 66.1% of the enterprises stated that the care of their cattle was carried out in the barn during the whole period, while 33.9% stated that they utilized pastures in different periods and processes. Pasture and grassland utilization are among the parameters taken into consideration in the planning of enterprises. In meeting the pasture and pasture needs for small ruminant breeding activities, 47.1% stated that 47.1% of the enterprises are used by the owner himself/herself, family members or shepherd/caregiver, 5.8% use the common herd manager method, 43% use the slaughter/impulse method and 4.1% use different options. The first reason for the preference of goat breeding enterprises is the low feed costs. In addition, easy maintenance (13.6%), being preferred in the region (13.6%) and convenient use and marketing of dairy products (9.1%) (Table 8).

Expenditure costs (feed costs, labor requirements and veterinary services) have an important place in the economic sustainability of livestock activities. Reducing feed costs is directly proportional to the planning and utilization of pasture and grazing lands of the enterprises. Participants stated that the highest expenditure among the operating expenses in animal husbandry activities is feed expenses. It is seen that the obtained data (87.6%) is above the results found in similar studies (Kara and Kızıloğlu, 2012; Özalp and Sayın, 2018; Unal and Dellal, 2023). In addition, 6.6% of caregiver services, 3.3% of veterinary services and 2.5% of other expenses (Table 9). It was concluded that 47.75% of the roughage need, which constitutes the important expense items of animal husbandry activities, was met by the enterprises. It was concluded that the rate of the enterprises that meet all of the roughage needs is 18.2%, the rate of the enterprises that meet some of it is 80.2%, and the rate of the enterprises that provide all of it by purchasing from outside is 1.6%. It was found out that 66.96% of the roughage amount that the producers had during the production period was used in cattle breeding and 63.25% of the concentrate feed assets were used in cattle breeding.

Table 8. Farming preferences and opinions of the enterprises

Taking Cattle to Pasture	n	%
Yes	41	33.9
No	80	66.1
Total	121	100
Reason to Prefer Goat Breeding		
Feed costs are low	14	63.7
Easy to maintain	3	13.6
More preferred in the region	3	13.6
Other	2	9.1
Total	22	100
Care Preference of Ovine Animals		
The Person Himself	57	47.1
Joint Herd Manager	7	5.8
Partner Herder	52	43
Other	5	4.1
Total	121	100

Table 9. Livestock expenses of enterprises

Expenditures on Livestock Activities	n	%
Feed	106	87.6
Veterinary Services and Medicine	4	3.3
Herder	8	6.6
Other Expenses	3	2.5
Total	121	100

3.4. Organization and Credit Utilization in Enterprises

Farmers stated that 74.4% of the producers are registered to one or more producer associations. Farmers stated that 9.9% of the enterprises were not registered because the dues and deductions were too high, not useful or not necessary. In the study, 71.1% of the producers stated that they were members of cooperatives and that they utilized their membership in terms of input supply such as feed, fuel, fertilizer, etc., while 4.7% of them utilized their membership in other ways.

In the study, 66.9% of the credit utilization of the enterprises in the study was from Ziraat Bank and Agricultural Credit Cooperatives and 4.1% from private banks. Of the loans used by the producers, 15.2% of them said that they used for the purchase of real estate, 23.3% for the payment of bank or private debt, 2.3% for the education, health, etc. expenses of family members, 17.4% for increasing the livestock of the enterprise, 22.1% for the purchase of equipment, and 8.1% for meeting other needs. In addition, 11.6% of the producers left this question unanswered (Table 10).

Table 10. Area of use of the loan

	n	%
Housing/Land/Fields etc.	13	15.2
Bank or private debt	20	23.3
My family's education/health etc. needs	2	2.3
Animal procurement	15	17.4
Equipment procurement	19	22.1
Other	7	8.1
Unanswered	10	11.6
Total	86	100

3.5. Utilization and Satisfaction of Enterprises with Subsidies

It was emphasized that the type and amount of agricultural support have an important place in the planning of enterprises, diversification or distribution of production and economic sustainability for producers. Although the rate of benefiting from diesel oil and fertilizer support is high (92.56%), it is seen that raw milk support stands out in terms of earnings. When the utilization of agricultural subsidies by the enterprises was evaluated, it was concluded that the distribution of raw milk support, calf support, and additional input support was at the same rate, and 54.54% of sheep and goat support. It was revealed that fodder crops support, diesel oil and fertilizer support, calf support, mother sheep and goat support, and additional input support have an important place in terms of income return among agricultural supports. Agricultural subsidies have an impact on the producers' orientation and planning of their production, continuity of production and increasing agricultural income. The opinions of the enterprises on agricultural subsidies are presented in Table 9. Accordingly, 19.8% of the producers think that agricultural subsidies are very important, 43% think that they are important, 16.5% think that they are unimportant, and 5% think that they do not have information about their importance. When the distribution of the enterprises' satisfaction with agricultural subsidies is analyzed, the presence of producers who think that the amount and type of agricultural subsidies are not sufficient ranks first with a rate of 44.6%. The number of enterprises that think that the enterprises are satisfied with agricultural subsidies and the number of enterprises that think that they cannot receive them on time have the same share (18.4%). The rates of producers who think that support policies are wrong and agricultural. It is concluded that the rate of farmers who are not interested in the type and amount of subsidies is equivalent to the same percentage. 52.2% of the farmers think that the types and amounts of agricultural subsidies will have a positive effect on changing the current production activities, while 33.1% of the enterprises think that they will not have a positive effect (Table 11). As a result of the research, it was also determined that the average duration of the enterprises benefiting from agricultural activities during their activity processes was 13.76.

Table 11. Opinions of enterprises on agricultural subsidies

	n	%
Importance of Agricultural Supports for Production Continuity		
Very Important	24	19.8
Important	52	43
Unimportant	20	16.5
I don't know	6	5
Unanswered	19	15.7
Total	121	100
Satisfaction of Enterprises with Agricultural Supports		
Yes, satisfied.	22	18.4
I can't get it on time.	22	18.4
It is not enough.	54	44.6
Support policies are wrong.	2	1.6
I am not interested in agricultural subsidies.	2	1.6
Unanswered	19	15.7
Total	121	100

In terms of the continuity of the activities of the enterprises, that is, in terms of the sustainability of the sector, it is important that the young members of the family continue production. In addition to this, the increase in the education level of the enterprise individuals and their participation in trainings related to the sector are effective in the development of the enterprises. 74.4% of the enterprises examined stated that they did not want their children to continue in the agricultural sector for different reasons. This result shows that the enterprises are not satisfied with their situation and pose a danger for the future of production activities.

4. Conclusion

Increasing labor problems in the agricultural sector and the perspective of the young population on the sector are among the most important factors affecting the satisfaction and sustainability of the enterprises. The high age of the enterprise individuals in the research (the age of the family members is mostly 45 years and above, the average age of the enterprise owners is 59.5 years / the average working period of the enterprises in the agricultural sector is 29 years) poses a risk for the future and current situation of the enterprises. The fact that the young population is not satisfied with the situation of the enterprises or that they turn to different sectors due to socio-economic reasons also poses a problem in terms of meeting the labor force required for the continuity of the activities. In addition, it was concluded that the fact that the educational level of the enterprises is mostly at the primary school level and that they do not have sufficient agricultural education negatively affects the transition of producers from traditional agricultural practices to modern agricultural practices, planned production and adoption of technology-based systems. This situation significantly affects the satisfaction dimension of the enterprises and the adaptation of the producers. In order to eliminate this problem, it is seen that it is necessary to guide the children of producers with projects and support tools in their educational life and later choices and to plan their future in terms of the sustainability of agricultural production and the satisfaction of enterprises. In the study, some of the problems encountered by the enterprises in their activities are seen as priority problems. The main problems are high production costs and agricultural inflation, fluctuations in agricultural markets and marketing problems. In addition, inadequate agricultural subsidies, climate change and the increase in its effects, inadequate rural infrastructure, ineffective and ineffective use of agricultural cooperatives or unions, and the negative effects of pasture problems have been observed to negatively affect the continuation of production and the current situation of the enterprises.

Land insufficiency, problems in the planning of land use and management are among the important problems affecting the situation of the enterprises. The continued transformation of agricultural lands into small and fragmented through inheritance also causes a decrease in production in terms of efficiency and quality. The fact that the average enterprise has 62.1 da and 6.2 pieces of land and 35.1% of them are rented and sharecropped explains the mentioned situation. In addition, the fluctuations in the livestock assets of the enterprises due to fluctuations in the markets, labor-related factors and regional basic problems reveal other reservations about the future of production. With the irrigation project of agricultural lands under construction and planning in the region, the desired yield and quality increase in the lands will be achieved. On the other hand, the protection of lands from climate change and drought problems that pose a risk in the future production periods, together with the new production pattern, will provide production opportunities that will have a high return and will have an impact on meeting the feed needs to a large extent. In this way, it is thought that the increase in agricultural income of the enterprises, planning in agricultural lands, alternative products and production continuity will have a positive impact on the sustainability of the enterprises and the satisfaction of the producers with their enterprises.

The majority of the enterprises stated that animal husbandry is the profession they can do due to the special conditions (education, health, age, etc.) and that they continue their activities because it is common in the region (72.9% - 92 enterprises). Participants stated that they prefer small cattle breeding in order to reduce feed costs, while those who focus on cattle breeding prefer it because it is easier to maintain and more profitable. Transhumance activities, pastures and grasslands are of great importance for cattle and sheep breeding in the region. The protection, planning and improvement of these areas have an important place in terms of continuity of production as they directly affect economic production and will contribute positively to the current situation of the enterprises.

Within the scope of the study, it was revealed that producers engaged in small ruminant breeding thought of terminating their activities or turning to different production branches as a result of the labor shortage (caretaker / shepherd / herd manager) they encountered. It is seen that migrant labor is employed in enterprises due to the higher need for permanent labor compared to other fields of activity. This situation leads to an increase in the use of unregistered labor, the formation of an uncontrollable wage market and different social and economic problems of enterprises. 76.6% of the enterprises that need and use unregistered/illegal labor force, which is defined as migrant shepherds, stated that they would quit small ruminant breeding when they reach suitable marketing conditions and would turn to different production areas (86 enterprises). This situation poses a great risk especially for the sustainability of small ruminant breeding in the region and the satisfaction of producers with their enterprises. It is thought that this risk will be eliminated or reduced by the agricultural support for herd management, the arrangements to be made in agricultural policies and the increase in support. As a result, it will enable the registration and control of the illegal and unregistered labor force, the regulation of the labor market and a direct increase in the agricultural income of the enterprises.

In the research, 74.4% of the enterprises stated that they were not satisfied with the situation and future of their agricultural enterprises (90 enterprises). Business owners want their children to turn to different sectors and not to continue in the agricultural sector. This situation poses a danger for the future of the enterprises. Producers stated that they consider agricultural supports important in terms of production and continuity (62.8%) and that they take into account the supports in their production planning. Producers who are satisfied with agricultural supports (18.4%) also stated that they are worried about the future of the enterprises and therefore want their children to work in different sectors with higher welfare and social life. In addition, it was concluded that most of the participants (64.6%) thought that they did not receive the supports on time, found them insufficient, and found the agricultural support policies wrong. It was concluded that agricultural supports are important for the sustainability of production activities, the adoption of the individuals in the enterprise and increasing their satisfaction. Re-evaluation of the support policies implemented, increasing the amount and credits will help to meet the expectations of the producers. In addition, it is necessary to increase the existing livestock assets of the enterprises and to put forward support tools that will facilitate the transition to technological production models with practices for planning and bringing the assets of tools and agricultural equipment to the desired level. In addition, it is thought that regulating the social-economic conditions of rural areas, developing cooperatives that are important for the region, supporting and developing them by different sectors will benefit the satisfaction and future of the enterprises.

Studies in areas such as labor needs of enterprises and migrant workers, climate change and its impact on the sustainability of production activities, irrigation projects and planning, different production methods and technological use are recommended as they will help guide the future and practices of enterprises in the region.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	F.Ç.	E.O.
C	70	30
D	80	20
S	20	80
DCP	80	20
DAI	70	30
L	80	20
W	80	20
CR	70	30
SR	80	20
PM	70	30
FA	80	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Permission to conduct the study was obtained with the decision of the Ankara University Research Ethics Committee (approval date: 20 November, 2021, protocol code: 17/189).

Acknowledgments

This study is based on the first author' doctoral dissertation.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1528904

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 542-547 / September 2024

EFFECTS OF OZONE TREATMENTS ON IN VITRO SEED GERMINATION OF *Ruscus aculeatus*, *Ruscus hypoglossum* AND *Danae racemosa*

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Abstract: *Ruscus aculeatus*, *Ruscus hypoglossum*, and *Danae racemosa* are cut foliage and valuable for ornamental plants; they are also very valuable in terms of their medicinal aromatic properties. Propagating these plants using their seeds is very challenging due to deep dormancy and required pre-treatments before sowing. Ozone is a colorless gas with a pungent odor, made up of three oxygen atoms, and it offers an eco-friendly solution to break dormancy in seeds. In this study, stored seeds for four years were treated to ozone gas for 0, 15, and 30 minutes using an ozone generator that has the capacity to produce 6 grams of ozone per hour. Then, the seeds were cultured in Petri dishes containing Murashige and Skoog medium without plant growth regulators. *In vitro* seed germination rates were recorded 30, 40, 50, and 60 days after culture initiation. According to statistical analysis, the effects of species, duration of ozone treatments, and interaction of species and duration of ozone treatments on *in vitro* germination rates of seeds were statistically significant. The highest *in vitro* germination rates of 42%, 28%, and 24% were recorded at 30 min ozone treatment in *R. aculeatus*, *R. hypoglossum*, and *D. racemosa*, respectively. These results indicate that ozone application positively affects the germination of seeds.

Keywords: Dormancy, Germination, In vitro, Ozone, Seed

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Received: August 06, 2024
Accepted: September 02, 2024
Published: September 15, 2024

Cite as: Tütüncü M, Andiç M, Kebeli F, Çelikel FG, Şimşek Ö. 2024. Effects of ozone treatments on in vitro seed germination of *Ruscus aculeatus*, *Ruscus hypoglossum* and *Danae racemosa*. BSJ Agri, 7(5): 542-547.

1. Introduction

Ruscus species are used as cut foliage in floral arrangements such as baskets, bouquets, wreaths, and arrangements to enrich the texture, create a green background, and highlight the cut flowers they are used with. Additionally, after it is dried and painted in different colors, it can be used in decorative indoor designs or as green vases. The *Ruscus* genus belongs to the Asparagaceae family. However, the genus fluctuated between families and by turns in Liliaceae, Convallariaceae and Ruscaceae (Veronese, 2014). The genus is represented by four species and totally five taxa in Türkiye, including. *R. aculeatus* var. *aculeatus*, *R. aculeatus* var. *angustifolius* Boiss., *R. hypoglossum* L., *R. colchicus* P. F. Yeo, *R. hypophyllum* (Güvenç et al., 2011). *R. aculeatus* originating from Mediterranean region is spread in western and southern Europe and Africa (Manole and Banciu, 2015). *R. hypoglossum* is native to the Euro-Mediterranean, North Italy to Austria and Slovakia, and east to Türkiye and Crimea (Ivanova et al., 2013). Plants belonging to the genus *Ruscus* are evergreen, shrub-like perennials that can grow up to approximately one meter tall. They form branched stem structures with numerous cladodes (flat, leaf-like stem tissue known as phylloclade). Cladodes can reach 2 to 18 cm long and 1 to 8 cm in width. The true leaves are small, scale-like structures and are not photosynthetic. The flowers are small, white with dark violet centers, and in the middle of the cladodes. The fruits are red and 5 to 10 mm in diameter. Some species are monoecious, while others are dioecious. They reproduce by seeds or underground rhizomes (Kebeli, 2021).

D. racemosa (L.) Moench (Syn. *R. racemosus*) commonly known as Alexandrian laurel or poet's laurel, belongs to the Liliaceae/Ruscaceae/Asparagaceae family. It is the only species in the genus *Danae* (Shen et al., 2013). *D. racemosa* is distributed in a narrow area between Istanbul and Adapazarı and in Mersin, Adana, Osmaniye and Hatay provinces in the Mediterranean part of the Türkiye (Kebeli, 2021). This species is also found in Iran, Syria and Italy. *D. racemosa*, an erect, evergreen shrub, thrives under the shade of forest trees. It features green shoots, glossy leaves, thick unarmed alternate cladophylls, and terminal racemes of white-yellow flowers that are followed by red berries. This plant is frequently used for its decorative green foliage in fresh flower arrangements (Masoudi et al., 2022).

These species are not only valuable as ornamental plants, but studies have reported that they are also precious in terms of their medicinal aromatic properties and contain active ingredients used to treat many diseases. Additionally, while the aerial parts of *Ruscus* species are edible, their underground organs, including rhizomes and root structures, are utilized as phytotherapeutic products in traditional medicine (Kebeli and Çelikel, 2024). For instance, *R. aculeatus* was used to treat skin diseases (Ali-Shtayeh et al. 1998), and its decoction was used to treat eczema, diarrhea, and nephritis (Tuzlaci and Aymaz, 2001). Hadžifejzović et al. (2013) reported that the fruits of *R. hypoglossum* species are boiled and used to treat skin disorders such as chilblains and warts. *D. racemosa*, abundant in powerful natural flavonoids such as quercetin and kaempferol, could be an attractive option as a valuable source of natural antioxidants. It can potentially replace synthetic antioxidants and neutralize stress-induced free radicals (Fathiazad and Hamedeyazdan, 2014).

In Türkiye, these species are traditionally harvested from the wild due to the ease and low cost of collection. However, cultivating and rapidly propagating these species from seeds is crucial. This approach protects their natural populations and satisfies sector demand (Özden et al., 2016). The production of these species can be done using vegetative and generative methods. Generative production using seeds obtained from plants is not at the desired level due to the deep dormancy seen in the seeds and harms the germination process (Halada and Erdelská, 2005). Therefore, various seed priming methods were applied previously to break seed dormancy. However, Ozone provides an eco-friendly technological method to improve seed germination and disinfestation, leaving minimal residual impact (Pandiselvam et al., 2020).

Ozone is a colorless gas with a pungent odor made up of three oxygen atoms. Unlike the stable oxygen molecule (O_2), ozone (O_3) is unstable. It was first used for disinfecting water treatment plants (Bocci, 2004; Uslu et al., 2022). Unlike other oxidizing agents, ozone breaks down into molecular and atomic oxygen and limited oxides during chemical reactions. These byproducts do not pollute the environment or form carcinogenic substances, unlike chlorine or fluorine oxidation products (Normov et al., 2019).

Despite the considerable amount of research on ozone, its stimulating properties on germination of dormant seeds remain insufficiently understood. Therefore, our study aimed to investigate the effect of ozone on the seed germination rates of *R. aculeatus*, *R. hypoglossum* and *D. racemosa*.

2. Materials and Methods

Kebeli (2021) gathered the plant materials of *R. aculeatus*, *R. hypoglossal*, and *D. racemose* (Figure 1) from their natural habitats around the Beykoz district of Istanbul. The samples were then planted in a greenhouse at the Agriculture Faculty of Ondokuz Mayıs University in 2018.

In 2019, the harvested seeds were first separated from the fruit flesh under laboratory conditions and then cleaned using tap water. This step was taken to prevent any fungal agent formation that could arise from fruit flesh residues during drying. The washed seeds were laid out on blotting paper to remove excess water (Figure 2). Then, the dried seeds were stored in the dark in a glass jar at room temperature for four years.

The stored seeds were pre-treated with ozone, and then *in vitro* germination rates were assessed. The ozone generator used in the study can produce 6 grams of ozone per hour. The ozone concentration varies between 3% and 10%. The reactor pressure ranges from 6.5 to 8.5 PSI, and the gas inlet flow rate averages between 0 and 4 liters per minute. The ozone production technique used is the Corona Discharge System. Cooling is achieved with air. The generator operates at 220 volts and 800 Hz, with an energy consumption of 250 watts (Haci, 2015). The seeds were soaked in water overnight before application. Then, they were placed in a beaker containing 500 ml of pure water and ozone gas was applied to the water for 15 and 30 minutes. No application was made to the control group.

Figure 1. The visual appearance of the species in their natural habitats.

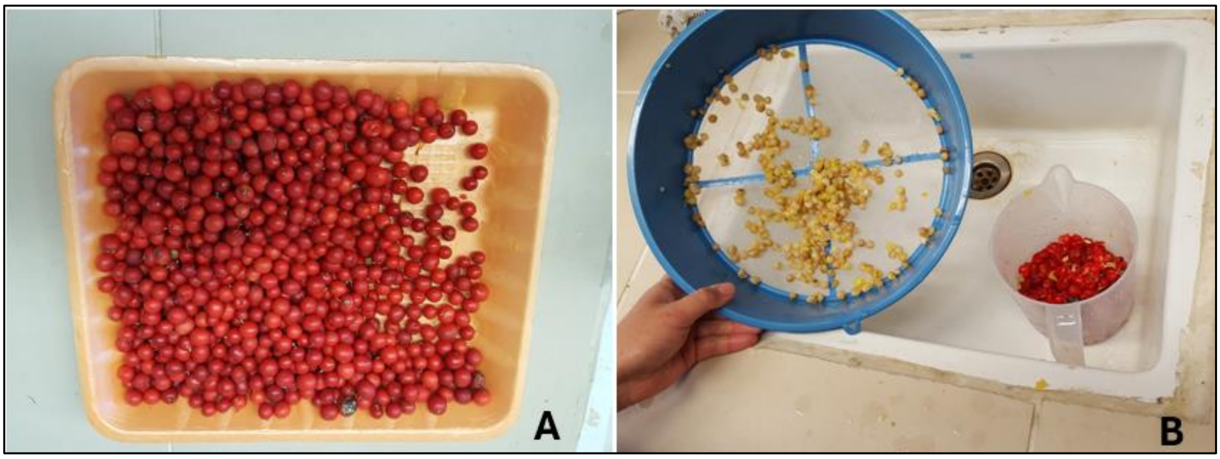

Figure 2. A) harvested fruits, B) separating fruit flesh from the seeds.

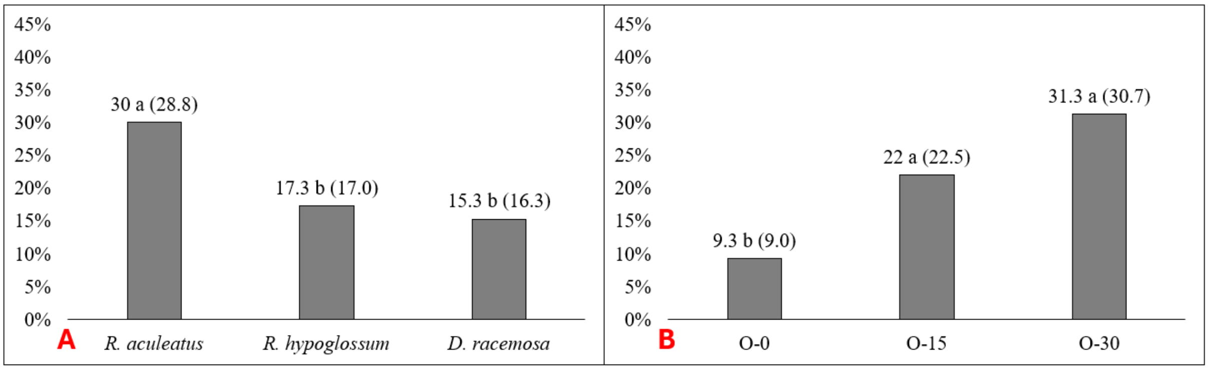

Figure 3. A: seeds germination rates of species, B: effects of ozone treatment time on seed germination. Levels not connected to same letter are significantly different (P<0.05), LSDspecies and LSDozone: 5.294, Arc-sin transformed values shown in parentheses.

The seeds were submerged in a 70% ethanol solution for 1-2 minutes and rinsed several times with sterile water to eliminate any remaining ethanol. They were soaked in a 25% commercial bleach solution (Domestos®, 4.5% v/v) for 15 minutes. The final step consisted of four additional washes with sterile distilled water. Seeds were cultured in Petri dishes (90 × 15 mm) containing 25 ml of full-strength MS medium (Murashige and Skoog, 1962), supplemented with 3% sucrose, and did not include plant growth regulators. The medium was solidified with 7 g/L agar, and its pH was adjusted to 5.6–5.8 before being autoclaved at 121°C and 15 psi for 15 minutes. All explants were maintained at 23 ± 2°C in complete darkness for 2 months and were subcultured every 4 weeks. The germination rates of the seeds were recorded. Data from the last observation were used to calculate *in vitro* germination rates of the species.

In vitro germination tests with ten replicates (Petri dishes) and each replicate containing five seeds were established based on a completely randomized design. Means of all data were separated by variance analysis, and significant differences (*p<0.05*) were evaluated with an LSD test using the JMP® program (SAS Institute, Cary, NC). The percentage values were arc-sin transformed before the variance analysis. *In vitro* germination of seeds was recorded 30 (D-30), 40 (D-40), 50 (D-50), and 60 (D-60) days after the culture initiation.

3. Results and Discussion

According to statistical analysis, the effects of species, duration of ozone treatments, and interaction of species and duration of ozone treatments on *in vitro* germination rates of seeds were statistically significant. The highest *in vitro* germination rate with 30% was recorded in *R. aculeatus*, while the lowest with 15.3% in *D. racemosa* (Figure 3A). A significant increase in the germination rate of seeds is observed as the duration of ozone application increases. Compared to the control group, a 15-minute ozone application increases the germination rate by approximately 2.4 times, while a 30-minute ozone application increases the germination rate by approximately 3.4 times. These results indicate that ozone application positively affects the germination of plant seeds (Figure 3B).

Table 1 provides significant information on the interactions between species and ozone treatments. The germination rates generally increased with longer ozone treatment durations and germination rates of untreated seeds were the lowest for all species. *R. hypoglossum* shown that the lowest seed germination rate with 2% in control group, while *R. aculeatus* had the highest seed germination rate with 42% at 30 minutes ozone treatment. *R. hypoglossum* has the lowest germination rate without ozone treatment but shows substantial improvement with increased ozone exposure. *D.*

racemosa shows moderate improvement with longer ozone treatments, but the changes are not as pronounced as in *R. aculeatus* and *R. hypoglossum* (Table 1).

Table 1. Effects of ozone treatment and species interaction on seed germination

Species	Ozone treatment (min.)	Germination rate (%)
	0	16.0 ^{bcd} (15.58)
R. aculeatus	15	32.0 ^{ab} (32.44)
	30	42.0 ^{a} (38.65)
	0	2.0 ^{d} (2.65)
R. hypoglossum	15	16.0 ^{bcd} (17.08)
	30	28.0 ^{ab} (31.52)
	0	10.0 ^{cd} (9.0)
D. racemosa	15	18.0 ^{bcd} (18.23)
	30	24.0 ^{abc} (21.92)

Levels not connected to same letter are significantly different ($P<0.05$), LSDspecies*ozone: 7.487 and Arc-sin transformed values shown in parentheses.

Overall, ozone treatments showed that the germination rates increase for all treatments by the D-60. The highest germination rate is observed with the O_3-30 treatment, followed by O_3-15, while O_3-0 remains the lowest. At day 50, the germination rates continued to rise, with O_3-30 showing the highest increase, followed by O_3-15, and O_3-0 showing the least increase. By day 60, the germination rates reached plateau. O_3-30 maintains the highest germination rate, followed by O_3-15, and O_3-0 shows the lowest rate. It was found that the longer ozone treatments lead to higher germination rates over time, with the most significant increases observed for the 30-minute treatment (O_3-30) (Figure 4A).

In germination rates of *R. aculeatus*, significant increases were observed by the D-40, with O_3-30 showing the highest rate, followed by O_3-15, and O_3-0 showing the least increase. At day 50, O_3-30 continues to show the highest germination rate, followed closely by O_3-15, with O_3-0 lagging. By day 60, the germination rates for O_3-30 and O_3-15 remain high, while O_3-0 shows minimal increase (Figure 4B). Similar results were recorded for *R. hypoglossum* (Figure 4C) and *D. racemosa* (Figure 4D).

R. aculeatus and *R. hypoglossum* exhibit significant improvements in germination rates with increased ozone treatment durations. *D. racemosa* shows a moderate response to ozone treatments, with the highest rates at 60 days. These findings highlight the positive impact of ozone treatments on seed germination rates, particularly for *R. aculeatus* and *R. hypoglossum*. The data suggests that ozone exposure can be a beneficial treatment to enhance germination, with optimal durations varying slightly among different species. After seed germination, the shoots continued to be cultured in the same nutrient medium and rooting and tillering were observed in the shoots at 8 months after seed germination (Figure 5).

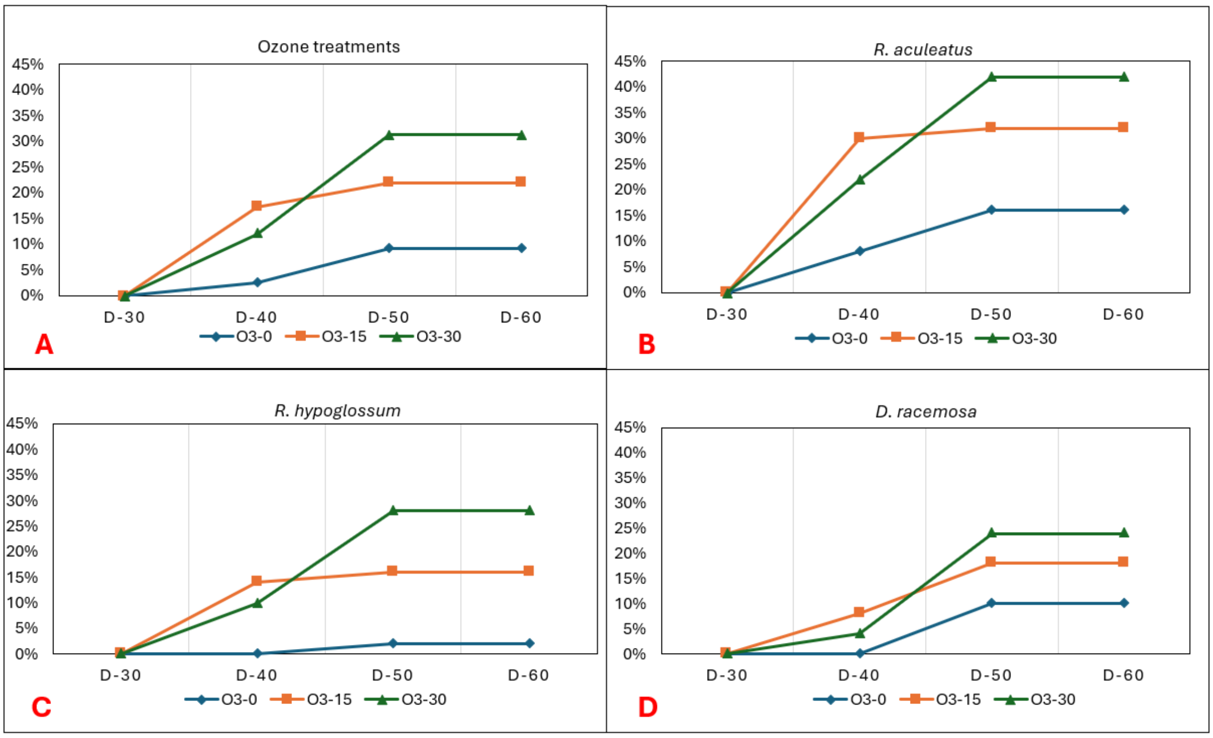

Figure 4. Effects of ozone treatments in *in vitro* germination 30, 40, 50, and 60 days after culture initiation, A) overall effects of ozone, B) seed germination by the time in *R. aculeatus*, C) in *R. hypoglossum*, D) in *D. racemosa*.

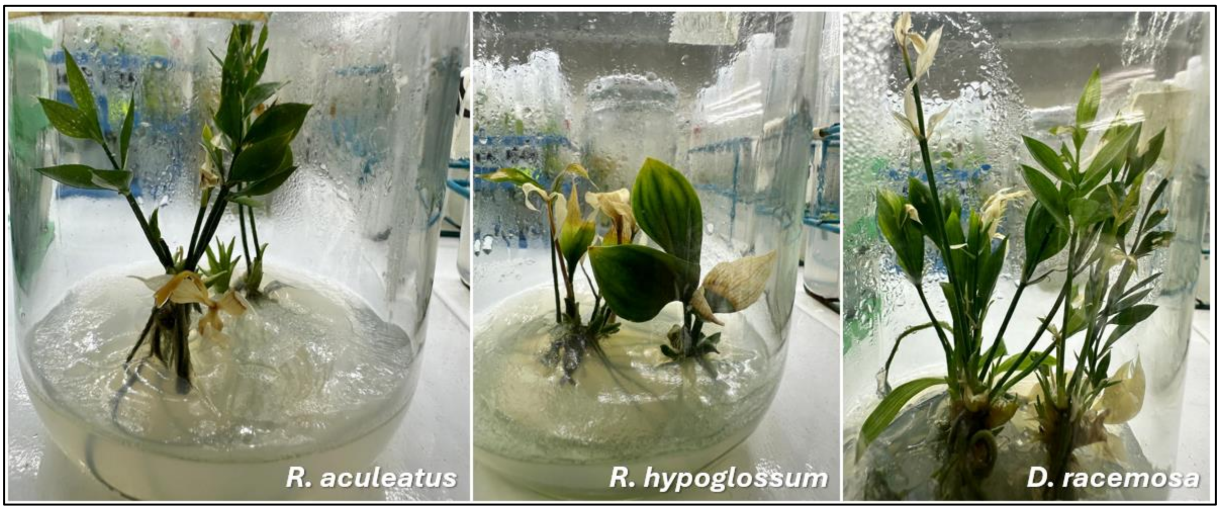

Figure 5. *In vitro* grown seedlings from left to right *R. aculeatus*, *R. hypoglossum* and *D. racemosa*.

The effects of different chemicals on seed germination of *Ruscus* species have been previously studied by many researchers. Research conducted by Kebeli (2021) reported that the germination rate, which was 50% in control, increased to 68% with 8 mg/L KNO_3 pre-application, to 65% with 100 µM SNP pre-application and to 64% with 48 h 1500 ppm permalink, immersion pre-application in *R. aculeatus*. The first germination was reported at 50th day after seed sown. Özden et al. (2016) reported that the highest germination rate of 65% was obtained from seeds kept in KNO_3 (4 mg) solution for 6 hours in *R. aculeatus*, while in *R. hypoglossum*, the highest germination rate of 58.7% was obtained from 1000 ppm GA_3 application. It was determined that there was no germination in both control and H_2SO_4 (2 and 4 minutes) applications. The researchers indicated that the first germinations were observed on the 50th day in *R. aculeatus* and on the 40th day in *R. hypoglossum*. Banciu and Aiftimie-Păunescu (2012), indicated that *in vitro* seed germination of *R. aculeatus* was notably slow, taking six months to occur in dark conditions. On MS medium without hormones, 60% of seeds germinated after this six-month period. However, when the MS medium was supplemented with GA_3, the germination rate increased to 85% over the same timeframe. These studies show that the germination rates and germination times of the species vary according to treatments. Halada and Erdelská (2005) reported that the dormancy of *R. hypoglossum* seeds lasts one year. However, in our study, germination rates were lower than in previous studies. This was probably due to some of the seeds losing their viability during the storage for 4 years. On the other hand, these findings highlight the positive impact of ozone treatments on seed germination rates, particularly for *R. aculeatus* and *R. hypoglossum*.

The beneficial effect of ozone treatment on seed germination were also reported in different species such as cyclamen (Tütüncü, 2022), barley (Dong et al., 2022), winter wheat (Avdeeva et al., 2018), corn (Violleau et al., 2007) and tomato (Sudhakar et al., 2011). It is hypothesized that the application of O_3 plays a crucial role in speeding up seed germination by prematurely breaking dormancy, which is linked to a decreased level of ABA in seeds treated with O_3 (Sudhakar et al., 2011). However, it is reported that ozone treatments can have negative effects depending on concentration in species such as vetch (Uslu et al., 2022) and alpine plants (Abeli et al., 2017). Therefore, it is crucial to determine optimum concentration and duration.

4. Conclusion

In this study, we assessed the effects of different durations of ozone treatments on stored seeds of *R. aculeatus*, *R. hypoglossum*, and *D. racemosa* for four years. The results of the study show that ozone treatments lasting 30 minutes result in increased germination rates for all species over time, while the control group (O3-0) consistently shows the lowest germination rates. The data suggests that ozone exposure can be a beneficial treatment to enhance germination, with optimal durations varying slightly among different species. In conclusion, ozone treatments can be suggested as environmentally friendly and inexpensive methods to break dormancy and enhance germination in the seed germination process.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.T.	M.A.	F.K.	F.G.Ç.	Ö.Ş.
C	40	20	10	10	20
D	50				50
S	40			30	30
DCP	40	30	10	10	10
DAI	50				50
L	20	20	20	20	20
W	50				50
CR	20	20	20	20	20
SR	20	20	20	20	20
PM	20	20	20	20	20
FA	20	20	20	20	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1522914

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 548-556 / September 2024

DETERMINATION OF BIOMASS YIELD, FORAGE QUALITY AND MINERAL CONTENT OF PEARL MILLET VARIETIES (*Pennisetum glaucum* (L.) Br.) UNDER SEMI-ARID CONDITIONS

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Abstract: With the continuous growth of the world population and Türkiye, researchers are investigating quality forage alternatives to satisfy the expanding demands of livestock. The promotion of pearl millet (*Pennisetum glaucum* (L.) R. Br.) as a summer crop and a potential green forage source during the summer season is significant. This study was conducted to determine the biomass yield and forage quality values of some pearl millet varieties under the second crop conditions in Akcakale/Sanlıurfa. Plants were harvested plants were harvested in the soft dough stage of the seeds in the panicle. In addition to biomass yield and components, quality traits in dry biomass were also determined this study. Significant statistical differences were found among all examined varieties in the yield components of biomass. Plant height (PH, cm), number of leaves per main stem (MSL, number), number of tillers per plant (PTN Number), biomass yield (BY, kg da^{-1}) and dry biomass yield (DBY, kg da^{-1}) varied between 198-341 cm, 11.1-15.7 number, 7.45- 12.30 number, 5938-12571 kg da^{-1} and 1847-3666 kg da^{-1} respectively. Moreover, significant and positive correlations were identified between DBY and BY, PH, MSL. As a result of the study, while, the White variety showed better performance in components of yield, the Ashana variety performed better in terms of forage quality. Moreover, Yellow and Tifleaf III varieties have CP values above 10%. Based on high biomass yield and moderate forage quality of the pearl millet varieties in the study, pearly millet could play an important role in closing the forage deficit of ruminant animals, especially in arid and semi-arid ecologies around the world and Türkiye.

Keywords: Pearl millet (*Pennisetum glaucum* (L.) R. Br.), Variety, Forage yield, Quality, Semi-arid

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Received: July 26, 2024
Accepted: September 04, 2024
Published: September 15, 2024

Cite as: Sayğıdar MN, Yücel C, Taş T. 2024. Determination of biomass yield, forage quality and mineral content of pearl millet varieties (*Pennisetum glaucum* (L.) R. Br.) under semi-arid conditions. BSJ Agri, 7(5): 548-556.

1. Introduction

In Türkiye, it was reported that the approximate roughage requirement for a year for 17 million cattle units is 76 million tons. About half of this feed has been obtained from field agriculture and pasture meadow areas. Additionally, if garden pastures, alternative roughages, and straw stubble residues are not considered, there is still a significant need for roughage (BUGEM, 2024). It is important to explore different roughage sources to close Türkiye's roughage deficit. According to the latest report published by the IPCC, the global average temperature has increased by approximately 1.45 °C in the last century. It was reported that this increase causes to climate change, warming the land and the seas. Furthermore, it has been stated that these temperature increases will continue to rise day by day, especially on land, with occasional droughts and high temperatures being experienced. The production and development of new plant species that can tolerate abiotic stress conditions such as drought and high temperatures will become increasingly important in future projections (IPCC, 2023)

Pearl millet is more tolerant to abiotic stress factors such as temperature, drought, and salinity compared to corn and sorghum species, and is more adaptable to marginal areas. Therefore, this plant is becoming prominent for arid and semi-arid regions (Yucel and Yucel, 2022). Moreover, compared to similar species like corn and sorghum, it was reported that the production of this plant will increase in the coming years due to its lower agricultural inputs and higher tolerance to diseases and pests (Devi et al., 2011; Yucel and Yucel, 2022). Pearl millet is utilized in very different forms such as grazing, silage, dry and green biomass (Newman et al., 2010). It has been reported that pearl millet can be harvested several times in a growing season, and about a week after harvest, the stems start to regrow from the root (Banks and Stewart, 1998). The dry biomass of pearl millet is emphasized to be rich in phosphorus (P) and calcium (Ca), have a high protein content, be of better quality than the forage of species like sorghum and Sudan grass, and not contain toxic secondary metabolites such as hydrocyanic and prussic acid for animals (Arya et al., 2009; Hassan et al., 2014; Malakar et al., 2024). C4 plants with summer characteristics, which have high biomass yield per unit area and silage quality that can be an alternative to silage corn, need to increase their agricultural production. If the production of alternative species with low inputs is not popularized, it is estimated that the roughage deficit in developing countries and Türkiye cannot be closed with the current production systems.

This study was conducted to determine the biomass yields of some pearl millet varieties, some important quality traits of dry biomass, and mineral element contents in the GAP region of southeastern Türkiye, which has arid or semi-arid conditions

2. Materials and Methods

Pearl millet varieties such as Tifleaf III from the United States and Yellow, Ashana, White, and Heveahri from Sudan were used in the study.

The study was carried out in 2020 pearl millet growing periods, at the GAP Agricultural Research Institute, Talat Demirören Research Station, Sanlıurfa/Türkiye (36 54′ 13″ N, 38 55′03″E, altitude 378 m). The field experiment used randomized block trial design with four replications. The inter row and intra row spacings were 70 and 25 cm, respectively. Each plot had four rows, 5 m in length, and the area of each plot was 14 m. Fertilizer was applied in the seedbeds at a rate of 8 kg^{-1} da in the form of compose (20%N - 20%P_2O_5 – 0 %K_2O) during sowing. Nitrogen (40 kg ha^{-1}) was applied as urea (46% N) at the fourth week of sowing (Mesquita and Pinto, 2000). Weed control was performed mechanically by hand. The plants were harvested for biomass yield when the soft dough period of the seed (Newman et al., 2006).

The traits such as plant height (PH, cm), number of leaves per main stem (MSL, number), number of tillers per plant (PTN, number), biomass yield (BY, kg da^{-1}), and dry biomass yield (DBY, kg da^{-1}) were determined using established methods (Rao and Bramel, 2000; Upadhyaya et al., 2008; Upadhyaya and Gowda, 2009). 500 g of fresh biomass was taken from each experimental plot. These plant samples were dried in incubator at 70°C until their weight stabilised, then, DBY (kg da^{-1}) was calculated from fresh and dry samples.

After drying process in incubator, the plant samples were ground in a specially designed mill with a sieve with a diameter of 1-2 mm and prepared for forage quality analysis. Forage quality traits were analyzed on NIRS (Near Reflectance Spectroscopy) and Foss XDS Rapid Content Analyser devices.

The forage quality traits which are crude protein (CP, %), neutral detergent fiber (NDF, %) and acid detergent fiber (ADF, %) analyzed by these devices. Moreover, dry matter digestibility Rate (DMD, %), dry matter intake (DMI, %), relative feed value (RFV) and net energy lactation (NEL, Mcal/kg) were also calculated from the equations (1-4) below (Schroeder, 1994; Anonymous, 2018). Furthermore, the mineral element content including P, K, Mg, and Ca of the same plant samples were also analyzed.

$$DMD\ (\%) = 88.9 - (0.779\ x\ \%ADF) \quad (1)$$

$$DMI = 120/\ \%\ NDF \quad (2)$$

$$RFV = (\%DMD\ x\ \%\ DMI)/1.29 \quad (3)$$

$$(NEL)\ (Mcal/kg, dry\ matter) = 1.892 - (0.0141 * ADF) \quad (4)$$

2.1. The Climate and Soil Characteristic of the Study Area

The study area was located in a semi-arid climate with high temperatures and low relative humidity during the summer season. The average temperature ranged from 23.9 °C to 33.0 °C during the growing period from June to October in 2020. The average temperatures for July and August were 32.4 °C and 33.0 °C respectively, while the average temperature for the growing period was recorded at 30.2 °C. Additionally, the average relative humidity ranged from 27.5% to 41.0% during the growing period. While, the highest relative humidity value recorded in October, lowest values recorded in June and July. The climate data of the study area recorded during the pearly millet growing seasons of 2020 are provided in Table 1 (MGD, 2020).

In addition, among the physical properties of the soil, it was determined that the silt, clay and sand contents were between 26-27%, 44-45% and 28-30%, respectively, and the soil texture was clayey. PH varies from 7.96 to 8.00 and electrical conductivity is between 1.05 and 1.40 dS m^{-1} which indicates no salinity problem.

Table 1. Some climate parameters of the study area (https://www.mgm.gov.tr/)

Months	June	July	August	September	October	Average
Mean temperatures(°C)	31.5	32.4	33.0	30.1	23.9	30.2
The lowest temperatures (°C)	23.0	25.4	23.7	22.2	18.8	22.6
The highest temperatures (°C)	39.2	39.0	41.7	36.9	30.7	37.5
Total precipitation (mm m^{-2})	0.0	0.0	0.0	0.0	0.0	0.0
Mean humidity (%)	29.3	27.5	31.2	32.6	41.0	32.3
Precipitation (day)	0.0	0.0	0.0	0.0	0.0	0.0

2.2. Statistical Analysis

Statistical analysis was performed using JMP (Version13.2.0) statistical software. The mean values for the varieties were compared using the LSD test at P≤0.05 probability level. A correlation test was performed between all traits determined in the study (Yurtsever, 2011).

3. Results and Discussion

3.1. Agronomic Traits

The difference between varieties was statistically significant (P≤0.05) for all parameters such as plant height (PH, cm), number of leaves on per main stem (MSL, number), and number of tillers per plants (PTN, number), biomass yield (BY, kg da^{-1}) and dry biomass yield (DBY, kg da^{-1})

3.1.1. Plant height (cm)

The average plant height of pearl millet varieties ranged from 198 to 341 cm. The lowest plant height was observed in the Tifleaf III variety, while the other varieties were statistically same grouped together (Table 2). PH varied between 137.4-377.0 cm (Izge et al., 2007; Angarawai et al., 2016), 150-219 cm (Medici et al., 2018), 143-262.4 cm (Shah et al., 2012; Hassan et al., 2014) and 30-490 cm (Upadhyaya et al., 2007; Govindaraj et al., 2011; Upadhyaya et al., 2013) in studies conducted with different pearl millet genotypes in countries such as Nigeria, Brazil, Pakistan and India. Moreover, pH of pearl millet varied between 174-449 cm in Cukurova (Dagtekin, 2019; Cakır et al., 2023; Donmez and Hatipoglu, 2024) and 146.5-352.0 cm in GAP conditions in Türkiye (Ozer, 2023; Yucel et al., 2023a). Although the region where the experiment was conducted is a semi-arid region with high temperatures, plant heights are taller than plants grown in other parts of the world, and the reason for this is that pearl millet may be tolerant to abiotic stress conditions. Additionally, it can be said that quantitative traits such as plant height are affected by genetic and environmental factors (climate and soil).

3.1.2. Number of leaves on per main stem (number)

The average MSL of varieties was ranged from 11.1 to 15.7 number. It was observed that the leaf numbers of Heveahri, White and Yellow varieties were close to each other, statistically in the same group, and had higher values than the remaining other varieties. Additionally, it was determined that varieties with high PH also had high MSL (Table 2). This could be explained by the fact that there are significant and positive correlations between PH and MSL (Table 5). The results of previous studies were similar to our findings (Dagtekin, 2019; Cakır et al., 2023; Yucel et al., 2023a). MSL ranged from 8.0 to 19.0 (Izge et al., 2007; Abdulhakeem et al., 2019), 6.9 to 9.7 (Abd-El-Lattief, 2011), and 11 to 15 number (Shah et al., 2012) in studies conducted with various pearl millet genotypes across countries such as Nigeria, Benin Republic, and Pakistan. In addition, the number of leaves varies between 8.4 and 22.2 (Dagtekin, 2019; Cakır et al., 2023) and 7.67 and 17.0 (Yucel et al., 2023a) in Cukurova/Türkiye and Sanlıurfa/Türkiye conditions respectively. It was thought that the most important reason for the difference between the MSL determined in the study in question and the number of leaves obtained in previous studies was due to environmental factors such as climate and soil and variety characteristics.

3.1.3. Number of tillers per plants (number)

PTN varied between 7.45 and 12.30 number and the White variety formed more tillers than the other varieties (Table 2). PTN is one of the important agronomic traits affecting unit area yield. Therefore, positive and significant relationships were found between PTN and BY, DBY (Table 5). Consistent with our study, significant and positive relationships between PTN and BY, DBY were reported by many researchers (Kumari and Nagarajan, 2008; Dagtekin, 2019; Aswini et al., 2022; Cakır et al., 2023; Yucel et al., 2023a). PTN values in different environments ranged from 1.7 to 2.3 number in Nigeria (Izge et al., 2007) and 0.55 to 6.4 number in India (Govindaraj et al., 2011; Athoni et al., 2016; Thomas et al., 2018). And also, 6 to 8 number in Ghana (Asungre, 2014) and 5.3 to 12.5 number in Algeria (Rahal-Bouziane and Semiani, 2016). In addition to international studies, PTN varied between 3.67 and 18.0 number under Cukurova conditions (Dagtekin, 2019; Cakır et al., 2023; Donmez and Hatipoglu, 2024) and 2.33 and 7.50 number under GAP conditions (Yucel et al., 2023a) in Türkiye. It was determined that the PTN of pearl millet varieties in the study were higher than PTN obtained from other national and international studies. In short, it was decided that the varieties used in the study were compatible with the regional conditions.

Table 2. Averages of forage yield and yield-related traits of pearl millet varieties

Varieties	PH (cm)	MSL(Number)	PTN(Number)	BY(kg da^{-1})	DBY (kg da^{-1})
Ashana	320 a	13.4 b	7.45 c	6384 cd	2108.3 bc
Heveahri	328 a	14.2 ab	9.94 b	8348 bc	2605.2 b
White	341 a	15.7 a	12.30 a	12571 a	3666.0 a
Yellow	325 a	15.4 a	9.57 b	9952.3 b	2576.2 b
Tifleaf III	198 b	11.1 c	8.17 c	5938 d	1847.0 c
Mean	303	14.0	9.48	8638	2560.5
CV (%)	4.61	7.44	9.27	16.72	15.89
Sig.	0.001**	0.0003**	0.001**	0.0002**	0.0004**

*,**= significant at 0.05 and 0.01 levels of probability respectively, NS= not significant, According to the LSD test, There is statistically significant difference at P≤0.05 level among the averages shown with different letters in the same column, PH= plant height (cm), MSL= number of leaves per main stem (number), PTN= number of tillers per plant (number), BY= biomass yield (kg da^{-1}), DBY= dry biomass yield (kg da^{-1}).

3.1.4. Biomass yield (kg da^{-1})

It was determined that the BY values of the varieties varied between 5938 and 12571 kg da^{-1}, and White variety had higher yields than other varieties in study (Table 2). It was observed that varieties with high PH, MSL and PTN also have high BY yields. Significant positive correlations were recorded between PH and PTN, MSL, and BY and DBY (Table 5). The findings obtained from the study are compatible with other studies (Dagtekin, 2019, Subbulakshmi et al., 2022; Yucel et al., 2023a; Cakır et al., 2023; Donmez and Hatipoglu, 2024). BY varied between 7744 and 8615 kg da^{-1} (Abd-El-Lattief, 2011) and 3300 and 10000 kg da^{-1} (Shah et al., 2012) in Benin Republic and Pakistan conditions respectively. Moreover, in studies conducted at national level, BY ranged from 4110 to 8400 kg da^{-1} in Cukurova/Türkiye (Donmez and Hatipoglu, 2024) and 5400 to 8076 kg da^{-1} in Sanlıurfa/Türkiye conditions. Although some findings obtained in previous studies were similar to the data in the study, it was observed that some varieties had higher yields.

3.1.5. Dry biomass yield (kg da^{-1})

DBY varied between 1847 and 3666 kg da^{-1} for all varieties. The highest DBY was obtained from the White variety, while the lowest DBY was obtained from the Tifleaf III variety (Table 2). Varieties with high BY are also high in DBY. Significant positive correlations were recorded between DBY and BY. In addition, significant and positive relationships were determined between DBY and BY, PH, MSL and PTN (Table 5). The data found in the study are in parallel with some previous studies (Dagtekin, 2019, Subbulakshmi et al., 2022; Cakır et al., 2023; Yucel et al., 2023a). DBY ranged from 750 to1250 kg da^{-1} in India (Sheahan, 2014), 390 to 520 kg da^{-1} in Brazil (Medici et al., 2018), 169 to 347 kg da^{-1} in Pakistan. (Hassan et al., 2014) and 216 to 276 kg da^{-1} under Mexican conditions (Morales et al., 2015). When the studies conducted in Türkiye were looked into, it was reported that DBY varied between 690 and 1800 kg da^{-1} in Cukurova conditions (Donmez and Hatipoglu, 2024) and 2322.0 and 3472.8 kg da^{-1} in Sanliurfa ecological conditions (Ozer, 2023). Although the results obtained in studies conducted in different ecology and different regions of Türkiye are similar to the results obtained from the study, the DBY results obtained from the study are slightly higher than other DBY results, the reason for this is that the temperature and soil characteristics of the region where the study was conducted were thought to be effective in creating this difference.

3.2. Quality Traits of Fodder

Except for crude protein (CP, %), the difference between varieties was not statistically significant ($P \leq 0.05$) for other quality parameters such as neutral detergent fiber (NDF, %), acid detergent fiber (ADF, %), dry matter digestibility (DMD, %), dry matter intake (DMI, %), relative feed value (RFV) and net energy lactation (NEL, Mcal/kg^{-1}).

3.2.1. Neutral detergent fiber (%)

NDF varied between 63.98% and 74.24% for all varieties. The highest NDF was obtained from the Tifleaf III variety, while the lowest NDF was obtained from the Ashana variety (Table 3). Different findings were obtained for NDF in studies conducted in different environments. NDF content ranged from 63.2% to 67.3% in Kentucky/ABD (Rasnake et al., 2005), 56.3% to 60.1% in Brazil (Buso et al., 2014) and 46.1% to 64.8% in Pakistan conditions (Heuzé et al., 2015). When the studies conducted in Türkiye were examined, it was reported that NDF varied between 78.3% and 87.5% in Cukurova conditions (Dagtekin, 2019), 47.19% and 66.85% in GAP (Yucel et al., 2023b), 44.49% and 52.09% in the region where the study was conducted (Ozer, 2023). It is seen that the NDF contents obtained in the study are higher than the NDF values obtained in previous studies. It is estimated that the reason for this difference is the genetic structure of the varieties and the harvest period. As biomass harvests are delayed or the plants get older, the cellulose ratio of biomass increases, thus, increasing the NDF content.

3.2.2. Acid detergent fiber (%)

ADF varied between 35.78% and 39.71% for all varieties. The highest ADF was obtained from the Tifleaf III variety, while the lowest ADF was obtained from the Ashana variety. Varieties with high NDF are also high in ADF (Table 3). Significant positive correlations were recorded between NDF and ADF (Table 5). Dagtekin (2019) and Yucel et al. (2023a) were declarated similar findings. In addition, ADF contents was reported 37.8%, 31.1% and 37.9% respectively by Rasnake et al., (2005) and Buso et al., (2014) and Heuzé et al., (2015). ADF values obtained in pearl millet in different regions of Türkiye varied between 42.6% and 51.5% (Dagtekin, 2019), 34.33% and 46.89% (Yucel et al., 2023b), 31.11% and 36.69% (Ozer, 2023). ADF values were obtained in the study are within more acceptable limits in terms of animal nutrition, cellulose and lignin contents compared to NDF values. As ADF values exceed 30%, feed digestibility and quality decrease.

3.2.3. Crude protein (%)

CP contents varied between 8.54% and 11.72% in the study. Tifleaf III variety has the highest CP rate, while Heveahri variety has the lowest CP rate. CP rates were varied in studies conducted with different environments and genotypes.

CP ranged from 7.4% to 9.6% in Benin Republic (Abd-El-Lattief, 2011), 6.73% and 10.35% in Pakistan (Hassan et al., 2014), 8.8% and 16.2% in Sudan (Babiker et al., 2015) and 6.24% and 11.63% in India conditions (Thomas et al., 2018). CP varied between 4.3% and 14.4% in Cukurova/Türkiye conditions (Dagtekin, 2019) and 6.45% and 14.75% in GAP/Türkiye ecological conditions (Ozer, 2023; Yucel et al., 2023b). Additionally, Pearl millet CP rates varied between 12% and 14%. This value was stated to be higher than the CP rate of corn silage by Sheahan (2014).

Table 3. Averages of some forage quality traits of pearl millet varieties

Varieties	NDF (%)	ADF (%)	CP (%)	DMD (%)	DMI (%)	RFV	NEL (Mcal kg^{-1})
Ashana	63.98	35.28	9.19 bc	61.42	1.890	90.29	1.395
Heveahri	69.61	37.89	8.54 c	59.39	1.729	79.74	1.358
White	68.68	37.89	8.87 c	59.78	1.749	81.12	1.365
Yellow	67.84	36.95	10.44 ab	60.12	1.776	82.51	1.371
Tifleaf III	74.24	39.71	11.72 a	57.97	1.624	73.13	1.332
Mean	68.87	37.44	9.75	59.73	1.754	81.43	1.36
CV (%)	6.96	7.89	9.23	3.85	7.13	10.86	3.06
Sig.	NS	NS	0.001**	NS	NS	NS	NS

*,**= significant at 0.05 and 0.01 levels of probability respectively, NS= not significant, According to the LSD test, There is statistically significant difference at P≤0.05 level among the averages shown with different letters in the same column, NDF= neutral detergent fiber (%), ADF= acid detergent fiber (%), CP= crude protein (%), DMD= dry matter digestibility rate (%), DMI= dry matter intake (%), RFV= relative feed value, NEL= net energy lactation (Mcal kg^{-1}).

Table 4. Some mineral element contents of pearl millet varieties

Varieties	Ca (%)	K (%)	Mg (%)	P (%)
Ashana	0.223 b	3.043	0.270 bc	0.290
Heveahri	0.333 a	3.443	0.240 c	0.270
White	0.250 b	3.518	0.363 a	0.300
Yellow	0.333 a	3.530	0.278 b	0.295
Tifleaf III	0.173 c	3.490	0.240 c	0.300
Mean	0.262	3.405	0.278	0.291
CV (%)	9.49	13.49	7.55	5.57
Sig.	0.001**	NS	0.001**	NS

*,**= significant at 0.05 and 0.01 levels of probability respectively, NS= not significant, According to the LSD test, There is statistically significant difference at P≤0.05 level among the averages shown with different letters in the same column, Ca= calcium (%), K= potassium (%), Mg= magnesium (%), P= phosphorus (%).

3.2.4. Dry matter digestibility (%)

DMD rate was varied between 57.97% and 61.42% in the study. Ashana variety had higher rates of DMD than the remaining varieties (Table 3). According to Hassanat et al. (2007), the digestibility rate of silage pearl millet ranged from 640 to 690 g kg^{-1} DM. DMD results of Kichel et al. (1999) are parallel to our study results. DMD rate was varied between 52.37% and 62.16% (Yucel et al., 2023b). In addition, Yucel (2020) was reported that digestibility rate of silage sorghum material is higher than dry material.

3.2.5. Dry matter intake (%)

DMI contents was varied between 1.624% and 1.890% in the study. Ashana variety had the highest DMI rate, while Tifleaf III variety had the lowest DMI rate (Table 3). DMI values varied was reported between 1.80% and 2.54% under GAP region conditions in Türkiye (Yucel et al., 2023b). Additionally, the results of this study are compatible with our study.

3.2.6. Relative feed value (RFV)

RFV values varied between 73.13 and 90.29 in the study. Ashana variety had the highest RFV values in study (Table 3). Ashana variety had medium-class feed quality reported by Rohweder et al. (1978). RFV values of dry biomass varied between 74.66 and 122.52 stated Yucel et al. (2023b).

3.2.7. Net energy lactation (Mcal kg^{-1})

NEL contents were varied between 1.332 and 1.395 Mcal kg^{-1} in the study. Ashana and Tifleaf III varieties had the highest and lowest values, respectively (Table 3). While, significant negative correlations were recorded between NEL and NDF, ADF, significant positive correlations were recorded between NEL and DMD, DMI and RFV (Table 5). In order to obtain feeds with high energy content, ADF and NDF contents must be low. In this aspect, Ashana variety had better energy value than the remaining varieties in study. NEL contents were varied between 1.23 and 1.41 Mcal kg^{-1} DM reported by Yucel et al. (2023b).

3.3. Mineral Element Contents

The difference between varieties were statistically significant (P<0.05) for Calcium (Ca) and Magnesium (Mg) (Table 4).

Ca contents was varied between 0.173% and 0.333% in the study. While the highest Ca value was obtained in the Heveahri and Yellow varieties, the lowest value was obtained in the Tifleaf III variety (Table 4). Ca contents was ranged from 0.42% to 0.51% (Weichenthal et al., 2003), 0.29% to 0.85% (Heuzé et al., 2015), 0.28 % to 0.56% (Dagtekin, 2019) and 0.01% to 0.40% (Yucel et al., 2023b).

K contents was varied between 3.043% and 3.530% in the study. While the highest K content was obtained in the Yellow variety, the lowest value was obtained in the Ashana variety (Table 4). Many different results were obtained in previous studies on the K content of pearl millet. K contents ranged from 3.2% to 4.3% (Weichenthal et al., 2003), 0.160% to 0.419% (Heuzé et al., 2015), 1.901% to 4.233% (Dagtekin, 2019) and 2.48% to 4.33% (Yucel et al., 2023b).

Mg contents was varied between 0.240% and 0.363% in the study. While the highest Mg content was obtained in the White variety, the lowest value was obtained in the Heveahri and Tifleaf III varieties (Table 4). Many different results were obtained in previous studies on the Mg content of pearl millet. Mg contents ranged from 0.320% to 0.330% (Weichenthal et al., 2003) and 0.24% to 0.45% (Heuzé et al., 2015). Moreover, Mg contents ranged from 0.201% to 0.343% in Cukurova/Türkiye (Dagtekin, 2019) and 0.04% to 0.255% in GAP/Türkiye (Yucel et al., 2023b). Mg data were obtained from the study are in line with past studies.

P contents was varied between 0.270% and 0.300% in the study. While the highest P content was obtained in the White and Tifleaf III varieties, the lowest value was obtained in the Heveahri variety (Table 4). Different regions, treatments and genotypes affected the P contents of pearl millet. P contents ranged from 0.191% to 0.240% (Weichenthal et al., 2003) and 0.04% to 0.45% (Heuzé et al., 2015). In addition, P contents was ranged from 0.270% to 0.434% in Cukurova/Türkiye (Dagtekin, 2019) and 0.290% to 0.540% in GAP/Türkiye (Yucel et al., 2023b). P data obtained from the study are in agreement with past studies.

3.4. Analysis of Relationships between Traits

All parameters of pearly millet varieties were determined via correlation test. The results of correlation test including correlation coefficients and level of significance was given in Table 5. Significant and positive correlational relationships were obtained in the study. Significant positive correlations were recorded between NDF and ADF (r=0.9652**, P<0.01), BY and DBY (r=0.9512**, P<0.01), DBY and PTN (r=0.4248**, P<0.01), K and CP (r=0.5228*, P<0.05). Significant positive correlations were recorded between DMD and DMI (r=0.9508**, P<0.01), RFV (r=0.9721**, P<0.01), NEV (r=1.0000**, P<0.01). Significant positive correlations were recorded between PH and MSL (r=0.6535**, P<0.01), BY (r=0.5316**, P<0.05), DBY (r=0.5790**, P<0.01).

There were significant and positive relationships between PH and BY and DBY of pearl millet was reported by Dagtekin, 2020, Yucel et al., 2023a, Cakır et al., 2023 and Donmez and Hatipoglu, 2024. Additionally, studies conducted in different environments were reported significant and positive correlations between biomass yield and plant height, number of leaves, number of nodes and number of fertile tillers (Berwal et al., 1996; Yadav et al., 2012; Singh et al. al., 2014; Kanwar and Shekhawat, 2015; Aswini et al., 2022).

Table 5. Correlation coefficients and significance levels of all traits in study

Traits	Ca	K	Mg	NDF	P	CP	DMD	DMI	RFV	NEL	PH	MSL	BY	DBY	PTN
ADF	-0.35	0.17	-0.23	0.97	-0.29	-0.32	-1.00	-0.95	-0.97	-1.00	-0.36	-0.09	-0.08	-0.13	0.09
Ca		0.02	0.73	-0.29	-0.34	-0.06	0.35	0.26	0.28	0.35	0.61	0.65	0.36	0.35	0.06
K			-0.16	0.24	0.36	0.52	-0.17	-0.30	-0.28	-0.18	-0.08	0.16	0.07	0.04	0.32
Mg				-0.12	-0.33	-0.01	0.23	0.11	0.15	0.23	0.32	0.31	0.20	0.28	-0.02
NDF					-0.26	-0.17	-0.97	-0.99	-0.99	-0.96	-0.46	-0.16	-0.11	-0.18	0.13
P						0.69	0.29	0.24	0.25	0.29	-0.18	-0.16	0.09	-0.01	0.02
CP							0.32	0.15	0.20	0.32	-0.35	-0.01	0.05	-0.02	0.16
DMD								0.95	0.97	1.00	0.36	0.09	0.08	0.13	-0.09
DMI									0.99	0.95	0.43	0.14	0.07	0.13	-0.15
RFV										0.97	0.41	0.12	0.06	0.12	-0.14
NEL											0.36	0.09	0.08	0.13	-0.09
PH												0.65	0.53	0.58	0.23
MSL													0.66	0.62	0.33
BY														0.95	0.34
DBY															0.42

PH= plant height (cm), MSL= number of leaves per main stem (number), PTN= number of tillers per plant (number), BY= niomass yield (kg da^{-1}), DBY= nry biomass yield (kg da^{-1}), NDF= neutral detergent fiber (%), ADF= acid detergent fiber (%), CP= crude protein (%), DMD= dry matter digestibility rate (%), DMI= dry matter intake (%), RFV= relative feed value, NEL= net energy lactation (Mcal kg^{-1}), Ca= calcium (%), K= potassium (%), Mg= magnesium (%), P= phosphorus (%).

4. Conclusion

As a result of the study, White variety was showed better performance than remaining varieties for forage yield and other yield components under second crop conditions in the GAP region. Ashana is the variety with the best relative feed quality in the trial and has middle class feed quality. It has also been determined that Yellow and Tifleaf III varieties are better than other varieties in terms of crude protein ratio. The biomass yield and feed quality traits of pearl millet varieties in the study conducted in the semi-arid climate zone was within acceptable limits for livestock farming. For this reason, pearl millet cultivation could be recommended in regions of the world with similar climate zones, especially in order to close the roughage gap. Pearly millet has the potential to be an alternative to silage corn, especially in arid regions.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.N.S.	C.Y.	T.T.
C	40	30	30
D	50	30	20
S		50	50
DCP	20	30	50
DAI	15	70	15
L	80	10	10
W	50	30	20
CR	30	40	30
SR	10	10	80
PM	20	50	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

This study was supported by Şırnak University Scientific Research Projects Unit with the project code 2020.FLTP.13.09.04 and was produced from the MSc (Master of Science).

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1528282

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 557-562 / September 2024

EFFECT OF DIFFERENT CHEMICAL INDUCERS ON MYCELIAL GROWTH OF *Neoscytalidium dimidiatum*

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Abstract: *Neoscytalidium dimidiatum* has become one of the most aggressive fungal pathogen that cause economical damage to plants with changing climatic conditions. Pathogen causes disease symptoms including dieback, canker, blight, root rot, leaf spot, and fruit rot at a wide range of plant species and significant yield losses and damages. Few studies have been conducted on the efficiency of different chemical fungicides against the pathogen, but no effective control method has been found. Also, comprehensive studies on different control methods were needed due to the disadvantages in the use of chemical fungicides. The aim of the study was to evaluate the effects of chitosan (1, 1.5, 2 mg/ml), metyl jasmonate (MeJA; 0.01, 0.1, 1 mM) and acibenzolar-S-methyl (BTH; 0.01, 0.1, 1 mM) on mycelial growth of *N. dimidiatum*. The results showed statistically significant differences among the inhibition rates of chemical inducers against *N. dimidiatum*, but also among different doses of chemical inducers as compared to control. Chitosan at 2 mg/ml concentration was the most effective with the inhibition rate of 45.2%, followed by 1.5 mg/ml and 1 mg/ml doses of chitosan that inhibited mycelial growth at the rates of 44.6 and 37.9%, respectively. BTH was the second most effective treatment after chitosan with the inhibition rate of 18.9% at 1 mM dose, while MeJA was sufficiently ineffective in inhibiting the mycelium growth of *N. dimidiatum* at the concentrations tested. The results indicated that chitosan could be an alternative to fungicides due to its high level of effectiveness and non-toxicity.

Keywords: Chemical inducer, Disease control, Mycelial growth, *Neoscytalidium dimidiatum*

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Received: August 05, 2024
Accepted: September 03, 2024
Published: September 15, 2024

Cite as: Palacıoğlu G. 2024. Effect of different chemical inducers on mycelial growth of *Neoscytalidium dimidiatum*. BSJ Agri, 7(5): 557-562.

1. Introduction

Neoscytalidium is a fungal genus that has emerged as a significant agricultural threat with global warming. The pathogen has a wide range of host species including trees, crops, vegetables, and shrubs and causes disease symptoms such as canker, blight, dieback, leaf spot, root rot, and fruit rot (Derviş and Özer, 2023). High temperatures, drought and plant stress promote infection by the pathogen and can significantly reduce the quality and value of fruit in some crops (Hong et al., 2020; Derviş and Özer, 2023). *Neoscytalidium* have been reported recently by many researchers from Türkiye and around the world (Derviş et al., 2020; Ören et al., 2022a; Ören et al., 2022b; Güney et al., 2022; Zaeimian and Fotouhifar, 2023; Çaplık et al., 2024; de Lima Costa et al., 2024).

Three species of this genus including *N. dimidiatum*, *N. novaehollandiae* and *N. orchidacearum* were first identified as plant pathogenic species (Crous et al., 2006; Phillips et al., 2013; Huang et al., 2016; Suwannarach et al., 2018). However, *N. dimidiatum* was recognized as a single species due to high nucleotide similarity between the species and the other two species were accepted as synonyms (Zhang et al., 2021; Crous et al., 2021). The fungus forms blackish-brown pycnidia and doliiform, oblong-obtuse, 0–2-septate, dark brown, powdery arthroconidia originating from aerial mycelium (Phillips et al., 2013). *Neoscytalidium dimidiatum* could infiltrate host plants through wounds, natural openings, and directly penetrate young plants via the formation of appressoria.

The high adaptability of the pathogen possessed important challenges in disease control. The most common and effective control strategy against *N. dimidiatum* was the use of chemical fungicides nowadays (Moral et al., 2019; Al Raish et al., 2020). However, the adverse effects of fungicides on human and environment, the absent of registered chemicals against the pathogen in some country and the risk of fungicide resistance in the future revealed the necessary of alternative control methods. The use of various synthetic non-toxic chemical compounds helps to inhibit pathogen development and to develop effective control methods by activating natural defense mechanisms in plants (Li et al. 2009; Varghese and Thomas, 2023). Among these compounds with non-toxic properties, chemicals such as chitosan, BTH (Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester) and jasmonic acid are known to play a role as secondary inducers in the activation of related genes and signaling mechanisms (Métrauxs, 2001; Walters et al. 2007). They were also used as a biological control agent against various plant pathogenic fungi, bacteria, and viruses (Johnson and Temple, 2016; Thomas-Sharma et al. 2017; Gutiérrez-Martínez et al. 2017). The inducers provide effective protection against various pathogens by regulating the defense-related enzymes such as peroxidase, β-1,3-glucanase, chitinase, catalase, superoxide dismutase, PR proteins and phytoalexin metabolism in the host plant, and showing antifungal activity against pathogens (Andrade et al., 2013; Gutiérrez-Martínez et al., 2017; de Souza et al., 2018; Varghese and Thomas, 2023). Various chemical ingredients have been used by the researchers in the control of *N. dimidiatum*, but the use of metyl jasmonate (MeJA), chitosan and Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester or acibenzolar-S-methyl (BTH) in disease management has not been adequately addressed (Du et al., 2019; Kılınç and Güldür, 2020; Sür and Oksal, 2021; Sakçı et al., 2022; Abdul-Karim et al., 2023; Mohammadi et al., 2024).

This study aimed to investigate the effects of chitosan, jasmonic acid or metyl jasmonate (MeJA) and Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester or acibenzolar-S-methyl (BTH) applications on mycelial growth of *N. dimidiatum* and to examine their potential as an alternative to chemical fungicides.

2. Materials and Methods

Neoscytalidium dimidiatum isolate M1, identified as pathogen in a previous study was utilized in the assay (Ören et al., 2022a). The isolate was grown on potato dextrose agar (PDA; Merck, Darmstadt, Germany) medium for seven days and stored on filter paper in 4 °C.

2.1. Preparation of Chitosan, Methyl Jasmonate and Acibenzolar-S-methyl

Chitosan (HiMedia; 75%-85% deacetylation degree, Pennsylvania, United States) with low molecular weight was used in the study. According to the manufacturer's protocol, the stock solution of chitosan at 10 mg/ml concentration was firstly prepared and adjusted to pH 5.6 using 0.1 M NaOH. The application doses of chitosan were adjusted to 1.0, 1.5, and 2.0 mg/ml by adding sterile distilled water. Stock solutions (1 mM) of methyl jasmonate 95% (MeJA; Sigma-Aldrich, Massachusetts, United States) and acibenzolar-S-methyl (BTH; Syngenta) were prepared considering the molecular weights of commercial products and the solutions were diluted to different doses ranging from 0.1 to 0.01 mM. These concentrations were selected considering the studies that reported that defense mechanisms were activated and disease development was prevented in different research (Johnson and Temple, 2016; Gutiérrez-Martínez et al., 2017; Thomas-Sharma et al., 2017; Palacıoğlu, 2024).

2.2. Evaluation of the Inhibitory Effects of Chitosan, MeJA and BTH on Mycelial Growth of *Neoscytalidium dimidiatum*

Potato dextrose agar medium was prepared separately in 250 ml Erlenmeyer flasks for each concentration of the chemical inducers, autoclaved at 121 °C for 20 min, and cooled to 50 °C. Different concentrations of each of compounds were mixed with sterile PDA medium and poured in Petri dishes about 20 ml in each dish. Agar disks with a diameter of 4 mm were taken from the fungal culture of the *N. dimidiatum* isolate grown for 7 days and placed on each PDA medium containing different chemical inducers. Petri dishes were incubated at 25 °C for 7 days. All treatments were performed as 3 replicates for each concentration. PDA medium without any chemical inducers was used as control. At the end of the incubation period, the diameters of the fungal colonies in each Petri dishes were measured from both directions and arithmetic mean was calculated to evaluate the effects of chemical inducers on mycelium growth of *N. dimidiatum*.

2.3. Statistical Analysis

Significant differences between mean values of mycelial growth were determined by analysis of variance (one-way ANOVA) using Least Significant Difference (LSD) method ($P<0.05$). The effectiveness of each compound at different concentrations was calculated using the Abbott formula (Karman, 1971).

3. Results and Discussion

Neoscytalidium dimidiatum is an important fungal pathogen that has a wide range of host and caused dieback, canker, blight, root rot, leaf spot, and fruit rot diseases in agricultural areas. The pathogen has recently caused serious economic losses due to climate change. Many studies have been conducted to assess the efficiency of various control methods, including some chemical fungicides, biocontrol agents and essential oils (Xian et al., 2018; Noegrohati et al., 2019; Taguiam et al., 2020; Ratanaprom et al., 2021; Sür and Oksal, 2021; Sakçı et al., 2022; Riska et al., 2023). This study aimed to investigate *in vitro* the efficiency of chitosan, MeJA, and BTH as alternative control methods against *N. dimidiatum*. These chemical inducers depending on their doses inhibited mycelial growth of the pathogen and significant differences were observed among both inducers and application doses (Figure 1). The results indicated that chitosan treatment was the most effective on pathogen development. Inhibition rates on mycelial growth increased with increasing chitosan dose (Figure 2). The application of 2 mg/ml chitosan allowed mycelial growth of 2.89 cm, while the growth at 1 mg/ml and 1.5 mg/ml doses were 3.27 cm and 2.92 cm, respectively (Figure 1). In MeJA and BTH applications, the lowest mycelial development was observed at 1 mM dose. BTH application was the second effective application, allowing lower mycelial growth than MeJA treatment. Mycelial growth values in BTH application ranged from 4.27 to 4.98 cm. Mycelial growth observed in Petri dishes treated with MeJA ranged from 5.05 to 5.21 cm.

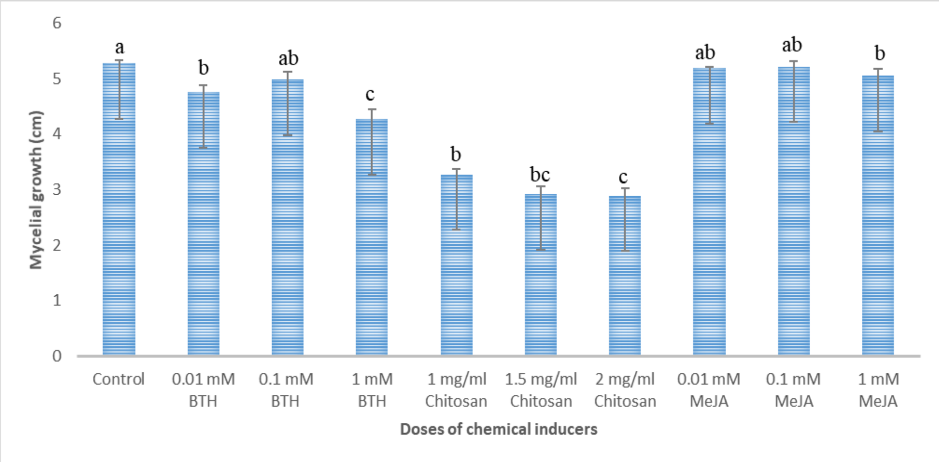

Figure 1. Effects of BTH, chitosan and MeJA on the mycelial growth of *Neoscytalidium dimidiatum* seven days after incubation.

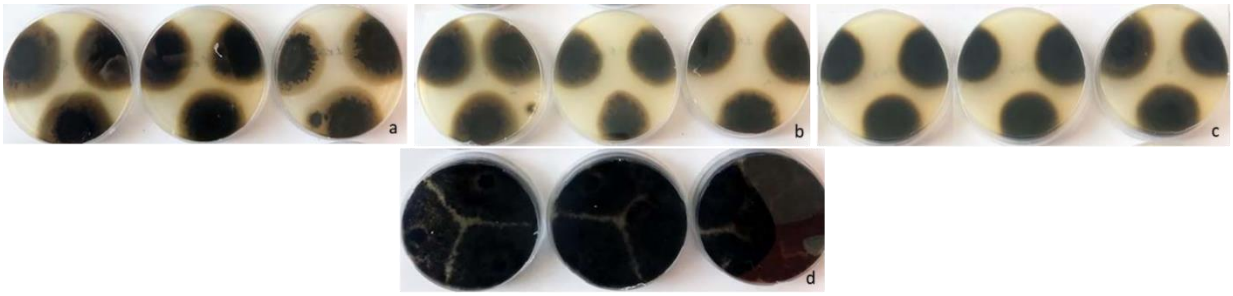

Figure 2. Effects of chitosan at 1 mg/ml, 1.5 mg/ml and 2 mg/ml on mycelial growth *Neoscytalidium dimidiatum* (a: 1 mg/ml, b: 1.5 mg/ml, c: 2 mg/ml, d: control).

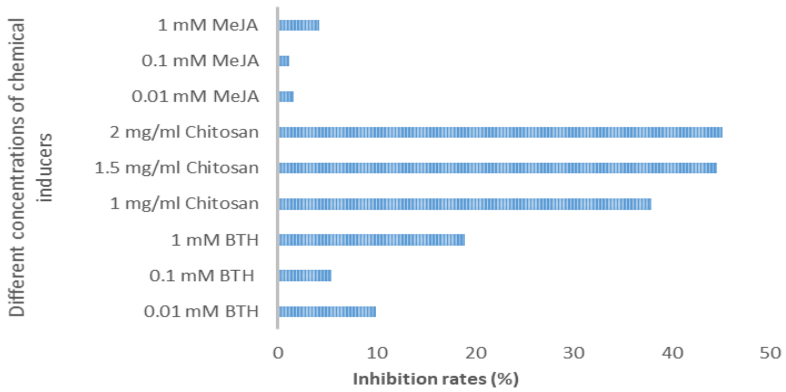

Figure 3. Inhibition rates (%) resulted from chitosan, BTH and MeJA applications at different doses on mycelial growth of *Neoscytalidium dimidiatum*.

When the inhibition rates of chemical inducers after incubation were examined, it was observed that the highest effect was provided by the 2 mg/ml chitosan (Figure 3). The application was quite effective on mycelial growth and provided 45.2% inhibition as compared to the control. This was followed by 1.5 and 1 mg/ml chitosan doses with the inhibition rates of 44.6% and 37.9%, respectively. Inhibition rates of BTH applications varied between 5.4 and 18.9%. MeJA showed less effect than chitosan and BTH with inhibition rates ranging from 1.1 to 4.2%.

Similarly, Mohammadi et al. (2024) who determined the effects of six different doses (100, 250, 500, 1000, 1500, 2000 ppm) of chitosan nanoparticles on *N. novaehollandiae* (syn. *N. dimidiatum*) *in vitro* conditions, reported that radial growth of the pathogen varied between 0 and 5.88 mm, and mycelial growth inhibited completely at doses of 1500 ppm and above. The researchers also stated that mycelial growth was inhibited by 7.1 to 60.34% at doses ranging from 100 ppm to 1000 ppm, respectively, while 100% inhibition was observed at the doses of 1500 and 2000. Similar findings were reported by Chun and Chandrasekaran (2019), who observed that 5.0 mg/ml CS and CNPs concentration maximally inhibited of radial mycelial growth of *Fusarium andiyazi* on tomato by 54.8% and 73.81%, respectively. Dodgson and Dodgson (2017), who 0.5% chitosan had a similar effect to fungicides in preventing anthracnose in cucumber. In an another study, conducted by Abdul-Karim and Aljarah (2023), the antifungal effect of kaolin and MgO nano-particles at 0.5%, 1% and 2% concentrations were investigated on *N. dimidiatum*. MgO nano-particles completely inhibited fungal growth at all doses, while 1 and 2% kaolin applications inhibited by 35.69 and 37.08%, respectively. Evaluating *in vitro* antifungal activity of alginate-stabilized Cu2O-Cu nanoparticles against *N. dimidiatum* causing brown spot disease on dragon fruits, Du et al. (2019) reported that 25.1, 22.5 and 100% inhibition on the pathogen growth was obtained at 15, 22.5 and 3 ppm doses, respectively. Similar results were obtained by Sür and Oksal (2021), who investigated the *in vitro* efficacy of seven different fungicides against *N. dimidiatum*, the causal agent of sudden shoot dryness in apricot trees. They reported that cyprodinil+fludioxonil at doses of 30 and 100 µg/mL, and floupiram+tebuconazole at doses of 10, 30 and 100 µg/mL completely inhibited the mycelium growth. The researchers also indicated that the efficacy rates of the other five fungicides used varied between 0 and 98.02%. In another study, the *in vitro* activities of five fungicides against *N. novaehollandiae* responsible for cancer and death symptoms in almonds were examined and the highest activity was obtained with fluazinam (EC_{50};0.002 µl ml^{-1}), thiophanate-methyl (EC_{50};0.3 µl ml^{-1}), and tebuconazole (EC_{50};0.4 µl ml^{-1}) treatments. The lowest effect was found in the application of trifloxystrobin (EC_{50}; 19.5 µl ml^{-1}) (Sakçı et al., 2022). Chemical compounds like chitosan, jasmonic acid and BTH are among the basic compounds that play a key role in the induction of plant resistance mechanisms against diseases and provides effective protection against many significant fungi species such as *Fusarium* spp., *Alternaria* spp., *Botrytis* spp. and *Phytophthora* spp. These inducers provided resistance in plants by affecting spore germination, germ tube elongation, mycelial growth of the pathogen and inducing defense related genes and pathways (Xu et al., 2007; Li et al., 2009; Al-Hetar et al., 2011; Silva et al., 2014; Siddiqi and Husen 2019).

4. Conclusion

Chitosan was identified as the most effective compound in inhibiting the mycelial growth of *N. dimidiatum* under *in vitro* conditions, achieving up to 45.2% inhibition, followed by BTH at a 1 mM dose with an inhibition rate of 18.9%. However, the tested doses of MeJA were insufficient in inhibiting the fungal growth. Using higher doses of MeJA and investigating different combinations of the compounds may be more useful in inhibiting fungal growth. Chitosan among the compounds could be evaluated as an alternative to fungicides due to its high level of effectiveness and non-toxic, environmentally-friendly properties.

Author Contributions

The percentage of the author(s) contributions is presented below. All author(s) reviewed and approved the final version of the manuscript.

	G.P.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1525082

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 5: 563-569 / September 2024

THE SINGLE AND INTERACTIVE EFFECT OF SALINITY AND TEMPERATURE ON GERMINATION CHARACTERISTICS OF ITALIAN RYEGRASS (*Lolium multiflorum* LAM.) SEEDS

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Abstract: Italian ryegrass (*Lolium multiflorum*) is a grass species within the *Lolium* genus of the Poaceae family. In recent years, annual ryegrass has shown excellent adaptability to the climatic and soil conditions of Türkiye. It serves as a good alternative forage source to bridge the forage deficit and is widely used as a low-growing native turf mixture in local landscaping. Abiotic stress factors are among the primary elements that hinder plant growth and development. Temperature and salinity significantly affect seed germination and development. This study aimed to investigate the germination and growth parameters of three different Italian ryegrass varieties (İlkadım, Kocayaşar, Zeybek) under different salt concentrations (Sodium chloride-NaCl) and temperatures. Three different salt doses (control, 5 EC, and 10 EC) and three different temperatures (15 °C, 20 °C, and 30 °C) were used in the study. Germination percentage, shoot and root lengths, fresh and dry weights of shoots, and ion leakage parameters were examined. The results showed that the highest germination rate, shoot and root lengths, and fresh and dry weights in all varieties were recorded at 20 °C with 0 EC and 20 °C with 5 EC salt treatments, while the lowest were observed at 15 °C with 10 EC salt treatments. The lowest ion leakage was determined in the control treatment at 15 °C, while the highest ion leakage was observed in the 10 EC treatment at 30 °C. Increasing temperature positively influenced growth parameters. It was determined that salt stress could be tolerated up to a certain level at higher temperatures. This study on different Italian ryegrass varieties highlights the importance of developing ryegrass varieties resistant to temperature and salt stress, which are significant issues in sustainable agriculture.

Keywords: Abiotic stress, Ion leakage, Italian ryegrass, Principal component analysis

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Received: July 31, 2024
Accepted: September 12, 2024
Published: September 15, 2024

Cite as: Okumuş O, Dalda Şekerci A, Uzun S. 2024. The single and interactive effect of salinity and temperature on germination characteristics of Italian ryegrass (*Lolium multiflorum* Lam.) seeds. BSJ Agri, 7(5): 563-569.

1. Introduction

Abiotic stress is a significant issue that greatly restricts agricultural production globally (Kopecka et al., 2023). One of the abiotic stress factors, salinity, is considered one of the most critical environmental stresses that significantly reduce yield and quality in crop production worldwide (Doruk Kahraman and Topal, 2023). More than 800 million hectares of land globally are affected by salinity, constituting over 6% of the world's total land area, and this proportion is increasing due to natural and anthropogenic activities (Munns and Tester, 2008; Hasanuzzaman and Fujita, 2022). Salt stress causes physiological, biochemical, and metabolic changes in plants, leading to a decrease in crop production. The main components of salt stress in plants are osmotic stress, ionic stress, and secondary stress, namely the excessive accumulation of reactive oxygen species (ROS) (Morton et al., 2019; Li et al., 2022).

The germination characteristics of seeds at different temperatures can vary significantly depending on the plant species and environmental conditions (Okumuş et al., 2023). Different temperature levels can have noticeable effects on germination rate, percentage, and post-germination seedling development (Kaya et al., 2006; Okumuş et al., 2024). Temperature stress significantly reduces the seed germination percentage, germination time, and seedling vigor in many plants (Lamichaney et al., 2021). High temperatures lead to cell dehydration, causing cell size reduction and ultimately leading to decreased growth (Arun-Chinnappa et al., 2017).

Italian ryegrass (*Lolium multiflorum* Lam.) is a plant species within the genus *Lolium* of the Poaceae family, belonging to the order *Poales* (Lale and Kökten, 2020). In recent years, annual ryegrass has shown excellent adaptability to the climate and soil conditions in Türkiye and is expected to be a good alternative forage source to address the forage deficit (Özkan et al., 2022). According to the Turkish Statistical Institute (TÜİK) statistics, in 2022, the sown area was 539,944 decares, and green fodder production was 2,122,105 tons (TÜİK, 2023). The climates it best adapts to are cool and humid. It can easily be grown in areas with an annual rainfall of over 400 mm (Açıkgöz, 2021). The optimum air temperature for the most productive growth is between 18-24 °C (Pişkin, 2007).

Grass plays a significant role in human life by adding elegance to the environment and forming the basis of many recreational sports. They constitute a large part of both residential and commercial landscapes. One native landscaping option is the use of native grass mixtures, which provide a turf appearance with low-growing native grasses (Pooya et al., 2013). Native plants are resilient as they are adapted to local conditions (Butler et al., 2012). Because native plants require less maintenance, they offer excellent options for large commercial landscapes and residential gardens. Turfgrass breeders are in search of varieties that can grow satisfactorily across a wide range of climates, soil, and environmental conditions (Pessarakli and Kopec, 2008). These grasses are excellent candidates for producing dwarf and turf-type varieties and are also used as turfgrass in arid and semi-arid regions worldwide to develop more attractive and low-maintenance varieties. Due to these reasons, there is increasing interest in selecting and producing native grass varieties that exhibit many other beneficial characteristics (Bormann et al., 2001). Because of their different growth patterns, a mixture of two or more grass varieties can complement each other to provide both functional and aesthetic improvements in turf-grassquality.

Therefore, it is crucial to conduct sustainable agriculture-focused studies that adapt to global climate change. Plants' responses to salt and temperature stress vary among species and even among varieties, and adaptation to stress conditions needs to be evaluated on a species-specific basis. This study aims to determine the responses of some annual grass varieties to salt stress at different temperatures and to examine germination and some seedling development characteristics during the germination period under increasing NaCl concentrations.

2. Materials and Methods

This study investigated the temperature and salinity tolerances of three different Italian ryegrass varieties. The experiment was designed with three factors and established according to the split-split plot design in a randomized complete block design. The varieties used as plant material in the study were İlkadım, Kocayaşar, and Zeybek. İlkadım and Kocayaşar varieties were registered by the Black Sea Agricultural Research Institute, and Zeybek variety was registered by the Aegean Agricultural Research Institute.

In the study, NaCl (Merck, Germany) was used to induce salt stress. The salt levels were set to control, 5 EC, and 10 EC. The study was conducted under controlled conditions at temperatures of 15 °C, 20 °C, and 30 °C. The seeds used in the study were sterilized with 10% sodium hypochlorite for 5 minutes and then rinsed three times with distilled water. The seeds were placed in groups of 25 between three filter papers, and the edges were sealed with zip lock bags to prevent moisture loss. Seven milliliters of solution were added to each filter paper. Seeds were considered germinated when the root length reached ≥2 mm, and germinated seeds were counted for 14 days. At the end of the 14-day period, the germination percentage (Number of germinated seeds/25 x 100) was calculated. Additionally, shoot and root length, fresh weight, dry weight, and ion leakage data were examined on 10 randomly selected seedlings.

Ion leakage was measured according to the method described by Aydın (2018). Fresh shoots (0.5 g) were washed with distilled water and incubated in 10 ml of distilled water at room temperature for 24 hours, after which the solution's EC was measured (O.D1). The samples were then autoclaved at 121 °C for 20 minutes, cooled, and the EC was measured again (O.D2). Ion leakage in leaf tissues was calculated using the following formula Equation 1;

$$\%\ \text{Ion leakage} = (O.D1 / O.D2) \times 100 \qquad (1)$$

2.1. Statistical Analysis

The research was established in a factorial design with four replications in randomized plots. The data obtained from the research were analyzed using the "JMP 13.2.0" software according to the factorial design in randomized plots. Treatment means were compared using the Tukey's Multiple Comparison Test (Snedecor and Cochran, 1967).

3. Results and Discussion

3.1. Germination Percentage

In this study, the germination percentages of three Italian ryegrass varieties at different salt doses and temperatures were determined. In terms of germination percentage, variety, salt dose, variety x salt dose, and variety x temperature were found to be statistically significant ($P<0.001$), while the interaction of variety x temperature x salt dose was found to be insignificant.

According to the results, the germination percentage ranged from 87.33% to 44.00% for the İlkadım variety, from 82.66% to 40.66% for the Kocayaşar variety, and from 92.00% to 51.33% for the Zeybek variety (Table 1). The differences in germination percentages among the varieties were statistically significant. The highest germination percentage for all three varieties was observed at 20 °C, while the lowest was at 15 °C. A decline in germination percentages was observed in the varieties with increasing salt doses. The results indicate that high temperature and salt levels delay the germination period. It is suggested that excessive salt ions, aided by temperature, may limit water uptake by the germinating seeds. As is well known, seed water uptake depends on the osmotic potential of the seed and its surrounding environment, and one of the reasons salinity adversely affects germination is osmotic stress (Doğan and Budaklı Çarpıcı, 2016).

Table 1. Germination rate (%), shoot and root length (cm), fresh and dry weights (mg), and ion leakage (%) of Italian ryegrass at different temperature and salt concentrations

°C	Salt Doses	Germination Rate			Ion Leakage		
		İlkadım	Kocayaşar	Zeybek	İlkadım	Kocayaşar	Zeybek
	Cont.	59.33±1.50hij	70.66±1.50	71.33±1.50fg	26.03±0.85n	29.63±0.85mn	32.33±0.85klm
15	5 EC	52.00±1.50ijk	60.00±1.50hi	64.00±1.50gh	34.27±0.85klm	39.47±0.85ij	41.80±0.85hi
	10 EC	44.00±1.50kl	40.66±1.50l	51.33±1.50jk	48.07±0.85c-f	48.37±0.85b-f	50.37±0.85a-e
	Cont.	87.33±1.50bc	82.66±1.50cd	99.33±1.50a	31.73±0.85lm	32.17±0.85lm	32.10±0.85lm
20	5 EC	83.33±1.50cd	70.66±1.50fg	92.00±1.50ab	42.70±0.85ghi	44.40±0.85fgh	44.40±0.85fgh
	10 EC	72.66±1.50ef	60.66±1.50h	81.33±1.50cd	51.90±0.85a-d	51.37±0.85a-e	51.36±0.85a-e
	Cont.	80.66±1.50cde	72.00±1.50fg	86.66±1.50bcd	34.17±0.85klm	35.50±0.85jkl	36.83±0.85jk
30	5 EC	71.33±1.50fg	56.00±1.50hij	78.66±1.50def	42.23±0.85ghi	47.07±0.85efg	47.93±0.85c-f
	10 EC	58.66±1.50hij	42.66±1.50l	70.66±1.50fg	52.80±0.85ab	52.37±0.85abc	53.46±0.85a
Mean Significant		TxC**	CxS **	TxCxS NS	TxC**	CxS *	TxCxS NS
°C	Salt Doses	Root Length			Fresh Weight		
		İlkadım	Kocayaşar	Zeybek	İlkadım	Kocayaşar	Zeybek
	Cont.	3.33±0.16ef	2.10±0.16hi	2.56±0.16 e-h	50.06±1.29ijk	50.43±1.29ijk	60.33±1.29fgh
15	5 EC	2.26±0.16gh	1.36±0.16ij	2.13±0.16hi	42.73±1.29l	43.63±1.29kl	57.13±1.29ghi
	10 EC	1.13±0.16j	0.90±0.16j	1.26±0.16ij	31.40±1.29n	29.27±1.29n	50.33±1.29ijk
	Cont.	3.13±0.16efg	5.50±0.16b	5.73±0.16b	73.10±1.29bc	66.43±1.29c-f	82.97±1.29a
20	5 EC	3.23±0.16ef	5.23±0.16b	4.23±0.16cd	64.60±1.29def	52.70±1.29ij	78.43±1.29ab
	10 EC	2.50±0.16fgh	3.40±0.16de	2.53±0.16e-h	51.33±1.29ij	44.17±1.29kl	61.30±1.29efg
	Cont.	4.86±0.16bc	5.57±0.16b	6.93±0.16a	64.60±1.29def	62.53±1.29efg	70.17±1.29cd
30	5 EC	2.86±0.16 e-h	3.37±0.16ef	5.30±0.16b	45.60±1.29jkl	53.93±1.29hi	68.30±1.29cde
	10 EC	2.50±0.16fgh	3.33±0.16ef	2.60±0.16 e-h	39.87±1.29lm	34.73±1.29mn	52.90±1.29i
Mean Significant		TxC**	CxS **	TxCxS **	TxC**	CxS **	TxCxS **

Table 1. Germination rate (%), shoot and root length (cm), fresh and dry weights (mg), and ion leakage (%) of Italian ryegrass at different temperature and salt concentrations (continue)

°C	Salt Doses	Shoot Length		
		İlkadım	Kocayaşar	Zeybek
	Cont.	4.03±0.18f-i	3.50±0.18hij	4.87±0.18ef
15	5 EC	3.60±0.18hij	3.26±0.18ij	4.37±0.18e-g
	10 EC	2.26±0.18kl	1.90±0.18l	2.83±0.18jkl
	Cont.	5.30±0.18de	6.37±0.18bc	6.77±0.18bc
20	5 EC	4.83±0.18ef	5.27±0.18de	5.13±0.18de
	10 EC	4.40±0.18e-h	3.50±0.18hij	3.63±0.18g-j
	Cont.	5.23±0.18de	7.20±0.18ab	8.00±0.18a
30	5 EC	4.60±0.18efg	5.97±0.18cd	4.80±0.18ef
	10 EC	2.98±0.18jk	2.83±0.18jkl	2.87±0.18jkl
Mean Significant		TxC**	CxS **	TxCxS **
°C	Salt Doses	Dry Weight		
		İlkadım	Kocayaşar	Zeybek
	Cont.	5.26±0.18h-k	5.43±0.18g-j	6.03±0.18fgh
15	5 EC	4.33±0.18kl	4.53±0.18jkl	5.43±0.18g-j
	10 EC	3.93±0.18l	2.50±0.18m	5.23±0.18 h-k
	Cont.	8.30±0.18ab	7.17±0.18cde	8.87±0.18a
20	5 EC	6.73±0.18def	5.60±0.18ghi	8.07±0.18abc
	10 EC	5.46±0.18g-j	5.10±0.18h-k	6.37±0.18efg
	Cont.	6.93±0.18def	6.73±0.18	7.73±0.18bcd
30	5 EC	4.97±0.18ijk	6.07±0.18fgh	7.30±0.18b-e
	10 EC	3.70±0.18l	3.70±0.18l	5.63±0.18ghi
Mean Significant		TxC**	CxS **	TxCxS **

Previous studies have also indicated a direct relationship between the inhibitory effect of salt ions on seed germination and the hindrance of embryo growth with increasing temperature.

In similar studies, Soysal et al. (2021) reported that salt stress predominantly had a reducing effect on germination in their experiment with an annual ryegrass variety. Kuşvuran et al. (2015) reported that 150 and 200 mM NaCl adversely affected germination in their study with *Lolium perenne*. Another study reported that tetraploid Italian ryegrass (GT) had higher germination percentage and germination energy under different salinity levels and temperatures compared to diploid Italian ryegrass, and the most suitable temperature for seed germination was determined to be 25°C (Özkan et al. 2022). In a related study, the optimal germination percentage and germination energy were determined to be at 25°C, indicating that grass species prefer relatively higher temperatures for seed germination (Lin et al., 2018).

3.2. Ion Leakage

In terms of ion leakage, variety and variety x temperature were found to be significant at the 0.01 level, while variety x salt dose was significant at the 0.5 level, and the interaction of variety x temperature x salt dose was found to be insignificant.

As seen in Table 1, ion leakage ranged from 52.80% to 26.03% for the İlkadım variety, from 52.37% to 29.63% for the Kocayaşar variety, and from 53.46% to 32.33% for the Zeybek variety. An increase in ion leakage was observed with increasing salt doses and temperatures. The high osmotic effect, ion toxicity, oxidative stress, and nutrient deficiencies in these areas adversely affect plant growth (Naeem et al., 2020; Okumuş and Dalda-Şekerci, 2024). High salt concentrations lead to membrane breakdown, increasing ion leakage (Kalisz et al., 2023).

3.3. Shoot and Root Lengths

The analysis revealed that the effects of variety, salt dose, variety x salt dose, variety x temperature, and variety x salt dose x temperature interactions on shoot and root lengths were statistically significant at the 1% level. According to Table 1, shoot lengths varied between 2.26 cm and 5.30 cm for the İlkadım variety, 1.90 cm and 7.20 cm for the Kocayaşar variety, and 2.83 cm and 8.00 cm for the Zeybek variety, with the highest shoot length observed at 30°C. For root length, the İlkadım variety ranged from 1.13 cm to 4.86 cm, the Kocayaşar variety from 0.90 cm to 5.57 cm, and the Zeybek variety from 1.26 cm to 6.93 cm, with the highest root length observed in the Zeybek variety at 30°C. Increasing salt doses negatively affected both shoot and root lengths, whereas higher temperatures had a positive impact on these parameters. Successful plant growth under stress conditions largely depends on effective shoot and root development. Following successful seed germination, a robust seedling stage is crucial, and identifying the optimal temperatures for different species under stress conditions is essential. Osmotic and ionic effects are considered the dominant factors that inhibit seed germination responses under salt stress (Debez et al., 2004). The detrimental effects of salinity generally decrease at optimal temperatures (Khan et al., 2002). Studies have reported that interactive effects of salinity and temperature linearly reduce root and shoot lengths in Lolium species (Marcum and Pessarakli, 2013; Guo et al., 2020). Another study highlighted that shoot length is more severely affected than root length under stress conditions, and tetraploid Italian ryegrass exhibited better average root and shoot lengths compared to diploid varieties at varying temperature and salinity levels (Özkan et al. 2022).

3.4. Shoot Fresh and Dry Weights

In terms of shoot fresh and dry weights, variety, salt dose, variety x salt dose, variety x temperature, and variety x salt dose x temperature interaction were found to be significant at the 0.01 level.

As seen in Table 1, shoot fresh weight ranged from 31.40 mg to 64.60 mg for the İlkadım variety, from 29.27 mg to 66.43 mg for the Kocayaşar variety, and from 50.33 mg to 78.43 mg for the Zeybek variety. The highest shoot fresh weight was observed in the Zeybek variety at 20 °C. For shoot dry weight, the İlkadım variety ranged from 3.70 mg to 6.93 mg, the Kocayaşar variety from 2.50 mg to 7.17 mg, and the Zeybek variety from 5.23 mg to 8.87 mg (Table 1). The highest shoot dry weight was also observed in the Zeybek variety at 20 °C. Like shoot fresh and dry weights, stress effects were tolerable with the provision of optimal temperature. Increasing temperatures and salinity levels reduced the fresh weight in all varieties. The findings are consistent with previous studies. Özkan et al. (2022) noted that increasing temperatures and salinity levels decreased fresh weight, and that tetraploid varieties had higher fresh weights compared to diploid varieties. Other similar studies have reported that increasing salinity negatively affects both fresh and dry weights. Zabihi-e-Mahmoodabad et al. (2011) reported a decrease in both shoot and root fresh and dry weights with increasing salinity, and this feature was noted as a key indicator of salinity tolerance. Additionally, Hussein et al. (2007) found a negative relationship between vegetative growth parameters and increasing salinity.

Osmotic and ionic effects are generally accepted as the dominant factors hindering seed germination behavior under salt stress (Debez et al., 2004). Various studies have shown that germination responses are significantly affected by salinity and temperature. Salt stress includes osmotic and ionic stresses, leading to suppressed growth. The harmful effects continue as decreased plant survival or yield reduction. Conversely, one study indicated that there was no significant difference in germination percentage of Italian ryegrass between four different temperatures, and temperature alone was not a limiting factor in the absence of salinity if soil moisture was present (Lin et al., 2018). Özkan et al (2022) stated that the optimal temperature for seed germination of Italian ryegrass is between 20-30 °C.

The correlation graph evaluating the interaction of variety, salt, and temperature applications on the germination parameters of Italian ryegrass seeds supports the findings (Figure 1). Although germination rates vary by variety, the germination parameters are positively affected under conditions where low salt and high-temperature applications are combined. Additionally, the effect of three different salt doses (control, 5, and 10 EC) and three different temperatures (15, 20, and 30 °C) on germination parameters is supported by PCA analyses. According to the calculated

PCA analysis, the graphs explain 86% of the applications (Component 1 73.5%; Component 2 12.9%). When the obtained findings are examined, it is observed that there is a linear relationship between temperature and the germination of Italian ryegrass seeds. As the temperature increases, the tendency to form roots and shoots also increases (Figure 2).

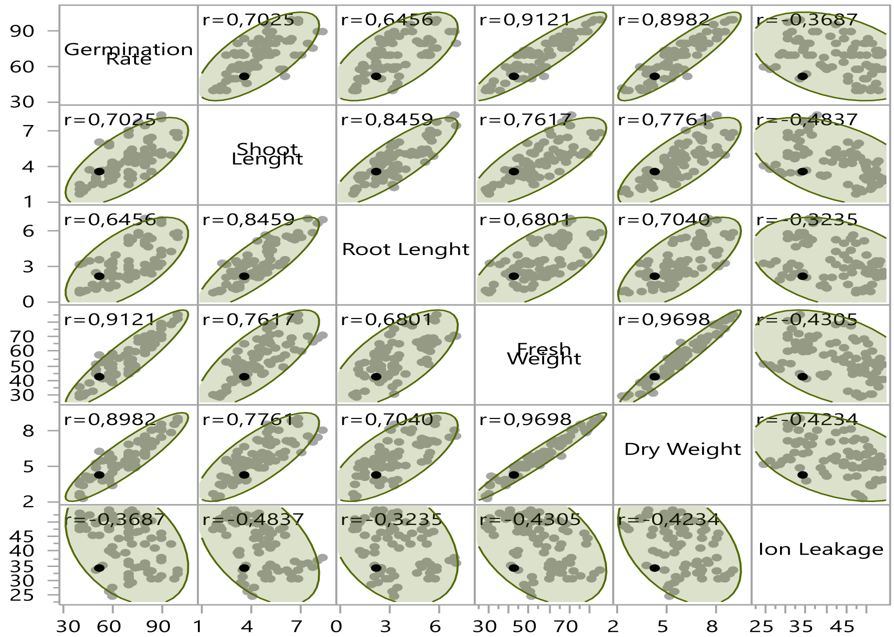

Figure 1. Scatterplot matrix and correlation of the germination parameters of Italian ryegrass with different salt and temperature treatments.

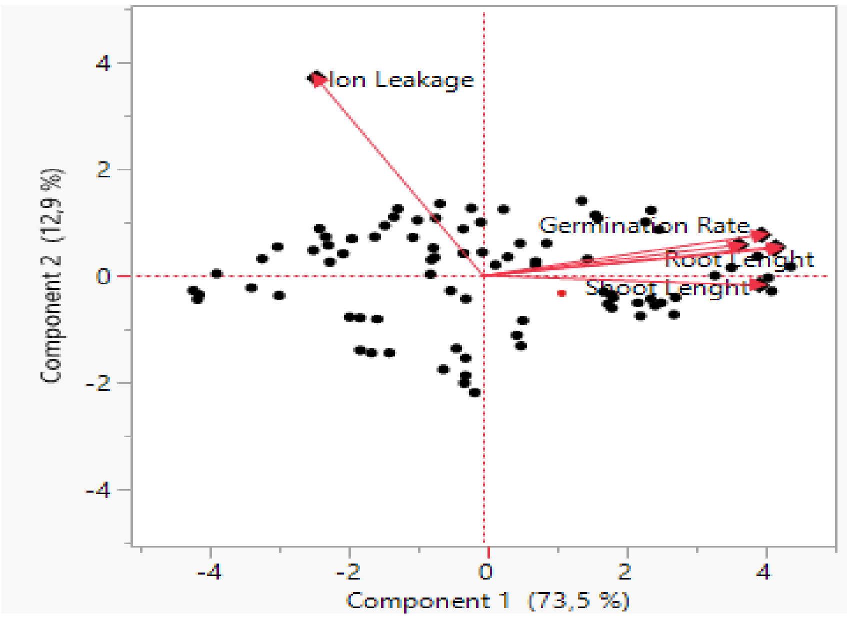

Figure 2. Principal Component Analysis (PCA) between germination parameters of Italian ryegrass with different salt and temperature treatments.

When looking at the ion leakage graph, it is seen that salt applications are positioned in the opposite direction to germination parameters. As the salt application in the environment increases, ion leakage also increases (Figure 2). The PCA graph evaluating the interaction of variety, salt, and temperature applications on the germination parameters of Italian ryegrass seeds also supports the findings obtained. Although germination rates vary by variety, the germination parameters are positively affected under conditions where low salt and high-temperature applications are combined. Under high-temperature conditions, similar results to the control were obtained when low salt doses were applied.

4. Conclusion

Salinity and high temperatures are significant abiotic stress factors that negatively impact plant growth and productivity, thereby limiting agricultural production. This study investigated the effects of different salt and temperature treatments on the germination parameters of Italian ryegrass seeds. The results indicated that salinity significantly inhibited the germination of Italian ryegrass seeds. As the salt concentration increased, a notable decrease in germination rate was observed. Increased salt stress also led to higher ion leakage, accompanied by ion toxicity. This is believed to obstruct water uptake in seeds, limiting germination as the environmental salt concentration rises. Similar results were obtained across the three varieties used in the study. Additionally, it was observed that the damage caused by salt stress decreased with increasing temperature. This study serves as a foundational investigation, and the grass species used here can be applied both as animal feed and for landscaping purposes. In the context of sustainable agriculture, responsible production and consumption, and addressing global climate issues, this research is valuable for breeding varieties that are resistant to salinity and temperature stress.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.O.	A.D.Ş.	S.U.
C	70	15	15
D	50	50	
S		30	70
DCP	60	30	10
DAI	60	30	10
L	30	40	30
W	30	40	30
CR	20	60	20
SR	20	60	20
PM	15	15	70
FA	10	10	80

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1519668

Open Access Journal
e-ISSN: 2618 – 6578

Review
Volume 7 - Issue 5: 570-579 / September 2024

ON VARIOUS SOIL MOISTURE MEASUREMENT TECHNIQUES AVAILABLE IN THE LITERATURE

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Abstract: The sustainability and health of soil associated with its productivity are closely related to soil moisture. The amount of water in the soil is an important parameter in the mobility of nutrients in the soil, in soil reclamation studies, and in the leaching-transportation and mixing of fertilizers and other chemicals applied to the soil into the groundwater. On the other hand, it has become important today not only to minimize water consumption but also to accurately measure the amount of moisture in the soil so that the proper amount of water that the plant needs can be circulated to the root zone of the plant. In this context, information was given about various measurement methods for determining soil moisture (direct and indirect - thanks to correlations) existing in the literature, and among these studies, especially the studies on microwave methods were examined. Comparison results of the dielectric constant values are obtained for three different soil samples with different gravimetric volumetric moisture rates by one of the calibration-independent non-resonant microwave measurement methods, which have a high application potential and is different from the existing calibration-requiring microwave measurement methods, and the dielectric constant values predicted by the Mironov model in the literature are presented. The comparison result showed that this new type of microwave measurement method has a high potential for measuring the moisture value of soil samples.

Keywords: Soil moisture, Direct and indirect methods, Microwave methods, Calibration-independent

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Received: July 20, 2024
Accepted: August 27, 2024
Published: September 15, 2024
Cite as: Hasar H. 2024. On various soil moisture measurement techniques available in the literature. BSJ Agri, 7(5): 570-579.

1. Introduction

The sustainability of the soil, which is related to the productivity and health of the soil, depends on the amount of water it contains (Orgiazzi et al., 2016). The productivity of soils is decreasing day by day due to salinity, desertification and destruction of agricultural lands (TOB, 2019). The World Meteorological Organization predicts that extraordinary weather events caused by climate change will increase day by day. In this context, sustainable and protectionist agricultural policies need to be brought to the fore for the future of our country (Atlı et al., 2021).

The amount of water in the soil is an important parameter in the mobility of nutrients in the soil, in soil reclamation studies, and in the washing-transportation and mixing of fertilizers and other chemicals applied to the soil into the ground water (Hupet and Vanclooster, 2002). Water in the soil can flow both horizontally and vertically. The flow of these two types of water provides not only the amount of water the plant can absorb, but also the mobility of nutrients in the soil and microbial diversity.

On the other hand, understanding the extent to which rainwater passes into the soil profile becomes important, especially in dry farming areas. The amount of water that needs to be given to the soil and the time of giving this water are important in terms of water saving. In this regard, it is necessary to measure the moisture content of the soil along the soil profile at different times in order to increase the soil water cycle. However, making measurements of this type (over different time and different soil layers) is not only costly for large agricultural lands (Moore et al., 1988), but also does not seem to be very profitable even in small lands in our country where agricultural soil properties change frequently.

In addition to the above reasons, it is important to accurately measure the amount of moisture in the soil in order to minimize water consumption and send as sufficient water as the plant needs to the root zone of the plant (Yetik and Aşık, 2021). This is also very important in creating and implementing a plant irrigation program, making fertilizer applications more effectively, saving water, and not putting the plant under water stress or leaving it dehydrated (Çetin, 2003).

When the literature studies on soil moisture measurement are examined (Öztaş, 1997; Uytun et al., 2013; Özbek and Kaman, 2014; Karaca, et al., 2017; Başdemir, 2020; Yetik and Aşık, 2021), it can be seen that these studies have no information about microwave measurement methods that have made significant progress in recent years. It contains no information or partial information. In this study, different methods will be briefly explained regarding the accurate determination of soil moisture, and then detailed information about microwave methods will be presented. Finally, unlike the calibration-requiring microwave measurement methods, the dielectric constant values obtained for three different soil samples with different gravimetric volumetric moisture ratios with a non-resonant measurement method that does not require calibration and has a high potential in determining soil moisture, which has recently entered the literature and has a high potential in determining soil moisture, were compared with the Mironov model in the literature. Comparison results with dielectric constant values obtained from a model known as are presented. The comparison shows that calibration-requiring microwave methods could have the potential to prevent the problems of low measurement sensitivity caused by calibration standards, and calibration-free microwave measurement methods could have a high potential in the accurate determination of soil moisture.

2. Various Direct and Indirect Methods Used in Soil Moisture Determination

There are different methods in the literature for faster and more reliable measurement of soil moisture. These methods can generally be divided into two groups: direct and indirect methods.

2.1. Direct Methods

These methods are gravimetric methods and are based on the principle of removing moisture from the soil sample by heating, washing, or chemicals and then finding the mass of water (Gardner, 1986). In the gravimetric method, samples are taken from different layers of the soil with the help of a simple hand auger (Figure 1).

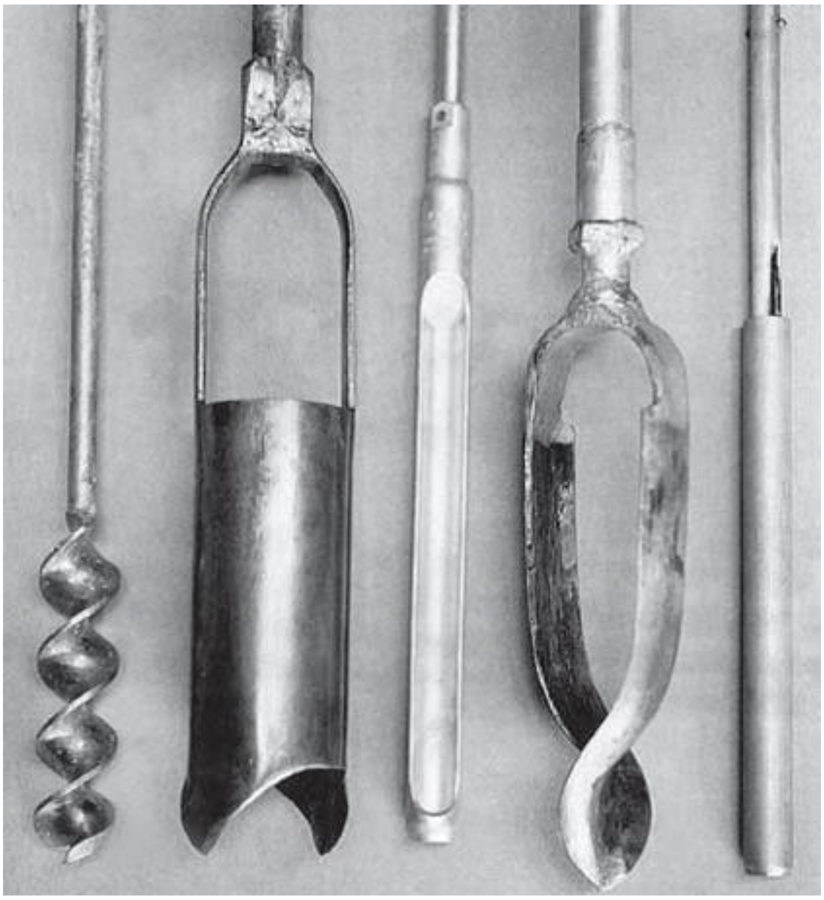

Figure 1. Image of various soil augers and tubes.

Sampling with a soil auger is both easy and allows taking soil samples of the sufficient quality. In measurements made with direct methods, soil moisture is made on a mass or volume basis. However, in cases where the soil area is large, requiring very different measurements, in fragmented lands where the soil has very different textures, and even in dry, hard, stony, and gravelly lands, sampling with a soil auger can be both challenging and time-consuming. At the same time, this situation causes the formation of macropores in the soil and consequently changes the moisture pattern of the soil (Kutilek and Nielsen, 1994).

2.2. Indirect Methods

Soil moisture measurements made by indirect methods are based on associating soil moisture with another property of the soil. In these methods, a correlation is generally tried to be established between the physicochemical properties of the soil and the moisture of the soil. One of the advantages of this method is that the applied sensors can remain in the soil for a long time during the analysis (they can provide information about soil moisture over a wide period of time). Another advantage is that the sensor placed in the soil does not adversely change the soil texture, compared to direct methods that are carried out multiple times. Additionally, measurements made with these methods are generally very fast (Öztaş, 1997).

There are indirect methods with different types and features in the literature: Time-space reflectometer, frequency-space reflectometer, time-space transmissometer, ground penetration radar, neutron meters, tensiometers, porous blocks, electrical resistance blocks and microwave methods (Uytun et al., 2013; Karaca, et al., 2017; Başdemir, 2020).

As one of the most frequently used indirect methods, the time-space reflectometer method determines the moisture content in the soil by means of multiple probes placed in the soil (Figure 2).

Figure 2. An image of a time-space reflectometer device (Soil Sensor, 2021a).

The lengths of the probes generally vary between 10-30 cm (Soil Sensor, 2021a). In this method, the process of determining soil moisture varies according to the principle of measuring the dielectric constant of the soil (Topp and Reynolds, 1998).

While time-space reflectometer measurements are based on the principle of reflection of short pulses within the soil texture in time space, frequency-space reflectometer measurements are based on reflection measurements in frequency space (Figure 3). Like time-space reflectometer measurements, frequency-space reflectometer measurements are based on dielectric constant measurements. The measurements made vary greatly with the volumetric water density in the soil. This is because radio frequency signals change significantly with the amount of moisture in the soil.

The working principle of the time-space transmissometer is parallel to the working principle of the time-space reflectometer. This method works on the response that occurs as a result of the signals transmitted from the soil sample propagating (travelling) along the length of the line containing the soil sample, as opposed to the response that occurs when the pulse generated in time-space is reflected from the soil sample (Figure 4). Although the shape of the transmitted waveform is not important, measuring the pulse travel time along the transmission line will allow an estimate of the dielectric constant of the medium. The speed of the pulse is related to the dielectric permittivity (Soil Sensor, 2021b).

As another indirect method, ground penetration radar is also frequently used. In this method, both transmission and reflection properties of electromagnetic signals in the range of 106-109 Hz in the soil are used (Figure 5). Operating frequency (center frequency), resolution and penetration depth are the basic parameters used in measurements made with this method (Daniels, 2007). Here, resolution means the ability to distinguish two signals in time space and generally increases with increasing operating frequency (Davis and Annan, 1989; Huisman and Bouten, 2001). On the other hand, the depth range decreases as the electrical conductivity of the soil increases. Measurements made in ground penetration radar measurements are highly affected by the penetration depth and electrical conductivity of the soil; because soil salinity and soil moisture content are two factors that strongly affect soil conductivity (Daniels et al., 1995; Huisman et al., 2003).

Figure 3. An image of a frequency-space reflectometer device (Experimental Hydrology, 2021).

Figure 4. An image of a time-space transmissometer device (Soil Sensor, 2021b).

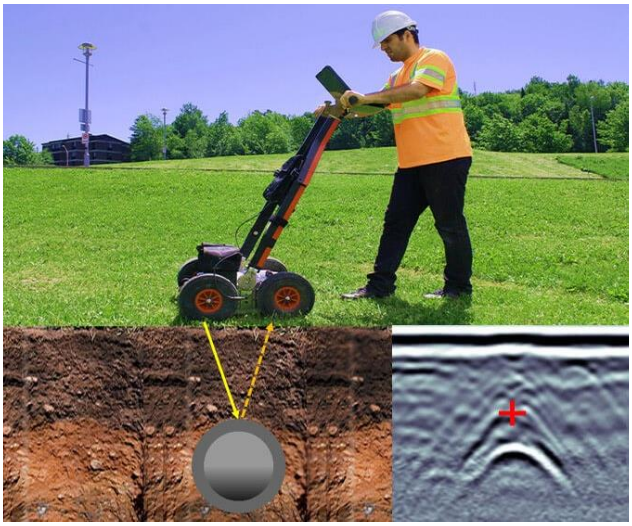

Figure 5. An image of a measurement made with a typical ground penetration radar device (Frimec, 2021).

In addition to the indirect methods mentioned above, neutronmeters are also among the methods used to determine the moisture content of the soil. The difference of this method from other indirect methods is that it provides soil moisture measurements at different times and layers in a simple, reliable, and fast way. Devices using this method consist of a radioactive source, a counter and a detector tube that counts the neutrons coming out of this source, which slow down after hitting the atoms in the soil (Figure 6). Calibration of neutronmeters is very important for reliable humidity determination.

Tensiometers, on the other hand, work with a principle based on soil water tension change. Tensiometers consist of cylindrical tubes with a diameter of approximately 2.54 cm, containing a vacuum at one end and a porous ceramic cup at the other end (Figure 7). The biggest advantage of tensiometers is that they provide data for long periods of time after being placed in the soil (Grattan and Oster, 2003).

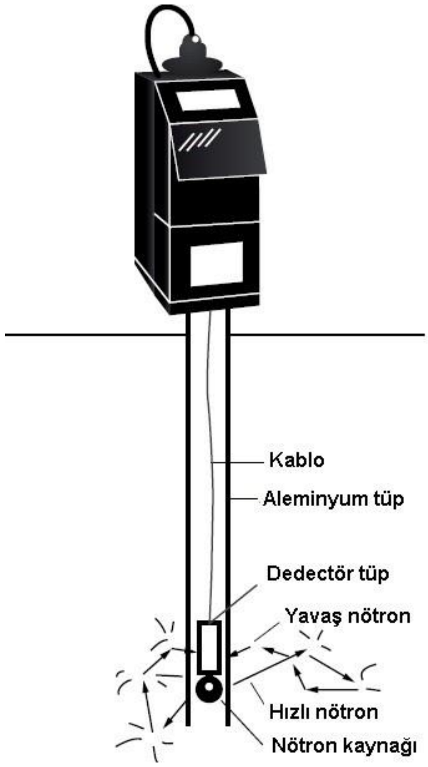

Figure 6. A typical image of the neutronmeter device (General Directorate of Meteorology).

Figure 7. Image of a tensiometer (Esi, 2021).

Another indirect method used to determine soil moisture is porous blocks made of nylon, fiberglass or ceramic. In this method, porous blocks are buried at a certain depth in the soil according to the physical, chemical and texture structure of the soil. In this method, the amount of soil moisture is determined according to the soil-water tension principle. In the indirect method of electrical resistance blocks, soil moisture determination is determined by the resistance value obtained from the principle of measuring the current passing through specific blocks and the voltage between these blocks against the current and voltage applied to the blocks. The sensitivity of the measurements is generally more suitable for determining soil waters with high resistance (Başdemir, 2020).

Another indirect method that can be used to determine soil moisture is measurements made with microwave signals. Microwave signals corresponding to the frequency range of 0.3-30 GHz are electromagnetic signals. The most important feature of microwave signals is that they include the 1-3 GHz frequency range, where the interaction of water in the soil is high (Pozar, 2011). Soil moisture determination using microwave signals is carried out by measuring the microwave signals reflected from and transmitted from the soil. Detailed information about these methods will be mentioned below.

Although they have advantages, the accurate results of indirect methods used in soil moisture determination directly depend on calibration and environmental factors (Topp and Ferre, 2002).

3. Information about Indirect Microwave Methods Used in Soil Moisture Determination

As mentioned above, microwave methods that use the correlation between the electromagnetic properties of soil samples and their moisture content are in the class of indirect methods. We can divide the various microwave measurement methods in the literature into two separate groups: resonant and non-resonant (Chen et al., 2004). In resonant-type microwave methods, the electromagnetic properties of the samples are determined by placing the sample, shifting the resonant frequency and using a combination of the magnitude of the resonant frequency. These methods, which generally work on the principle of the low-loss sample assumption (low perturbation), are more suitable for determining the electromagnetic properties of low-loss samples. Another disadvantage of these methods is that measurements are limited to only a single resonance frequency or frequency range in a narrow band (Lonappan et al., 2009; Sheen 2009).

On the other hand, non-resonant microwave techniques allow electromagnetic characterizations to be made in a much wider frequency range compared to resonant microwave techniques. This is especially important in terms of determining the dielectric constant of soil samples with different moisture contents. In addition to this advantage, unlike resonant microwave methods, non-resonant microwave methods require less sample preparation in determining the moisture of soil samples (Afsar et al., 1986; Baker-Jarvis et al., 1995; Zoughi, 2000; Carriveau and Zoughi, 2002; Chen et al., 2004; Kharkovsky and Zoughi, 2007; Kaatze, 2010;)

Non-resonant microwave measurement methods with various features are also generally included (Nicolson and Ross, 1970; Weir, 1974; Chen et al., 2004; Chalapat et al., 2009; Hasar and Westgate, 2009), wave-directed measurement techniques (Nicolson and Ross, 1974). 1970) and free-space measurement techniques (Ghodgaonkar et al., 1990). In wave-directed methods, a wave-emitting medium is needed to direct the microwave signals generated from the source in a certain direction. Examples of this medium are waveguide or coaxial cables used to transmit microwave signals. In free-space methods, measurements are carried out by transmitting these signals directly in the air (without the need for any physical medium) without the need to direct the generated microwave signals in a certain direction (Varadan et al., 1991).

Non-resonant microwave techniques can be divided into two groups: calibration-requiring and (ordinary) calibration-free methods. As the name suggests, calibration-requiring non-resonant microwave methods require the calibration of the measurement system before performing sample-related measurements (characterization processes). Since calibration standards are used in calibration processes, the slightest deviation from the ideal in these standards may negatively affect the sensitivity of the calibrations (Wan et al., 1998a; Wan et al., 1998b). The calibration process performs the calibration by including the calibration environment in which the measurements are made. This means that microwave measurements performed with calibration will be affected by environmental factors such as humidity and pressure in the measurement environment. To simultaneously eliminate these two disadvantages of calibration-requiring methods, calibration-free microwave methods can be used in sample characterization processes (Wan et al., 1998a; Wan et al., 1998b; Janezic and Jargon, 1999; Reynoso-Hernandez et al., 1999; Huynen et al., 2001; Guoxin, 2015; Hasar et al., 2023).

By way of example, Figure 8 shows a schematic view of the soil sample placed on the sample holder inside the coaxial cable from non-resonant microwave coaxial cable measurements. Under normal circumstances, before non-resonant microwave coaxial cable measurements, it is necessary to calibrate the measurement system, the schematic of which is shown in Figure 8. For example, the calibration method suggested in the recent study (Lewandowski et al., 2019) can be used for this calibration. Figure 9 shows a photograph of the non-resonant microwave coaxial cable measurement setup proposed in (Hasar et al., 2023).

Figure 10 illustrates dielectric constant values for three different soil samples (SI, SII, and SIII) with different gravimetric volumetric moisture rates (θ_V), at a frequency of 1.4 GHz with the calibration-free non-resonant microwave coaxial cable measurement method proposed in the study of (Hasar et al., 2023). All the soil samples have 90% or more sand content and were taken from various places in Gaziantep in 2021. The realized dielectric constant values (ε'_{rs}) are reported in the literature by Mironov et al. (2013) shows the comparison ($\Delta\varepsilon'_{rs}$ - difference in determined ε'_{rs} values) with the dielectric constant values obtained from the model developed. As can be seen from this figure, ε'_{rs} values measured for 3 different soil samples with different gravimetric volumetric moisture rates and those values predicted by the Mironov et al., model developed are in good agreement with each other (less than 5% difference). In light of the information given in Figure 10, it can be said that the sensitivity of microwave non-resonant methods that do not require calibration is at a good level.

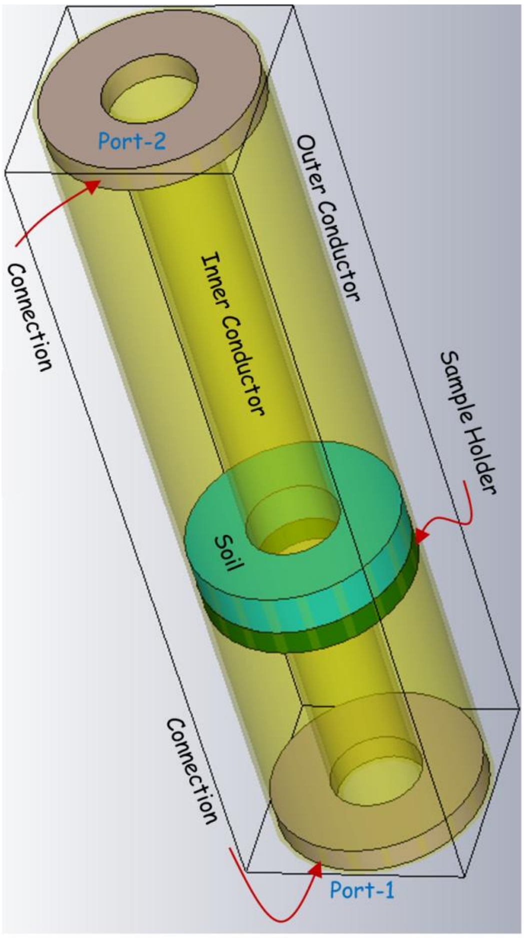

Figure 8. The schematic of a non-resonant microwave coaxial cable measurement setup (Hasar et al., 2023).

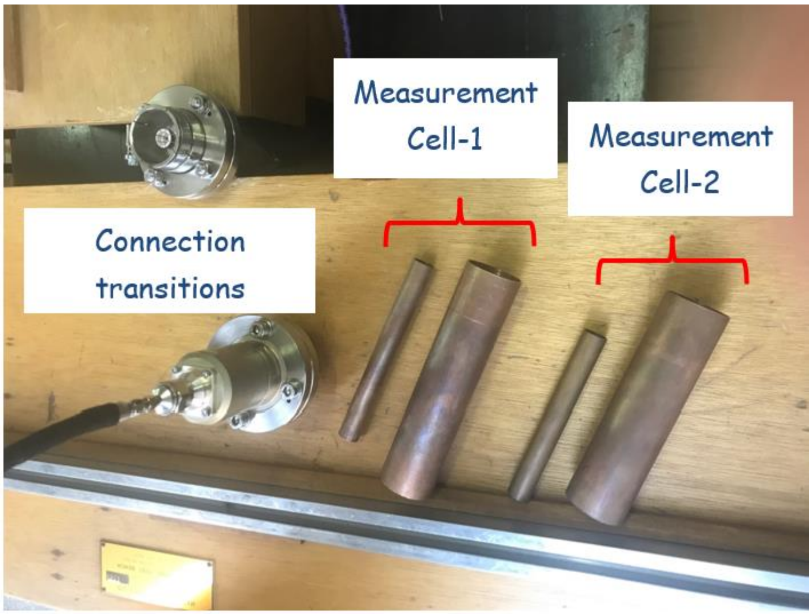

Figure 9. The photograph of non-resonant microwave coaxial cable measurement setup (Hasar et al., 2023).

S_{I}	$\theta_V = 0,31$			$\theta_V = 0,38$		
	Meas	Model	$\Delta\varepsilon'_{rs}$ (%)	Meas	Model	$\Delta\varepsilon'_{rs}$ (%)
	18, 272	19, 155	4, 610	24, 951	25, 027	0, 304
S_{II}	$\theta_V = 0,25$			$\theta_V = 0,34$		
	Meas	Model	$\Delta\varepsilon'_{rs}$ (%)	Meas	Model	$\Delta\varepsilon'_{rs}$ (%)
	14, 918	14, 746	1, 166	21, 529	21, 576	0, 218
S_{III}	$\theta_V = 0,21$			$\theta_V = 0,42$		
	Meas	Model	$\Delta\varepsilon'_{rs}$ (%)	Meas	Model	$\Delta\varepsilon'_{rs}$ (%)
	11, 732	12, 126	3, 249	27, 334	28, 735	4, 876

Figure 10. The dielectric constant values obtained at 1.4 GHz frequency with the calibration-free non-resonant microwave coaxial cable measurement method proposed in the study of (Hasar et al., 2023) are similar to those in the literature by Mironov et al., Comparison with the dielectric constant values obtained from the model developed (Mironov and Fomin, 2009; Mironov et al., 2013).

4. Conclusion

In this study, information is given about indirect and direct measurement methods used to determine soil moisture. In this regard, firstly the gravimetic method, which is a direct method, is explained, and then the time-space reflectometer, frequency-space reflectometer, time-space transmissometer, ground penetration radar, neutron meters, tensiometers, which are frequently used in the literature as indirect methods (thanks to the provision of correlations) for the determination of soil moisture. Information is given on porous blocks and electrical resistance blocks. Subsequently, general information is presented about microwave measurement methods, which have just begun to take their place in the literature and have great potential for the determination of soil moisture. Finally, information about non-resonant microwave measurement methods that do not require calibration, which constitute a special class of microwave measurement methods, is presented. The dielectric constant values obtained by one of these methods for 3 different soil samples with different gravimetric volumetric moisture rates were compared with those in the literature. It is compared with the dielectric constant values obtained from the developed model. As a result of the comparison, it was observed that for three different soil samples with different gravimetric volumetric moisture rates, the dielectric constant value obtained from the measurements and the dielectric constant values obtained from the model were in agreement with each other (less than 5% difference). This shows that calibration-free microwave measurement methods, which have the potential to avoid the problems of low measurement sensitivity caused by calibration standards, have high potential in the accurate determination of soil moisture.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	H.H.
C	100
D	100
S	100
L	100
W	100
CR	100
SR	100
PM	100

C=Concept, D= design, S= supervision, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The author declare that there is no conflict of interest.

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Black Sea Journal of Agriculture

doi: 10.47115/bsagriculture.1495873

Open Access Journal
e-ISSN: 2618 – 6578

Review
Volume 7 - Issue 5: 580-595 / September 2024

A REVIEW OF THE EFFECT OF CLEANER ENERGY TRANSITION ON FOOD PRICES AND CLIMATE CHANGE MITIGATION IN SUB-SAHARAN AFRICA: NIGERIA AS A CASE STUDY

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Abstract: Access to clean energy is crucial for achieving sustainable development goals, but investment in renewable energy has been unevenly distributed between developed and developing countries. Developed nations have seen significant growth in renewable energy investment since the Paris Agreement, while developing countries struggle to secure the necessary funds. This study focuses on Sub-Saharan Africa and explores the relationship between the transition to cleaner energy and its impact on food prices. By conducting a systematic literature review, the study highlights the challenges posed by investment disparities, particularly in Sub-Saharan Africa, where financial constraints and infrastructural deficits hinder progress in clean energy infrastructure. The findings suggest that, without appropriate policy reforms and consistent implementation, the transition to cleaner energy in the region may contribute to rising food prices and exacerbate food insecurity. Effective integration of agricultural and energy policies is essential to ensure that the energy transition supports food security objectives. Governments in Sub-Saharan Africa should prioritize policy reforms that promote renewable energy adoption while considering food security. Additionally, reintroducing transparent subsidy programs can help mitigate the impact of high energy costs during the transition to cleaner energy. This review emphasizes the importance of equitable investment and comprehensive policy strategies to balance renewable energy adoption with food security and economic equity in Sub-Saharan Africa.

Keywords: Renewable energy investment, Sub-Saharan Africa, Food prices, Policy reforms, Energy transition

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Received: June 04, 2024
Accepted: August 26, 2024
Published: September 15, 2024

Cite as: Ikuemonisan E. 2024. A review of the effect of cleaner energy transition on food prices and climate change mitigation in sub-Saharan Africa: Nigeria as a case study. BSJ Agri, 7(5): 580-595.

1. Introduction

According to the Global Hunger Index, there has been a steady increase in global hunger since 2014. The number of undernourished individuals increased from 628.9 million in 2014 to 687.8 million in 2019. The statistics remained unchanged in 2022, but there was a slight increase to 735 million in 2023. There is ample evidence indicating that severe hunger is particularly prevalent in South Asia and Sub-Saharan Africa (SSA), both of which have registered a Global Hunger Index of 27.0. This has posed an increasingly daunting challenge to improving productivity, especially in SSA. The 2020 Global Report on Food Crises highlighted SSA as the site for five of the ten worst food crises globally, with the number of victims of chronic hunger increasing from 234.7 million people in 2019 to 282 million people in 2023 according to IFPRI's 2023 Global Food Policy Report. This region, despite its abundant water and arable land resources, continues to grapple with rising food insecurity, accentuated by factors such as postharvest losses, inadequate deployment of ICT along agricultural value chains, conflict, climate change, economic downturns, pest infestations, increased food import costs, reduced agricultural investment, and Ebola and COVID-19 outbreaks, and the repercussions of the Russia-Ukraine war have collectively intensified in recent years (Matthew et al., 2023; Akpa et al., 2023; Li et al., 2024). The evidence that these shocks have led to soaring food prices and diminished food availability and accessibility and have driven millions into hunger and poverty is well documented in the literature (Swinnen et al., 2023).

In addition, the prevalent reliance on biomass fuels due to limited access to clean energy sources not only hampers economic growth and poverty alleviation efforts but also contributes to health and environmental hazards (Edafe et al., 2023; Li et al., 2024). HLPE (2021) and von Grebmer et al. (2023) reported that youth in this subregion are the most impacted by deteriorating and increasingly vulnerable food systems. The interplay of these factors sets a precarious stage for food security in SSA, amplifying challenges related to poverty, nutrition, health, and economic stability.

Therefore, the general consensus among experts and scholars is that understanding the intricate drivers behind food price fluctuations is paramount for designing effective policies that can safeguard food security, mitigate inflationary pressures, and promote sustainable development in SSA and beyond. Many scholars have explored the intersections between food prices and institutional quality to advance relevant policies and resolve conflicts (Rossignoli and Balestri, 2018); food prices and climate-related challenges (Brown, 2014); integrating climate change, food prices and population health (Bradbear and Friel, 2013); and food prices and the combined effects of global financial, energy and food crises (Lagi et al., 2012). Despite these and many policies developed to address the associated challenges, many puzzles around food security, particularly as it concerns rising food prices, in the subregion remain unresolved. More than 20% of the population remains significantly malnourished (FAO, IFAD, UNICEF, WFP, WHO, 2022). This poses a threat to the realization of sustainable development goals, especially those associated with poverty alleviation (SDG 1) and food security (SDG 2).

At the centre of the efforts of governments in the subregion is the compelling need to resolve the problem of availability and affordable sources of energy. Africa, despite being one of the regions most affected by climate change and home to one-sixth of the global population, accounts for less than 6% of global energy consumption and 2% of cumulative global emissions (International Energy Agency (IEA), 2014). However, extending clean energy access to millions is crucial for economic growth and achieving sustainable development goals. The continent's geographic diversity offers significant potential for renewable energy, particularly solar, wind, hydropower, and geothermal energy, which could propel Africa towards fulfilling the Paris Agreement and limiting the global temperature rise to 1.5 °C. However, the current state of energy security in the region shows a large energy deficit (Alemzero et al., 2021), and requires huge capital outlay to erect necessary infrastructure to achieve the desired results on energy for all in the sub-region.

This debate on cleaner energy and food prices receives significant attention recently because of its ecological and socioeconomic dimensions. Take for instance, lack of energy and power increases the potential for food waste. Food waste is a critical challenge that exacerbates food insecurity and environmental degradation. There are staggering statistics that one-third of all food produced for human consumption is lost or wasted, amounting to 1.3 billion tonnes annually. Global food waste has reached alarming 40%. And this loss not only represents a missed opportunity to feed nearly nine billion people by 2050 but also contributes significantly to greenhouse gas emissions, further aggravating climate change. The case of food waste in SSA is pathetic because of very low access to energy and power. Consequently, the potential for food wastes and/or costs of proper storage using cleaner energy create avenue for food producers and other actors along the food value chains to include risk premium in food prices. This puts pressure on countries in SSA, even as the population is rising, to provide energy infrastructure that will reduce the overhead cost of food production and distribution in order to increase food output.

Countries such as South Sudan, Burundi, Chad, and Malawi have the lowest electricity access rates in sub-Saharan Africa, ranging from 7.7% to 14.2%, and face significant challenges in providing clean cooking facilities (IEA, 2021a). In contrast, Ghana, Kenya, and Rwanda have made notable progress towards achieving full electricity access by 2030 (Climate Analytics, 2022b; IEA, 2021b). However, the COVID-19 pandemic has adversely affected the progress of energy and power access across the region. In Nigeria, despite a population increase from 120 million to over 200 million in the last decade, power generation has stagnated at 4000 MW over two decades. The country's national grid connects only 40% of households, with weak distribution infrastructure causing approximately 35% of generated power to be lost during distribution. This gap between electricity supply and demand is widening, exacerbated by an unstable grid prone to sabotage, leading many to rely on personal generators (Nucho, 2022). The overall situation underscores the significant disparities in energy access and the compounded difficulties in achieving reliable and sustainable electricity infrastructure in the region.

The Nigerian energy situation is presented in Figure 1. The International Energy Agency's data on Nigeria's energy consumption, with 77.9% used residential energy, 8.71% used industrial energy, and only 0.34% used agricultural energy, highlight significant energy security challenges. High residential demand strains infrastructure, leading to frequent outages and limited energy for crucial sectors such as industry and agriculture, hindering economic growth and development. Insufficient industrial energy allocation stifles expansion and increases reliance on imports, while negligible energy use in agriculture severely limits productivity and rural development. This disproportionate energy distribution increases vulnerability to disruptions and raises sustainability concerns (Olujobi et al., 2023). However, a number of countries have addressed these disruptions by implementing policies to balance energy distribution, invest in renewable sources, enhance residential energy efficiency, and allocate more energy to industry and agriculture, supported by targeted subsidies and incentives for renewable energy adoption (Alemzero et al., 2021).

Figure 2 reveals the per capita energy consumption and energy generation trends in selected sub-Saharan African countries, comparing them with continental and global averages. Energy consumption and generation are essential for economic development and quality of life, yet sub-Saharan Africa (SSA) exhibits significant disparities compared to global standards. In 2022, the global average per capita energy consumption was 21039 kWh, with South Africa having a slightly greater average of 22351 kWh, highlighting its advanced energy infrastructure. In contrast, Africa's average was only 3944 kWh, indicating widespread energy poverty. Ghana's consumption was 3483 kWh, while Nigeria's consumption fluctuated from 1250 kWh in 2009 to 2458 kWh in 2021, reflecting its energy instability. Globally, energy generation totalled 167788 TWh in 2022, with Africa contributing just 5627 TWh. South Africa generated 1339 TWh, whereas Nigeria's output was only 544 TWh, underscoring its inadequate generation capacity. This imbalance in SSA, with low per capita consumption and insufficient generation, hampers economic growth and quality of life (International Energy Agency, 2022). To improve energy security, SSA must invest in renewable energy sources and enhance energy infrastructure, particularly in transmission and distribution, to ensure sustainable economic development (IEA, 2022).

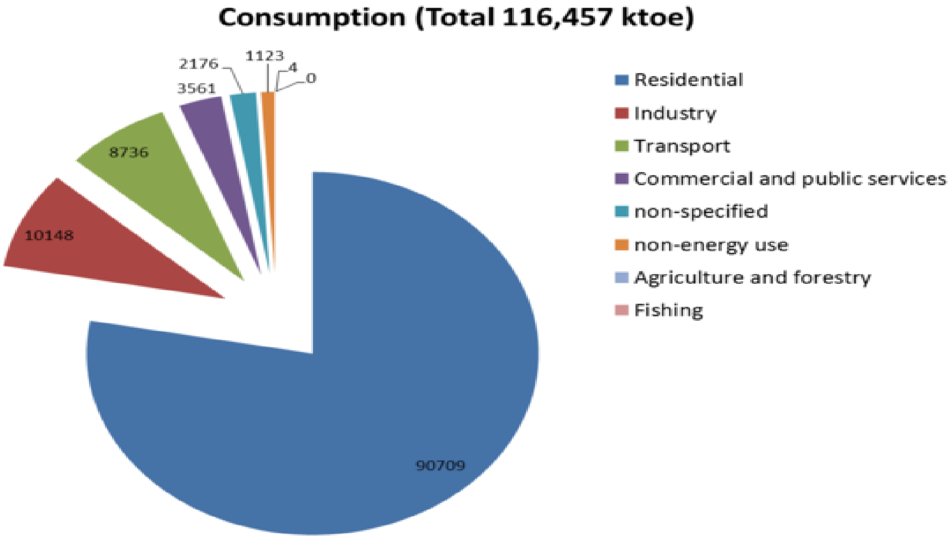

Figure 1. Nigerian energy situation (International Energy Agency, 2022).

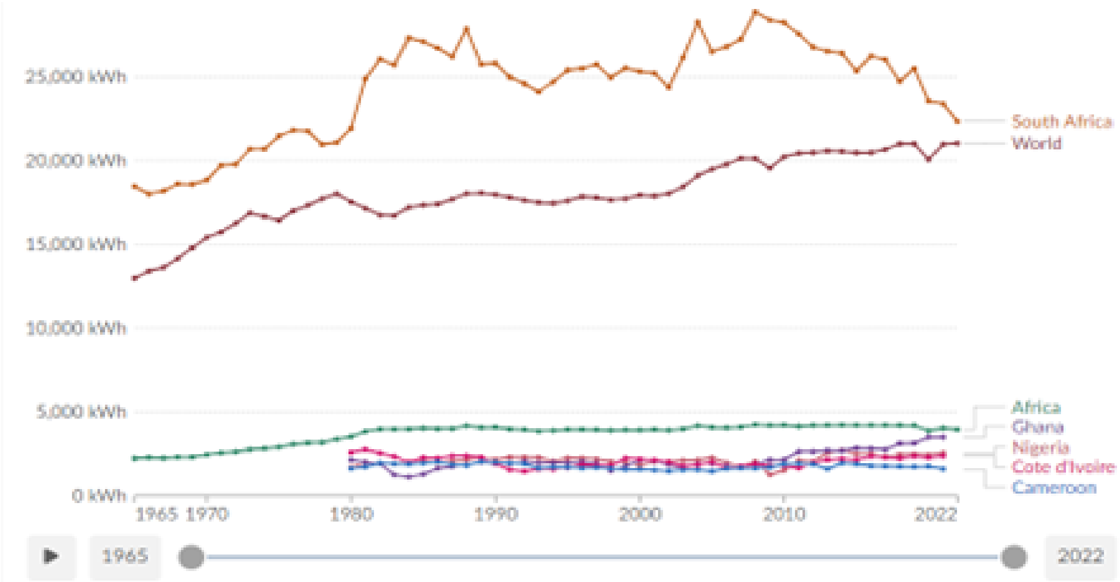

Figure 2. Trend in average energy consumption per person (Energy Information Administration, 2023).

The disparities in energy consumption and generation between sub-Saharan Africa and global averages have profound implications for sustainable development in the subregion (IEA, 2022). In view of this, there is concern that limited energy access, particularly in Nigeria and Ghana, hinders economic activities, educational opportunities, and overall quality of life. The stark contrast in energy generation between South Africa and Nigeria highlights the potential investment opportunity for substantial investment in energy infrastructure across the region. In Nigeria, the demand for energy significantly outweighs the energy supply (Olaoye et al., 2016); hence, the current reliance on traditional energy sources necessitates a strategic shift towards renewable energy (Nnaji et al., 2013; Climate Analytics, 2022a). Effective energy policies and regulatory frameworks are essential for enhancing energy production and distribution, reducing losses, and improving energy access, thereby driving economic growth and development (IEA, 2014). Enhancing energy infrastructure and expanding renewable energy capacities can attract foreign investment, boost industrial productivity, and create jobs, positioning sub-Saharan Africa as a competitive player in the global economy (Climate Analytics, 2022b).

The extensive body of literature and numerous advocacy efforts regarding the global shift from non-renewable energy, which poses threats to human habitats, to renewable and cleaner energy underscore the emerging paradigm in the energy sector. However, this transition is significantly impeded by high capital costs, which have widespread implications for the entire energy system (IEA, 2022). The UNCTAD's World Investment Report 2023 reveals a significant disparity in the distribution of international investment in renewable energy. Since the Paris Agreement's inception in 2015, investment in renewable energy has almost tripled, but the bulk of this growth has been concentrated in developed nations. In contrast, developing countries, which require approximately $1.7 trillion annually for renewable energy investments, only managed to secure $544 billion in foreign direct investment for clean energy in 2022. Moreover, the overall financial requirements for transitioning to sustainable energy systems in developing countries are considerably greater. These needs encompass investments in power grids, transmission lines, storage solutions, and energy efficiency improvements. UNCTAD Secretary-General Rebeca Grynspan emphasized that boosting investment in sustainable energy systems within developing nations is critical for achieving global climate targets by 2030.

Therefore, adopting renewable energy is a pressing concern in the global pursuit of a sustainable future. According to Demetrios Papathanasiou, Global Director of Energy and Extractives at the World Bank, "Poorer countries are caught in a vicious cycle where they end up paying more for electricity, cannot afford the substantial initial investment required for clean energy, and remain dependent on fossil fuel projects. Essentially, these countries face a triple penalty during the energy transition." This situation creates a poverty trap that transforms into an energy trap and, ultimately, a climate trap. The consequences are increasingly being felt in the agricultural industry. Interestingly, numerous scholarly works have demonstrated that varying energy costs, depending on the source, result in higher operational costs for agricultural production (Samson et al., 2005; Pimentel et al., 2008; Wood et al., 2010; Intergovernmental Panel on Climate Change [IPCC], 2022), while climate change increases the frequency of extreme weather events, alters growing conditions, and reduces crop yields, which ultimately influence rising food prices.

Similarly, there has been a concentration of studies on the positive effects of renewable energy adoption on enhancing people's welfare and economic well-being (Arraiz and Calero, 2015; Chakravorty et al., 2016; Bonan et al., 2017), leaving a critical gap in the implications of the unintended effects of the transition. For instance, little is known about the relationship between the transition to cleaner energy and food prices. However, the points of divergence and convergence in the narrative of sub-Saharan Africa's transition to cleaner energy, including the move to embrace biofuel energy as alternatives to fossil fuels, can be a necessary inflexion for policy considerations. This study delves into the complex relationship between the transition to cleaner energy and food prices in sub-Saharan Africa, shedding light on climate change mitigation. Therefore, by examining the related literature, this review aims to contribute to a comprehensive understanding of the impact of renewable energy adoption (cleaner energy) on rising food prices in sub-Saharan Africa.

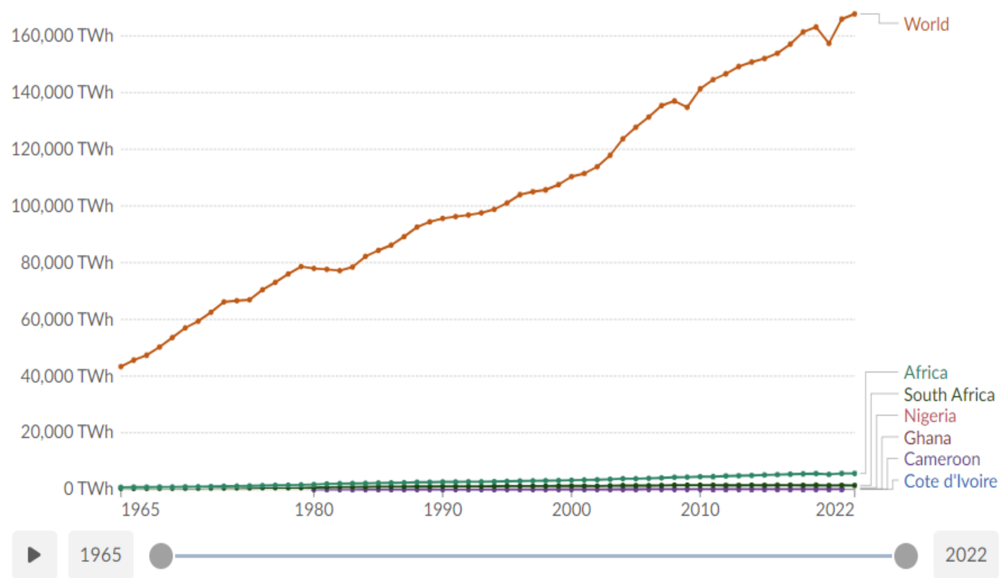

Figure 3. Trends in the quantity of energy generated each year across selected countries in sub-Saharan Africa

compared to Africa and the world average (Energy Information Administration, 2023).

1.1. Conceptual Framework

This conceptual framework illustrates the interactions and feedback loops between various sectors and their impacts on food prices and climate change mitigation in Sub-Saharan Africa. The diagram is centered on four main components: Food Production & Food Value Chain, Fossil Fuel, Renewable Energy, and Government Policy, with additional elements considering the economic and social effects of energy use.

Efficient farming practices and food systems are critical in determining the productivity and sustainability of food production. The outcomes of efficient farming and food systems are pivotal in reducing food prices and enhancing food security (Godfray et al., 2010). The economic and social effects of using fossil fuels in food production include increased costs, environmental degradation, and health impacts, which all play significant roles in the overall viability and sustainability of food production systems (Tilman et al., 2002; FAO, 2011).

Government policies influence the use of fossil fuels through regulations, subsidies, and taxes, directly affecting the fossil fuel industry and its integration into the power and energy sector (IEA, 2020). The fossil fuel industry supplies essential energy to the power sector, which supports various activities within the food production and food value chain. However, the economic and social effects of fossil fuel use are broad, including higher production costs, adverse environmental impacts, and negative social welfare implications (World Bank, 2014).

Policies aimed at promoting renewable energy can incentivize the transition away from fossil fuels, including investments, subsidies, and regulatory support for renewable energy sources (IRENA, 2022). The integration of renewable energy into the power sector can significantly reduce dependency on fossil fuels, leading to cleaner energy production and use (REN21, 2020). The shift to renewable energy sources brings substantial economic and social benefits, such as potential reductions in energy costs, creation of green jobs, and improved public health outcomes (UNEP, 2019).

Government policies play a critical role in supporting renewable energy development, driving the transition to cleaner energy and impacting the power sector and overall energy mix (IRENA, 2022). Regulatory frameworks can either constrain or promote the use of fossil fuels, affecting their role in the energy market (OECD, 2020; Lankoski and Thiem, 2020). Policies also influence food production through agricultural subsidies, food safety regulations, and support for sustainable practices, thereby shaping the sustainability and efficiency of the food production and value chain (FAO, 2018).

As renewable energy is integrated into the power sector, it can progressively replace fossil fuels, leading to cleaner energy production (IEA, 2021a). Traditionally reliant on fossil fuels, the power sector's transition to renewable energy can significantly impact the sustainability and efficiency of energy use within the food production and food value chain (IRENA, 2022).

The economic and social effects of fossil fuel use include increased costs due to environmental damage, health impacts, and social disparities (World Bank, 2014). Conversely, the economic and social benefits of renewable energy use are substantial, including lower greenhouse gas emissions, reduced energy costs, and improved public health, all contributing to a more sustainable and equitable energy and food production system (UNEP, 2019).

Efficient farming and food systems lead to more sustainable and cost-effective food production, reducing prices and improving food security (Godfray et al., 2010). The positive outcomes of efficient farming practices are critical for achieving a sustainable and resilient food production system in Sub-Saharan Africa (FAO, 2011).

Feedback loops between government policy and the use of fossil fuels or renewable energy show that policies influence the energy mix, which in turn affects economic and social outcomes, feeding back into policy adjustments (IEA, 2020). The interaction between the power and energy sector and the food production and value chain indicates that the efficiency of energy use impacts food prices and availability (FAO, 2018). Furthermore, the economic and social effects of energy use influence farming practices and food systems, leading to more sustainable and efficient outcomes (Tilman et al., 2002).

This conceptual framework highlights the complex interactions between energy use, government policy, and food production, emphasizing the need for a multidisciplinary approach to address food prices and climate change mitigation in Sub-Saharan Africa. By transitioning to cleaner energy sources and promoting efficient farming practices, it is possible to achieve significant economic, social, and environmental benefits (UNEP, 2019; IRENA, 2022).

1.2. Significance of the Review

The proposed framework highlights crucial areas where our understanding of the food system and its energy use needs enhancement. Despite significant research, there are notable gaps, particularly in regional variability of energy inputs and the indirect contributions from fertilizers, pesticides, and machinery (Pelletier and Tyedmers, 2010; Finley and Seiber, 2014). Integrating renewable energy sources into agricultural practices remains underexplored, especially in developing countries, where it could significantly reduce costs and increase productivity (FAO, 2023). Additionally, most studies have focused on large-scale operations, neglecting the impact on small-scale and subsistence farmers (Haggblade et al., 1989; Cleaver, 1993). Developing robust economic and policy frameworks to

support the transition to cleaner energy in agriculture is also essential (Stoneman and Robinson, 1987).
Agriculture's dependency on energy inputs varies significantly between industrialized and developing countries, impacting productivity and sustainability (Pelletier and Tyedmers, 2010). Mechanization has boosted productivity but increased energy consumption, requiring a balance between energy efficiency and environmental sustainability (Finley and Seiber, 2014). Conservation agriculture practices, such as reduced tillage and maintaining soil cover, can enhance soil health and reduce energy use but necessitate changes in traditional farming methods (FAO, 2023). Addressing these gaps involves comprehensive research, data collection, and the integration of renewable energy systems, alongside policies that consider both agricultural and rural energy needs (Stoneman and Robinson, 1987; Cleaver, 1993). This approach can enhance agricultural productivity and food security while minimizing environmental impacts.

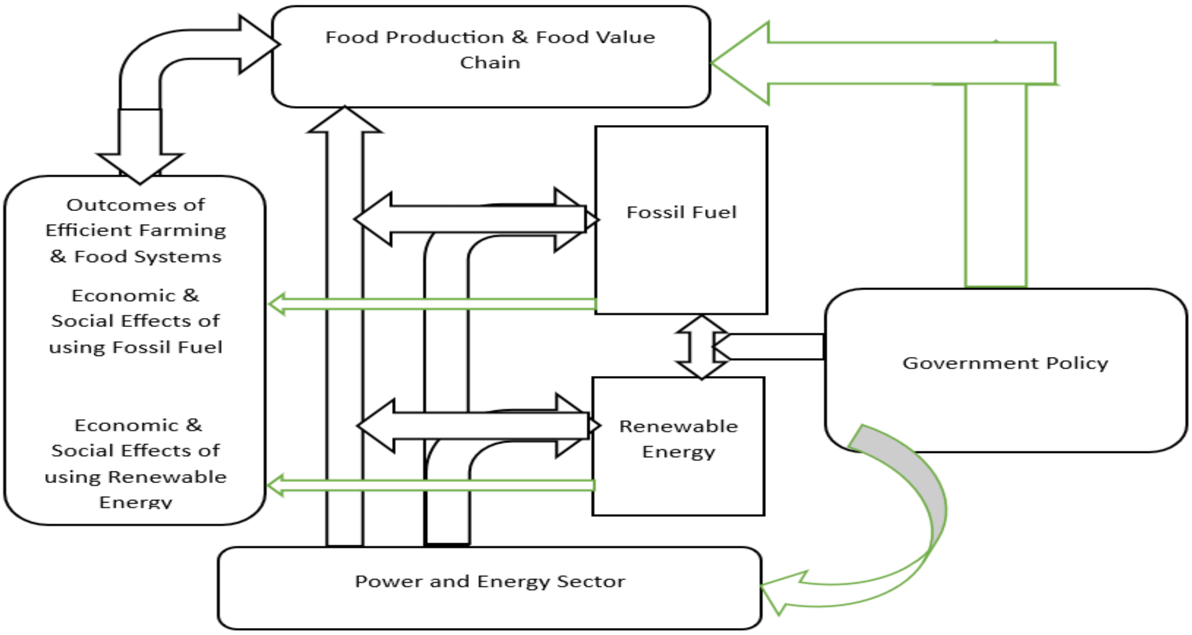

Figure 4. Conceptual framework.

This study addresses a critical gap in the literature concerning the relationship between the transition to cleaner energy and food prices in Sub-Saharan Africa (SSA). While existing research has extensively examined food security, energy access, and climate change individually, there is a lack of integrated studies that explore their combined effects on food prices in SSA. Previous studies have predominantly focused on the intersections between food prices and institutional quality, climate-related challenges, the integration of climate change with food prices and population health, and the impacts of global financial, energy, and food crises on food prices. However, the specific interplay between renewable energy transition and food prices remains underexplored, which is significant for policy-making and sustainable development.
The study aims to fill this gap by understanding how investments in energy infrastructure and the shift to cleaner energy sources affect agricultural productivity and food prices. It also seeks to assess the broader socio-economic consequences of energy transition policies on food security in SSA and provide insights into how renewable energy adoption can either mitigate or exacerbate food insecurity, considering the region's unique challenges. By exploring this complex relationship, the study intends to contribute to a comprehensive understanding of how renewable energy adoption impacts rising food prices in SSA, integrating themes of renewable energy investment disparities and food security issues to offer a novel perspective.

2. Methodology

The research question for the study was meticulously formulated and addressed using a structured approach. Initially, the study checked for existing reviews and protocols to determine if the review was still needed. This involved a comprehensive search of databases such as PubMed, Scopus, and Google Scholar to identify any recent reviews on the topic. Although there were some related reviews, none comprehensively covered the specific impacts of cleaner energy transitions on both food prices and climate change mitigation in Sub-Saharan Africa, highlighting a significant gap in the literature (Peters et al., 2015; Smith et al., 2018). This affirmed the necessity of this review and precluded duplicating previous efforts.
The study then clearly identified the research question: How does the transition to cleaner energy sources affect food prices and climate change mitigation in Sub-Saharan Africa? This question was formulated to be specific,

focused, and of appropriate scope. To define our terminology, the study outlined key concepts such as "cleaner energy transition," "food prices," "climate change mitigation," and "Sub-Saharan Africa" (Jones et al., 2017). This step ensured clarity and consistency throughout the review process.

Next, the study defined inclusion and exclusion criteria to establish a review protocol. Studies were included if they focused on Sub-Saharan Africa, examined the transition to renewable energy sources, and assessed impacts on food prices or climate change mitigation. Exclusion criteria included studies not in English, those not peer-reviewed, or studies focusing on regions outside Sub-Saharan Africa (Higgins and Green, 2011). This step was crucial in ensuring the relevance and quality of the included studies.

The study conducted comprehensive searches in relevant databases, collaborating with a librarian to develop robust search strategies across various platforms (Briscoe, 2019). Gray literature was methodically approached to ensure comprehensive coverage. All retrieved records were collected into Endnote for organization and de-duplication before screening.

Study selection for inclusion involved a two-step process: an initial title and abstract screening to remove irrelevant studies, followed by a full-text screening based on our predefined criteria. Two independent reviewers conducted this process to ensure objectivity, resolving any disagreements through consensus (Liberati et al., 2009).

Data extraction from included studies was systematically performed using a spreadsheet. The study piloted the data extraction tool to refine and ensure all relevant data fields were included (Moher et al., 2009). This allowed for consistent and thorough data collection across studies.

Evaluating the risk of bias in included studies was done using the Cochrane Risk of Bias Tool, adapted as necessary to suit the study designs encountered (Higgins and Thompson, 2002). This assessment was crucial to understanding the potential biases and reliability of the study findings.

The results were then clearly presented, detailing our methodology including search strategies, selection criteria, and risk of bias assessments. Based on the evidence gathered, the study provided recommendations for practice and policy-making, emphasizing areas where high-quality evidence was sufficient. Additionally, the study outlined directions for future research to address existing knowledge gaps or strengthen the current body of evidence.

This structured approach ensured a thorough, objective, and comprehensive review of the impacts of cleaner energy transitions on food prices and climate change mitigation in Sub-Saharan Africa. By clearly defining our research question, establishing robust criteria, and systematically synthesizing the data, we provided valuable insights and recommendations to inform policy and future research in this critical area.

3. Discussion

3.1. Theoretical Argument Linking Transition to Cleaner Energy with Rising Food Prices in Sub-Saharan Africa

The energy sector in Sub-Saharan Africa (SSA) faces critical challenges that significantly impact long-term economic development. Rural electrification rates are as low as 14%, making access to energy a priority for governments and investors who see it as a means to alleviate poverty and boost productivity (Pueyo and Maestre, 2019a; Peters and Sievert, 2016). Inadequate power supply has been a major constraint on economic development across SSA. For example, many companies have cited poor power supply as a primary reason for relocating their factories from Nigeria. The limited number of companies operating in Nigeria and many commercial farm enterprises rely heavily on off-grid power generated by diesel, gas, or petrol-powered electric generators (Olaoye et al., 2016). This dependence results in substantial overhead costs and contributes significantly to greenhouse gas emissions and other environmental issues (Pueyo, 2018a).

The relationship between energy prices and food prices is critical in understanding the broader economic implications. The global shift from fossil fuels to cleaner energy sources is driven by the urgent need to address environmental, climate, and health concerns. While this transition is widely supported, SSA faces significant challenges in implementing it, including inadequate human capital, low levels of technology, insufficient funding, inconsistent policy support, insecurity, and corruption just as echoed by Gregory and Sovacool (2019). Despite these hurdles, the commitment to cleaner energy remains strong. However, the literature lacks substantial evidence on how the transition to cleaner energy exacerbates economic challenges, particularly rising food prices in SSA. This study aims to bridge that gap by exploring the theoretical underpinnings of this issue.

The transition to cleaner energy in SSA necessitates significant financial investments, which have implications for other critical sectors, particularly agriculture. This economic phenomenon can be critically examined using the theories of opportunity cost articulated by Spiller (2011), Buchanan (1978), and Harberger (1972). According to these theoretical perspectives, the transition to cleaner energy in SSA can be seen as a complex economic decision with significant opportunity costs. The substantial investments required for cleaner energy infrastructure and technology divert critical resources from agriculture, leading to decreased productivity and increased food prices. This is line with the thoughts of evidence in literature about economies in technology transition (Harberger, 1972; Buchanan, 1978; Spiller, 2011). Understanding these dynamics is crucial for policymakers in SSA to balance the urgent need for cleaner energy with the equally critical need for food security and agricultural development.

In distressed economies, budget constraints are a significant barrier to balanced development. The increased expenditure on cleaner energy projects inflates the budget without a corresponding increase in revenue, often due to stagnant or shrinking revenue portfolios. Theoretical insights from Pueyo (2018b), Gregory and Sovacool (2019), and Singh and Ru (2022) collectively demonstrate the complex relationship between renewable energy investment and food insecurity in SSA. Budget constraints, inefficient revenue collection, financial risks, and policy challenges intersect to limit investment in critical sectors such as agriculture. The resulting low food production exacerbates food insecurity (Pueyo, 2018a; Gregory and Sovacool, 2019; Singh and Ru, 2022).

The transition to cleaner energy in SSA is hindered by inadequate technology and infrastructure. The focus on developing energy infrastructure diverts resources from agricultural infrastructure, such as irrigation systems, storage facilities, and transportation networks. According to the theory of production, such deficits reduce the efficiency and productivity of agricultural inputs, leading to lower agricultural output and higher food prices (Harberger, 1972).

The shift to cleaner energy often entails high initial costs, which can translate into higher energy prices. Agriculture, an energy-intensive sector, is directly affected by these increased costs. Higher energy prices lead to increased costs for the production, processing, and transportation of food products. This situation aligns with cost-push inflation theory, where increased production costs result in higher prices for the final goods, in this case, food (Meyer and von Cramon-Taubadel, 2004).

Meyer and von Cramon-Taubadel (2004) discuss asymmetric price transmission, where changes in input costs do not always result in proportional changes in output prices. This theory highlights the potential for unequal price adjustments along the supply chain, often leading to greater price volatility and inefficiencies in the market. In the context of SSA, the high initial costs of transitioning to cleaner energy can increase energy prices, which in turn increase the costs of agricultural production. Due to asymmetric price transmission, these increased costs may not be fully absorbed by intermediaries and could be passed on to consumers, resulting in higher food prices.

Fiscal policies in SSA are strained by the need to balance increased expenditures on cleaner energy with limited revenue streams. Efforts to increase revenue through higher taxes can lead to reduced disposable income and lower economic activity. In some SSA countries, such attempts have failed to generate sufficient revenue while exacerbating economic distress among the population. As disposable incomes decrease, the demand for food remains inelastic due to its necessity, but higher production and distribution costs due to increased energy prices lead to higher food prices (Singh and Ru, 2022).

To boost domestic industries and cope with economic pressures, some SSA countries have imposed restrictions on food imports. While these policies aim to support local farmers, they can worsen food shortages, especially when domestic production is insufficient to meet demand due to reduced agricultural investment. Basic economic principles of supply and demand suggest that such imbalances lead to higher food prices (Gregory and Sovacool, 2019).

3.1.1. Energy costs and agricultural production

The interplay between food prices and energy costs is a significant concern, particularly in the context of agricultural production. The reliance on fossil fuels in traditional agricultural practices underscores the vulnerability of the sector to energy price fluctuations. Research by Majeed et al. (2023b), Li et al. (2020), and Zhao et al. (2022) highlights the extensive use of fossil fuels for machinery, irrigation, and transportation in agriculture. This dependency is notable, as approximately 30% of global energy consumption occurs within the agricultural and food sectors, with primary agriculture accounting for about 20% of this energy use (FAO, 2011).

The transition to renewable energy sources represents a potential shift in the cost dynamics of agricultural production. Renewable energy, such as solar and wind, can provide more stable and predictable energy costs compared to the volatility associated with fossil fuels (Griggs et al., 2013). This stability is crucial as it can help mitigate the risk of sudden increases in food prices driven by energy cost spikes. Moreover, decentralized renewable energy systems, such as solar panels on farms, offer significant advantages. They can reduce dependency on external energy supplies, thereby enhancing the resilience of food production systems. By generating their own energy, farmers can lower operational costs and protect themselves from market fluctuations in energy prices.

The integration of renewable energy into agriculture aligns with broader environmental and economic goals. For instance, reducing reliance on fossil fuels not only decreases greenhouse gas emissions but also supports sustainable agricultural practices. The potential for renewable energy to stabilize and even reduce energy costs in agriculture could lead to lower food prices, benefiting consumers and enhancing food security.

Furthermore, the literature suggests that the adoption of renewable energy in agriculture is not without challenges. Initial investment costs for renewable energy infrastructure can be high, which may pose a barrier for small-scale farmers. However, long-term benefits, such as reduced energy costs and increased energy security, can offset these initial expenditures. Policy support, in the form of subsidies or incentives, can play a critical role in facilitating this transition.

3.1.2. Energy inputs in food processing and distribution

Beyond production, the processing and distribution stages of the food supply chain also consume substantial amounts of energy. Together, food processing and transportation consume approximately 40%, making a substantial contribution to global energy consumption throughout agricultural value chains (FAO, 2011). Renewable energy can lower the operational costs of food processing facilities by reducing reliance on grid electricity and fossil fuels (Majeed et al., 2023b). This reduction in energy costs can translate to lower food prices for consumers. This is corroborated by Li et al. (2024). Furthermore, the integration of renewable energy in cold storage and transportation systems can help maintain the quality and safety of perishable goods, reduce food waste and improve overall food security.

3.1.3. Bioenergy and land use competition

The production of bioenergy, derived from organic materials, introduces significant competition for land and resources between energy crops and food crops. This dual-use scenario, as highlighted by Searchinger and Heimlich (2015), presents substantial challenges to food security by diverting food crops for biofuel production, such as ethanol and biodiesel. The literature outlines two primary ways this competition can elevate food prices. First, when arable land is repurposed from food production to bioenergy crops, the reduced supply of land for food crops can drive up prices. Second, diverting food crops like maize and sugarcane for bioenergy reduces their availability for human consumption, further increasing food prices. Currently, biofuels constitute about 2.5% of global transportation fuel, a figure projected to remain stable until 2050 by the FAO. This stability, however, exacerbates the crop calorie gap—the shortfall between future foods needs and projected crop production. The literature suggests that phasing out crop-based biofuels could reduce this gap from 70% to 60% by 2050, thereby enhancing food security. Conversely, ambitious biofuel targets, such as those set by the US and Europe, if adopted globally, could widen the gap to 90%, significantly undermining food security. Evidence from the literature emphasizes the importance of eliminating crop-based biofuels to narrow the crop calorie gap and ensure a sustainable food future. This provides critical insights for policymakers who must balance energy production and food security demands. In scenarios where bioenergy becomes inevitable, policymakers are advised to promote it judiciously, ensuring adequate land and resources remain available for food production.

3.1.4. Climate change mitigation and resilience

To combat climate change and mitigate its unequal impacts on societies, it is crucial to move away from unsustainable economic models reliant on fossil fuels (IPCC, 2022). This shift requires a well-planned industrial policy, as advocated by UNCTAD (2023), to expedite the transition to cleaner energy sources and restructure industries. Such a policy should prioritize innovation that supports sustainable development, ensuring economic growth without excessive use of natural resources or harmful environmental impacts. Renewable energy, as highlighted by Majeed et al. (2023a), plays a vital role in mitigating climate change, which has both direct and indirect effects on food prices. Climate change exacerbates extreme weather events like droughts and floods, disrupting food production and supply chains, leading to price volatility (Haile et al., 2017; Busnita et al., 2017). By reducing greenhouse gas emissions, renewable energy contributes to climate stabilization, helping to mitigate adverse effects on agriculture. Additionally, renewable energy systems can enhance the resilience of rural communities by providing reliable and sustainable energy sources, thereby reducing their vulnerability to climate-related disruptions.

3.1.5. The 2030 agenda for sustainable development and the Paris Agreement

The 2030 Agenda for Sustainable Development and the Paris Agreement address the interconnected challenges of climate change, social exclusion, and uneven economic development, necessitating a new development paradigm that respects planetary limits (UNCTAD, 2023). Central to this paradigm is a socioeconomic shift towards decarbonizing the economy, addressing distributional concerns, and investing in public goods. Economic policies and incentives for renewable energy development, such as subsidies and tax incentives, can significantly impact food prices by stimulating rural investment, creating jobs, and boosting local economies. However, poorly designed or implemented policies can lead to unintended consequences, such as increased land prices and displacement of small-scale farmers (Yang et al., 2020). Coordinated policy approaches that integrate agricultural and energy policies are essential to ensure the equitable distribution of renewable energy benefits and support food security goals (Arbex and Perobelli, 2010; Majeed et al., 2023a).

3.2. Current State of Energy Security in Sub-Saharan Africa

Access to energy is essential for addressing malnutrition and achieving broader development goals in sub-Saharan Africa (Pondie et al., 2023). The availability of modern energy services, particularly electricity, profoundly impacts economic development and quality of life (Bonan et al., 2017; IEA, 2014). The literature underscores that higher per capita energy consumption is associated with improved living standards and well-being (Sambodo and Novandra, 2019; Pondie et al., 2019). This research aligns with the broader understanding that enhancing energy access in sub-Saharan Africa is critical for advancing economic development, reducing malnutrition, and achieving the Sustainable Development Goals. Effective energy policies must therefore prioritize expanding access to clean and reliable energy sources to support sustainable development and improve quality of life across the region.

However, Sub-Saharan Africa remains the world's least electrified region, with only 48% of the population having access to electricity and a mere 17% having access to clean cooking facilities (IEA et al., 2022). The region's energy mix is heavily reliant on traditional biomass, which constitutes nearly half of its primary energy consumption (IEA, 2022; IRENA, 2022). The remaining energy needs are predominantly met by fossil fuels, with significant contributions from oil, coal (this is common in South Africa), and an increasing share of natural gas. Renewable energy sources, excluding traditional biomass, account for approximately one-fifth of the primary energy mix. Among renewables, solar, wind, and geothermal energy together represent approximately 1% of the energy mix, while hydropower contributes approximately 1.7% (IEA, 2021b). This outlook has been implicated as one of the significant drivers of rising food prices in the sub region.

3.2.1. Sectorial electrification rates

The low electrification rates across various sectors in sub-Saharan Africa significantly impact economic development and food security. As of 2017, the industrial sector had the highest electrification rate at 26%, while the transport and building sectors lagged behind with 1% and 4%, respectively (IRENA, 2022). Despite slight improvements in electricity access—from 46% in 2018 to 48% in 2020—the region's growing population has increased the number of people without electricity (IEA et al., 2022). Countries with the lowest electricity access, such as South Sudan, Burundi, Chad, and Malawi, experience higher food prices due to the high costs of production and transportation, which rely on inefficient and expensive energy sources. Conversely, countries like Ghana, Kenya, and Rwanda have made strides towards full electrification, which helps stabilize food prices by reducing production and transportation costs. The slow overall electrification rates, further exacerbated by the COVID-19 pandemic, continue to strain food supply chains and elevate food prices, underscoring the urgent need for comprehensive energy policies that address these challenges to improve food security and economic stability.

3.2.2. Regional disparities in energy access

Regional disparities in energy access within sub-Saharan Africa significantly impact food prices, reflecting the uneven distribution of infrastructure and resources. Southern Africa, which boasts the highest clean cooking access rate at 37% and an average electricity access rate of 49%, benefits from lower energy-related food production costs, contributing to more stable food prices (IRENA, 2022). In contrast, East and West Africa, with electricity access rates of 46% and 53%, respectively, suffer from very low clean cooking access rates of 7% and 13% (IRENA, 2022). This disparity forces reliance on inefficient cooking methods, driving up food prices due to higher energy consumption and costs. Central Africa, with the lowest electricity access rate at 32% and clean cooking access at 17%, faces the highest food prices (IRENA, 2020; IEA et al., 2022). The compounded challenges of inadequate energy infrastructure severely impact food production and preservation, exacerbating food insecurity. These regional disparities underscore the critical need for targeted energy policies to address infrastructure gaps, promote clean cooking technologies, and enhance overall energy access to stabilize food prices and improve food security across the region (IEA, 2022).

3.3. Transitioning to a Cleaner Energy and Cleaner Environment

3.3.1. Subsidy removal, a breach of social contact, and its implications for food prices

The removal of fossil fuel consumption subsidies, which globally amounted to over $400 billion in 2018, presents significant implications for food prices and social stability, especially in regions like sub-Saharan Africa (International Energy Agency, 2019; McCulloch et al., 2021). These subsidies, which often exceed key sources of domestic revenue, such as in the median country of sub-Saharan Africa where more than 1% of GDP is allocated to energy subsidies, have historically led to increased fossil fuel consumption, elevated levels of air pollution, and higher greenhouse gas emissions (McCulloch and Dom, 2019). Furthermore, fossil fuel subsidies contribute to societal inequities, disproportionately benefiting the wealthy while fostering environmental and public health issues, such as the 3 million premature deaths annually attributed to outdoor air pollution from fossil fuels (Coady et al., 2015). In nations like Nigeria, subsidies are part of an implicit social contract, providing affordable fuel in exchange for public support (Beblawi and Luciani, 1987; Hertog, 2017). The abrupt removal of these subsidies without adequate social protection measures can lead to public unrest and economic instability, exacerbating food prices by increasing production and transportation costs in the absence of affordable energy. This underscores the need for careful policy design that balances the environmental benefits of subsidy removal with measures to protect vulnerable populations and maintain social stability.

3.3.2. Economic implications of subsidy removal for food prices

The removal of fossil fuel subsidies in sub-Saharan Africa (SSA) has profound implications for food prices, primarily through increased production and transportation costs. Fossil fuel subsidies help keep the cost of energy, and by extension, the cost of agricultural production and transportation, artificially low (Ikuemonisan and Akinbola, 2019). When these subsidies are removed, fuel prices sharply increase, leading to higher costs for operating farm machinery, irrigation systems, and transporting goods to markets (Coady et al., 2015). In Nigeria, for example, the removal of petrol subsidies significantly raises the cost of agricultural inputs and transportation, thereby increasing the overall cost of food production and distribution (Alghalith, 2010; Okorie and Wesseh, 2024). This situation is further compounded by inadequate infrastructure in many SSA countries, making the energy supply less reliable and more expensive (McCulloch and Dom, 2019). Consequently, the increased cost of energy exacerbates existing challenges in the agricultural sector, resulting in higher food prices for consumers.

3.3.3. Social and political ramifications

The removal of subsidies on Petrol Motor Spirit (PMS), a major fossil fuel extract, presents significant social and political challenges in sub-Saharan Africa (SSA). In many SSA countries, these subsidies form a crucial part of the social contract, providing citizens with affordable fuel (Beblawi and Luciani, 1987; Luciani, 1990). Removing subsidies without enhancing social welfare protections can be seen as a breach of this contract, leading to widespread public protests and political instability (McCulloch et al., 2021; van Asselt et al., 2022). For example, previous attempts to remove fuel subsidies in Nigeria have faced strong popular opposition, underscoring the deep reliance on these subsidies for economic relief (Akov, 2015). To mitigate the adverse effects on food prices and maintain social stability, it is essential for governments to implement comprehensive social protection measures and transparently reinvest the savings from subsidy reforms into sectors directly impacting households, such as health, education, and infrastructure (Bodea and LeBas, 2016). This approach could help garner public support for subsidy reforms by demonstrating tangible benefits from the reallocation of resources.

3.4. The Need to Address Rising Food Prices in the Transition to Sustainable Agriculture in Sub-Saharan Africa

The transition to sustainable agriculture in sub-Saharan Africa is essential for addressing the region's rising food prices, which are exacerbated by an unsustainable reliance on fossil fuels, leading to high operational costs and adverse environmental impacts (Peters and Sievert, 2016; Pueyo and Maestre, 2019b). Jeuland et al. (2021) emphasized the need for agricultural practices to adopt renewable energy and efficient nutrient recycling to mitigate these impacts. Investing in renewable energy infrastructure, policy reforms, and enhancing farmer knowledge can reduce fossil fuel dependence, lower energy costs, and boost agricultural productivity (Lee et al., 2020). This aligns with sustainable development goals, helping to stabilize food prices and enhance resilience (Bernard, 2012). Empirical assessments by Okou et al. (2022) highlight the crucial role of staple foods in the region's caloric intake and the urgent need for investment in agricultural infrastructure. Long-term strategies focusing on agricultural development and economic diversification are vital for stabilizing food prices and ensuring sustainable growth (Vincent and Okowa, 2022). Balancing economic development with environmental quality through a renewable energy transition and effective environmental policies is essential for achieving this balance (Chem and Taylor, 2019; Udi et al., 2020; Adedoyin et al., 2020).

3.5. The Transition from Traditional Non-Renewable Energy Sources

The transition from traditional non-renewable energy sources to cleaner alternatives such as biofuels and green energy is imperative in Sub-Saharan Africa to mitigate carbon dioxide emissions, combat climate change, and promote sustainable power generation (Barreto et al., 2003; Bockris, 2003; Sangster, 2011; Kalyani et al., 2015; Hussain, 2015; Eluwa and Kilanko, 2024). However, the lack of coherent policies integrating renewable energy with agriculture has created a complex scenario. While renewable energy projects may benefit farmers economically, consumers often face higher food prices due to the associated costs, highlighting a policy gap that must be addressed to ensure equity (Eluwa and Kilanko, 2024). Renewable energy is crucial not only for reducing greenhouse gas emissions and air pollutants but also for improving air quality, stabilizing energy prices, and creating job opportunities (Midilli et al., 2006). Green energy, sourced from natural elements such as sunlight and wind, offers an environmentally friendly alternative to fossil fuels. However, concerns persist regarding the potential impact of this transition on food security and economic equity in Sub-Saharan Africa, where limited technology use and postharvest losses contribute to high production costs and food prices (FAO, IFAD, UNICEF, WFP, WHO, 2022; Akpa et al., 2023; Matthew et al., 2023). Therefore, comprehensive policies are essential for navigating the delicate balance between the economic benefits for farmers and the potential adverse effects on consumers during the transition to renewable energy. These policies should prioritize sustainability, inclusivity, and affordability to ensure a harmonious and equitable shift towards renewable energy adoption in sub-Saharan Africa.

3.6. Challenges to Clean Energy Transition

3.6.1. Investment disparities and their implications

The stark contrast in investment flows between developed and developing countries, as highlighted by the UNCTAD report, underscores a critical challenge in the global transition to cleaner energy. This investment gap has profound implications for sub-Saharan Africa (SSA), where financial constraints and infrastructural deficits are already significant hurdles. The limited foreign direct investment (FDI) in renewable energy for developing countries impedes their ability to make necessary advancements in clean energy infrastructure. This limitation directly impacts the capacity of these countries to mitigate climate change effectively and transition away from fossil fuels.

3.6.2. Impact on food prices

The financial burden of transitioning to cleaner energy in sub-Saharan Africa (SSA), in the absence of substantial foreign direct investment (FDI), can strain public finances and divert resources away from other vital sectors such as agriculture. Various theoretical frameworks, including opportunity cost considerations and cost-push inflation, suggest that redirecting funds to energy projects may reduce agricultural productivity. The high initial costs of renewable energy projects, combined with inadequate investment in agricultural infrastructure (such as irrigation and storage), can increase production costs and subsequently elevate food prices. This scenario aligns with cost-push inflation theory, where higher production costs lead to increased prices for end consumers, exacerbating food insecurity in the region. Such findings underscore the critical need for balanced investment strategies that simultaneously address the energy transition and support agricultural development to mitigate adverse impacts on food prices and security.

3.6.3. Climate change mitigation

From a climate change mitigation perspective, the emphasis on renewable energy investments in developed countries, as opposed to developing ones, poses a global risk. Developing countries, including those in sub-Saharan Africa (SSA), are often more vulnerable to the adverse effects of climate change, such as extreme weather events, which can exacerbate food insecurity and economic instability (Peters and Sievert, 2016; Pueyo and Maestre, 2019b). Therefore, a significant increase in investments in these regions is crucial not only for local sustainability but also for global climate resilience. Rebeca Grynspan's call for enhanced investment in sustainable energy systems in developing countries is pivotal, as it underscores the need for a more equitable distribution of resources to ensure these regions can contribute effectively to global climate goals. Such investments would help SSA mitigate climate change impacts while promoting economic growth and stability, aligning with sustainable development goals and addressing both local and global challenges. This approach advocates for integrated policy frameworks that support renewable energy adoption while ensuring economic and food security in vulnerable regions.

4. Conclusion and Recommendations

4.1. Conclusion

The transition to cleaner energy in sub-Saharan Africa (SSA) is essential for climate change mitigation but introduces significant economic challenges that impact food prices across the region. The high initial and operational costs associated with renewable energy projects strain public finances and divert resources from critical sectors such as agriculture, leading to increased production costs. These costs are then transferred to consumers through higher food prices, exacerbating food insecurity. Similarly, the intersection of food waste, cleaner energy transition, and food prices is a complex but critical area of study. Addressing food waste can contribute to food security and climate change mitigation, central to the study's goals. By adopting a holistic approach that incorporates energy and agricultural policies, SSA can advance towards sustainable development while ensuring food security and economic equity. This study provides a novel perspective by linking these interconnected issues and highlighting the importance of integrated policy solutions. The exploration of the interplay between the transition to cleaner energy and food prices in SSA presents an innovative approach to addressing sustainable development challenges in developing countries. This dual focus on energy and food security underscores the necessity of comprehensive and coordinated policies to achieve long-term sustainability and resilience in SSA.

4.2. Recommendations

The study highlights the critical role of food waste in climate change. Decomposing food waste in landfills produces methane, a potent greenhouse gas. By reducing food waste through better management and policies, greenhouse gas emissions can be significantly reduced. This aligns with the study's objective of climate change mitigation through cleaner energy transitions. Integrating food waste reduction strategies with energy policies can create synergies that enhance both food security and environmental sustainability. Therefore, a clearly defined timeline and transparency for the transition to cleaner energy can significantly reduce apprehension in the food and agriculture sector in sub-Saharan Africa. Establishing a well-communicated and realistic roadmap ensures that stakeholders, including farmers and agribusinesses, are aware of the upcoming changes and can plan accordingly. Transparency in the process fosters trust and allows for the anticipation and mitigation of potential disruptions. By outlining specific milestones and providing regular updates on progress, governments can demonstrate their commitment to a structured and manageable transition, which helps to stabilize market expectations and reduce the uncertainty that can lead to price volatility.

To mitigate the negative effects of the energy transition on food prices, governments across sub-Saharan Africa should adopt several practical measures. First, they should invest in modernizing agricultural infrastructure to improve efficiency and reduce energy costs. This includes developing irrigation systems, storage facilities, and transportation networks that are compatible with renewable energy sources. Second, governments should provide subsidies or financial incentives to help farmers and agribusinesses offset the initial costs of adopting cleaner energy technologies. Third, agricultural and energy policies must be integrated to ensure that the transition benefits of both sectors are crucial. Policies should aim to enhance food production capabilities while promoting sustainable energy use. Additionally, governments should facilitate access to affordable financing and technical assistance to support small-scale farmers in adopting new technologies. A more transparent subsidy regime should be reintroduced to cushion the effects of high energy costs resulting from the process of transitioning to cleaner energy.

4.3. Areas for Further Research

Based on the findings of this study, several areas for further research should be explored. One critical area is the long-term economic impact of the transition to cleaner energy on food security in sub-Saharan Africa. Research should examine the potential benefits of integrated policy approaches that simultaneously address energy and agricultural development. Another important area is the exploration of scalable and cost-effective renewable energy solutions specifically tailored to the agricultural sector in SSA. Finally, studies should investigate the effectiveness of various financial instruments and policy incentives in supporting smallholder farmers and agribusinesses during the transition period. Understanding these dynamics will provide valuable insights for policymakers and stakeholders to develop strategies that ensure a balanced and equitable transition to cleaner energy while safeguarding food security.

Limitations

The study could not conduct meta-analysis to synthesize data quantitatively. Although, the study considered some relevant literature and data across countries in Sub Saharan Africa, however, Nigeria being the most populous country in the subregion was more focused.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	E.I.
C	100
D	100
S	100
L	100
W	100
CR	100
SR	100
PM	100

C=Concept, D= design, S= supervision, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The author declare that there is no conflict of interest.

Acknowledgments

The author appreciates the cooperation of the Department of Agricultural Economics, Adekunle Ajasin University, Akungba-Akoko, who encouraged me in this review.

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Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1535854

Open Access Journal
e-ISSN: 2618 – 6578

Review
Volume 7 - Issue 5: 596-602 / September 2024

POTENTIAL BIOENERGY CROPS: SWEET SORGHUM AND GLOBE ARTICHOKE

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Abstract: The growing international demand for petroleum-based fuel and the related environmental issues, such as greenhouse gas emissions, global warming, and changes in the climate, have redirected global focus toward the development of sustainable, eco-friendly, and renewable fuels derived from energy crops. The production of biofuel utilizing fast-growing and very effective bioenergy crops is becoming a dependable substitute for fossil fuels. Bioenergy crops refer to specific plants that are cultivated and managed at reduced expenses for the purpose of producing biofuels. Among these, globe artichoke and sweet sorghum are significant bioenergy crops that can expedite the shift towards a low-carbon economy. Both plants are important crops that serve multiple purposes as food, animal feed, and bioenergy sources. Moreover, they are highly adaptable to harsh conditions. The potential for ethanol production from sweet sorghum is a minimum of 6000 L per hectare. Globe artichoke, on the other hand, has high biomass and energy production even with limited external management sources. These traits make them highly desirable as bioenergy plants. This review demonstrates the potential of global artichoke and sweet sorghum as bioenergy sources. A comprehensive understanding of the bioenergy potential of globe artichoke and sweet sorghum will better allow us to exploit these crops.

Keywords: Biomass, *Cynara cardunculus*, Fossil fuels, *Sorghum bicolor*

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Received: August 19, 2024
Accepted: September 12, 2024
Published: September 15, 2024

Cite as: Güden B, Özsan Kılıç T. 2024. Potential bioenergy crops: Sweet sorghum and globe artichoke. BSJ Agri, 7(5): 596-602.

1. Introduction

By 2050, there will probably be 9.1 billion people on the planet, 550 parts per million of CO_2 in the atmosphere, 60 parts per billion of ozone, a 2 °C rise in temperature (Jaggard et al., 2010), and fossil fuel resources will likely be depleted (Saidur et al., 2011). This suggests that action needs to be taken to save the environment and enhance the production of food and energy to fulfill the requirements of the growing population.

Fossil fuels which provide our current energy needs, are considered to be among the most important resources on Earth. In other words, about fifty-eight percent of the fuels utilized for energy are derived from fossil fuels, which make up 80% of the fuel supply (Escobar et al., 2009; Gaurav et al., 2017). However, in recent years, there has been an extraordinary and uncontrolled use of fossil fuels worldwide. Using these fuels increases the amount of dangerous chemicals in the atmosphere, such as nitrogen oxide, carbon dioxide, and greenhouse gases. For instance, the emission of greenhouse gases by coal, such as carbon dioxide, particle ash, and substances containing sulfur, causes acidity in the soil. Nuclear fission produces enormous amounts of infrastructure-related energy that are detrimental to both the ecosystem and human health (Gresshoff et al., 2017; Yadav et al., 2019). Because fossil fuels are used excessively, it has been found that the sources of oil reserves and fossil fuels are major contributors to air pollution and harmful gas emissions. This in turn causes changes in the climate and biodiversity brought on by globalization, among other effects such as melting glaciers and rising water levels (Shweta et al., 2024). Long-term environmental effects linked to the usage of fossil fuels include land degradation and desertification of rich soils (Karp and Shield, 2008; Yadav et al., 2019). The consequences of the explosion in the use of fossil fuels are now evident in the form of diseases connected to pollution in the environment, changing the climate, and excessive precipitation.

With the growing worldwide worries about climate change, the relationship between bioenergy and agriculture has become increasingly important and demands careful investigation (Bibri et al., 2024; Soyombo et al., 2024). Collaborative efforts are facilitated by the fact that the production of biofuel and the storage of carbon in bioenergy crops both contribute to the overall objectives of sustainable agriculture (Welfle and Röder, 2022; Soyombo et al., 2024). The use of organic resources to produce bioenergy presents a promising substitute for traditional fossil fuels that are low-carbon and renewable. Sustainable agriculture posits that to fulfill the increasing demands of an expanding global population, farming techniques should be both resource-efficient and ecologically conscious (Athuman, 2023; Soyombo et al., 2024).

Conventional fuels continue to be the primary energy source in a large number of countries. Due to the widespread recognition of the detrimental effects of using fossil fuels, efforts have been made to find alternate fuel sources. A number of countries have switched from using non-renewable to using renewable energy alternatives as their top concern when it comes to energy supply. Only a restricted amount of energy sources, nevertheless, are environmentally friendly and sustainable. Using "bioenergy crops" to generate energy is one such viable option with promising long-term results (Yadav et al., 2019).

Bioenergy crops acquire their energy from biomass that is derived from both plants and animals. Crop products used in bioenergy production include ethanol, biodiesel, biogas, and others (Yuan et al., 2008). Increased soil carbon, decreased greenhouse gas emissions, decreased soil erosion, increased transpiration, and the potential to produce heat and power are all benefits of bioenergy crops (Wang et al., 2012; Kim et al., 2013; Yadav et al., 2019). The phytoremediation of soil polluted with heavy metals is another benefit of bioenergy crops (Barbosa et al., 2015). Crops grown for bioenergy on huge scales may also benefit wildlife.

Bioenergy crops are gaining global interest due to their renewable and environmentally favorable characteristics. Nevertheless, the global market mostly utilizes bioenergy crops for food purposes, which therefore raises concerns over food safety when used for energy production. In addition to using bioenergy plants as food, it is very important to use their biomass as a source of bioenergy. There are a lot of potential bio-energy plants of this type. This review describes the features of potential bioenergy crops, particularly globe artichoke and sweet sorghum.

2. Bioenergy Crop Types

Traditional bioenergy crops have the added benefit of reducing global climate change, which might enhance the production of food as well as fodder. The five primary categories into which they are divided are first-, second-, and third-generation crops, specialized energy crops, and halophytes (Figure 1).

Although the energy from specialized bioenergy crops is now minimal, it is anticipated that in coming years, it will account for a significant portion of the overall biomass potential. Selecting the best bioenergy crop to cultivate is a difficult decision that depends on several aspects such as, soil and environmental conditions, market accessibility, transporting and harvesting challenges, and more. Six main categories may be used to group bioenergy crops according to their structural makeup, conversion method, and bioenergy utilization (Karp and Shield, 2008);

1. Using second-generation crop/fuel chains, woody lignocellulosic plants such as poplar, willow, and eucalyptus may be used to produce wood chips, pellets, or bioethanol*;
2. Using a second-generation crop/fuel chain, herbaceous lignocellulosic plants such as miscanthus, switchgrass, Cynara, fiber sorghum, and kenaf can be used to produce agro-pellets, biogas, or bioethanol;
3. Oil crops such as sunflower, soybean, rapeseed, and oil palm are used to produce agro-pellets utilizing crop leftovers and biodiesel based on a first-generation crop/fuel chain;
4. Depending on a first- or second-generation crop/fuel chain, sugar crops including sugarcane, sweet sorghum, and sugar beet are used to produce bioethanol;
5. Utilizing agricultural leftovers and first-generation crop/fuel cycles, starch crops such as wheat, rye, triticale, and maize are used to produce agro-pellets and bioethanol;
6. Biogas may be produced for fuel, heat, or power by processing Leguminous plants and grasses with manure or waste.

*Biofuels classified as a first, second, or third generation are fuel/crop cycles that rely on current, emerging, and future conversion technologies, in that order.

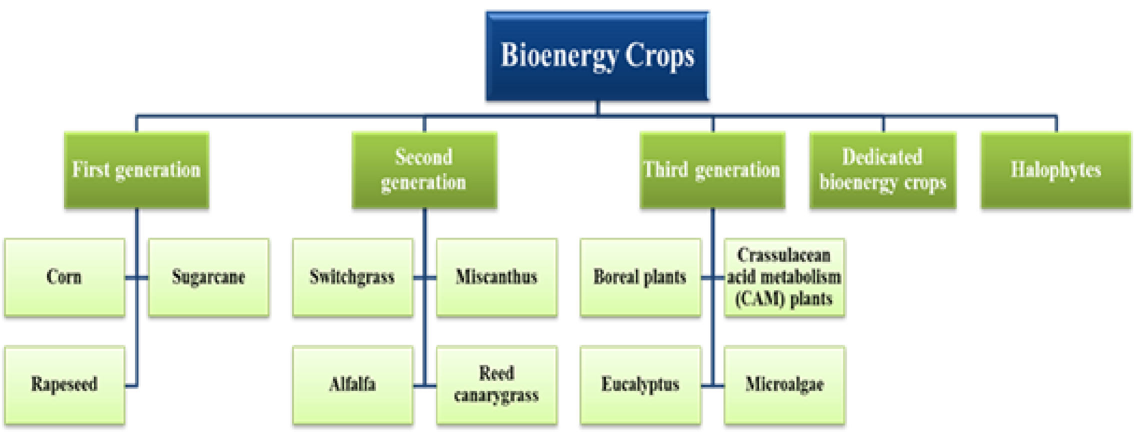

Figure 1. Bioenergy crop types (Adapted from Yadav et al., 2019).

The main energy crops grown worldwide are sugarcane, oil palm, rapeseed, and to a much lesser extent, miscanthus, poplar, willow, and eucalyptus. Now employed for both solid and gaseous biofuel manufacturing, miscanthus, willow, and poplar are the top three crops in the EU-27 for biodiesel production, with rapeseed and sunflower coming in second and third respectively (Christou et al., 2010).

Certain energy crops, like Cynara cardunculus and Sorghum bicolor, have several uses and purposes. Indeed, many other crops, including globe artichoke and sweet sorghum are being extensively researched currently (Archontoulis, 2011; Gominho et al., 2011; Olweny et al., 2013).

3. Globe Artichoke

The Asteraceae species *Cynara cardunculus* L. (Figure 2) comprises both the wild (var. sylvestris) and cultivated cardoon (var. altilis), in addition to the well-known edible globe artichoke (var. scolymus). It is a Mediterranean-native perennial C3 species that avoids the warmest and driest period of the year by starting its growth cycle in the fall and finishing it in the early summer (Sonnante et al., 2007, Mauro et al., 2012, Pesce et al., 2017). The 'Cynara' genus, which includes the globe artichoke [*Cynara cardunculus* var. scolymus (L.)], has a history dating back to the Ice Age. It is thought to be endemic to the Mediterranean region, which includes Southern Europe and Northwest Africa. Its predecessor is the thistle. During this time, wild artichokes, or "cardoons," were found across the Mediterranean region, extending from the southern portion of the basin to the Sahara. Towards the end of the ice age, they also reached the eastern and western regions of the Mediterranean, where they are now widely cultivated (Ciancolini, 2012). Nowadays, an important crop and source of alternative medicine, globe artichoke has been cultivated extensively, particularly in several countries around the Mediterranean.

Cynara is a perennial C3 plant with yearly cycles that may be utilized to produce bioethanol (lignocellulosic biomass), biodiesel (from seeds), or combined heat and power. Cynara is harvested dry in the summer, with the production of biomass ranging from 6 to 30 t dry matter ha-1 y-1, and cultivated as rainfed during the fall, winter, and spring seasons. *Cynara cardunculus* has been recognized as one of the number potential candidate species for bioenergy manufacturing in the Mediterranean region since it meets a variety of cropping approaches (the length of the growing season and whether irrigation is used or not, etc.). Also, previous studies have demonstrated favorable findings in regard to their capacity for production (Archontoulis, 2011).

3.1. Agronomic Characteristics of Globe Artichoke as a Bioenergy Feedstock

The morphology of the perennial herbaceous plant artichokes is examined in two sections. Its above-ground parts (shoot, leaves, and flowers) are annual and can grow up to 1 m tall and cover a 1 m^2 area. Depending on the variety, the rosette-shaped leaves can have segmented or unsegmented structures. A growth tip is located where the leaves merge, and the flower stem grows when the air temperature is between 13 and 17 °C (Ekbiç, 2005).

It has been cultivated for its immature blooms or heads, and because of the high bioactive content of its body and leaves -which are still regarded as waste- it has lately started to be employed in a variety of sectors, particularly in medicines.

Figure 2. *Cynara cardunculus* var. *scolymus* (L.).

The heads (inner bracts and 'heart') and the base of the flowers are the components of the perennial globe artichoke plant that are consumed. Nonetheless, outer bract leaves on the heads, stem, and leaves are regarded as non-food components (Ruiz-Aceituno et al., 2016). Although it fluctuates based on genotype and harvest time variances, the ratio of edible sections to the entire plant is generally recognized to be between 35 and 55 percent (Abu-Reidah et al., 2013).

The primary use of Cynara as a crop for energy is in the production of solid biofuel. The following crop attributes lend support to this application: a high biomass productivity with a comparatively little amount of crop input conditions of a Mediterranean climate, low biomass moisture content at harvest, mostly lignocellulosic biomass composition, and a high heating value. From a botanical standpoint, Cynara and sunflowers are related. Both produce oil fruits, which are commonly referred to as "seeds."

The following characteristics also indicate Cynara's potential as an oil crop: heating value, fatty acid composition, seed yield, and seed oil content (Fernandez et al., 2006). Within the scope of a European project, the potential of Cynara biomass for the production of paper pulp was investigated and demonstrated; the hemicellulose, cellulose, and lignin contents of the various plant sections of a Cynara crop harvested at the end of the cycle (summer) and evaluated. Additionally, a number of studies have shown the potential of the Cynara crop for producing green feed (Fernandez et al., 2006).

3.2. Production of Globe Artichoke

It is a herbaceous perennial plant that can withstand droughts and is easily grown from seed, which has significant benefits for crop management. Because it can be grown without irrigation (rainfed), it is especially suited to Mediterranean climates, where water is the primary problem restricting productivity (Rana et al., 2016). The globe artichoke is grown for its juvenile inflorescence, which can be eaten raw or cooked (Pesce et al., 2017). The germination of seeds, which typically occurs in early autumn, initiates the first growth cycle of artichoke. After the first two new cotyledons appear, a number of leaves quickly sprout and eventually form a leaf rosette. Typically, the leaf rosette grows quite slowly but steadily. The plant experiences winter and early spring when it is in the rosette stage. The plant produces a leaf-branched flower scape with many heads by late April. The fruits ripen following full bloom and flower fertilization, and the aerial biomass eventually dries up in the summer.

Perennating buds on the basal plant portion sprout and a new development cycle begins when the weather gets milder. It may continue for a few years. There have been reports of this annual growth cycle succession lasting more than 15 years (Fernandez et al., 2006).

In the last twenty years, rainfed countries in Mediterranean climates have come to regard the cultivated cardoon as a possible energy crop. Since both wild and cultivated cardoon forms present a significant amount of biomass regardless of being given very little input, they have been suggested as potential bioenergy crops since they may be grown on land that isn't often utilized for cropping (Mauromicale et al., 2014; Mauro et al., 2015). The biomass may be burned directly to provide energy, and the oil that collects in the achenes can be used as a fuel to make biodiesel (Encinar et al., 2002; Fernandez et al., 2006; Pesce et al., 2017). On the other hand, the cardoon biomass, including its high carbohydrate and low lignin concentration, offers the potential for fermentation to produce ethanol or biomethane (Cotana et al., 2015; Fernandes et al., 2015; Pesce et al., 2017); currently, the majority of this is achieved using maize silage, while triticale and bread wheat biomass are also used to a lesser extent (Dressler et al., 2012; Pesce et al., 2017). This crop's strong biomass and energy production under minimal external management energy sources are what draw attention to it (Ierna et al., 2012a; Acquadro et al., 2013; Mauromicale et al., 2014). The exceptional adaptation of Cynara to the Mediterranean climate indicates this feature. This includes the ability to uptake nutrients from deep soil layers, photosynthesize during the winter, and maintain an ideal equilibrium between the stages of the plant's life cycle and variations in the Mediterranean climate. In fact, the extremely deep root system makes it possible to explore the soil at a deeper level and to take water and nutrients that have accumulated throughout the soil profile (Ierna et al., 2012b; Mauromicale et al., 2014).

4. Sweet Sorghum

Sorghum bicolor (L.) Moench belongs to the Andropogoneae tribe, which is a part of the panicoideae tribe of the grass family, poaceae (Kellogg, 2013). The earliest cultivated sorghums were discovered in Neolithic populations in Sudan about the fourth millennium BC (Winchell et al., 2017). Domesticated sorghum, originating from its earliest predecessor in Africa, was spread worldwide via numerous methods, with trading routes being the most prevalent (Ananda et al., 2020). *Sorghum bicolor* (L.) Moench, commonly referred to as sorghum, is classified as one of the most prominent five cereal crops globally (Venkateswaran et al., 2014). It has a crucial function in the production of food worldwide and serves as the main source of sustenance for billions of people (Mace et al., 2009). Sorghum is a versatile crop that is used for several purposes, including grain, sweet, fodder, and broomcorn (Ananda et al., 2020). In addition, it functions as a fuel source, providing bioethanol. It is a crucial staple crop in arid and semi-arid areas worldwide, whereas in wealthy countries it is mostly cultivated for animal feed and forage purposes (Venkateswaran et al., 2019).

Sweet sorghum is a C4 plant species characterized by its broad, flat leaves and a rounded or oval head filled with mature grains. This is a short-day plant, and its blooming process is accelerated by shorter days and longer nights. Sorghum grows in arid and semi-arid regions, exhibiting a temperature range of 12–37 °C, with its optimal range being 32–34 °C (Rao et al., 2009). The ideal conditions for optimal growth and maximum stem juice yield are loam and sandy loam soils with soil temperatures above 18°C and pH levels around 5.8 (Mask and Morris, 1991).

4.1. Agronomic Characteristics as a Bioenergy Feedstock

As a bioenergy crop, sweet sorghum cultivars must have many desirable traits (Appiah-Nkansah et al., 2019):

1. The sweet sorghum cultivars have a high amount of biomass production.
2. The stalk of this plant is thick and can withstand.
3. The juice extracted from cultivars has a high content of total soluble brix.
4. These cultivars have a high percentage of extractable juice.

Sweet sorghum refers to sorghum cultivars that produce juice which comprises up 78% of the total plant biomass. This juice contains 15 to 23% fermentable sugar, which mostly consists of sucrose (70–80%), along with fructose and glucose (Appiah-Nkansah et al., 2019) that can be easily converted into ethanol (Vinutha et al., 2014).

In the past, sweet sorghum juice had been utilized as a natural sweetening agent by concentrating it into a syrup. Additional research has shown that sweet sorghum juice may be transformed into granulated sugars, granul syrups, and jaggery. Furthermore, it can serve as a base material for the production of hydrogen and methane (Antonopoulou et al., 2008). The utilization of sweet sorghum as a substrate for ethanol production emerged in the late 1970s (Umakanth et al., 2019) and is currently gaining attention due to its notable characteristics, including its high productivity, strong resistance to stress, and ability to easily integrate into existing agricultural systems as a bioenergy crop.

Furthermore, the requirements for desirable traits in sweet sorghum, such as tolerance to environmental and biological stresses and high grain yield, differ across different production systems. After extracting the juice, the remaining bagasse, which is a dry fibrous material made of lignocellulose, can be utilized for various purposes including paper manufacturing, animal feed, production of cellulosic ethanol (Appiah-Nkansah et al., 2019).

4.2. Production of Sweet Sorghum

Sweet sorghum has better drought tolerance compared to the majority of other C4 grasses, making it a highly efficient crop with minimal resource requirements. The productivity in a particular region is determined by climatic conditions, soil type, and agronomic methods (Rooney et al., 2007). For instance, research performed in central Iowa, United States, suggested planting sweet sorghum early to produce a biomass production of 26 to 29 tons per hectare, with a theoretical ethanol potential of 14,500 liters per hectare (Khawaja et al., 2014). In India, the cultivation of sweet sorghum is suggested during the rainy season, post-rainy season, and summer season, contingent upon the accessibility of water resources (Appiah-Nkansah et al., 2019).

The cultivation of sweet sorghum is possible in several soil types, but the most productive soils for this crop is well-drained and have a well-structured composition, namely red or black clay loam soils (Reddy et al., 2005). Sweet sorghum needs a well-balanced application of fertilizers in order to produce a high-yielding crop. The specific quantities of fertilizers required depend on the existing levels of nitrogen (N), phosphorus (P), and potassium (K) in the soil profile. While sweet sorghum constitutes a crop that can withstand drought conditions, the availability of water also has a substantial impact on its yield.

Early planting often enhances sugar yield and had a substantial positive impact on agricultural productivity (Teetor et al. 2011). Moreover, it has been shown that the yield increases when radiation levels rise during the reproductive period. For example, Ricaud and Arenneaux (1990) found that the average stalk yields in Louisiana were 56 and 49 Mg ha-1 when planting was carried out on 26 April and 25 May, respectively, across several cultivars.

The stalk sugar content varies depending on the cultivar and the growing stage of the plant. Early cultivars often have the highest concentration of sugar in their stalks just before they blossom, while late-maturing cultivars with longer growing seasons continue to accumulate sugar in their stalks until they reach maturity (Shukla et al., 2017). According to Lingl (1987) and Regassa and Wortmann (2014), the sugar concentration in the stalk is often at its lowest during the boot stage and reaches its maximum level during the soft dough stage. Ricaud et al. (1979) discovered that the sugar level in stalks varied from 8.3% to 14.0% during the blooming stage and from 12.8% to 16.6% during the soft dough stage. The optimal development stage for harvesting sweet sorghum might vary depending on the cultivars, ranging from the early milk stage to the hard dough stage (Oyier, et al., 2017).

The potential for ethanol production from sweet sorghum is 6000 L per hectare, with an energy yield on investment of more than three units each unit invested. Sweet sorghum has a Brix percentage ranging from 13 to 24, with a juice sucrose concentration of 7.2 to 15.5%. It yields a total stalk sugar yield of 12 mg ha-1, a fresh stalk yield of 24 to 120 mg ha-1, and a biomass yield of 36 to 140 t ha-1 (Regassa and Wortmann, 2014).

5. Conclusion

The urgent need to combat climate change motivates the investigation of potential synergies between sustainable agriculture and bioenergy. The scientific community is interested in the idea of bioenergy crops because of their eco-friendliness and renewability. By providing a competitive substitute for traditional fossil fuels, bioenergy from renewable organic substances helps to lower the emissions of greenhouse gases. Globe artichoke and sweet sorghum are perfectly in line with the goals of sustainable agriculture, which stresses eco-friendly methods to lessen the effects of climate change. With these plants, by recognizing and utilizing the synergistic impact of sustainable agriculture and bioenergy, we may shift to a low-carbon economy and make major progress against climate change.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	B.G.	T.Ö.K.
C	50	50
D	50	50
S	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50

C=Concept, D= design, S= supervision, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declare that there is no conflict of interest.

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