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RESEARCH ARTICLE

Factors Influencing Farmers' Attitude to Adopt Drought Tolerant Maize Varieties in Ondo State, Nigeria

Jonathan Akinsola Akinwale[1] • Patience Chinonso Justine[2] • Foluso Ojo[2]

[1]Federal University of Agriculture, College of Agricultural Management and Rural Development, Department of Agricultural Extension and Rural Development, Abeokuta, Ogun State/Nigeria
[2]Federal University of Technology, School of Agriculture and Agricultural Technology, Department of Agricultural Extension and Communication, Akure, Ondo State/Nigeria

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ABSTRACT

The study investigated factors influencing farmers' attitude towards adoption of drought-tolerant maize varieties. One hundred maize farmers were selected using a multi-stage sampling procedure and data were collected through structured questionnaires. Information was collected on the socio-economic characteristics of the maize farmers, farmers' level of awareness of drought-tolerant maize varieties, farmers' attitudes towards agricultural innovation, constraints to adoption of drought-tolerant maize varieties and, factors influencing farmers' attitude towards adoption of drought-tolerant maize varieties. Data were analyzed using frequencies, percentages, Pearson Product Moment Correlation and Logit regression. The findings revealed that 76% of the maize farmers had limited awareness regarding the benefits and characteristics of drought-tolerant maize varieties. Also, the majority (74%) of the farmers lacked knowledge about the quality and advantages of these varieties. Despite positive attitude (mean = 4.28) which was measured at ordinal level, several constraints to adoption were identified, mainly low output of drought tolerant maize varieties (mean = 2.30). The findings also showed the substantial impact of two key factors (that is, sex and income) as factors influencing farmers' attitude to adopt drought-tolerant maize varieties. Farmers are yet to take advantage of drought-tolerant maize varieties as a mitigating strategy against climate change. The need for extension services and non-governmental organizations to step up awareness creation and training on best agronomic practices in growing the maize varieties is recommended.

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1. Introduction

Agriculture continues to play a crucial role in sustaining the livelihoods of the majority of rural households in sub-Saharan Africa. Despite the significant prominence of the petroleum sub-sector in Nigeria's economy, it is imperative to underscore the essential contribution of the agriculture sector to the country's Gross Domestic Product. According to Food and Agriculture Organization (FAO, 2023), over 70% of Nigeria's population depends on agriculture for their employment and livelihoods. Despite the significant portion of the population in Nigeria relying on agriculture for their livelihoods, the country continues to face food scarcity. Approximately 20 percent of Nigeria's population endured food insecurity between 2018 and 2020 (Statista, 2023). This percentage of food-insecure households in Nigeria is expected to increase further due to population growth that is outpacing food production. In order to stem the tide against food insecurity, it is imperative to promote the widespread adoption of agricultural innovations. Agricultural innovation stands at the forefront of transformative

Correspondence
E-mail address: akinwaleja@funaab.edu.ng

change within the farming sector, acting as a pivotal force in driving heightened productivity and sustainability. Technological advancements in agriculture encompass a spectrum of innovations, ranging from precision farming and data analytics to the development of genetically modified crops and sustainable practices. These innovations not only enhance the efficiency of agricultural processes but also contribute to mitigating challenges such as climate change, resource scarcity, and evolving global food demands. Jamilu et al. (2014) asserted that farmers' socio-economic status plays a pivotal role in shaping their inclination towards adopting innovative agricultural practices. The socio-economic landscape of farming communities is diverse and complex, encompassing factors such as household income, education, access to resources, and land tenure. Understanding how these elements interplay with the adoption of innovative agricultural practices is crucial for designing effective interventions and policies that resonate with the realities of farmers.

Abhijeet et al. (2023) underscore the pivotal role of effective agricultural extension services in the diffusion of knowledge and technologies to farmers.

Agricultural extension services act as a crucial intermediary between research institutions and farmers, facilitating the transfer of up-to-date agricultural technologies, best practices, and scientific knowledge to enhance farm productivity. However, the efficacy of these services is contingent on various factors, including accessibility, adaptability, and the ability to cater to the diverse needs of farmers.

Climate change is impeding agricultural growth and affecting crop production in several parts of the country. It is expected to increase in the coming decades in the country where the adaptive capacity is weaker and its impacts on agriculture threaten both food security and agriculture's pivotal role of livelihoods development (Alemu & Mengistu, 2019). Adoption of improved crop varieties has a substantial impact on yield and contributes significantly to food security. This review delves into the realm of crop-specific innovations, with a focus on key crops such as maize, rice, and wheat. It scrutinizes the adoption rates of these innovations and evaluate their overarching impact on agricultural sustainability. Climate change poses unprecedented challenges to global agriculture, necessitating a paradigm shift towards climate-resilient practices. Recent studies by IPCC (2022) underscore the urgency of addressing climate change effects on food production.

However, it has been observed that Agricultural Extension service has been lacking in both adequacy and effectiveness when it comes to fulfilling this crucial role (Uguru et al., 2015). Consequently, many agricultural technologies that could have significantly enhanced the productivity of smallholder farmers are still inaccessible. Moreover, various barriers impede adoption of agricultural innovations, including limited resources, the incompatibility and complexity of new technologies, a shortage of technical training and information, as well as socio-economic and cultural constraints (Silva & Broekel, 2017). Hence, there is an urgent requirement to tackle these obstacles to enhance farmers' production performance and work towards the goal of achieving food security.

Maize (*Zea mays L.*) stands as the world's most cultivated cereal crop and ranks third in terms of global consumption, following wheat and rice (FAO, 2021). Worldwide, maize production totals approximately 1,127 million tons (OECD/FAO, 2019). Africa contributes about 75 million tons of maize to the world production, which represents 7.5% of the total global output. Nigeria leads the continent in maize production, producing 33 million tons, followed by South Africa, Egypt, and Ethiopia (International Institute for Tropical Agriculture, 2021). Maize is produced in virtually every part of Nigeria by smallholder farmers. This cereal has emerged as a crucial crop for ensuring food security, serving both human and animal consumption needs in the country (Global Agricultural Information Network, 2017). However, when it comes to maize yield, Nigeria is positioned at 117th place, averaging 2.1 tons per hectare. In contrast, the United States leads with a remarkable yield of 28.5 tons per hectare, while Egypt holds the highest yield in Africa at 7.1 tons per hectare (FAO, 2020). Heavy reliance on rain-fed agriculture and limited adoption of improved varieties, particularly those resistant to drought, are contributing factors to the existing yield gap. This study was therefore designed to: describe the socio-economic characteristics of the maize farmers, determine farmers' level of awareness of drought tolerant maize varieties, ascertain farmers' attitude towards agricultural innovation, identify constraints to the adoption of drought tolerant maize varieties and examine factors influencing farmers' attitude toward adoption of drought tolerant maize varieties. The study hypothesized that there is no significant relationship between selected farmer's socio-economic characteristics and farmers' attitudes to adopting agricultural innovation and that there is no significant relationship between farmers' level of awareness and farmers' attitudes to adopting agricultural innovation.

2. Materials and Methods

Primary data were used for this study. Data were collected from maize farmers using well-structured questionnaire. The study adopted multi-stage sampling procedure. The first stage was random selection of two local government areas (LGAs) in Ondo State, Nigeria. The LGAs are: Owo and Ifedore. The LGAs were selected randomly because, it helps to mitigate the risk of bias in the selection. If the local governments were chosen based on specific characteristics, it might introduce bias if those characteristics are related to variables being studied. In the second stage, five rural farming communities, that is: Ohuze, and Ibeje community in Owo Local Government Area and Ikota, Ipogun and Ijare in Ifedore Local Government Area were randomly selected. The aforementioned communities were selected in order to help in achieving a more representative sample and minimizes the risk of selecting communities that might not be typical of the larger population. In the third stage, twenty maize farmers were randomly selected from each community to give a sample size of one hundred (100) farmers with a 95% confidence level. This means that we are 95% confident that the characteristics and responses observed in the sample represent the entire population of maize farmers in the selected communities. The total sample size of one hundred (20 farmers x 5 communities) is determined by considerations such as available resources and time constraints. The maize farmers' level of awareness about drought tolerant maize varieties was measured by asking the farmers to indicate Yes or No to a list of awareness statements on drought tolerant maize varieties. The Yes response was coded one (1) and No coded as zero (0). Farmers' attitude towards agricultural innovation was measured on a five-point Likert-type scale of Strongly agree = 5, Agree = 4, Undecided = 3, Disagree = 2, Strongly disagree = 1. The grand mean was 2.05. Any item with a mean score of 2.05 and above was regarded as positive attitude and items with less than 2.05 were regarded as negative attitudes. Constraints to the adoption of drought tolerant maize varieties was measured on a three-point Likert-type scale of Major constraint = 3, Minor constraint = 2, and No constraint = 1. The grand mean is 2.06. Any item with a mean score of 2.06 and above was considered to be a constraint, and items equal to a mean score of 2.06 is considered to be a minor constraint, while any item less than 2.06 was not. Data collected were analyzed using descriptive statistics such as frequencies and percentages. Pearson Product Moment Correlation and Chi-square were used to test the study hypotheses. Logit regression was used to determine factors influencing farmers attitude to adopt agricultural innovations, the attitude was classified into positive attitude, coded as (1) and negative attitude coded as (0). A threshold value of 48 was used as the demarcation point. This calculation was derived by multiplying the total number of items (16) by the maximum obtainable points per item (5), resulting in 80. The total number of items (16) was then added to 80, yielding a sum of 96. Subsequently, this total of 96 was divided by two (2) to distinguish between positive and negative attitudes. Where any farmer with an attitude score above 48 was categorized as having a positive attitude, while those with scores below 48 were categorized as having a negative attitude.

The Logit regression analysis is explicitly represented below:

$$Y = b0 + b1X1 + b2X2 + b3X3 + b4X4 + b5X5 + b6X6$$

Where:

Y = Farmers' attitude to adopt agricultural innovation (Positive (1), Negative (0)

X_1 = Sex (Male 1, Female 0)

X_2 = Income (Naira)

X_3 = Age (Years)

X_4 = Farm size (Hectares)

X_5 = Years of Farming Experience (Years)

X_6 = Highest Level of Education (Number of years)

3. Results and Discussion

3.1. Socioeconomic Characteristics of the Maize Farmers

The socioeconomic characteristics of the maize farmers were presented in Table 1. Majority (69.0%) of the maize farmers were male while 31.0% were female. This is in agreement with the findings of Akinwale et al. (2020), that majority of maize farmers in Akure South and Akoko North West Local Government Area of Ondo state were predominantly male. Average age of the maize farmers in the study area was 42.0. This indicates that most of the maize farmers were still within the productive age with strength and agility to engage in agricultural practices. Most (67.0%) of the maize farmers were married. Also, a significant majority (57%) of maize farmers reported having household sizes ranging from 6 to 10 individuals, followed by 23% having household sizes between 1 and 5 members. The calculated mean of the household size stood at 8 persons, highlighting relatively large household sizes among maize farmers. This contradicts the results from Adebisi et al. (2017), which observed that majority (65.7%) of farmers had between 4 and 6 household size. According to the data collected, 43% of the maize farmers had secondary education, 27% had primary education, 25% had no formal education, while 5% had tertiary education. This result indicates that most of the farmers have attained appreciable level of education that will enhance their grasp of improved agronomic practices in maize production. Furthermore, most (72%) of the maize farmers had farm sizes between 1-4 hectares, 21% had 5-8 hectares and 7% had above 8 hectares farm size. The mean farm size was 4.0 hectares. This corroborates with the findings obtained by Owoeye et al. (2017), who reported that maize farmers in Ekiti state typically work on an average farm size of 3.4 hectares. This suggests that maize farmers in the study area are predominantly engaged in small-scale farming practices.

Table 1. Socioeconomic characteristics of the maize farmers.

Socio-economic characteristic	Frequency	Mean	Standard Deviation
Sex			
Male	69		
Female	31		
Age (Years)			
<20	3		
21-30	18	42.0	1.224
31-40	26		
41-50	32		
51-60	14		
>60	7		

Table 1. (continued)

Socio-economic characteristic	Frequency	Mean	Standard Deviation
Marital Status			
Single	11		
Married	67		
Widowed	18		
Divorced	4		
House Hold Size (Number of persons)			
1-5	23		
6-10	57	8.0	0.681
11-15	19		
16-20	1		
Level of Education			
No Formal Education	25		
Primary Education	27		
Secondary Education	43		
Tertiary Education	5		
Farm Size (Hectares)			
1-4	72	4.0	0.609
5-8	21		
9-12	7		

3.2. Maize Farmers' Level of Awareness of Drought Tolerant Maize Varieties

The result in Table 2 shows that majority (76%) of the maize farmers were not aware of maize varieties that can grow despite variability in rainfall patterns. This implies that there is lack of awareness among the maize farmers about certain aspects of maize production. This lack of awareness can undermine efforts at achieving food security and sustainable food production. Also, 74% of the maize farmers were not aware that some maize varieties can grow and produce even if rain fails. This situation may lead to poor yields and low economic returns for the farmers. This could also have broader implications on food security, as low yields could lead to food shortages, high food prices, and increased reliance on imports. Moreover, 71% of the maize farmers lacked awareness that certain agronomic practices, such as no-till maize production, could contribute to the reduction of greenhouse gas emissions. That the farmers were unaware of these agronomic practices may make them to be dependent on synthetic fertilizers which may not environmentally sustainable. On the other hand, majority (70%) of the maize farmers exhibited awareness regarding the existence of maize varieties resistant to pests and diseases. This awareness stands as an encouraging indicator for maize farming, as the recognition of pest and disease-resistant varieties bears the potential to diminish crop losses and enhance overall productivity.

Table 2. Maize farmers' level of awareness of drought tolerant maize varieties.

S/N	Statement	Yes F(%)	No F(%)
1.	Are you aware that some maize varieties can grow and produce even if rain fails	26(26)	74(74)
2.	Do you know that there are maize varieties that are pest and diseases resistant	70(70)	30(30)
3.	Are you aware you can plan your planting period with some maize varieties	46(46)	54(54)
4.	Do you know there are some maize seedlings that improves early seedling growth during the germination stage	45(45)	55(55)
5.	Do you know for best management against drought, maize seeds for planting must be bought every year and not from previous planting	44(44)	56(56)
6.	Are you aware you can determine yourself when to plant with some maize varieties	32(32)	68(68)
7.	Do you know with some best agronomic practices such as no-till, maize production can reduce greenhouse gas emission	29(29)	71(71)
8.	Are you aware of new improved maize varieties that is early maturing	41(41)	59(59)
9.	Are you aware of maize varieties that can still grow even when there is increase in variability of rainfall patterns	24(24)	76(76)

3.3. Farmers' Attitude Towards Agricultural Innovation

Table 3 shows that the strongest attitude of the respondents towards agricultural innovations was that they are willing to embrace any agricultural innovation provided the innovation was practicalised ($\bar{x}$ = 4.28). This suggests that the respondents recognize the importance of innovation in agriculture and are open to adopting new technologies and practices. This is a positive sign for the agricultural sector, as innovation can help to increase productivity, reduce environmental impact, and improve food security. This contradicts the findings of (Kazeem et al., 2017), who found out that farmers attitudes had an insignificant impact on technology adoption, but identified that negative perceptions of extrinsic factors, such as constraints on technology training, had a stronger influence on farmers' adoption of novel technology. Next is the belief that agricultural innovation is better than traditional practices ($\bar{x}$ = 4.08). This suggests that the respondents recognize the potential benefits of adopting new technologies and practices in agriculture. They always wish to receive information about agricultural information (x̄ = 4.07), which implies that the respondent has positive attitude towards receiving information about agricultural innovation. This suggests that the respondents are interested in learning about new technologies and practices in agriculture which will in turn increase their productivity. I often take my time before making a decision to adopt an agricultural innovation (x̄ = 3.89), this implies that the respondents tend to deliberate and carefully consider before deciding to adopt new agricultural innovations. Other identified attitudes of the farmers include, their farmer friends who use new agricultural innovation influence them to do the same (x̄ = 3.84). This suggests that social networks play an important role in the diffusion of agricultural innovations. Farmers may be more likely to adopt new technologies and practices if they see their peers doing the same, as this can provide social proof and reduce the perceived risk of experimentation. According to farmers opinions, the use of any agricultural innovation increases effectiveness in their farm (x̄ = 3.68). This suggests that farmers believe that new technologies and practices can help to increase their productivity, reduce costs and improve the quality of their produce. They may have had positive experiences with previous innovations or seen the benefits of innovation in the farms of their peers. Policy makers have acknowledged that farmers' responses to changes in agricultural policy are influenced in part by their attitudes and mindsets. The implication here is that the attitudes and mindsets of farmers play a role in shaping how they react to shifts or modifications in agricultural policies. This understanding is crucial for policymakers as they design and implement changes, recognizing that the success and acceptance of new policies may hinge, at least in part, on aligning them with the attitudes and beliefs of the farming community. Furthermore, farmers' attitudes can be more positive when they possess knowledge about diversification, make informed choices regarding suitable technologies, and receive financial support to maximize returns while minimizing risks (Adegebo et al., 2016).

Table 3. Farmers' attitude towards agricultural innovation.

Statements	S	D	U	A	S	Mean
I always wish to receive information about agricultural innovation	5	8	6	37	44	4.07
I believe that agricultural innovation is better than traditional practices.	5	5	8	41	41	4.08
I will not forgo the traditional varieties no matter the information I hear of any agricultural innovation	37	5	14	21	23	2.88
I am willing to embrace any agricultural innovation provided the innovation is practicalized	3	4	20	48	25	4.28
The uncertainty of not knowing how successful an agricultural innovation will be in the long-term would make me uncomfortable to adopt	22	7	17	28	26	3.29
The use of agricultural innovation increases effectiveness in my farm	1	12	18	56	13	3.68
The use of any new agricultural innovation makes me popular among my peers	25	12	16	24	23	3.08
My farmer friends who use new agricultural innovation influence me to do the same	18	8	20	30	24	3.84
I enjoy reading/listening about the different agricultural innovation currently in use	17	10	9	44	20	3.40
I enjoy discussing about current agricultural innovation currently promoted by extension services	7	11	16	52	14	3.55
I often take my time before making a decision to adopt an agricultural innovation	15	8	19	39	19	3.89
I am always skeptical about new agricultural innovation	19	13	10	36	22	3.29
Friends always use me as a point of reference for new innovations	33	6	17	22	22	2.94
I have keen interest in new agricultural innovations	17	12	17	41	13	3.21
I am always skeptical when it comes to new agricultural innovations	22	10	9	34	25	3.30
I prefer to stick to existing agricultural practices that I am familiar with	39	9	11	22	19	2.73

2.05 is significant.

3.4. Constraints to the Adoption of Drought Tolerant Maize Varieties

Table 4 shows that the major constraints that hindered the respondent from adopting drought tolerant maize varieties were low output of drought tolerant maize varieties (x̄ = 2.28). This could be due to lack of compliance with agronomic practices associated with the cultivation of drought-tolerant maize varieties. Next is technicalities of innovation (lack of technical know-how) (x̄ = 2.27), this is due to various reasons such as inadequate information on usage and management of drought-tolerant maize varieties and limited access to extension services. Also, non-accessibility of drought tolerant maize varieties (x̄ = 2.26). This due to reasons such as limited availability of drought-tolerant maize varieties in local markets, high prices of such varieties, or inadequate distribution channels. Other identified constraints encountered by the maize farmers include: lack of adequate information/warning about drought (x̄ = 2.17). This is due to various reasons such as limited access to weather forecasts, inadequate communication channels for disseminating weather information, or low awareness of the risks associated with drought. Farmers unwillingness to adopt drought-resistant maize varieties due to distrust arising from previous experience ($\bar{x}$ = 2.09). This is due to various reasons such as farmers' bad experiences with previously adopted drought-tolerant maize varieties, poor performance of such varieties, low yield, fragility to pests and diseases, inferior product quality and false promises from seed companies.

Table 4. Constraints to the adoption of drought tolerant maize varieties.

Statement	Major Constraint	Minor Constraints	No constraints	Mean	Ranking
Low output of drought tolerant maize varieties	44	40	16	2.28	1st
Technicalities of innovation (lack of technical know-how)	43	41	16	2.27	2nd
Non-accessibility of drought tolerant maize varieties	44	38	37	2.26	3rd
Lack of adequate information/ warning about drought	37	43	20	2.17	4th
Farmers unwillingness to adopt due to distrust arising from previous experience	27	55	18	2.09	5th
Uncertainty/fear of failure of the drought tolerant maize varieties	32	36	32	2.00	6th
Lacking sufficient skills to adopt the drought tolerant maize varieties	30	36	31	1.96	7th
Inadequate extension agent to provide essential information	26	40	34	1.92	8th
Socio-cultural limitation to the adoption of drought tolerant maize varieties in my locality	20	45	35	1.85	9th

Major constraint = 3, Minor constraint =2, No constraint =1.

3.5. Factors Influencing Farmers' Attitude to Adopt Agricultural Innovation

Logit regression analysis indicated that the model holds statistical significance, underscoring its efficacy in predicting farmers' attitudes toward agricultural innovation (Table 5).

Sex: An exploration of the analysis revealed that the coefficient for sex (male = 1, female = 0) was estimated at 1.398 (SE = 0.585). This estimate was accompanied by a Wald chi-square statistic of 5.716, yielding a p-value of 0.017. The odds ratio for sex was determined to be 4.043 (95% CI: 2.054-6.924), suggesting that male farmers were notably more inclined to exhibit a positive attitude toward agricultural innovation compared to their female counterparts. This gender-based distinction significantly influenced farmers' attitudes toward agricultural innovation.

Income: The coefficient pertaining to income was observed to be 0.000 (SE = 0.000). This coefficient resulted in a Wald chi-square statistic of 5.025 and a corresponding p-value of 0.025. The odds ratio for income was computed as 1.000, indicating that with each unit increase in income, the odds of adopting a favorable attitude toward agricultural innovation increased by approximately 2.5%. The effect of income on farmers' attitudes was statistically significant, reinforcing its role as a contributing factor.

These findings are in agreement with the findings of Adarkwa et al., (2017), that male farmers exhibited a higher propensity for embracing agricultural innovations compared to their female counterparts, while farmers boasting elevated income levels demonstrated a greater likelihood of adopting these advancements. Furthermore, research revealed that farmers who had undergone training in agricultural innovations exhibited a heightened inclination toward their adoption (Adarkwa et al., 2017).

Non-Significant Predictors: In contrast, the predictor variables of age, marital status, farm size, and years of farming experience exhibited no substantial impact on farmers' attitudes toward agricultural innovation.

Model Fit: The Nagelkerke R^2 value of 0.226 for the model indicates that approximately 22.6% of the variance observed in farmers' attitudes toward agricultural innovation can be attributed to the variables integrated into the model.

Table 5. Factors influencing farmers' attitude to adopt agricultural innovation.

Variable	Coefficient	SE	Wald	p-value
Sex (male =1, female =0)	1.398	0.585	5.716	0.017
Income	0.000	0.000	5.025	0.025
Age	-0.048	0.031	2.394	0.122
Farm size	-0.756	0.487	2.412	0.120
Years of Farming Experience	-0.035	0.058	0.362	0.548
Level of Education	-0.823	1.445	0.325	0.569

3.6. Test of Hypotheses

Two hypotheses were tested in this study and were stated in null form at 0.05% level of significance.

H_{01}: There is no significant relationship between farmer's socio-economic characteristics and Farmers attitude to adopt Agricultural innovations. The result of the Pearson correlation analysis presented in Table 6 indicates that there is a statistically significant relationship between level education and Farmers attitude to adopt Agricultural innovations, p-value <0.05. While there is no significant relationship between other selected socio-economic characteristics and farmers' attitude towards agricultural Innovation.

Table 6. Correlation analysis of relationship between the socio-economic characteristics and farmers attitude to adopt agricultural innovations.

Socio-economic characteristics/ farmer's attitude to adopt Agricultural innovation	Correlation Coefficient	P-Value	Decision
Age	-0.181	0.071	NS
Household size	-0.109	0.280	NS
Level of Education	0.200	0.046	NS
Farm Size	0.027	0.787	NS
Years of Farming Experience	-0.082	0.419	NS

Correlation is significant at the level 0.05(1-tailed). NS: Not significant.

H_{02}: There is no significant relationship between farmers' level of awareness on drought tolerant maize varieties and farmers attitude to adopt Agricultural innovation.

Table 7 presents the results of Chi-square analysis to show relationship between the level of awareness on drought maize tolerant varieties and farmers attitude to adopt Agricultural innovation. Statistically, there is a significant relationship between the level of awareness on drought tolerant maize varieties and farmers attitude to Agricultural innovation ($p < 0.05$). This implies that farmers who are more aware of drought maize tolerant varieties as Agricultural innovation, have a positive attitude towards adopting them. This is because awareness help farmers to understand the benefits of drought tolerant maize varieties as Agricultural innovation and to see how they can help them to improve their yields and incomes in drought-prone areas.

Table 7. Chi-square analysis of relationship between farmers level of awareness and farmers attitude to adopt agricultural innovation.

Hypothesis	Chi-Square value (χ^2)	Sig. Value	Decision
There is no significant relationship between farmers level of awareness on drought tolerant maize varieties and farmers attitude to adopt agricultural innovation	100.000	0.000	Significant

4. Conclusion

The study's results indicate a noteworthy gap in farmers' awareness when it comes to comprehending the advantages and features of drought-tolerant maize varieties. A significant portion of farmers exhibited a lack of understanding regarding the qualities and benefits associated with the drought tolerant maize varieties. Several impediments to the actual adoption of these varieties were identified. These challenges encompassed issues such as low output of drought-tolerant maize varieties and the technical complexities related to their integration into farming practices due to a lack of requisite technical expertise. The research also highlighted the substantial impact of two key factors, namely, (sex and income) on farmers' decisions regarding the adoption of drought-tolerant maize varieties. These factors emerged as pivotal influencers in shaping the inclinations and choices of maize farmers towards adopting drought tolerant maize varieties. Level of education and level of awareness was correlated.

Based on the findings of the study on factors influencing farmers' attitude to adopt drought-tolerant maize varieties in Ondo State, Nigeria, the following recommendations are suggested:

1. Farmers maize output was low probably due to high effects of increased pest and diseases and drought. High yielding drought tolerant varieties could be massively deployed to drought prone area. This would go a long way in improving the low yield of maize production in the study area.

2. Level of awareness for drought tolerant maize varieties was very low. It is therefore recommended that information constraints by agricultural extension services / information dissemination services in the state, should be strengthened to increase farmers' awareness of the benefits of drought-tolerant maize varieties. This can be achieved through targeted awareness campaigns and training programs.

3. Maize farmers were saddled with so many maize production constraints all of which were either: socio-economic, institutional and technological factors. These could

be avoided through appropriate policies and better research approaches which will be critical to the future of maize producers and of the maize industry in Nigeria.

4. Getting appropriate feedback on farmers technology adoption behavior is crucial in designing a well-tailored intervention that could result in rationalizing scarce resources required for used by stakeholders in agricultural sector of the economy to avoid misappropriation of financial resources to develop varieties that would not be adopted by the end users.

5. The government and agricultural development organizations should work together to provide access to extension services to farmers in Ondo State, Nigeria.

Conflict of Interest

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Agronomic Performance of Young Cashew Trees Cultivated in Association with Groundnuts

Ange Kouakou Yann Cédric Letto[1]✉ • Lassina Fondio[2] • Yapo Yves Olivier Adiko[2] • Akadié Jean-Baptiste Djaha[2] • Florent Jean Haba[1] • Tanoh Hilaire Kouakou[1]

[1]Nangui Abrogoua University, UFR of Natural Sciences, Laboratory of Biology and Improvement of Plant Production, Abidjan/Côte d'Ivoire

[2]National Agricultural Research Center, Korhogo Regional Directorate, Abidjan/Côte d'Ivoire

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ABSTRACT

Cultural association is widely practiced in rural areas by cashew nut producers. These producers typically cultivate annual plants between the rows of young cashew trees. However, there are interactions between these associated crops, which can either result in complementarity or competition among the plants for environmental resources such as water and nutrients. Consequently, young cashew seedlings newly planted were associated with groundnuts during their first two years. The aim of this study was to investigate the compatibility of the cashew/groundnut system. The results showed that there is competition between groundnuts and young cashew trees in the first year, leading to poor cashew growth in association. Nevertheless, from the second year onwards, cashew trees cultivated in association exhibited similar growth to those cultivated in monoculture. Therefore, it would be advisable to plant groundnut seeds sufficiently far from cashew tree seedlings to avoid potential competition in the first year of cultivation.

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1. Introduction

Crop association is defined as the simultaneous cultivation of two or more plant species on the same surface for a significant period of their growth (Perrin & Lefevre, 2019). It is a widely practised farming system in tropical regions and is adopted by the majority of cashew nut producers worldwide (Adiga & Kalaivanan, 2017). These associations are typically established with food crops during the early stages of cashew tree growth (Konan & Ricau, 2010). According to Penot and Feintrenie (2014), this farming system enables farmers to generate substantial income through agricultural product diversification. Consequently, crop association appears to be a solution to the low incomes earned by farmers during the initial establishment and production phases of their orchards, as well as to the fluctuations in cashew nut yields and prices.

✉ Correspondence

E-mail address: lettoange@yahoo.com

However, the plants involved in these associations interact with each other. This interaction can result in either complementarity or competition between the crops. Practices that involve intercropping cashew trees with other crops may therefore carry risks such as competition for water, mineral elements, light, etc. Consequently, only a well-thought-out crop association that takes these risks into account and aims for a more efficient use of natural resources would be appropriate (Keli et al., 2005). According to Mansaray et al. (2022), the benefits of crop association are observed when the components of the system exhibit different morphological growth patterns and strongly compete for natural resources at different times. In the context of cashew farming, the recommended cropping system by Visalakshi et al. (2015) involves associating cashew trees with herbaceous plants, including legumes. The rationale behind intercropping cashew trees with legumes is based on the principle that trees can utilize the nitrogen fixed by the legume. In this relationship, the legume can either increase the available nitrogen content or compete with the non-fixing plant. However, non-leguminous plants typically do not benefit from associated legumes unless the non-fixing plants continue to absorb nitrogen after the senescence or death of the legumes (Mansaray et al., 2022).

Furthermore, in Côte d'Ivoire, the results of the survey conducted by Letto et al. (2022) have shown that groundnuts are the leguminous crop most commonly associated with cashew trees in the northern part of the country. However, the agronomic performance of cashew trees cultivated in association with this legume is not yet known. The present study was therefore conducted following this survey with the aim of investigating the growth and development of young cashew trees cultivated in monoculture and in association with groundnuts in northern Côte d'Ivoire.

2. Materials and Methods

2.1. Study Site

The present study was conducted at the research station of the National Centre for Agronomic Research (CNRA) in Ferkessédougou (longitude 5.22° W, latitude 9.59° N). This locality is the capital of the Tchologo region in the Savanes District, Northern Côte d'Ivoire (Figure 1). The soil at the site is of remodeled ferritic type, with a sandy-clayey texture, and is characterized by a moderate-depth lateritic induration (70-75 cm) (Akanza & N'Guessan, 2017). The soil has an acidic pH (6.45) and is poor in organic matter (Akanza & N'Da, 2018). The climate of the Tchologo region is of the Sudanese type, characterized by a dry season extending from November to April. The rainy season lasts from May to October, with the highest precipitation occurring in August. The Tchologo region has an average annual temperature of 26.4 °C and a rainfall of 1260 mm (Soro et al., 2020).

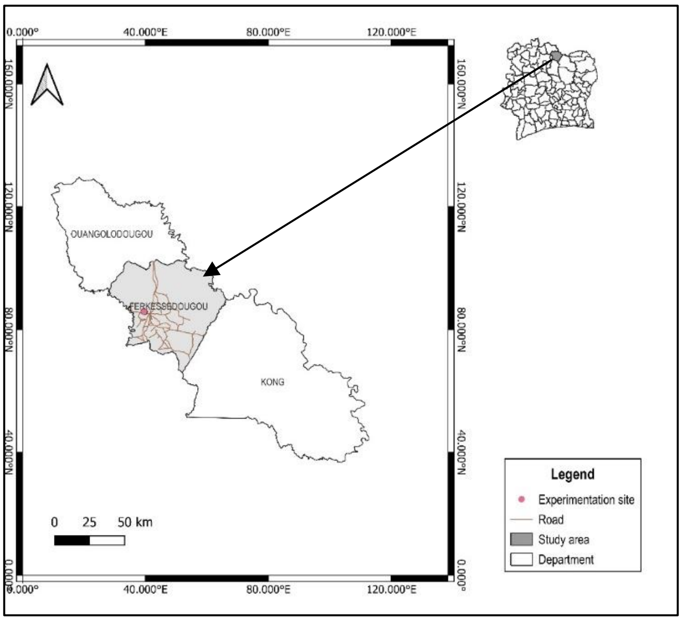

Figure 1. Study site.

2.2. Plant Material

The plant material used consisted of 45-day-old grafted cashew tree (*Anacardium occidentale* L.) seedlings. In addition, groundnut seeds commonly cultivated in rural areas of the Tchologo region, with a vegetative cycle of 120 days, were included.

2.3. Experimental Procedure

The effect of three levels of planting spacing on cashew tree growth was evaluated. The cashew trees were planted at three different spacing configurations: 10 m × 10 m, 12 m × 12 m, and 14 m × 14 m. As for groundnuts, they were sown between the rows of cashew trees, 7 days after the cashew tree planting. Groundnut seeds were sown in pairs (2 seeds per pocket), with a spacing of 0.50 m between rows and 0.20 m between pockets. Additionally, some cashew trees were grown in monoculture and served as control.

In this cashew tree and groundnut association, no mineral or organic fertilizers were applied throughout the two-year experimental period.

2.4. Experimental Design

The experimental design used was a split-plot with three replications. The main factor, cashew tree spacing, had three levels: 10 m × 10 m, 12 m × 12 m, and 14 m × 14 m. The secondary factor, the cropping system, had two levels: cashew tree monoculture and cashew tree/ groundnut association. With three levels of the main factor and two levels of the secondary factor, a total of six elementary plots were used per replication. Each elementary plot consisted of 12 cashew trees.

2.5. Parameters Studied

2.5.1. Mortality rate

The mortality rate of cashew trees was evaluated based on the cropping system employed. Dead cashew trees were counted on a weekly basis for two years. After counting the cashew trees, the mortality rate was calculated using the following formula:

$$MR = \frac{\text{number of dead cashew trees}}{\text{total number of cashew trees}} \times 100 \qquad (1)$$

Where: MR = Mortality Rate.

2.5.2. Growth rate

The growth rate was used to assess the percentage of elongation of cashew trees compared to their initial state. Height and stem diameter at the collar were determined monthly for two years. Height was measured from the collar to the apex of the stem using a measuring tape. Collar diameter was measured at the base of the plants using a caliper. The growth rate was then calculated as follows:

$$GR = \frac{m1 - m0}{m0} \times 100 \qquad (2)$$

Where: GR = growth rate (%), m0 = initial measurement (cm), m1 = final measurement (cm).

2.5.3. Vigor

Height and collar diameter of trees are commonly used parameters in tree cultivation. The ratio of these two dendrometric parameters was used to determine the vigor of cashew trees according to the following formula:

$$V = \frac{H}{D} \qquad (3)$$

Where: V = vigor, H = plant height (cm), D = collar diameter (cm).

2.5.4. Number of leaves

The number of leaves was used to assess the vegetative development of cashew trees. This parameter involved counting the leaves of cashew trees based on the cropping system during the first year of the study.

2.5.5. Canopy spread

Canopy spread is the (average) measurement of the lateral distance between the two outermost leaves of the cashew tree canopy in the east-west and north-south directions. It was measured to evaluate the lateral growth of cashew trees according to the cropping system. Canopy spread was calculated using the following formula:

$$CS = \frac{\text{N−S measurement} + \text{E−W measurement}}{2} \qquad (4)$$

Where:

CS = canopy spread (m), N-S measurement = measurement of canopy spread in the north-south direction (m), E-W measurement = measurement of canopy spread in the east-west direction (m).

2.6. Statistical Analysis

The cropping systems, cashew tree spacing, and their interaction were each subjected to a multivariate analysis of variance (MANOVA) to assess their overall influence on the measured parameters. This helped identify factors that showed a significant effect. The student's t-test was then used for mean comparison. When the probability (P) was ≥ 0.05, it was concluded that there was no significant difference, whereas when P < 0.05, at least one significant difference existed among the means. Finally, the chi-square (χ^2) test for independence was performed to evaluate the relationship between variables. The analyses were conducted using the XLSTAT software.

3. Results

3.1. Combined Effect of Plant Spacing and Cropping System on Mortality Rate

Figure 2 presents the mortality rates of cashew trees according to the studied treatments. The highest mortality rates were observed in cashew trees grown in association with groundnuts. Specifically, cashew trees grown in the intercropping system, with spacing configurations of 10 m × 10 m and 12 m × 12 m, recorded respective mortality rates of 31.25% and 25.64%. These rates were lower in cashew trees grown in monoculture at the same spacing, which recorded respective mortality rates of 20.31% and 17.95% for the 10 m × 10 m and 12 m × 12 m spacing. As for cashew trees spaced at 14 m, the mortality rate was the same for both cropping systems at 20.51%.

Furthermore, the chi-square (χ^2) test of independence conducted to determine the relationship between cashew tree mortality and the cropping system, on the one hand, and the plant spacing on the other hand (Table 1), showed that cashew tree mortality is not related to either the cropping system or the spacing between trees (P > 0.05).

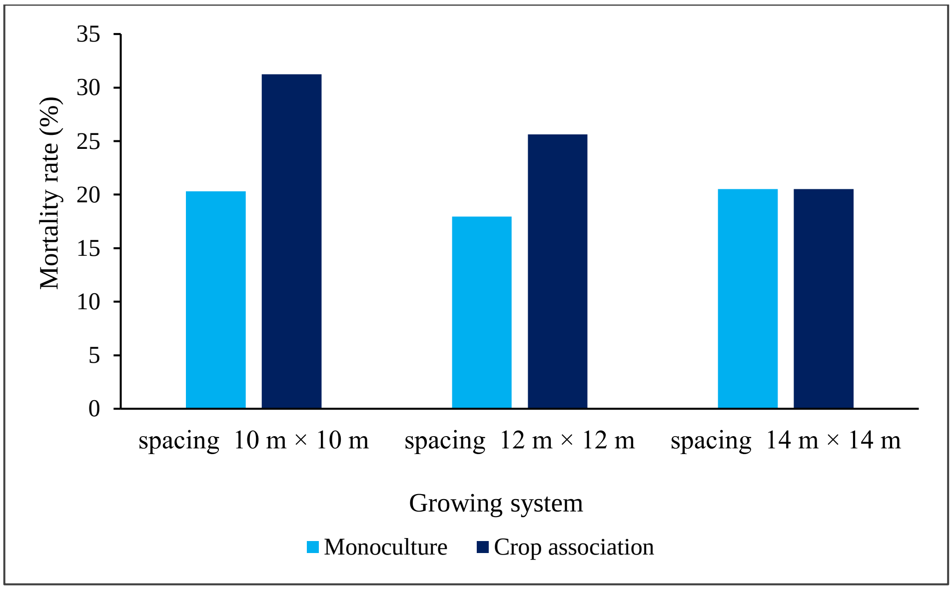

Figure 2. Variation in cashew mortality rate according to cropping system and plant spacing.

Table 1. Results of the chi-squared (χ^2) independence test regarding cashew tree mortality.

Variables	**Cashew trees spaced 10 m × 10 m**		**Cashew trees spaced 12 m × 12 m**		**Cashew trees spaced 14 m × 14 m**	
	X_{obs}	X_{theo}	X_{obs}	X_{theo}	X_{obs}	X_{theo}
Monoculture	13	14	7	7.21	8	6.79
Crop association	20	19	10	9.79	8	9.21
Dl			2			
P			0.77			

X_{obs}: observed value; X_{theo}: theoretical value; Dl: degree of freedom; P: probability.

3.2. Overall Effect of Cropping Systems and Spacing on Cashew Tree Growth Parameters

Table 2 presents the results of the multivariate analysis of variance (MANOVA) examining the overall effect of cropping systems and spacing on cashew tree growth parameters. This test revealed that only the cropping system had a significant effect ($P < 0.05$) on the studied parameters. Consequently, the comparison of means for agro-morphological parameters was conducted based solely on the cropping system through the student's t-test.

Table 2. The overall effect of cropping systems and spacing on growth parameters of cashew trees.

Factors	**Dl**	**P**
Spacing	8	0.071
Growing system	4	< 0.0001
Spacing × growing system	8	0.182

Dl: degree of freedom, P: probability.

3.3. Effect of Cropping System on Cashew Tree Growth Parameters

The results of the impact of the cropping system on the growth parameters of cashew trees are presented in Table 3. The Student's t-test indicated a highly significant effect ($P < 0.001$) of the cropping system on all the agronomic parameters studied in the first year of cultivation. Indeed, the growth of cashew trees cultivated in monoculture was faster than that of plants cultivated in association with groundnuts. In comparison to cashew trees cultivated in crop association, the highest values for vigor (37.54), number of leaves (36.01), diameter growth rate (82.30%), and height (116.83%) were observed in cashew trees cultivated in monoculture. The average value for vigor, number of leaves, diameter growth rate, and height of cashew trees cultivated in crop association were 33.83, 18.64, 49.96%, and 60.01%, respectively.

From the second year onward, the growth rates (diameter and height) of cashew trees cultivated in association and in monoculture did not differ significantly ($P > 0.05$). The diameter growth rate of cashew trees cultivated in monoculture and crop association was 43.53% and 40.86%, respectively. As for the height growth rate of the plants, it was 47.12% for cashew trees cultivated in monoculture and 49.71% for those cultivated in association with groundnuts. The Student's t-test also revealed that the vigor of cashew trees cultivated in crop association was higher than those cultivated in monoculture ($P < 0.001$). The average vigor values were 28.84 and 31.98, respectively, for cashew trees cultivated in monoculture and in association with groundnuts. Finally, a significant difference (P < 0.001) was observed in the canopy size of the trees. Cashew trees cultivated in monoculture had a significantly larger canopy (148.39 cm) compared to their counterparts cultivated in association with groundnuts (115.51 cm).

Table 3. Average values of cashew tree growth parameters according to cropping system.

	Variables	Monoculture	Crop association	*Statistics*	
				|t|	**P**
Year 1	**Diameter growth rate (%)**	82.30 ± 3.87[a]	49.96 ± 2.75[b]	6.80	< 0.001
	Height growth rate (%)	116.83 ± 6.51[a]	60.01 ± 4.42[b]	7.20	< 0.001
	Vigor	37.54 ± 0.78[a]	33.83 ± 0.68[b]	3.56	< 0.001
	Number of leaves	36.01 ± 2.15[a]	18.64 ± 1.27[b]	6.95	< 0.001
	Canopy spread (cm)	-	-	-	-
Year 2	**Diameter growth rate (%)**	43.53 ± 1.65[a]	40.86 ± 1.82[a]	1.08	0.277
	Height growth rate (%)	47.12 ± 4.32[a]	49.71 ± 3.33[a]	0.47	0.635
	Vigor	28.84 ± 0.60[b]	31.98 ± 0.65[a]	3.51	< 0.001
	Number of leaves	-	-	-	-
	Canopy spread (cm)	148.39 ± 4.59[a]	115.51 ± 4.10[b]	5.33	< 0.001

Values bearing the same letters horizontally are statistically equal; |t|: value of the t-test; P: probability of occurrence.

3.4. Growth of Cashew Trees According to the Cultivation System

The growth curve shows two distinct phases in the first year of cultivation. The first phase is observed during the first 30 days, where cashew trees exhibit similar growth for both cropping systems. Beyond this period, cashew trees grown in monoculture show more accelerated growth compared to those grown in the intercropping system with groundnuts (Figure 3). This trend differs in the second year of the experiment, where almost identical growth of cashew trees is observed regardless of the practiced cropping system (Figure 4).

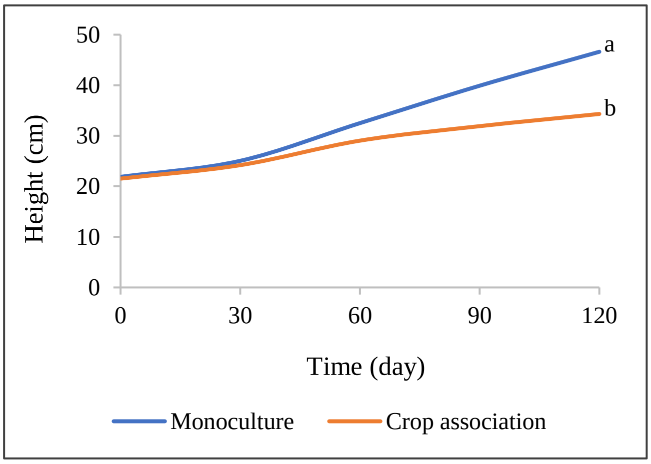

Figure 3. Evolution of cashew tree growth under the cultivation system in the first year of growth. Different letters mean significant differences between the treatments according to student's t-test (P < 0.05).

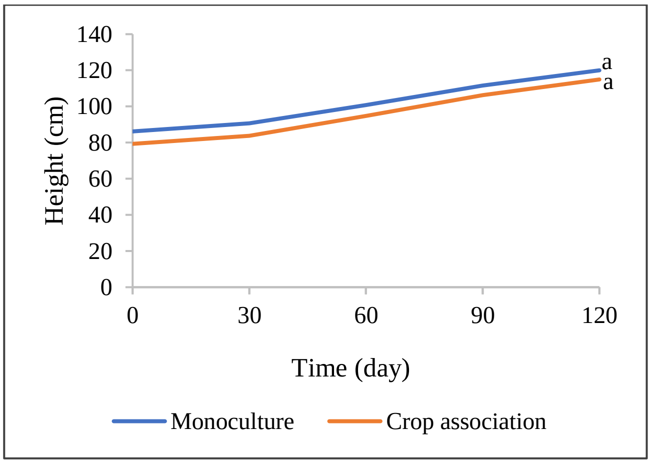

Figure 4. Evolution of cashew tree growth under the cultivation system in the second year of growth. Identical letters mean statistical equality between treatments according to the student t-test (P > 0.05).

4. Discussion

The lack of a significant difference observed in this study between the cropping systems regarding cashew tree mortality may be attributed to certain physical factors. Indeed, the taproots of the cashew trees may have broken during their transport to the field or during handling before planting, which could have led to their mortality. Working on the vegetative growth and grafting ability of two cashew genotype, Djaha et al. (2012) also indicated that root breakage in young cashew trees is the primary cause of their mortality after transplantation. Cashew tree mortality could also be attributed to the low quantity of organic matter and poor fertility of the experimental site. Indeed, the soils in the experimental area

(Tchologo region, northern Côte d'Ivoire) are generally deficient in organic matter (Akanza & N'Guessan, 2017). Consequently, young and delicate cashew trees, which had not received any amendments, were unable to withstand the edaphic conditions of the environment. This observation is in line with the findings of Tokore Orou Méré et al. (2022), who revealed that newly planted cashew trees fertilized with organo-mineral fertilizers had a lower mortality rate than those cultivated without fertilizer inputs.

The slow growth of cashew trees cultivated in association with groundnuts in the first year indicates potential interspecific competition. This result is largely attributed to the timing of groundnut planting. Indeed, planting the legume one week after establishing the cashew tree may have been a short interval. The young plants may not have had sufficient time to adapt and establish their roots properly to utilize the available resources. The intercropping may have consequently caused water and/or nutrient stress in the young cashew trees, observed starting from the 30th day of association. This date corresponds to the flowering period of groundnuts, during which they require significant amounts of water and mineral nutrients for pods and seed formation (Raphiou et al., 2020; Civil, 2022). Djè Bi et al. (2017) also highlighted that the planting timing is a factor that influences the growth of associated crops. These authors further reported that cassava's growth and development are limited when cultivated concurrently with groundnuts. However, the best agronomic performances of cassava (number of leaves, stem diameter, stem length) were observed when the cucurbit was planted at least 15 days after cassava planting. Similarly, the work of Legodi and Ogola (2020) showed that legumes (cowpea, chickpea, and pigeon pea) planted later between cassava plants do not exert significant competition on them.

One of the reasons for the improved growth of the trees observed in the second year of this study could be attributed to the fact that cashew trees had a greater competitive advantage for mineral elements compared to groundnuts. Indeed, at 12 months of age, cashew trees could explore a large volume of soil through the development of numerous lateral roots. These roots likely exerted sufficient competition for water and mineral elements present in the soil. This observation aligns with the findings of Mansaray et al. (2022), who indicated that well-established cassava plants (at 5 weeks of age) develop enough roots to compete with secondary crops like groundnuts, cowpeas, and soya beans. Consequently, a harmonious growth of cassava plants (height, stem diameter, and crown diameter) was observed under these cultivation conditions.

On the other hand, cashew trees cultivated in association may have benefited from the residual effects of the legume in the second year of cultivation, thus promoting the growth of their stems. Furthermore, plants with large stem diameters typically have a well-developed root system (Day et al., 2010). This correlation may have allowed the intermingling of cashew tree roots with those of groundnuts. This mechanism enables the transfer of nitrogen fixed by the legume during the crop association (Akanza & N'Guessan, 2017). This potential nitrogen nutrition would have accelerated the growth of cashew trees cultivated in association, helping them catch up in height with those cultivated in monoculture. Additionally, groundnuts may behave like some legumes that initially compete during the early years of association but eventually benefit the main crop. This is the case with alfalfa, which can hinder tree growth in the early years of cultivation. The benefits of this legume are observed after a few years, promoting tree growth (Coulon et al., 2000). The growth results of cashew trees obtained in the second year of this study corroborate those of Opoku-Ameyaw et al. (2011), who showed no significant difference between cashew tree association with groundnuts and cashew tree monoculture in terms of stem base circumference and tree height. The results of Santimaitree (2010) also revealed that the height and stem circumference growth of young rubber trees cultivated in monoculture and in association with groundnuts were similar.

5. Conclusion

At the end of this study, we conclude that the mortality of cashew trees is not linked to any of the factors studied (crop system and planting density). Additionally, there is competition between groundnuts and young cashew trees in the first year of their combined cultivation. This leads to a slowdown in the development and growth of cashew trees in association, unlike those grown in monoculture, which exhibited the highest values in terms of vigor, leaf count, and growth rates (height and stem diameter). However, the growth delay of young cashew trees grown in combined cultivation is compensated for from the second year of cultivation, characterized by a height and stem diameter similar to those grown in monoculture. Hence, based on the findings of this study, it would be advisable to sufficiently separate the groundnut planting points from the cashew tree plants to avoid potential competition in the first year of cultivation.

Conflict of Interest

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Potential Health Risk Assessment for Nitrate Contamination in the Groundwater of Mersin Province, Türkiye

Özgür Özbay✉

Mersin University, Faculty of Fisheries, Mersin/Türkiye

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ABSTRACT

Though groundwater is one of the most significant natural water sources, its quality is deteriorating due to the anthropogenic pressures that poses health risks for people. In this study, potential health risk assessment for nitrate pollution in groundwater of Mersin Province was determined by commonly using health indices. Study findings indicated that the calculated Hazard Index (HI) values varied between 0.001 and 17.89 for the adults whilst the HI values ranging from 0.001 to 29.87 for the children. The HI values in the groundwater of Erdemli, Göksu and Anamur regions showed low chronic health risk for the adults and children (HI≤1). However, the calculated health risk indices indicated significant health hazards for the children inhabited between Tarsus and Çeşmeli regions (HI≥4.00) due to severe nitrate contamination originated from terrestrial sources. The findings of this study performed in the Mersin Province showed the sustainable management of groundwater policies is needed to reduce nitrate contamination and potential health hazards of the groundwater of studied and other regions in Türkiye.

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1. Introduction

Though surface waters are critical sources of water supply, the amount of groundwater is about 100 times that of surface waters (Fitts, 2013; Yue et al., 2017; Ba et al., 2022). Groundwater is one of the most significant natural water supply that has been widely used for drinking water needs and irrigation purposes (Adimalla et al., 2018; Said et al., 2021). It was reported that larger than 1.5 billion people use groundwater for drinking purposes in the world directly or indirectly (Shen et al., 2008; Adimalla & Qian, 2019). Therefore, an increasing number of studies have focused on groundwater in recent years (Kuyumcu, 2023).

Though groundwater is the most significant natural water resources for human being, its quality is deteriorating due to the anthropogenic perturbations posing health risks for people who use groundwater for consumption (Li et al., 2012; Wu et al., 2012; Wu & Sun, 2016; Adimalla, 2020). For example, the increase in population growth, intense urbanization, intensive technological developments and excessive use of nitrogen-containing fertilizers have led to nitrate contamination in Türkiye (Erşahin & Bilgili, 2023).

Mersin province, located at the southern Türkiye, is an economically important tourism region for cultural heritage and

✉ Correspondence

E-mail address: ozgurozbay@mersin.edu.tr

coastal beaches (Özbay, 2023) and it was reported that its population of increases dramatically in the summer periods due to beach tourism (Özgüler et al., 2022). It was reported by the World Health Organization that approximately a quarter of mortalities have been caused by environmental risk factor (Radfard et al., 2019). Although water sources have been contaminated by nitrate and nitrite in Türkiye (Erşahin & Bilgili, 2023), their adverse effects on human health in drinking water are currently unclear (Picetti et al., 2022). This study, therefore, aimed to determine potential health risk assessment for nitrate pollution in groundwater of Mersin Province. Many studies have been reported to determine groundwater nitrate contamination in Mersin Province (Hatipoğlu & Bayarı, 2005; Güler, 2009; Demirel et al., 2011; Güler et al., 2012; Güven et al., 2022; Kuyumcu, 2023). However, this study is a first attempt to determine the health risk (non-carcinogenic risk) for inhabitants originated from nitrate pollution.

2. Materials and Methods

In order to assess potential health risk assessment for nitrate pollution in groundwater of Mersin Province, previously reported data sets that obtained from the different groundwater samples in the Mersin Province. The nitrate data were obtained from the studies of Hatipoğlu and Bayarı (2005), Güler (2009), Demirel et al. (2011), Güler et al. (2012), Güven et al. (2022), and Kuyumcu (2023) (Table 1). The studied groundwater sampling sites were shown on the map presented in Figure 1.

Table 1. The groundwater sampling sites studied between 2001 and 2022.

Region	Tarsus - Karaduvar	Tarsus - Karaduvar	Karaduvar	Karaduvar -Çeşmeli	Erdemli	Göksu	Anamur
Date	2008	2020	2006-2007	2001-2003	2022	2006-2008	2022
# of sampling locations	193	87	43	15	1	24	1
Reference	Güler et al. (2012)	Güven et al. (2022)	Güler (2009)	Hatipoğlu and Bayarı (2005)	Kuyumcu (2023)	Demirel et al. (2011)	Kuyumcu (2023)

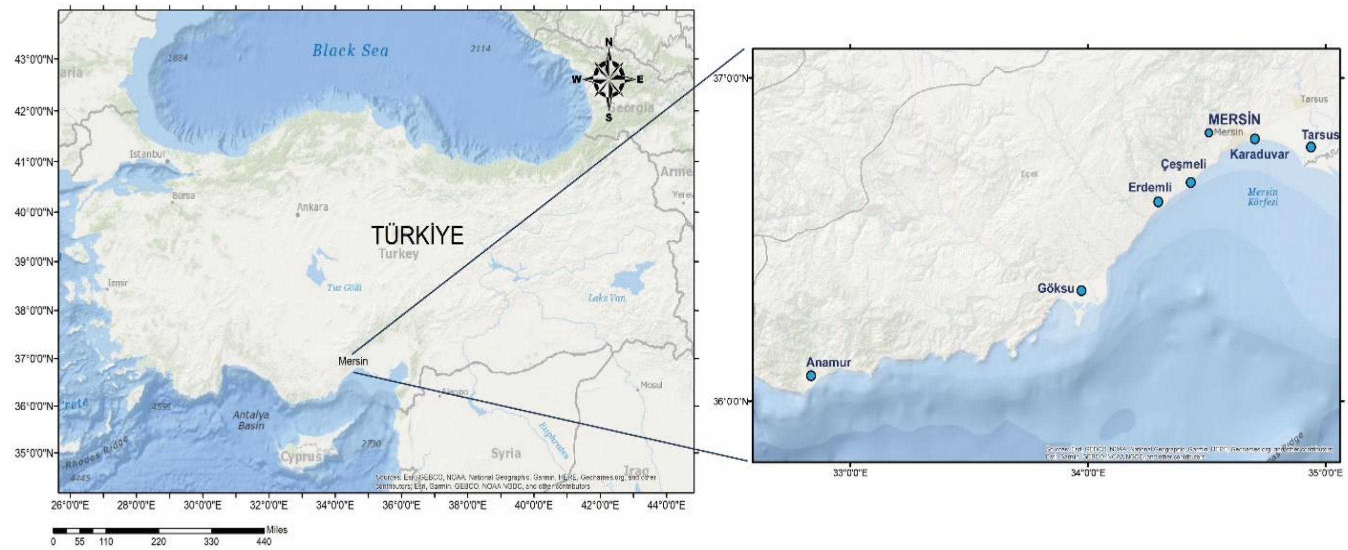

Figure 1. The studied groundwater samples between 2001 and 2022 (Sampling strategies were presented in Table 1).

In this study, health risk assessment for nitrate contamination were determined from the minimum, maximum and mean values of nitrate concentrations reported by the previous studies presented in Table 1. Potential health risk assessment can be determined by the basis of carcinogenic or non-carcinogenic effects. In this study, non-carcinogenic risk for nitrate was determined by the calculation of Chronic Daily Intake (CDI), Health Quotient (HQ) and Health Hazard (HI) for the adults and children (USEPA, 2004; USEPA, 2014; Zhang et al., 2020; IRIS, 2023). The chronic health risk assessment was performed by the classification reported by Kusa and Joshua (2023) (Table 1). Since oral ingestion and dermal contact are the main exposure pathways of nitrate, Chronic Daily Intake (Ingestion and Dermal) ($CDI_{ing.}$ and $CDI_{der.}$) values for the studied groundwater samples were calculated in this study (Table 2).

Table 2. Calculation of $CDI_{ing.}$, $CDI_{der.}$, HQ and HI values, and potential health risk assessment (USEPA, 2004; Muhammad et al., 2011; USEPA, 2014; Zhang et al., 2020; Habesoglu & Atici, 2022; IRIS, 2023; Kusa & Joshua, 2023).

$CDI_{ing.} = (C_{nitrate} \times IR \times EFq \times EDr/(BWt \times AvT)$

$CDI_{der.} = (AD \times Evf \times SSA \times EFq \times EDr)/(BWt \times AvT)$

$AbsD = K_p \times {}^{*}C_{nitrate} \times t_{event}$ — ${}^{*}C_{nitrate}$ (mg cm^{-3})

$HQ_{ing.} = CDI_{ing.}/RefD_{ing.}$

$HQ_{der.} = CDI_{der.}/RefD_{der.}$

$HI = HQ_{der.} + HQ_{ing.}$

		ADULT	CHILD
IR (L day^{-1})	Intake rate	2.5	0.78
EFq (day y^{-1})	Exposure Frequency	350	350
EDr (y)	Exposure Duration	26	6
BWt (kg)	Body Weight	80	15
SSA (cm^2)	Skin Surface Area	19652	6365
AvT (day)	Averaging Time (ED*365)	9490	2190
Evf (day)	Event Frequency	1	1
K_p (cm h^{-1})	Dermal permeability coefficient for water	0.001	0.001
t_{event} (h day^{-1})	Event Duration	0.58	1
$RefD_{ing.}$	Reference Dose for Ingestion (mg kg^{-1} day^{-1})	1.6	
$RefD_{der.}$	Reference Dose for Dermal (mg kg^{-1} day^{-1})	1.6	
$C_{Nitrate}$	Nitrate Concentration (mg L^{-1})		
CDI_{ing}.	Chronic Daily Intake for Ingestion (mg kg^{-1} day^{-1})		
CDI_{der}.	Chronic Daily Intake for Dermal (mg kg^{-1} day^{-1})		
AbsD	Absorbed Dose (mg cm^{-2} day^{-1})		
$HQ_{der.}$	Hazard Quotient for Dermal		
$HQ_{ing.}$	Hazard Quotient for Ingestion		
HI	Hazard Index		

Chronic Health Risk Classification			
HI<0.1	Negligible	4>HI≥1	Medium
1>HI≥0.1	Low	HI≥4	High/Severe
HQ<1	Safe for human health		

3. Results and Discussion

Previous studies performed in the groundwater of Mersin Province showed that nitrate concentrations displayed drastic spatial and temporal variations (Table 3). Groundwater nitrate concentrations varied from 0.03 mg L^{-1} in Anamur region to 950.8 mg L^{-1} in the region between Tarsus and Karaduvar. Maximum groundwater nitrate concentrations were consistently measured in between Tarsus and Karaduvar regions in 2020 (Table 3). Intensive irrigation in agriculture, rapid urbanization and the terrestrial inputs from the domestic wastewater and industrial discharges have led to nitrate pollution in surface waters and groundwater in Mersin Province (Özbay et al., 2012; Akçay et al., 2022; Akçay, 2023; Akçay et al., 2023; Erşahin & Bilgili, 2023; Türkeri et al., 2023). Study findings indicated that highest concentrations were consistently measured in the regions where human-induced pressures are very intense (Güler et al., 2012; Erşahin & Bilgili, 2023). According to WHO, the upper nitrate concentration limit is 50 mg L^{-1} for drinking waters (WHO, 2017). Findings of this study showed that the mean groundwater nitrate concentrations measured in the Mersin Province between 2001 and 2022 ranged from 22.9 to 210.4 mg L^{-1} and the mean nitrate concentrations in contaminated groundwater samples in Tarsus, Karaduvar, and Çeşmeli regions were much greater than the upper limit value of drinking water strongly suggested that groundwater in Mersin Province may pose significant health hazards for people (Table 3).

Table 3. Concentrations of nitrate (mg L^{-1}) in the groundwater of Mersin Province.

Region	Tarsus-Karaduvar	Tarsus-Karaduvar	Karaduvar	Karaduvar-Çeşmeli	Erdemli	Göksu	Anamur
Min.	1.77	4.87	13.73	17.91	0.12	3.54	0.03
Max.	890.59	950.81	715.57	561.43	40.27	141.23	45.62
Mean	-	210.4	147.3	205.7	27.1	34.3	22.9
Median	49.16	117.36	-	196.38	29.11	29.41	30.33
N	193	87	43	45	12	91	10
Reference	Güler et al. (2012)	Güven et al. (2022)	Güler (2009)	Hatipoğlu and Bayarı (2005)	Kuyumcu (2023)	Demirel et al. (2011)	Kuyumcu (2023)

The HQ value that is higher than 1 indicates that water contaminants may have non-carcinogenic effects whilst a calculated HQ, lower than 1, shows health effects on human health is very small (Zhang et al., 2021). By using the minimum, maximum and mean groundwater nitrate concentrations, potential health risk assessment was determined by the calculation of CDI, HQ and HI values for the adults and children, presented in Table 4 and Figure 2. It was shown that though the $HQ_{der.}$ values were less than 1, considered safe for human health (Muhammad et al., 2011; Habesoglu & Atici, 2022), $HQ_{ing.}$ values were much greater than the calculated $HQ_{der.}$ values indicating that the oral ingestion are the main exposure pathways of nitrate (Figure 2). Study findings indicated that the HQ values calculated for the children ($HQ_{ing.}$:0.001-29.63, $HQ_{der.}$:0.000-0.242) consistently greater than those calculated for the adults ($HQ_{ing.}$:0.001-17.81, $HQ_{der.}$:0.000-0.081). The study results showed the health risk due to excessive intake of nitrate contaminated groundwater for the children were higher than those for the adults, suggesting a greater health risk for the children in the Mersin province as also experienced by the previous studies performed in groundwater of different regions in the World (Wu & Sun, 2016; Zhang et al., 2020; Pazhuparambil Jayarajan & Kuriachan, 2021).

The HI values, calculated by the sum of $HQ_{ing.}$ and $HQ_{der.}$ values, in the groundwater of Erdemli, Göksu, and Anamur regions suggested low chronic health risk for the children and adults (HI≤1). The calculated HI values showed significant health hazards in the study region. The mean HI values for the children varied between 0.001 and 29.87 whilst the HI values for the adults ranged from 0.001 to 17.89 for the study region with the maximum values calculated for the region between Tarsus and Çeşmeli. Health risk assessment based on the calculated HI values also showed that some calculated HI values in the groundwater of Tarsus, Karaduvar, and Çeşmeli were greater than 4.00 suggesting "High/Severe" health risk for the adults and children (Figure 2). It was reported by the study of Güler et al. (2012) that Tarsus coastal plain was highly contaminated by the terrestrial discharges originated from anthropogenic activities as industrial and agricultural wastewater inputs. Study findings indicated that the highest groundwater nitrate concentrations were consistently measured in these regions due to the anthropogenic pressures caused to nitrate contamination that would lead to potential health hazards for the people that use groundwater of these nitrate contaminated regions (Table 1, Figure 2).

Table 4. The calculated CDI, HQ and HI values for the adults and children in the groundwater of Mersin Province.

		Tarsus-Karaduvar (2012)	**Tarsus-Karaduvar (2022)**	**Karaduvar**	**Karaduvar-Çeşmeli**	**Erdemli**	**Göksu**	**Anamur**
ADULT	**$CDI_{ing.}$**	1.4730	6.3035	4.4125	6.1639	0.8118	1.0293	0.6866
	$CDI_{der.}$	0.0067	0.0287	0.0201	0.0281	0.0037	0.0047	0.0031
	$HQ_{ing.}$	0.9206	3.9397	2.7578	3.8524	0.5074	0.6433	0.4291
	$HQ_{der.}$	0.0042	0.0180	0.0126	0.0176	0.0023	0.0029	0.0020
	HI	0.9248	3.9577	2.7704	3.8700	0.5097	0.6462	0.4311
CHILD	**$CDI_{ing.}$**	2.4511	10.4890	7.3423	10.2567	1.3508	1.7127	1.1425
	$CDI_{der.}$	0.0200	0.0856	0.0599	0.0837	0.0110	0.0140	0.0093
	$HQ_{ing.}$	1.5320	6.5557	4.5890	6.4104	0.8442	1.0704	0.7140
	$HQ_{der.}$	0.0125	0.0535	0.0374	0.0523	0.0069	0.0087	0.0058
	HI	1.5445	6.6091	4.6264	6.4627	0.8511	1.0792	0.7199

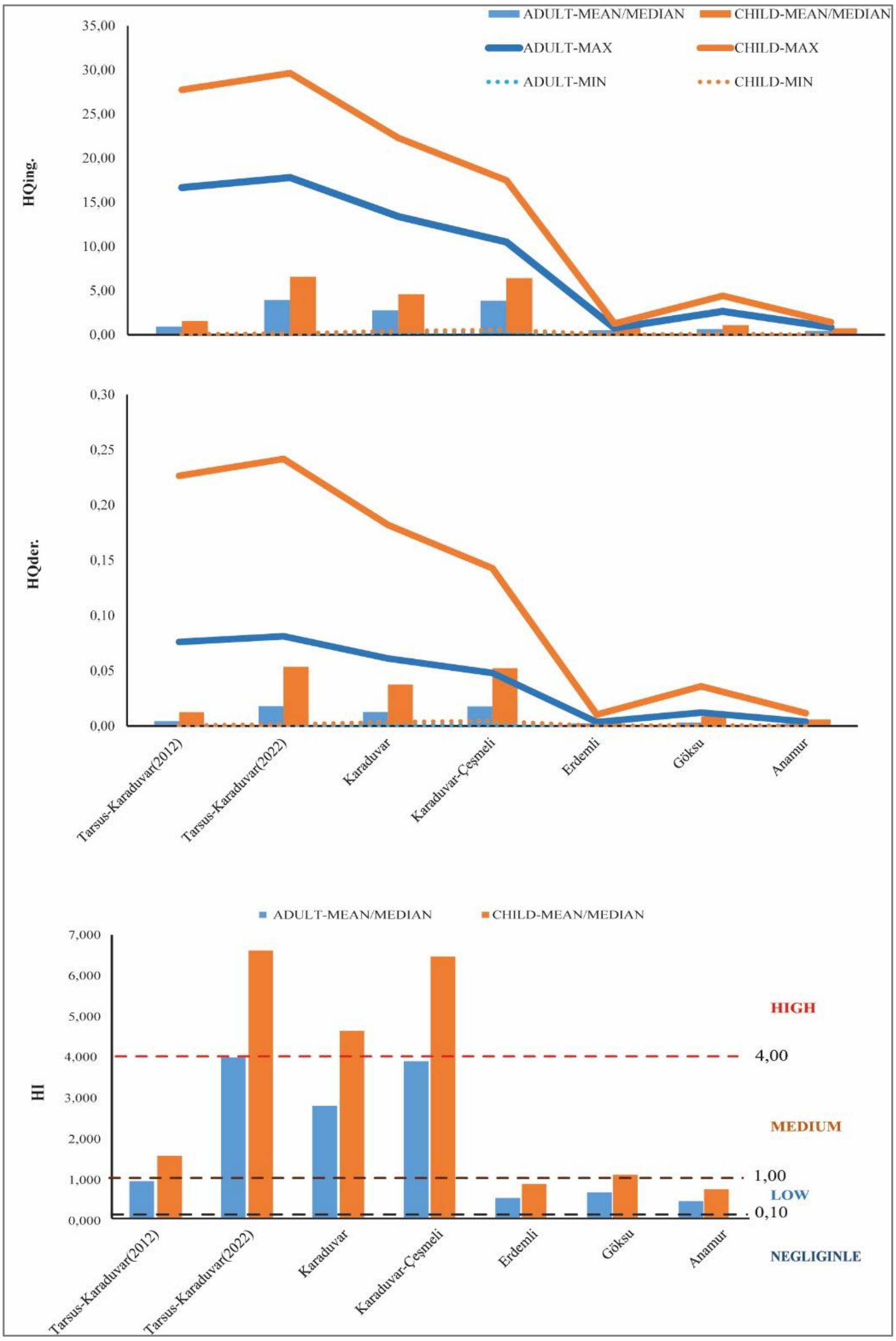

Figure 2. Health risk assessment of groundwater for nitrate in Mersin Province based on $HQ_{ing.}$, $HQ_{der.}$ and HI values calculated for the children and adults.

Anthropogenic perturbations such as the increase in population and hence domestic sewage, and the excess use of chemical fertilizer in the agriculture highly enhances nitrate pollution in the groundwater that can cause to health risks for people who use groundwater for consumption (Güler et al., 2012; Li et al., 2012; Wu et al., 2012; Wu & Sun, 2016; Adimalla, 2020; Erşahin & Bilgili, 2023). Previous studies performed in the groundwater in Mersin Province showed an apparent nitrate pollution due to intensive irrigation in agriculture and industrial activities as well as rapid urbanization in Mersin Province have led to nitrate pollution in groundwater in Mersin Province (Erşahin & Bilgili, 2023). In a recent study performed by Moeini and Azhdarpoor (2021), a sensitivity analysis was carried out to determine the most effective parameter in health risk assessment and it was shown that decreasing the nitrate concentrations can reduce the level of health risk for nitrate contamination by the reduction of HQ values for all age groups. The high levels of nitrate pollution in the groundwater of economically important Mersin Province pose to significant health hazards for the adults and children (Figure 2, Table 4), that should be reduced for the low possibility of adverse health effects for the people living in the region.

4. Conclusion

In this study, potential health risk assessment for nitrate pollution in groundwater of Mersin Province was determined. The study findings showed that nitrate concentrations ranged between 0.03 and 950.8 mg L^{-1} with the maximum values greater than the upper limit of 50 mg L^{-1} for drinking waters set by WHO recorded in the groundwater of the Tarsus, Karaduvar, and Çeşmeli regions. Health risk assessment showed that the mean HI values for the children varied between 0.001 and 29.87 whilst the HI values for the adults ranged from 0.001 to 17.89 for the study region with the maximum values calculated for the region between Tarsus and Çeşmeli. The low HI values (HI≤1) in the groundwater of Erdemli, Göksu, and Anamur regions suggested low chronic health risk for the children and adults. However, the calculated HI values in the groundwater of Tarsus, Karaduvar, and Çeşmeli regions were greater than 4.00 suggesting "High/Severe" health risk for the adults and children (Figure 2). The health risk assessment showed that the calculated HQ and HI values for the children consistently higher than the calculated HQ values for the adults, strongly suggesting that children were potentially at higher risk for health hazards from nitrate. The findings of this study indicated the sustainable management of groundwater policies for the reduction of nitrate pollution in the groundwater supplies is needed to reduce potential health hazards of the groundwater of studied and other regions in Türkiye.

Conflict of Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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RESEARCH ARTICLE

Free Fatty Acid Profile of Köy Cheese Consumed in Erzurum and Its Region

Emine Macit✉

Atatürk University, Faculty of Tourism, Department of Gastronomy and Culinary Arts, Erzurum/Türkiye

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ABSTRACT

Köy cheese is a type of cheese with white interior, slightly yellowish exterior, a slightly firm texture, and a mild salty taste. It is produced and consumed in some regions in the Marmara and Aegean regions of Turkey, as well as in Bayburt, Yusufeli and Erzurum regions. The present study was conducted to determine the free fatty acid profile of Köy cheeses consumed in Erzurum and its surrounding areas. Analyzes were carried out by GC-MS using 10 cheese samples, and as a result, the presence of 25 free fatty acids was determined. Among these, short-chain fatty acids accounted for 2%, medium-chain fatty acids for 7%, and long-chain fatty acids for 91%. Palmitic acid (36.3563±5.4150%), oleic acid (22.0077±5.3136%), myristic acid (12.3104±2.1054%) and stearic acid (11.9837±3.1320%) were the prominent fatty acids among total free fatty acids. This study is considered to be significant in terms of providing data about the free fatty acid profile of Köy cheese produced and consumed widely in Türkiye.

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1. Introduction

There are numerous types of cheese worldwide, each with distinct flavors and tastes. The diversity of cheeses varies based on the vegetation, cultural practices, types of milk-producing animals, and production methods of a country (Kubícková & Grosch, 1998). Köy cheese, which is the subject of our research, is a type of cheese that is slightly hard, white on the inside, slightly yellowish on the outside, slightly salty, and is generally not over-ripened and consumed fresh. In Türkiye, it is produced and consumed in various regions, including Mengen, Bolu, and Assos in Marmara region, as well as Bayındır, Ödemiş, Tire, Seferihisar, Söke, and Torbalı in and around Izmir. Additionally, it is produced and consumed in Yusufeli, Bayburt, and Erzurum region. Köy cheese is primarily made from cow, sheep, or goat milk, or a combination of these in certain proportions. It contains 16-22% milk fat (Kesenkaş et al., 2012).

Free fatty acids are components released as a result of lipolysis during ripening in cheese. The enzymatic hydrolysis of milk glycerides into free fatty acids is essential for flavor development in cheese (Thierry et al., 2017). In particular, short and medium-chain fatty acids directly contribute to the aroma of the cheese. They also serve as precursor molecules for a series of catabolic reactions leading to the production of taste and aroma compounds, such as lactones, esters, methyl ketones, alkanes, and secondary alcohols (Woo et al., 1984; Collins et al., 2003).

✉ Correspondence
E-mail address: emine.macit@atauni.edu.tr

As a result of the literature review, it was seen that although Köy cheese is produced and consumed in a wide area in Turkey, scientific research on it is insufficient. This study was conducted to obtain data on the free fatty acid profile of Köy cheese consumed in Erzurum and its surrounding areas.

2. Materials and Methods

2.1. Köy Cheese Production

According to the information obtained from manufacturers and literature, this cheese is produced as follows. Köy cheese is primarily made from cow, sheep, or goat milk, or a combination of these in certain proportions, depending on the characteristics of the region where it is produced. In Erzurum and its surrounding areas, it is possible to use raw or pasteurized milk in the production of Köy cheese. The milk brought to the facility for cheese production undergoes heat treatment at 85 °C for 15-20 minutes and is then cooled to 45-50 °C. Starter culture is not used in the production of Köy cheese. After the addition of $CaCl_2$, rennet is added in a specific amount that allows coagulation within 45-60 minutes. Following coagulation, the curd is broken, drained, and pressed (pressed in fine-pored cloths at 5-6 kg pressure) before being salted in brine for one night (15-degree Brix salt concentration for 10 hours). The salted cheeses are then filled into plastic buckets, brine is added on top, and they are transferred to storage rooms. They are stored at 4 °C in these rooms. Köy cheese is a cheese consumed fresh without over-ripening (Kesenkaş et al., 2012; Çayır & Güzeler, 2020). However, producers report that brine-salted Köy cheeses can last approximately one year (Kesenkaş et al., 2012).

Cheese samples obtained from 10 different sales points were used to determine the total free fatty acid profile of Köy cheese consumed in Erzurum and its region.

2.2. Free Fatty Acid Analysis

The method provided by Durna (2019) was modified and utilized for the analysis. The composition of free fatty acids in cheese samples was determined as fatty acid methyl esters (FAME). For the extraction of fat, approximately 3 g of cheese sample was weighed into a 50 ml Falcon tube, and 25 ml of a chloroform-methanol mixture (2:1, v:v) and 20 ml of a 10% NaCl solution were added. The mixture was vortexed for 15 minutes. Subsequently, it was centrifuged at 10,000 rpm for 20 minutes at 4 °C, and the chloroform+fat mixture collected in the lower phase was carefully pipetted. It was then filtered through filter paper containing Na_2SO_4 as a moisture-absorbing substance. The chloroform was removed using a rotary evaporator to obtain pure fat. For esterification, 0.2 g of fat was mixed with 4 ml of hexane and 0.4 ml of 2 N methanol KOH, vortexed for 2 minutes, and then centrifuged at 5,000 rpm for 5 minutes. A clear phase (1.5-2 ml) from the top was injected into a Shimadzu-GC 2010 Ultra series gas chromatograph equipped with an automatic sample injector and a flame ionization detector. Separation was performed on an Agilent J&W DB-Fast FAME, 30 m x 0.25 mm, df 0.25 fused-silica capillary column. The injector temperature was maintained at 250 °C, and the detector temperature at 260 °C. The oven program was initiated at 50 °C for 0.5 minutes, increased to 194 °C at a rate of 30 °C/min for 3.5 minutes, and then raised to 240 °C at a rate of 5 °C/min for 2 minutes. Helium was used as the carrier gas. Before analyzing the samples, a fatty acid standard (Supelco 37 Component FAME Mix, Supelco Inc., USA) was analyzed to determine the peak times for the fatty acids in the samples. Fatty acid methyl esters were expressed as a percentage (%) (Kocaman, 2017).

2.3. Statistical Analysis

The obtained data were analyzed statistically using the SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

Table 1 presents the free fatty acids and their proportions detected in the Köy cheese samples. The classification of fatty acids based on chain lengths was performed based on Huang et al. (2011) and Running and Mattes (2014). A total of 25 free fatty acids were identified. It seems there are no studies that focus on fatty acid profile of Köy cheese. Therefore, the results obtained from this research will be interpreted taking into account studies conducted on other cheese types.

As short and medium chain fatty acids in cheese samples; butyric acid was detected at the rate of 0.8776 ± 0.3740%, caproic acid at the rate of 1.1540 ± 0.3366%, caprylic acid at the rate of 1.1520 ± 0.5767%, capric acid at the rate of 3.6310 ± 2.5917%, undecanoic acid at the rate of 0.0361 ± 0.0184% and lauric acid at the rate of 2.6375 ± 1.2367%. Şengül et al. (2011) determined the percentages of butyric acid as 1.823%, caproic acid as 1.243%, caprylic acid as 0.618%, capric acid as 1.217%, and lauric acid as 1.529%. The ratio of butyric and caproic acid was higher than the values the present study obtained, while the ratio of caprylic, capric, and lauric acid was observed to be low. While Köy cheese is a cheese that is consumed mostly fresh without over-ripening, Karın Kaymağı cheese is a cheese that is consumed after being ripened for 2-3 months at an average temperature of 5-10 °C and 70-80% relative humidity (Şengül et al., 2011). In studies on free fatty acids in cheeses, it has been reported by various researchers that the butyric acid (C4:0) ratio increases during ripening (Georgala et al., 2005; Katsiari et al., 2000; Oluk & Güven, 2015).

Table 1. Free fatty acids and their percentages (%) detected in Köy cheeses consumed in Erzurum and its region.

	Köy Cheese Samples												
Short-chain fatty acids	1	2	3	4	5	6	7	8	9	10	Min	Max	Mean (x ± Sx)
Butyric Acid (C4:0)	1.1064	1.0807	0.0605	0.8790	0.7253	0.6497	0.9038	1.0586	1.4835	1.0398	0.0605	1.4835	0.8776±0.3740
Caproic Acid (C6:0)	1.5039	1.1404	0.4269	1.0660	0.9947	1.6015	1.4517	1.0810	1.3730	1.1805	0.4269	1.6015	1.1540±0.3366
Medium-chain fatty acids													
Caprylic Acid (C8:0)	1.1981	0.7989	0.6818	0.7941	0.7191	2.5909	1.2922	0.7345	0.8915	0.8497	0.6818	2.5909	1.1520±0.5767
Capric Acid (C10:0)	3.1503	2.2028	2.6751	2.0379	1.7979	10.3497	3.4611	1.6283	2.1642	2.1264	1.6283	10.3497	3.6310±2.5917
Undecanoic Acid (C11:0	0.0538	0.0233	0.0271	0.0290	0.0233	0.0721	0.0531	0.0174	0.0234	0.0216	0.0174	0.0721	0.0361±0.0184
Lauric Acid (C12:0)	0.0000	2.7658	4.1757	2.8443	2.3333	4.1501	3.9106	1.9325	2.7020	2.6596	0.0000	4.1757	2.6375±1.2367
Long-chain fatty acids													
Tridecanoic Acid (C13:0)	0.1174	0.0884	0.0869	0.0926	0.1048	0.0750	0.0929	0.0735	0.1037	0.0880	0.0735	0.1174	0.0928±0.0135
Myristic Acid (C14:0)	14.9848	12.1103	15.9735	12.6268	11.2943	10.1412	13.5422	9.0623	11.5229	11.4307	9.0623	15.9735	12.3104±2.1054
Myristoleic Acid (C14:1)	1.4442	0.8828	1.7776	1.3393	0.8146	0.0718	0.8876	0.3872	1.1025	0.8555	0.0718	1.7776	0.9510±0.4975
Pentadecanoic Acid (C15)	1.6446	1.7762	1.6821	1.8743	1.9326	0.9206	1.4262	1.5732	1.8766	1.9493	0.9206	1.9493	1.6271±0.3116
Palmitic Acid (C16:0)	42.3424	33.4262	45.8247	40.3936	37.0843	31.5876	31.6372	28.5372	37.2424	33.8380	28.5372	45.8247	36.3563±5.4150
Palmitoleic Acid (C16:1)	1.9255	1.3801	1.5397	2.3198	1.2471	0.3039	0.8797	1.0048	1.5839	1.3703	0.3039	2.3198	1.3482±0.5586
Heptadecanoic Acid (C17:0)	0.8502	1.1912	0.7667	1.0410	1.0782	0.6996	0.4685	1.3914	1.1833	1.3579	0.4685	1.3914	0.9907±0.2998
Stearic Acid (C18:0)	7.7292	12.7447	8.3345	8.8121	11.9448	15.6818	16.1641	13.7050	9.9710	14.8234	7.7292	16.1641	11.9837±3.1320
Oleic Acid (C18:1n9c)	18.7491	24.3688	13.5282	20.2383	24.2692	17.3643	19.1754	33.2088	23.7240	22.7291	13.5282	33.2088	22.0077±5.3136
Linoleic Acid (C18:2n6c)	1.7572	1.9733	1.1460	1.5759	1.7392	1.7213	2.4305	2.1867	1.2885	1.5839	1.1460	2.4305	1.7483±0.3858
Alfa-Linolenic Acid (C18:3n3)	0.6834	0.8793	0.6383	1.0133	0.7568	1.0561	1.2173	1.0925	0.4221	0.9186	0.4221	1.2173	0.8598±0.2426
Arachidic Acid (C20:0)	0.2505	0.3528	0.1818	0.2381	0.4118	0.3734	0.3985	0.4429	0.4509	0.3675	0.1818	0.4509	0.3417±0.0922
Henicosanoic Acid (C21:0)	0.0630	0.0850	0.0556	0.0751	0.0941	0.1035	0.0710	0.1094	0.0971	0.1228	0.0556	0.1228	0.0879±0.0215
cis-8,11,14-Eicosatrienoic Acid (20:5n-3)	0.0470	0.0576	0.0226	0.0573	0.0437	0.0106	0.0376	0.0449	0.0548	0.0390	0.0106	0.0576	0.0403±0.0152
Arachidonic Acid (C20:4n6)	0.1353	0.1411	0.0879	0.1229	0.1040	0.0879	0.0820	0.1537	0.1338	0.0850	0.0820	0.1537	0.1141±0.0270
Behenic Acid (C:22:0)	0.1031	0.2073	0.1184	0.2075	0.2158	0.1650	0.1696	0.2298	0.2544	0.2526	0.1031	0.2544	0.1901±0.0523
cis-5,8,11,14,17-Eicosapentaenoic acid (20:5n-3)	0.0591	0.0882	0.0414	0.0845	0.0495	0.0596	0.0844	0.0931	0.0735	0.0654	0.0414	0.0931	0.0694±0.0176
Tricosanoic Acid (C23:0)	0.0401	0.1053	0.0614	0.1159	0.0870	0.0718	0.0768	0.1013	0.1035	0.1102	0.0401	0.1159	0.0858±0.0244
Lignoceric Acid (C24:0)	0.0614	0.1296	0.0853	0.1215	0.1339	0.0909	0.0859	0.1501	0.1735	0.1350	0.0614	0.1735	0.1168±0.0347

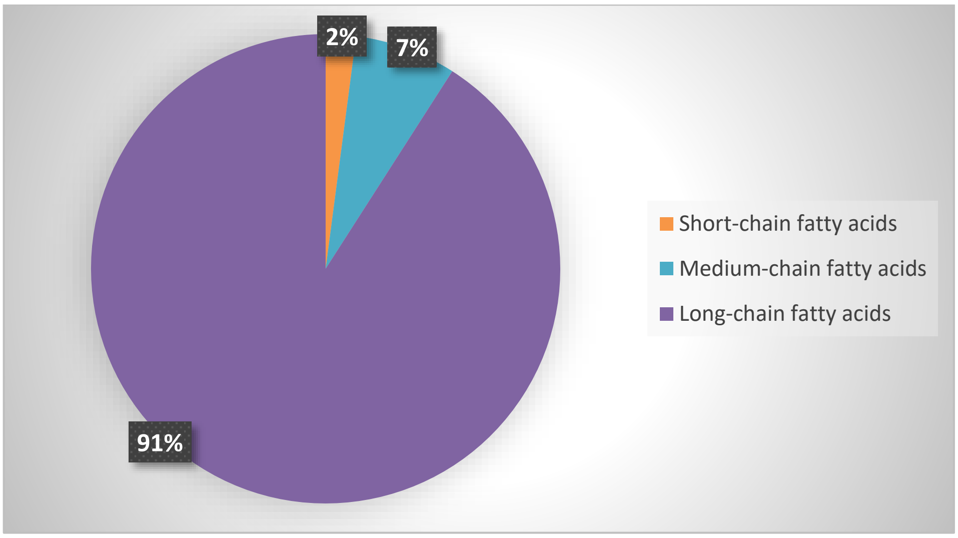

Figure 1. Distribution of free fatty acids identified in Köy cheese based on chain lengths.

Butyric acid (C4:0) is characterized by a rancid and cheesy taste and odor (Durna, 2019). The highest butyric acid rate was detected in sample number 9, and sample number 9 may be the most ripened among the cheese samples. Additionally, cheeses produced using traditional methods have been reported to contain significantly higher amounts of butyric acid compared to industrially produced cheeses (Georgala et al., 2005).

Released through the action of lipases on milk fat, free fatty acids contribute to the flavor of matured cheese, with certain fatty acids, notably caproic acid (C6:0), considered as an index for measuring cheese lipolysis (Güler, 2005; Durna, 2019). Atasoy and Türkoğlu (2008) found that the volatile free fatty acid (C4:0–C10:0) content of raw milk cheese was significantly higher than that of cheeses heat-treated at 65 °C and 72 °C, also reported that there were differences in butyric, caproic, caprylic and capric acid levels between raw milk cheese and low and high heat treated cheeses on the 90th day. In a study evaluating lipolysis development in 11 different cheese varieties sold in Türkiye, Hayaloglu and Karabulut (2013) determined the profiles of free fatty acids in cheese samples. They reported that, except for Çanak cheese, concentrations of short-chain fatty acids (C4 and C6 free fatty acids) were similar among all cheeses.

The highest levels of caproic (1.6015%), caprylic (2.5909%), capric (10.3497%), and undecanoic (0.0721%) acids in the cheese samples were detected in sample number 6, indicating that this sample may be produced from either sheep or goat milk. The type of milk has a significant impact on the formation of free fatty acids, with sheep and goat milk fats containing higher amounts of caproic, caprylic, and capric acids. These fatty acids contribute to a pleasant bitterness and a peppery taste in cheeses (Abd El-Salam et al., 1987; Durna, 2019).

Short and medium-chain free fatty acids have relatively low detection thresholds and directly contribute to cheese aroma (Güler, 2005). The short and medium-chain fatty acids identified in Köy cheese constitute 9% of the total free fatty acids (Figure 1). Atasoy and Türkoğlu (2008) determined these rates in Urfa cheese as 7-8% and 20-23%, respectively. It is thought that these values are higher than ours due to the use of starter culture and the maturation period. Georgala et al. (2005) detected the percentages of short-chain fatty acids in total free fatty acids as 44% and 33% in samples of Feta cheese made with traditional rennet from sheep and goat abomasum (Sample A) and in samples made by blending this rennet with a certain amount of commercial rennet made from calf abomasum (Sample B) after a 120-day storage period, respectively. Enzymes responsible for lipolysis in cheese include lipoprotein lipase naturally found in raw milk, pregastric esterase in cheeses made with rennet, and those originating from the starter or non-starter microbiota (Georgala et al., 2005; Erdoğan et al., 2012; Thierry et al., 2017). It is thought that this difference with Georgala et al. (2005) is mainly due to the ripening time and the lipolytic activity of rennet.

In the cheese samples, palmitic acid (36.3563 ± 5.4150%), oleic acid (22.0077 ± 5.3136%), myristic acid (12.3104 ± 2.1054%), and stearic acid (11.9837 ± 3.1320%) were prominent in terms of long-chain fatty acids. Güler (2005) identified the dominant free fatty acids in Kaşar cheese as palmitic, oleic, myristic, and stearic acids, while Hayaloglu and Karabulut (2013) reported the prominent free fatty acids in 11 different cheese varieties as palmitic (C16) and oleic (C18:1) acids. Şengül et al. (2011) reported average palmitic acid content as 24.639%, oleic acid as 9.384%, myristic acid as 7.346%, and stearic acid as 2.028%. These values appear to be lower than the results the present study revealed. It is thought that this may be due to the use of milk from different animal breeds in cheese production, the cheeses being at different maturity levels or different ripening conditions (Temiz et al., 2009; Hayaloglu & Karabulut, 2013). In addition, Karın

Kaymağı cheese is a ripened cheese and during this process, the breakdown of fatty acids into their sub-components may occur. Hayaloglu and Karabulut (2013) reported significant differences among samples for other free fatty acids, including C8 and C18:2, in various cheese varieties. The concentration of salt in the cheese also influences the formation of free fatty acids, with lipolysis levels decreasing as salt concentration increases. This effect is attributed to the inhibitory impact of salt on bacterial growth and enzymatic activity (Abd El-Salam et al., 1987; Katsiari et al., 2000).

Sample 3 was remarkable for its high levels of palmitic and myristic acids, sample 8 for oleic acid, and sample 7 for stearic acid. These variations could be attributed to the use of different animal milks or their blends in cheese production. The variation in fatty acid profile of milk depends on various factors, including dietary fatty acids, the species of the animal, season, lactation stage, lactation number, age, and the biohydrogenation process observed in the rumen (Djordjevic et al., 2019). Durna (2019) reported the descending order of palmitic acid levels as cow-goat-sheep milk, myristic acid levels as goat-cow-sheep milk, oleic acid levels as goat-sheep-cow milk, and stearic acid levels as sheep-cow-goat milk.

Pentadecanoic acid was determined to have an average ratio of 1.6271 ± 0.3116%, palmitoleic acid at 1.3482 ± 0.5586%, and linoleic acid at 1.7483 ± 0.3858% in the cheese samples. In addition, 12 minor long-chain free fatty acids were identified. Long-chain fatty acids constituted 91% of the total free fatty acids. Atasoy and Türkoğlu (2008) determined this rate as 70-72% in Urfa cheese. In Van Otlu cheese, although most short-chain fatty acids (C3, C4, C5, C6, C7, and C8) had a significant increase during maturation, their proportions in both fresh and mature samples were lower than those of medium and long-chain fatty acids (Ocak et al., 2015). While long-chain free fatty acids make up a significant proportion of the total free fatty acids, they have no substantial impact on cheese aroma (Attaie & Richter, 1996; Freitas & Malcata, 1998).

4. Conclusion

In conclusion; with this study, the free fatty acid profile of milk fat in Köy cheese, which increases production and consumption day by day, was determined and distribution rates were determined according to chain lengths. It was observed that the ratio of short and medium chain fatty acids, which have a significant effect on the taste and aroma of cheese, in total free fatty acids was quite low compared to cheeses ripened or produced using starter culture. In general, not many studies on Köy cheese have been found in the research. This study is important in terms of revealing the free fatty acid composition of Köy cheese and providing data for future studies.

Conflict of Interest

The author has no conflict of interest to declare.

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RESEARCH ARTICLE

Genome-Wide Analysis and Characterization of *FBA* (Fructose 1,6-bisphosphate aldolase) Gene Family of *Phaseolus vulgaris* L

Sümeyra Uçar[1] • Şeyma Alım[2] • Ayşe Gül Kasapoğlu[1] • Esma Yiğider[2] • Emre İlhan[1] • Murat Turan[1] • Aysun Polat[1] • Neslihan Dikbaş[2] • Murat Aydın[2]✉

[1]Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum/Türkiye
[2]Atatürk University, Faculty of Agriculture, Department of Agricultural Biotechnology, Erzurum/Türkiye

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ABSTRACT

Fructose-1,6-biphosphate aldolase (*FBA*) genes have important roles in plant stress responses. At the same time, these genes positively affect growth and development in plants. FBA is involved in gluconeogenesis, glycolysis, and the Calvin-Benson cycle, and it is an enzyme that plays an important role in signal transduction of these stages. This study aims to determine and characterize the *FBA* gene family in the bean genome. As a result of the study, 7 *Pvul-FBA* genes were determined in the bean (*Phaseolus vulgaris* L.) genome. The highest amino acid number of Pvul-FBA proteins was determined in the *Pvul-FBA-1* gene (1374), and the highest molecular weight (43.03 kDa) was determined in the *Pvul-FBA-7* gene. Again, the highest isoelectric point (8.03) was determined in the Pvul-FBA-3 gene. It has been determined that the *Pvul-FBA-6*/*Pvul-FBA-7* genes are segmental duplicated genes. The main four groups were obtained according to the phylogenetic analysis consisting of FBA proteins of three plants (*P. vulgaris*, *Glycine max*, and *Arabidopsis thaliana*). As a result of interproscan analysis, Motif-1, 2, 3, 4 and 5 were found to contain the fructose-bisphosphate aldolase domain. According to in silico gene expression analysis, it was determined that the expression rates of *Pvul-FBA* genes increased or decreased under salt and drought stress conditions. Synteny analyses of *FBA* genes in common bean and *A. thaliana* plants showed that these three plants have a relationship in terms of *FBA* genes. The results of this research will allow a better designation of the molecular structure of the *FBA* gene family in common bean.

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a species of plant belonging to the Fabaceae/ Leguminosae family (730 genera and 19400 species) (Broughton et al., 2003). Meanwhile, the most diverse vegetable crop among the legumes. Common bean is rich in protein, carbohydrates, fiber, and minerals and is a potential food source in the diet of the world's population (Blair et al., 2003; Schmutz et al., 2014; Cichy et al., 2015). Unfortunately, bean production is limited by abiotic and biotic stress factors (Fujita et al., 2006; Carvalho et al., 2011). Abiotic stresses such as salinity, drought, heavy metals, and cold are the main factors that negatively affect product productivity, quality, and sustainability (Sharma et al., 2019; Waqas et al., 2019). Because the bean is a glycophyte plant, it is not tolerant of high salt concentrations. Especially salinity and drought affect cell metabolism significantly (Taïbi et al., 2016).

✉ Correspondence
E-mail address: maydin@atauni.edu.tr

Plants respond to the stresses they are exposed to at molecular, biochemical, and physiological levels. This response varies according to species, genotype, organ, and cell type (Barnabás et al., 2008; Basu et al., 2016). Fructose-1,6-biphosphate aldolase (*FBA*) genes, one of these genes, have an important role in biotic and abiotic (high salt, drought, heavy metal, and low temperature, etc.) stress answer and regulate growth in plants (Michelis & Gebstein, 2000; Sarry et al., 2006; Khanna et al., 2014; Murad et al., 2014; Shu et al., 2014). FBA is involved in gluconeogenesis, glycolysis, and the Calvin-Benson cycle, and it is an enzyme that plays an important role in signal transduction of these stages (Anderson et al., 2005; Cho & Yoo, 2011; Cai et al., 2018). The translatable aldol division of fructose-1,6-biphosphate (FBP) into glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP) is catalyzed by the *FBA* gene, a metabolic enzyme. There are two classes of FBAs: class I and class II (Rutter, 1964; Cai et al., 2016). While class I FBAs are generally found in plants, animals, and protists, class II FBAs are found only in fungi (Rutter, 1964; Marsh & Lebherz, 1992; Lv et al., 2017). In addition, there are two isoforms (chloroplast /plastid FBA (CpFBA) and cytosolic FBA (cFBA) of FBA in plants (Lu et al., 2012). Chloroplast FBA is involved in starch biosynthesis, while cytosolic FBA is involved in the production of FBP, and sucrose. Inhibition of cytosolic FBA results in decreased sucrose synthesis (Fan et al., 2009; Strand et al., 2000). Some studies have determined that overexpression of the *FBA* gene in tobacco supports growth (Yamada et al., 2000). The decline in the expression of the *FBA* gene showed that the biomass of the tomato decreased (Cai et al., 2018). Diverse insider of the *FBA* gene family have been defined and characterized in spinach (Pelzer-Reith et al., 1993), rice (Kagaya et al., 1995), corn (Dennis et al., 1988), tobacco (Yamada et al., 2000), *Sesuvium portulacastrum* (Fan et al., 2009), Arabidopsis (Lu et al., 2012), tomato (Cai et al., 2016), *Triticum aestivum L*. (Lv et al., 2017), *Brassica napus* (Zhao et al., 2019), and cotton (Li et al., 2021). As a result of the research, no study was found related to the genome-wide analysis of the *FBA* gene members in the common bean plant.

Determining and describing the members of the *FBA* gene family in silico at the genome-wide level in beans is the primary goal of this work. Once more, the goal of this research is to determine the ortholog-paralogous link between *A. thaliana, G. max, and P. vulgaris L.* and the differences in *FBA* gene expression under salt stress and drought.

2. Materials and Methods

2.1. Identification of *Pvul-FBA* Genes

Using the Pfam database, Pfam accession numbers (class I: PF00274, class II: PF01116) in the bean genome were obtained. The protein, transcript, genomic, and CDS sequences in the genomes of *P. vulgaris* [7], *A. thaliana* [9], and *G.max* [15] of the *Pvul-FBA* gene family with accession numbers PF00274 and PF01116 were obtained using Phytozome database v13 (https://phytozome-next.jgi.doe.gov/). The blastp of the Phytozome database v13 and the Hidden Markov Model (HMM) (http://www.ebi.ac.uk) were used to identify FBA proteins present in the genomes of the three plants used. On the other hand, the presence of the *Pvul-FBA* domain was investigated with the HMMER (http://www.ebi.ac.uk) database. The instability index, amino acid number, and molecular weight, theoretical isoelectric point (pI), of the FBA proteins in the common bean were defined by the "ProtParam tool" (https://web.expasy.org/protparam/). In addition, intracellular localizations were estimated with the help of the WoLF SPORT database (Horton et al., 2007).

2.2. Identification of Gene Duplication, Physical Locations, Structure, and Conserved Motifs of *Pvul-FBA* Genes

Intron and exon regions of Pvul-FBA proteins in *A. thaliana*, *P. vulgaris*, and *G. max* were detected by the GSDS (Gene Structure Display Server v2.0) database (Guo et al., 2007). Using genomic sequences and CDS, the *Pvul-FBA* genes structure was discovered. Using by Phytozome v13, the chromosomal locations/sizes of the *Pvul-FBA* genes were ascertained. The MapChart tool was utilized to mark and depict the locations of *Pvul-FBA* genes on *P. vulgaris* chromosomes (Voorrips, 2002). *FBA* gene duplications between *P. vulgaris*, *G. max*, and *A. thaliana* were determined by MCScanX (Wang et al., 2012). Nonsynonymous ratios (Ka), synonymous ratios (Ks) and evolutionary strains (Ka/Ks) between binary pairs of *Pvul-FBA* genes were calculated using the PAML (Yang, 2007) with the help of PAL2NAL (Suyama et al., 2006). Conserved motifs of *Pvul-FBA* genes were detected with the tool "MEME Suite" (Bailey et al., 2006). The width of the motifs was recorded as minimum 6 and maximum 50 and the maximum number of motifs as 10. Possible domains were found by scanning the acquired motifs through the InterProScan database (Quevillon et al., 2005).

2.3. Phylogenetic Analysis

Phylogenetic and molecular evolutionary analyzes were realized by using the MEGA v11 (Tamura et al., 2021). Multiple sequence alignment of Pvul-FBA proteins was performed using ClustalW. To create phylogenetic trees, the bootstrap value of 1000 was repeated and the Neighbor-joining method was applied and the phylogenetic tree was designed (Thompson et al., 1997). Then, the obtained phylogenetic tree was shaped with the help of the iTOL (Letunic & Bork, 2011).

2.4. Promoter Analysis of the bean *FBA* Gene Family

First, of Phytozome database v13, the first 2000 bp upstream areas of the *Pvul-FBA* genes were obtained. The PlantCARE database was then utilized to identify cis-acting elements. Phenogram was then generated with TBtools (Lescot et al., 2002; Chen et al., 2020).

2.5. Synteny Analysis

The orthologous protein sequence information of *G. max, P. vulgaris, and A. thaliana* was retrieved using Phytozome v13. Synteny map was then drawn with the help of "TBtools" (Chen et al., 2020).

2.6. Homology Modeling of Pvul-FBA Proteins

The 3-dimensional structures of the Pvul-FBA protein sequences were determined with the help of the Phyre[2] (Kelley et al., 2015).

2.7. Pvul-FBA protein-protein interactions (PPI)

Help was taken from the STRING database (https://string-db.org/) to determine functional and physical aspects of protein-protein interactions. The software Cytoscape (Shannon et al., 2003) was utilized to categorize and present the collected data.

2.8. Gene Expression Analysis (In-silico)

Illumina RNA-seq data were acquired from the Sequence Reading Archive (SRA) data bank of the NCBI database in order to investigate the *Pvul-FBA* genes. Relevant RNA-seq data SRR957668 (salt stress treatment decontaminated leaf), SRR958469 (leaf salt control) (Hiz et al., 2014), and SRR8284480 (leaf drought control), SRR8284481 (drought stress treated leaf) accession numbers used and in silico expression profiles RPKM (Reads per kilobase: Transcripts per kilobase, expression of transcript normalized form) (Mortazavi et al., 2008) values log2 transform was calculated and CIMMiner (http://discover.nci.-nih.gov/cimminer) algorithm with heat graph (heatmap) was obtained.

3. Results

FBA gene family members were scaned in the common bean genome available in the Phytozome v13 with the PFAM accession number (class I: PF00274, class II: PF01116). As a result, were detected 7 genes in the common bean genome. Chromosome locations (Figure 1), molecular weights, theoretical isoelectric points and amino acid numbers of *Pvul-FBA* genes obtained from the common bean genome were determined and shown in Table 1. The amino acid numbers of *Pvul-FBAs* between 129 to 1374. The highest amino acid number was determined in the Pvul-FBA-1 protein (1374), while the lowest amino acid number was determined in the *Pvul-FBA-6* gene (129). Again, when the molecular weights of *Pvul-FBAs* were evaluated within themselves, the lowest molecular weight was determined by *Pvul-FBA-6* with a value of 14.15 kDa, while the highest molecular weight was detected in *Pvul-FBA-7* with a value of 43.03 kDa. In addition, the theoretical isoelectric points vary between 5.56 and 8.02, and the highest isoelectric point *Pvul-FBA-3* was obtained.

Table 1. Information on PvNPR-like proteins found in the common bean genome.

Gene Name	Phytozome ID	Chr No	Chromosome Location	aa length	MW (kDa)	pI	Instability index	Stability
Pvul-FBA-1	Phvul.004G150100	Chr04	45186912-45212757(+)*	1374	14.80	5,56	31.27	Stable
Pvul-FBA-2	Phvul.007G033800	Chr07	2745002-2747868(+)	382	41.82	7,6	44.69	Unstable
Pvul-FBA-3	Phvul.007G222900	Chr07	34634308-34636200(-)*	357	38.34	8,02	35.51	Stable
Pvul-FBA-4	Phvul.008G189200	Chr08	52718648-52721317(-)	358	38.60	6,73	31.47	Stable
Pvul-FBA-5	Phvul.008G282000	Chr08	62278473-62281022(+)	357	38.55	5,77	32.81	Stable
Pvul-FBA-6	Phvul.009G003100	Chr09	574960-575965(-)	129	14.15	6,73	41.19	Unstable
Pvul-FBA-7	Phvul.011G039100	Chr11	3560197-35630828(-)	398	43.03	7,62	35.35	Stable

*(-): reverse strand, (+): forward strand

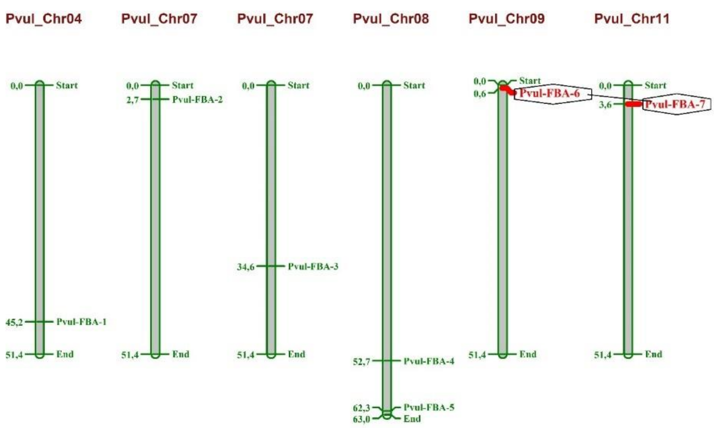

Figure 1. *Pvul-FBA* gene distribution in bean chromosomes (red indicates segmental duplicated genes).

Gene duplication events among *Pvul-FBA* genes were examined and the results are shown in Table 2. According to the analysis, while tandem duplication could not be detected in *Pvul-FBA* genes, segmental duplication was detected between *Pvul-FBA-6*/*Pvul-FBA-7* gene pairs. It was determined that the Ka/Ks ratio of genes showing segmental duplication was less than 1.

Table 2. Ka, Ks and Ka/Ks values of *Pvul-FBA* segmental duplicate genes.

Gene 1	Gene 2	Ka	Ks	Ka/Ks
Pvul-FBA-6	*Pvul-FBA-7*	0.4562	0.0695	0.1523

As a result of the information obtained from the WoLF PSORT database, it was identified that the *Pvul-FBA* genes are located in different intracellular regions such as the mitochondria, cytoplasm, and chloroplast, and are shown in Table 3. It has been determined that all *Pvul-FBA* genes in beans are located in the cytoplasm.

A phylogenetic tree was formed with the FBA protein sequences of *A. thaliana* and *G. max* plants and the relationship between Pvul-FBA proteins was determined. The phylogenetic tree was drawn utilizing the Neighbor-joining system, depending on the amino acid sequence of the FBA proteins, with a bootstrap value of 1000 repetitions. FBA proteins are clustered in 4 main groups as A, B, C, and D as shown in Figure 2.

Table 3. Intracellular localization of *Pvul-FBA* genes.

Gene	WoLF PSORT
Pvul-FBA-1	pero: 9, cyto: 3, nucl: 1, golg: 1
Pvul-FBA-2	chlo: 14
Pvul-FBA-3	cyto: 5, E.R.: 4.5, E.R._plas: 3.5, mito: 2, plas: 1.5, nucl: 1
Pvul-FBA-4	cyto: 11, chlo: 1, nucl: 1, E.R._vacu: 1
Pvul-FBA-5	chlo: 4, cyto: 5, pero: 2, nucl: 1, mito: 1, cysk: 1
Pvul-FBA-6	nucl_plas: 5.5, nucl: 7mito: 3, plas: 2, chlo: 1, cyto: 1
Pvul-FBA-7	hlo: 8, cyto: 2, mito: 2, nucl_plas: 2

*chr: chromosome, chlo: chloroplast, nucl: nucleus, mito: mitochondria.

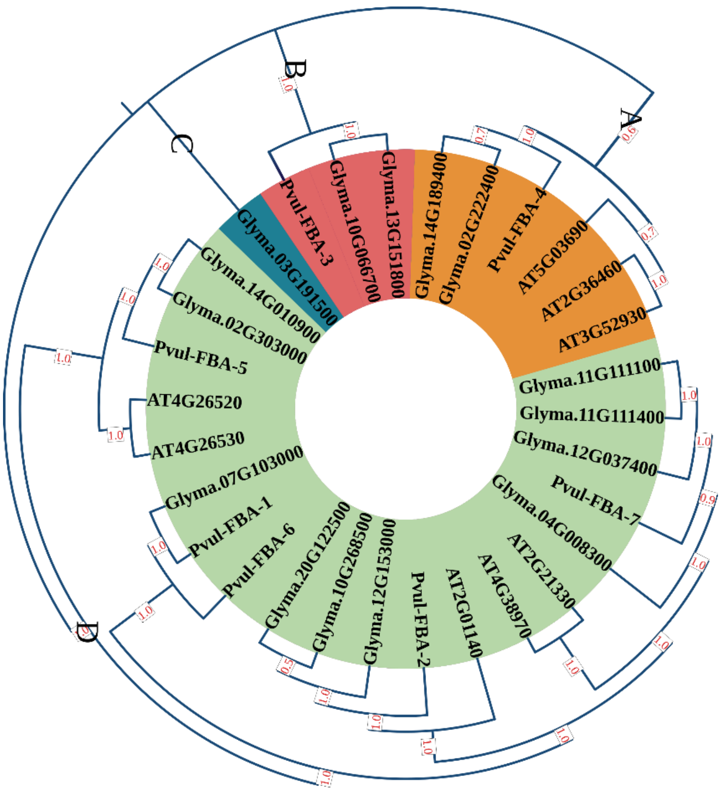

Figure 2. Phylogenetic tree constructed with Pvul-FBA proteins from *P. vulgaris*, *A. thaliana* and *G. max*.

As a result of the conserved motif analysis performed on Pvul-FBA proteins with the MEME suite v5.4.1 program, 10 conserved motifs were established. The length of the obtained motifs was in the range of 14-50 amino acids (Table 4). While 10 motifs were specified in *Pvul-FBA-2*, *Pvul-FBA-3*, *Pvul-FBA-4*, *Pvul-FBA-5* and *Pvul-FBA-7* genes, 3 motifs were specified in *Pvul-FBA-6* gene (Figure 3). The best possible match sequences corresponding to the motifs are shown in Table 4. In addition, it was identified that Motif-1, 2, 3, 4, and 5 were fructose-bisphosphate aldolase domains.

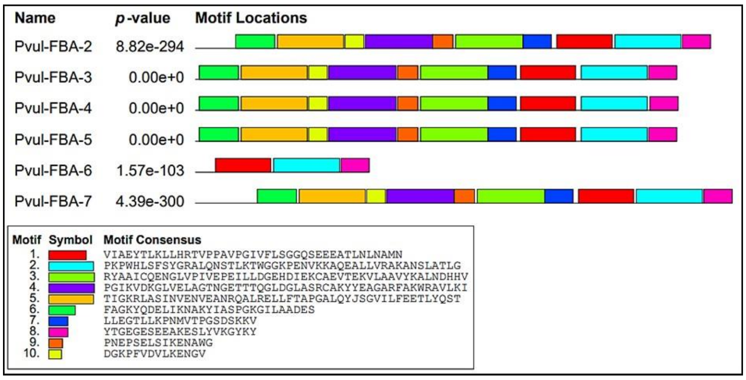

Figure 3. Conserved sequence regions of *Pvul-FBA* genes.

Table 4. Sequence information of possible motifs in Pvul-FBA proteins.

Motif id	WIDTH	Possible best match	Contains domain
1	41	VIADYTVRMLHRRVPPAVPGIMFLSGGQSEEEATLNLNAMN	Fructose-bisphosphate aldolase
2	49	PNPWHVSFSYGRALQQTTLKTWGGKPENVKKAQDALLFRCKA NSEAQLG	Fructose-bisphosphate aldolase
3	50	RYAAICQENGLVPIVEPEILLDGDHDIHKCAEVTERVWAECYFY LNDHHV	Fructose-bisphosphate aldolase
4	50	PGIKVDKGTVPLAGTNGETWCQGLDGLAQRCAKYYEQGARFA KWRTVLKI	Fructose-bisphosphate aldolase
5	49	TCGKRLASINVENVEANRQAYRELLFTAPGCLQYLSGVILFEET LYQST	Fructose-bisphosphate aldolase
6	29	FKGKYQDELIKNAKYIASPGKGILAMDES	N/A
7	21	LFEGTLLKPNMVTPGSDSKKV	N/A
8	21	YTGEGESDEAKESMFVKGYKY	N/A
9	15	PNEPSELSIHENAWG	N/A
10	14	DGKPFVDVLKENNV	N/A

Exons and introns of *Pvul-FBA* genes were identified according to structural analysis with GSDS (Figure 4). According to the results, it was found that the exon numbers of *Pvul-FBA* genes varied between 2 and 42, and the intron numbers varied between 1-41. The highest number of exons was determined in *Pvul-FBA-1* (42) genes, while the lowest number of exons was determined in *Pvul-FBA-3* and *Pvul-FBA-6* (2) genes. On the other hand, while the highest intron number was designated in the *Pvul-FBA-1* gene, the lowest intron number was designated in the *Pvul-FBA-6* gene (Figure 4).

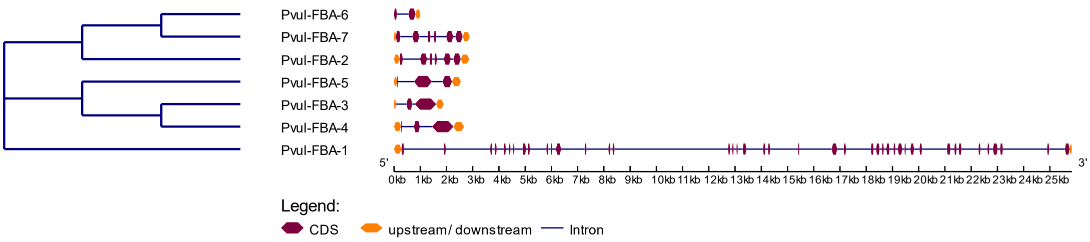

Figure 4. Number, position, and length of introns and exons in *Pvul-FBA* genes.

It was discovered that the *Pvul-FBA* genes' promoter regions aid in plant growth, the molecular reaction to abiotic stressors, and environmental adaptation. As a result of the analyzes performed in the PlantCARE database, the cis-acting elements in the *Pvul-FBA* gene sequences were shown using the TBtools program (Figure 5). The information demonstrated that all *Pvul-FBA* genes had elements linked to photosensitive elements, including the CAT-box, W box, and AE Box.

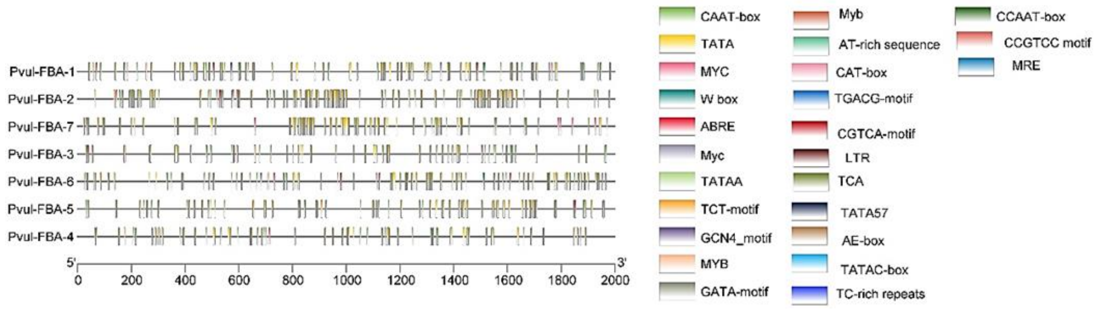

Figure 5. Promotor regions of *Pvul-FBA* genes.

Using FBA proteins from *P. vulgaris* and *A. thaliana* plants, a synteny map was produced. The synteny analysis revealed a connection between *P. vulgaris* and *A. thaliana* (Figure 6). Eight syntenic relationships were identified among *P. vulgaris* and *A. thaliana FBA* genes. Orthology was found between the *Pvul-FBA-1*/AT1G18270.3, *Pvul-FBA-2*/AT2G01140.1, *Pvul-FBA-3*/AT3G52930.1, *Pvul-FBA-3*/AT2G21330.1, *Pvul-FBA-6*/AT4G38970.1, *Pvul-FBA-6*/AT2G21330.1, *Pvul-FBA-7*/ AT4G38970.1 and *Pvul-FBA-7*/ AT2G21330.1 genes.

The structure and function of the proteins were predicted with the help of the EzMol database. Three-dimensional homology modeling of the identified Pvul-FBA proteins is shown in Figure 7. The Cytoscape tool was utilized to show the protein-protein interactions of the identified FBA proteins, as a consequence of the data obtained from the STRING database (Figure 8).

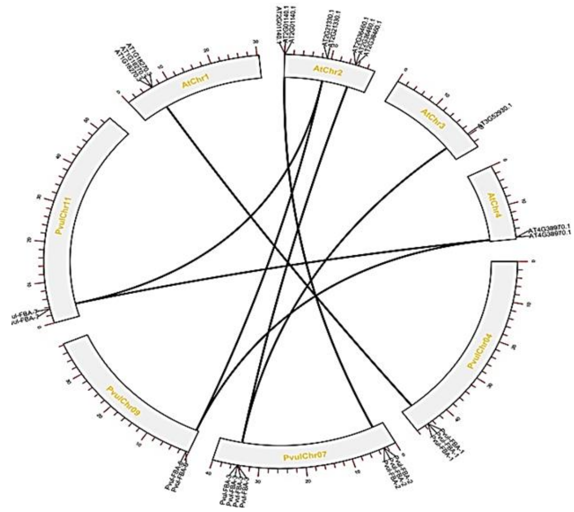

Figure 6. Syntenic relationship among *P. vulgaris* and *A. thaliana FBA* genes.

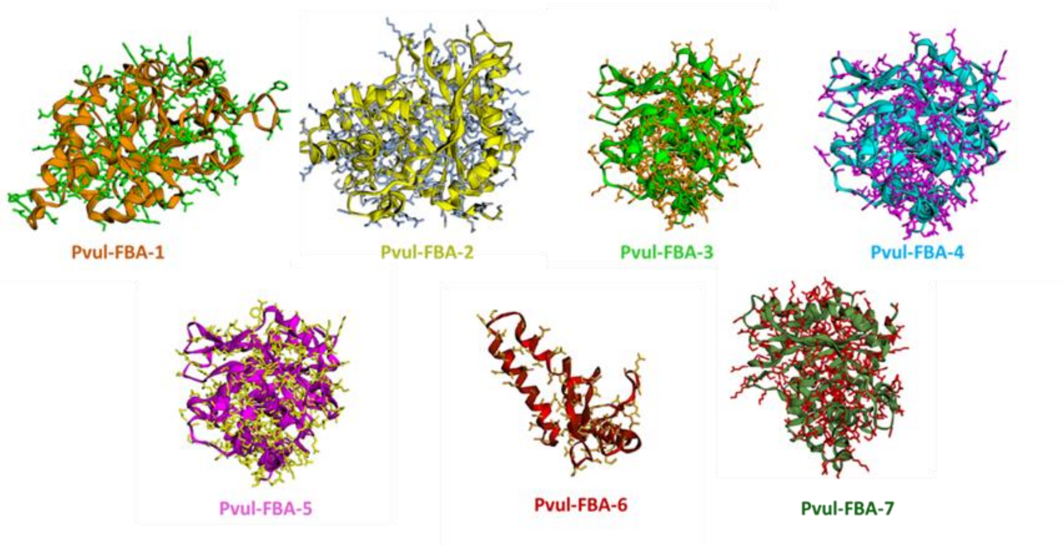

Figure 7. 3D structure modeling of Pvul-FBA proteins.

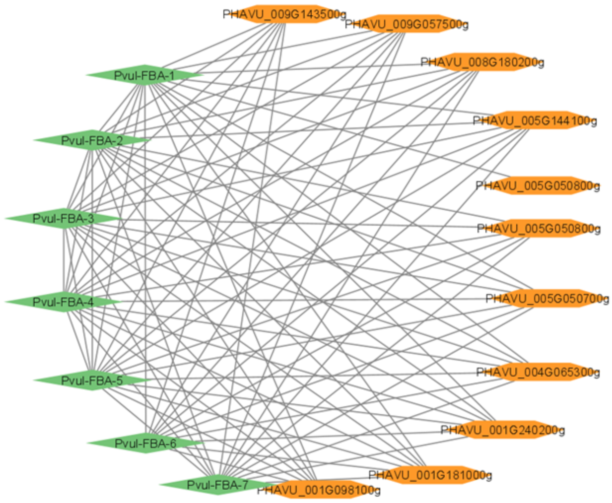

Figure 8. Protein-protein interactions (PPI) of the determined FBA proteins.

For the in silico evaluation of the expression of *Pvul-FBA* genes under drought and salt stress, RNAseq from the NCBI SRA database (Sequences Read Archive), SRR957668 (salt-stressed leaf), SRR958469 (leaf salt control), SRR8284480 (leaf drought control) data and SRR8284481 (drought stressed leaf) were used. The expression levels of the *Pvul-FBA* genes under drought and salt stresses were shown Figure 9 according to the heatmap constituted by the log2 transformation of the RPKM values based on the results obtained from the RNAseq data. Expression profiles of *Pvul-FBA* genes were identified in the shoot tissue of beans under drought and salt stress compared to the control (Figure 9). In salt application, the highest expression profile was determined in the *Pvul-FBA-7* gene, and it was determined that this increase was higher than the control group. The lowest expression profile was found in the *Pvul-FBA-6* gene in salt applications. On the other hand, when drought application was evaluated, the highest expression was detected in the *Pvul-FBA-7* gene.

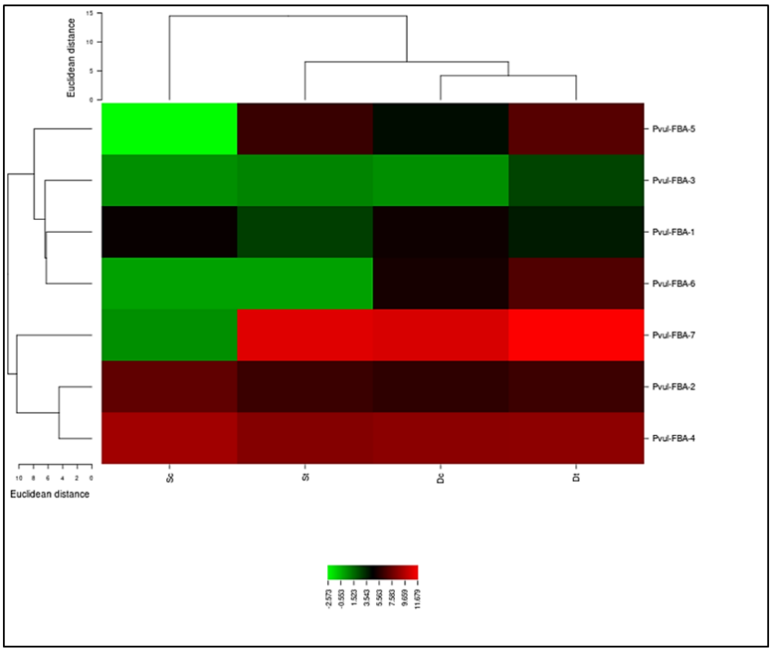

Figure 9. Heatmap of expression of *Pvul-FBA* genes in leaf tissue under drought and salt stress.

4. Discussion

Legumes are essential for human nutrition and the agricultural systems of poor nations since they are the primary source of protein and minerals and a fundamental component of agricultural production systems (Akibode & Maredia, 2012; Yeken et al., 2018). Common bean is the richest vegetable crop in terms of species diversity among legumes (Graham & Ranalli, 1997; Cichy et al., 2015). FBA is involved in gluconeogenesis, glycolysis, and the Calvin-Benson cycle and is the enzyme that plays an essential role in signal transduction of these stages (Cai et al., 2018). *FBA* genes have essential roles in plant growth and have been determined in many plant species (Lv et al., 2017).

As a result of our study, *Pvul-FBA* genes carrying the FBA domain were determined in beans. Diverse *FBA* gene members have been found in *Spinach oleracea* (Pelzer-Reith et al., 1993), *Oryza sativa* (Kagaya et al., 1995), *Zea mays* (Dennis et al., 1988), *Nicotiana tabacum* (Yamada et al., 2000), *A. thaliana* (Lu et al., 2012), *S. portulacastrum* (Fan et al., 2009) *Solanum lycopersicum* (Cai et al., 2016), *Triticum aestivum* L. (Lv et al., 2017), *Brassica napus* (Zhao et al., 2019), and *Gossypium hirsitum* (Li et al., 2021). Whole Genome Folds or polyploidy is a common phenomenon in nature (Wendel, 2000). In this case, tandem and segmental gene duplications occur, which allow new members of existing gene families to arise. Gene duplication, including tandem and segmental duplications, is one of the essential driving strengths in the evolution of genomes (Kasapoğlu et al., 2022). When Ka/Ks<1, it indicates purifying selection, when Ka/Ks=1, it indicates natural selection, and when Ka/Ks>1, it indicates positive selection during the evolution of the gene sequence (İlhan, 2018; Aygören et al., 2022; Oner et al., 2022). As a result of our research, the Ka/Ks ratios of segmentally duplicated genes were found to be less than 1. This indicates that the evolutionary process of the bean has involved purifying selection. In our study, the relationship between the FBA proteins of *P. vulgaris*, *A. thaliana*, and *G. max* was determined by forming a phylogenetic tree and 4 main groups were obtained. In another study, they determined the phylogenetic relationship between FBA proteins tomato, *A. thaliana*, and rice (Cai et al., 2016). Salt and drought stress, which is one of the abiotic stresses, affects all the main processes in plants such as germination, photosynthetic pigments, growth, nutrient imbalance, water relationship, oxidative stress, and yield. Molecular responses of plants to salt stress are primarily mediated by transcriptional induction of specific genes (Carpici et al., 2009). *FBA* genes, one of these genes, have a vital role in biotic and abiotic (high salt, drought, heavy metal, and low temperature, etc.) stress responses and regulate growth and development in plants (Murad et al., 2014; Shu et al., 2014). As a result of our findings, it was determined that *FBA* genes generally increased more in salt and drought applications than the control. It has been reported that the *FBA* expression level increased under cold, salt, and drought stress in other studies (Abbasi & Komatsu, 2004).

5. Conclusion

This study supplied a thorough investigated of the *FBA* gene family in common beans (*P. vulgaris* L.). Therefore, 7 *Pvul-FBA* genes were obtained in the *P. vulgaris* genome as a result of our study. The gene structure, Ka/Ks findings, and motif suggest that *Pvul-FBA* were significantly protected. This suggests that the *FBA* gene family, has an essential role in the development stages of plants, was examined. This study mentions function of *FBA* genes in beans will be elucidated and will lay the groundwork for many future studies.

Conflict of Interest

The authors have no financial or non-financial interests that would influence the research in this study.

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RESEARCH ARTICLE

The Effect of Pre-Applied Titanium Dioxide Nanoparticles on Germination in *Carthamus tinctorius* L. Varieties

Volkan Gul[1]✉ • Burcu Seckin Dinler[2] • Fırat Sefaoglu[3] • Hatice Cetinkaya[2] • Fatma Nur Koc[2]

[1]Bayburt University, Aydıntepe Vocational School, Food Technology Program, Bayburt/Türkiye
[2]Sinop University, Faculty of Arts and Sciences, Department of Biology, Sinop/Türkiye
[3]Kastamonu University, Faculty of Engineering and Architecture, Department of Genetic and Bioengineering, Kastamonu/Türkiye

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ABSTRACT

In the present study, to promote sustainable nano-farming, the apparent effects of different concentrations (0, 100, 200, 300, 400, 500 ppm) of titanium dioxide nanoparticle (TiO_2NPs) solutions on the germination percentage, index and duration of seeds belonging to Balcı, Dincer, Hasankendi, Koc, Olas, and Zirkon safflower varieties were investigated. Moreover, scanning electron microscopy (SEM) was employed to analyze TiO_2NPs in germinated safflower varieties. Germination performance was TiO_2NPs concentration and variety depended. It was determined that the seed samples displayed different responses to TiO_2NPs concentrations; germination percentages were between 20.0±1.15 and 82.9±0.44%, germination durations were between 2.01±0.021 to 3.82±0.017 days, and germination indices were between 9.97±0.606 and 38.97±0.959. While the highest germination percentage (82.9±0.44%) was obtained from Dincer variety with 100 ppm TiO_2NP pre-application, the lowest germination percentage (20.0±1.15% and 20.0±1.92%) was obtained from Balcı and Hasan Kendi varieties with 100 and 300 ppm TiO_2NP pre-application. According to this result, although the highest germination percentage based on variety was obtained from the Dincer variety, the Balcı variety with the lowest germination percentage provided the most significant increase in the 200 ppm TiO_2NPs application dose compared to the control. According to the germination percentage, it can be said that the most effective TiO_2NPs application dose in Safflower varieties is 200 ppm. Further research on nanoparticles is needed to determine both the economical doses of TiO_2NP pre-application and its uptake by the plant.

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1. Introduction

Fats and lipids are sources of energy storage and important in human and animal nutrition (Gürsoy, 2019). Vegetable oils are the sources of the most widely used lipids in human nutrition as they are rich in polyunsaturated omega fatty acids and do not contain cholesterol (Roccisano et al., 2016; Zhou et al., 2020). Safflower (*Carthamus tinctorius* L.) is an important oil seed plant for oil production due to its deep root system, which helps its tolerance to drought where dry farming is carried out (Omidi et al., 2012). Safflower seeds contain 30-50% oil and provide quality edible oil owing to its high (63-75%) linoleic acid (omega-6) content (Toma et al., 2014; Conte et al., 2016). The growth and yield of crop plants depend on rapid seed germination and emergence and good seedling formation. Seed germination is the first growth stage of a plant

✉ Correspondence
E-mail address: volkangul555@gmail.com

to limit its development under stress (Jabeen & Ahmad, 2013). Therefore, it is important to investigate effective seed treatment methods such as TiO_2NP to improve the germination percentage and seedling growth is of great importance.

Titanium dioxide nanoparticles (TiO_2NPs) are widely used catalysts in areas such as sewage treatment, food preservation, and environmental improvement, thanks to their low toxicity and high chemical stability (Arezoo et al., 2020; Li et al., 2021; Wu et al., 2018). Titanium dioxide nanoparticles are the most used nanomaterials in agricultural production thanks to their wide range of benefits in plants, such as promoting nutrient absorption in plants, increasing stress resistance, stimulating antioxidant enzyme activity, and increasing product yield and quality (Torres et al., 2020; Kamal & Mogazy, 2021; Shoja et al., 2022). Because nanoparticles (NP_S) are very small, they affect very wide areas in chemical and biological applications (Khalil et al., 2017; Rizvi & Saleh, 2018). Nanotechnology is the production of structures with completely new physical, chemical and biological properties, developed by working at the level of atoms with sizes of 1-100 nm. Since nanomaterials can be produced from many different materials, such as metals (Ni, Pt, Au), semiconductors (Si, InP, GaN) and insulators (SiO_2, TiO_2), they can be used in various industries, including the mechanical, medical, pharmaceutical, manufacturing and environmental sectors (Ateş et al., 2015; Castillo et al., 2020). In recent years, many studies have indicated that they are involved in the main metabolic events that control plant growth and development (Arora et al., 2012; Regier et al., 2015). Seed applications are an important field for the potential usage of NPs (Mahakham et al., 2016), where NPs can affect seed germination, seedling establishment, root and shoot growth, and photosynthesis (Paparella et al., 2015; Guha et al., 2018). They stated that titanium dioxide nanoparticle (TiO_2NP) applied to spinach leaves improved the growth of spinach (*Spinacia oleracea* L.) by promoting the reduction of nitrogenous fertilizer in the form of NH_3 to ammonium (NH_4) and nitrate (NO_3) for nitrogen (N) adsorption (Yang et al., 2008). They stated that TiO_2NPs significantly reduce salt stress and H_2O_2 concentration, increase antioxidant enzyme activity, and reduce disease index and biological stress in plants (Gohari et al., 2020; Satti et al., 2021). It was also reported that titanium application from seed reduced cadmium (Cd) stress in safflower plants (Sardar et al., 2022). Pre-treatment methods (such as a nanoparticle, gibberellic acid and salicylic acid) have been developed for seeds to minimize environmental stresses by increasing the germination quality of seeds (Liu & Lal, 2015). The pre-application method applied to seeds is determined by factors such as the osmotic state of plants, water potential, temperature, light conditions, oxygen availability, soil moisture, and seed quality (Hussain et al., 2016). Nanoparticle applications in the seed are promising techniques in the agricultural sector under normal conditions (Rastogi et al., 2019), and it was tested under environmental stress factors (Ahmad & Akhtar, 2019).

To increase the yield in safflower production, one of the most important considerations is that the seeds show good germination and emergence. Therefore, it is necessary to develop different methods against unfavorable environmental factors affecting the germination and exit process. it was added also above. In the study, it was investigated how titanium dioxide (TiO_2) nanoparticle pretreatment in different doses will have an effect between varieties to break the dormancy and increase productivity that occurs depending on genetic characteristics and promote germination in the germination performance of safflower seeds.

2. Materials and Methods

2.1. Material Procurement

Safflower seeds of six varieties; Balcı (Average yield: 1500-2500 kg ha^{-1}; 1000 grain weight: 40-48 g; oil content is 38-40%), Dincer (Average yield: 1000-2500 kg ha^{-1}; 1000 grain weight: 45-49 g; Oil content: 25-28%), Hasankendi (Average yield: 1600-2800 kg ha^{-1}; 1000 grain weight: 36-48 g; oil rate: 36-38%), Koc (Average yield: 2000-4000 kg ha^{-1}; 1000 grain weight: 40-50 g; oil content: 37-39%), Olas (Average yield: 1500-2800 kg ha^{-1}; 1000 grain weight: 45-50 g; oil content: 39-40%), and Zirkon (Average yield: 2500-3500 kg ha^{-1}; 1000 grain weight: 34-53 g; oil content: 28-35%) were obtained from institutes affiliated with the Turkish Republic Ministry of Agriculture and Forestry. Titanium dioxide nanoparticles (20 nm in size, 99.9% purity) were purchased from a company (Nanografi Nanotechnology, Ankara/Türkiye).

2.2. Experimental Setup

The seeds were kept in a 1% sodium hypochlorite (HClO) solution for 10 min. Then washed five times with distilled water for surface sterilization. After washing, the seeds were placed on filter papers and dried at room temperature. Titanium oxide concentrations at different doses applied to safflower seeds were determined by a preliminary experiment (0-500 ppm no germination) and the most appropriate doses were selected according to this range. Sterilized seeds were incubated in 0, 100, 200, 300, 400, and 500 ppm NP solutions for 18 h. Control treatment was incubated in distilled water without TiO_2 and NP solutions were prepared using distilled water. To prevent heat increases due to ultrasonic treatment, the seeds were subjected to "ultrasonic vibration (Frequency: 40 kHz and power: 180 W)" twice, 1 h at the beginning of the incubation and 30 min before at the end of the incubation. After 18 h, the seeds were washed three times with distilled water and left to dry on filter papers. Filter papers were placed in the Petri dishes and 3 ml of distilled water was added. The experiment was set up with three replications and each replication contained 50 seeds in petri dishes. The seeds were placed in a dark environment with a temperature of 25±2 ºC and a relative humidity of 50±5% for germination. Initial germination data were obtained two days after planting (germination durations differed among varieties). The experiment was terminated seven days after sowing when germination data were obtained (Figure 1).

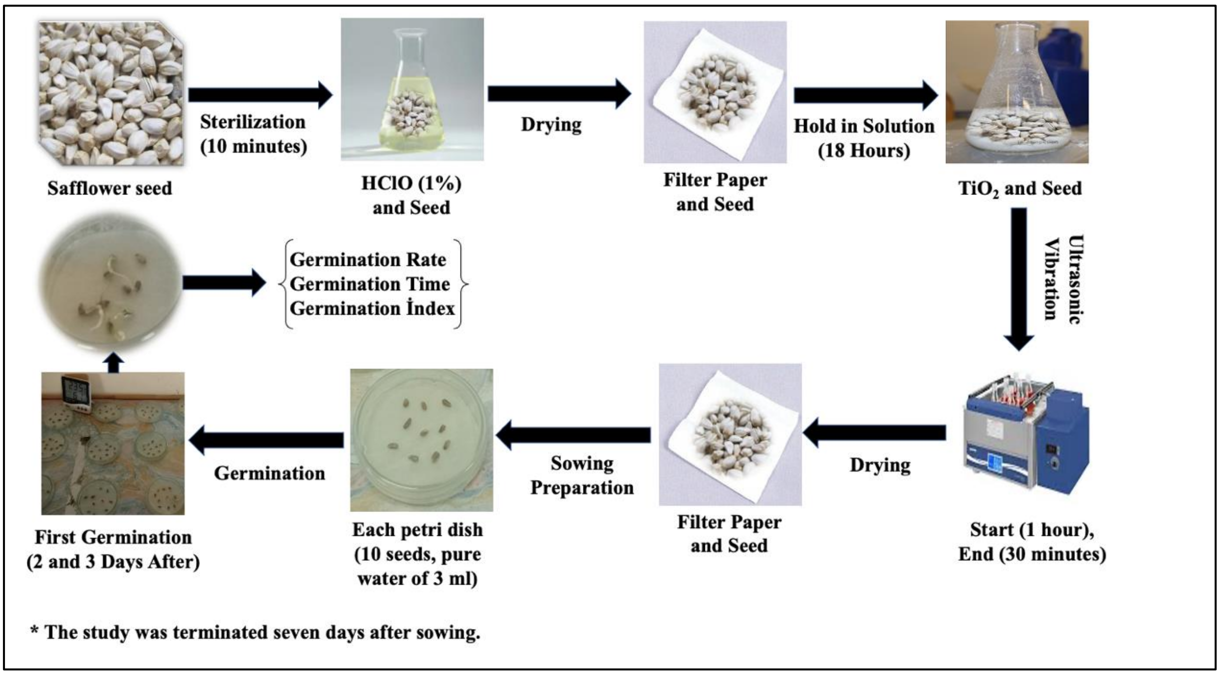


Figure 1. Scheme of preparation of the experiment.

2.3. Germination Percentage (%)

The germinated seeds were counted at the same time every day, and the seeds were considered as germinated when the radicle reached 2 mm and germination percentage was calculated according to Sanoubar et al. (2018).

$$Germination\ percentage\ (\%) = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds}\ x\ 100 \quad (1)$$

2.4. Mean Germination Time (day)

Mean germination time was determined following the formula of Ellis and Roberts (1981).

$$Mean\ germination\ duration = \frac{n_1 x\ d_1 + n_2 x\ d_2 + n_3 x\ d_3 + \cdots + n_n x\ d_n}{Total\ number\ of\ days} \quad (2)$$

Where, n = the number of germinated seeds, d = day.

2.5. Germination Index

The germination index was calculated according to Czabator (1962).

$$Germination\ index = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \cdots + \frac{n_n}{d_n} \quad (3)$$

Where, n = number of germinated seeds, d = day.

2.6. Scanning Electron Microscopy (SEM) And Energy-Dispersive X-Ray Spectroscopy (EDX) Analysis

For SEM imaging, the Examples of germinating safflower were left to dry in the air, fixed on carbon tapes, coated with Au-Pd on the Cressington Sputter Coater for 60 s and then placed on the FEI Quanta FEG 250 SEM (operating at 10 kV and 20 kV) immediately. The elemental analyses of the samples were done by area and point analysis with an EDX detector (Javed & Mashwani, 2020; Javed et al., 2020).

2.7. Statistical Analysis

The two-year data obtained from the research were subjected to analysis of variance with the help of JMP 5.0.1 (SAS institute 2002) package according to the Randomized Complete Blocks Desing. Significant differences among treatments were compared and grouped according to the Duncan Multiple Range Test at the $p<0.05$ probability level.

3. Results and Discussion

Significant differences were detected at the statistically $p<0.01$ level in terms of germination percentage, germination duration and germination index variety x TiO_2NPs pre-application. While significant differences were obtained in terms of TiO_2NPs pre-application at the germination index statistically level of $p<0.05$, significant differences were obtained at the germination percentage and germination duration statistically level of $p<0.01$ (Table 1).

Table 1. Variance analysis material of the development parameters of safflower varieties germinated in different titanium dioxide nanoparticles pre-applications.

Source Variation	d.f	Germination Percentage	Germination Duration	Germination Index
Variety	**5**	30.529**	54.316**	11.062**
Pre-Application	**5**	2.325*	2.361*	6.040**
Variety x Pre-Application	**25**	116.296**	13.626**	14.506**

**, * Statistically significant at P<0.01 and P<0.05, d.f: degrees of freedom, the difference between the averages denoted by different letters is significant.

3.1. Germination Percentage

The mean germination percentages of safflower varieties pre-treated with different concentrations of TiO_2NPs varied between 20.0±1.15 and 82.9±0.44 %. While the highest germination percentage (82.9±0.44%) was obtained from Dincer variety with 100 ppm TiO_2NP pre-application, the lowest germination percentage (20.0±1.15% and 20.0±1.92%) was obtained from Balcı and Hasan Kendi varieties with 100 and 300 ppm TiO_2NP pre-application. TiO_2NP pre-application doses had a positive effect on the varieties at different rates compared to the control (Table 2). The mean germination percentages of safflower varieties pre-treated with different concentrations of TiO_2NPs varied between 20.0±1.15 and 82.9±0.44%. This significant difference between varieties may be due to genetic, climate, and environmental factors. It can also be said that the storage life of safflower seeds varies depending on their storage life and conditions (Kumari, 2009). The highest germination percentage on a variety basis was obtained in the Dincer variety at a pre-application dose of 100 ppm TiO_2NP. It was observed that TiO_2NP pre-application doses had a positive effect on other varieties compared to the control. The administration dose of TiO_2NPs may have increased the germination rate by fully penetrating the seed coat and endosperm and acting on the embryo (Song et al., 2013). Dogaroglu and Köleli (2016), stated that the increase in TiO_2 and TiO_2Ag nanoparticle dose increases the germination percentage in lettuce seeds, so the same kind of nanoparticles may have different effects on other plants. (Ravindran et al., 2012), due to AgNP applications to tomato and corn plants, stated that tomato seeds are more sensitive than corn seeds and cause inhibition of 20-40% in germination percentages depending on the increase in concentration. The seed coat protects the embryo against adverse environmental conditions and may not always allow the inhibition of nanoparticles into the seed with its semi-permeable structure (Boonyanitipong et al., 2011; Song et al., 2013). TiO_2NPs, which do not ionize in water, can see the seed coat and endosperm as effective barriers, especially when they become clumped. The germination percentage results we found show similarities with the results of some researchers.

Table 2. The average germination percentage, germination duration and germination index of safflower varieties germinated in different titanium dioxide nanoparticles pre-applications.

Varieties	Germination Percentage (%)					
	Control	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**500 ppm**
Balcı	24.0±1.15qr	20.0±1.15r	47.0±0.58gi	43.0±2.89ij	46.3±0.33gi	37.0±0.58km
Dinçer	78.4±0.96b	82.9±0.44a	63.6±1.94d	70.7±0.67c	81.6±0.96ab	48.6±0.98gh
Hasankendi	47.6±1.24gh	34.8±1.13mo	46.4±0.22gi	20.0±1.92r	28.2±0.97pq	33.1±0.59mo
Koç	50.4±0.44fg	40.01±0.00jl	31.4±0.99op	54.8±0.98e	48.2±0.98gh	36.8±1.93kn
Olas	48.0±1.15gh	36.01±1.55ln	40.3±3.48jk	46.0±2.31hi	54.0±0.00ef	32.7±0.67no
Zirkon	61.4±0.98d	46.7±1.26gi	65.0±2.89d	40.0±3.85jl	73.3±0.87c	50.0±1.92fh
	Germination Duration (day)					
Balcı	3.04±0.151de	3.19±0.097bd	3.36±0.125bd	3.38±0.095bc	3.51±0.017ab	3.82±0.017a
Dinçer	2.39±0.205gi	2.31±0.124gj	2.41±0.011gi	2.33±0.049gj	2.62±0.130fg	3.04±0.119ce
Hasankendi	2.01±0.021j	2.11±0.032hj	2.23±0.071hj	2.30±0.087gj	2.34±0.023gj	2.35±0.115gj
Koç	2.16±0.023hj	2.24±0.017hj	2.20±0.115hj	2.31±0.055gj	2.36±0.031gi	2.38±0.035gi
Olas	2.09±0.060ij	2.16±0.081hj	2.24±0.062hj	2.36±0.086gi	2.34±0.172gj	2.40±0.226gi
Zirkon	2.32±0.072gj	2.40±0.160gi	2.39±0.144gi	2.45±0.072gh	2.45±0.358gh	2.83±0.192ef
	Germination Index					
Balcı	12.94±0.727qr	9.97±0.606r	24.10±0.02in	22.16±1.385ko	24.87±0.069im	18.09±0.382oq
Dinçer	37.48±0.149ab	35.73±1.299ac	27.09±0.823fk	30.59±0.247ch	32.60±1.566be	15.57±0.658pq
Hasankendi	28.42±1.727ei	28.34±1.213ei	22.19±0.206ko	31.41±0.535cf	27.19±7.459fk	18.99±2.994np
Koç	23.94±0.050in	18.96±0.147np	14.71±0.618pr	26.23±0.461gl	21.76±1.404lo	14.33±0.919pr
Olas	33.63±1.307bd	27.37±0.637fj	22.34±5.502jo	31.77±0.564cf	38.97±0.959a	25.50±0.316hm
Zirkon	26.79±0.267fl	18.65±0.352op	28.72±1.668di	22.91±1.411jo	30.74±0.399cg	20.84±0.584mo

The difference between the averages denoted by different letters is significant.

3.2. Germination Duration

According to the results of the variance analysis, the effect of variety and TiO_2NP applications on germination duration was significant, while the effect of variety x TiO_2NP interaction was insignificant (Table 1). The shortest mean germination duration among TiO_2NP doses was obtained from the control group (2.01±0.021 days) and the shortest germination duration was obtained from the 500 ppm TiO_2NP dose (3.82±0.017 days). Although the effect of different TiO_2NP doses on the germination duration was significant, the germination duration values recorded at the application doses were close (Table 2). The highest germination duration (3.82±0.017 days) was obtained from the 500 ppm TiO_2NP pre-application group in the Balcı variety. In comparison, the shortest germination duration (2.01±0.021 days) was obtained from the control group in the Hasankendi variety. In some cases, adverse effects were observed on some growth parameters, which may indicate a toxic effect of TiO_2NPs doses. The increase in different TiO_2NPs doses have restricted the germination duration compared to the control (Table 2). In this case, the seed pods and endosperm may have acted as filters that absorb metals but pass through the water. Indeed, Song et al. (2013) determined that 2.2 ± 0.6 mg kg^{-1} TiO_2 passed through the TiO_2-treated corn seeds (*Zea mays* L.) and that the nanoparticles did not penetrate the seed coats. (Younes et al., 2020), stated that the mean germination time of seeds belonging to the *Solanaceae* family was reduced when coated with 100 mgbL^{-1} TIO_2NPs. According to the results we obtained, like some researchers, the increase in the pre-application dose of TiO_2NP_s extended the germination duration.

3.3. Germination Index

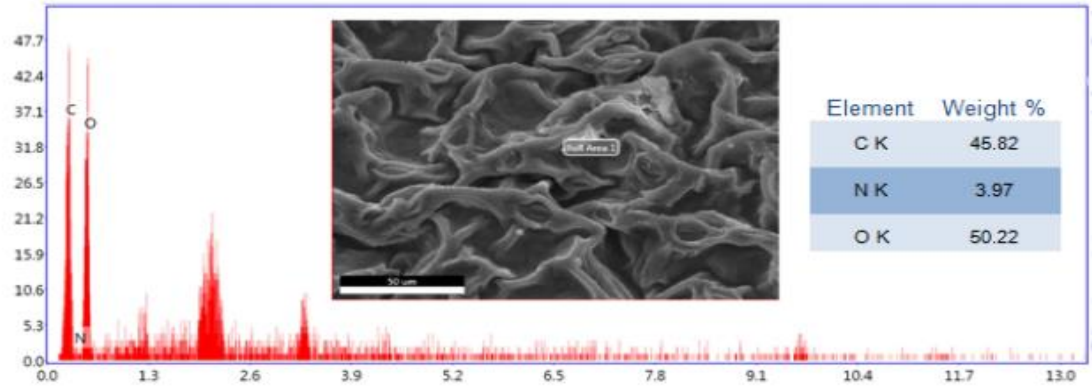
In the present study, 300 and 400 ppm concentrations of TiO_2NPs applied to safflower seeds showed a positive effect by increasing the germination index compared to normal conditions by 8.6%, respectively. However, 100, 200, and 500 ppm concentrations negatively affected the germination index by 14.76, 14.72, and 30.03%, respectively. The germination indexes of safflower seeds varied, and the highest mean index was obtained in Dincer (37.48±0.149) and Olas (38.97±0.959) varieties, whereas the lowest mean germination index was obtained in Balcı (9.97±0.606) varieties. Although the germination indexes of the Dincer and Olas varieties were the highest, as the pre-treatment concentrations of TiO_2NPs increased, the germination indexes decreased to a certain extent. Furthermore, although there were increases in 300 and 400 ppm concentrations, these were not extremely high compared to the standard conditions. In the Balcı variety, which had the lowest germination index, a 23.1% decrease occurred in the 100 ppm treatment compared to the control (12.94±0.727) group. In contrast, increases of 84.6, 69.2, 93.3 %, and 38.5% occurred in the pre-treatments of 200, 300, 400, and 500 ppm TiO_2NP, respectively. Differences were determined between varieties regarding their germination indices depending on the titanium dioxide nanoparticle concentration increase. Different responses to the pre-treatment concentrations of TiO_2NPs caused variety x pre-treatment interaction to be significant in terms of the germination index. Although the germination index of Dincer and Olas cultivars was the highest, germination indexes decreased at certain rates as TiO_2NPs pre-application doses increased. Although there are very few studies on the effect of TiO_2NPs on seed germination, the results of different studies show that each plant is affected differently by TiO_2NPs. Differences were determined in terms of the germination index of varieties depending on the increase in TiO_2NPs dose. (Mehrian et al., 2016), reported that the germination index of some tomato seeds increased with AgNP application, while there was no change in some. (Hatami et al., 2014), reported that applying different doses of TiO_2NPs to five different medicinal species seeds stimulated seed germination, but this response was dependent on the application of TiO_2NP_S and plant species (Faraji et al., 2018).

3.4. Scanning Electron Microscopy (SEM-ED) Analysis

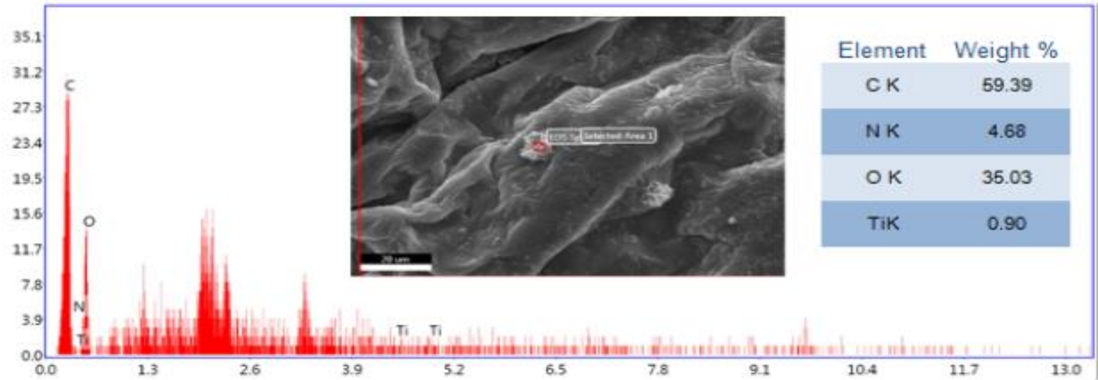
Surface morphology and topography of the TiO_2NPs were observed by using SEM. When the SEM images in Figure 2 were examined, it was observed that the particles appeared in cubic shapes and were located in the radicle and plumule of the seeds that were treated with TiO_2NPs. While nanoparticles were spread on the surface in both the radicle and plumula in the Balcı variety, it was determined that there was an agglomeration in the Dincer variety.

As seen in Figure 2, it shows that the morphology of TiO_2NP residues in the germinating safflower samples is close to spherical in shape and is in an aggregated state. Elemental detection of TiO_2NPs was observed with an energy-dispersive X-ray. Energy dispersive X-ray detector confirms the presence and purity of TiO_2NPs. While TiO_2NPs were detected at a rate of 0.90% in the Balcı variety and 0.28% in the Dincer variety in the applied groups, TiO_2NPs could not be seen on the surface of control plants. TiO_2NPs are less detected in Dincer than Balcı variety, which can be interpreted as the better development of this variety.

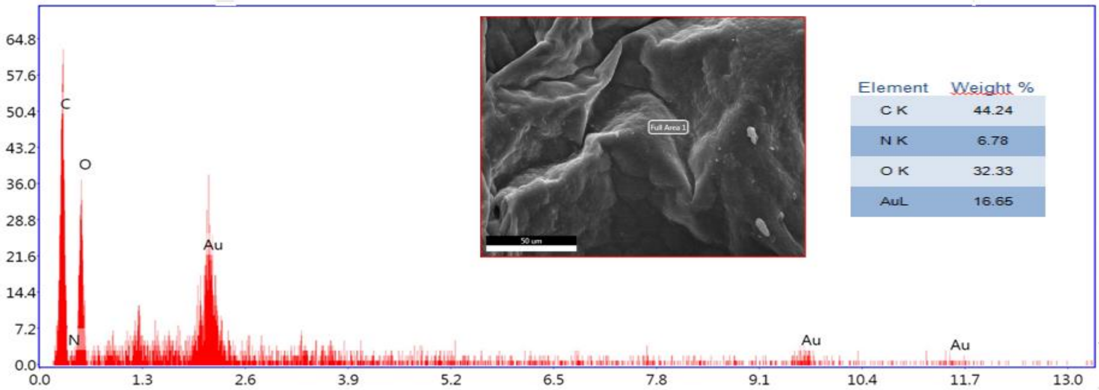
EDS spectra recorded from the TiO_2 nanoparticles are shown in Figure 2, respectively. SEM image showed that the TiO_2NP_S were spherical in shape or cubic (Figure 2). The qualitative and quantitative elemental profile involved in materialized synthesis was ascertained using EDS microanalysis of numerous distinct particles. EDS profile shows a strong Ti signal along with oxygen and carbon peak, which may originate from the biomolecules bound to the TiO_2 nanoparticles' surface. Observation of some peaks in the vicinity of the Ti and O other than C and O were Au peaks used in the coating of plant material.



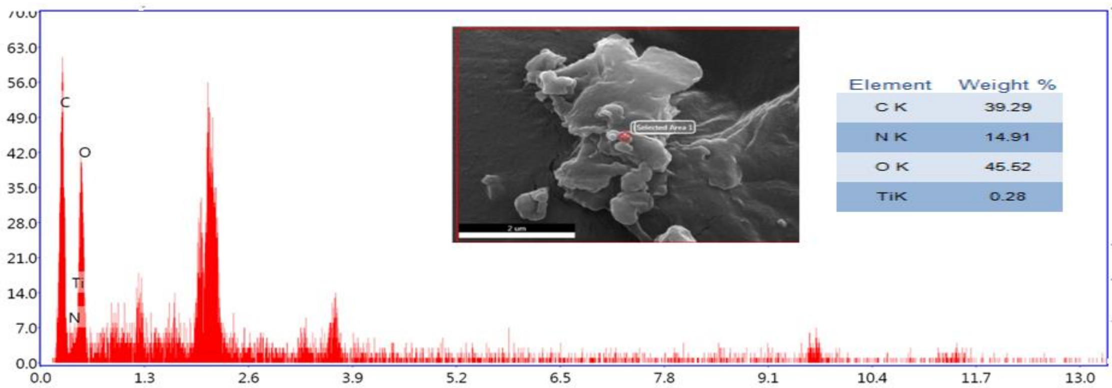
a. Balcı-Control



b. Balcı-Nano



c. Dincer-Control



d. Dincer-Nano

Figure 2. SEM and EDX-based analysis of safflower varieties of Balcı and Dincer (**a.** Balcı-Control, **b.** Balcı-TiO_2NPs, **c.** Dincer-Control, **d.** Dincer-TiO_2NPs). The difference between the averages denoted by different letters is significant.

4. Conclusion

Based on the results obtained, the germination performance of safflower seeds has shown changes depending on the doses of TiO_2NPs and the variety. According to TiO_2NPs concentrations and the variety of safflower seed samples, germination percentages varied between 20.0±1.15-82.9±0.44%, germination duration varied between 2.01±0.021-3.82±0.017 days and germination indexes varied between 9.97±0.606-38.97±0.959. It can be said that the significant differences between safflower varieties in terms of germination rate vary depending on the genetic, climate, and environmental factors of the varieties, as well as the storage life and conditions of the seeds. The highest germination percentage (82.9±0.44%) was obtained from Dincer variety with 100 ppm TiO_2NP pre-application, while the lowest germination percentage (20.0±1.15% and 20.0±1.92%) was obtained from Balcı and Hasankendi varieties with 100 and 300 ppm TiO_2NP pre-application. Different TiO_2NP pre-application doses had a positive effect on the germination percentage of safflower varieties compared to the control. According to this result, although the highest germination percentage based on variety was obtained from the Dincer variety, the Balcı variety with the lowest germination percentage provided the most significant increase in the 200 ppm TiO_2NPs application dose compared to the control. According to the germination percentage, it can be said that the most effective TiO_2NPs application dose in Safflower varieties is 200 ppm. Further research on nanoparticles is needed to determine both the economical doses of TiO_2NP pre-application and its uptake by the plant.

Conflict of Interest

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Effect of Plant Growth-Promoting Bacteria (PGPB) on the Development of Pea crop (*Pisum sativum* L.)

Esin Dadasoglu

Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum/Türkiye

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ABSTRACT

Microorganisms are of great importance in agriculture in terms of plant nutrients by reducing the need for chemical fertilization. In recent years, plant growth-promoting bacteria (PGPB) have been widely used as biological fertilizers (BF) in agriculture. This study was conducted to determine the effect of plant growth-promoting bacteria on the development of pea plants. Firstly the phosphate solubilization and nitrogen fixation potentials of the bacteria used in this study were determined. In the study, the effects of 4 different combinations, F1 [(*Rhizobium* sp. (FR-13) and *Pseudomonas alcaligenes* (FDG121)], F2 [(*Pseudomonas fluorescens* biotype F (FDG-7), *Rhizobium* sp. (FR-18) and *Bacillus-megaterium*-GC subgroup B(FDG-134)], F3 [*Arthrobacter oxydans* (FDG-72), *Bacillus-megaterium*-GC subgroup B (FDG-146), *Rhizobium* sp. (FR-11)] and F4 [*Acinetobacter genospecies* 9 (FDG-116), *Brevibacillus agri* (FDG-118), *Methylobacterium zatmanii* (FDG-123) and *Bacillus-megaterium*-GC subgroup A (FDG-153)] were investigated. Formulations made with bacteria that were found to be the best in terms of the properties specified among these strains were tested against pea plants under greenhouse conditions and their effects on the plant's total fresh and dry weight were investigated. The study was set up to have 3 replications. As a result of the statistical analysis made with the data obtained, the formulations used compared to the control; F2, F3 and F1 applications were important in total fresh weight, respectively, and F2 and F3 applications were important in total dry weight. As a result, these 3 formulations are especially effective on the yield of pea plants and can be used as potential biofertilizers.

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1. Introduction

Peas are farmed in about 84 countries all over the world. The pea crop (*Pisum sativum* L.) is a valuable leguminous crop and is a rich source of protein (Poblaciones & Rengel, 2016), phenolics, tannins and flavonoids, and antioxidants (Singh et al., 2017). Pea seeds have massive nutritional aspects, including high protein, carbohydrate, vitamin, phosphorus, and calcium contents (Maharjan et al., 2019; Janusauskaite, 2023). Pea crops are considered critical crops in sustainable agriculture (Powers & Thavarajah, 2019). This high predominance of peas is connected to their extraordinary growth, yield, and importance for human nutrition livestock.

Microorganisms are present near every living thing on the planet since they are ubiquitous in nature. When microorganisms connect with rhizosphere soil as biofertilizers, they colonize it and increase the take-up of plant nutrients (Demir et al., 2023). Nitrogen, phosphorus, and potassium are important macro-elements and are restricted nutrients for the expansion and evolution of plants. Utilizing microbial biofertilizers is a more eco-friendly strategy as they are ecologically gentler to plants and give plants the benefits of all the soil nutrients (Abeed et al., 2022).

Correspondence
E-mail address: edadasoglu@atauni.edu.tr

Bacteria that are free-living, promote plant growth, and are, used in biological control or as biological fertilizers (BF) are called plant growth promoting bacteria (PGPB). PGPB are able to exert a beneficial effect upon plant growth, N_2 fixing and P solubilizing and play a significant role as PGPB in the biofertilization of crops. These microorganisms are found in several genera including *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Burkholderia, Enterobacter, Flavobacterium, Rhizobium and Serratia*. Although the mechanisms of PGPB are not fully understood, are thought to include: the ability to produce plant hormones; such as auxins, cytokinins and gibberellins, asymbiotic N_2 fixation, solubilization of inorganic phosphate and mineralization of organic phosphate and mineralization of organic phosphate and/or other nutrients and antagonism against phytopathogenic microorganisms by production of siderophores the synthesis of antibiotics enzymes and/or fungicidal compounds, and competition with detrimental microorganisms (Burdman et al., 2000; Bloemberg & Lugtenberg, 2001; Vessey, 2003; Antoun & Prevost, 2006; Çakmakçi et al., 2006; Fuentes-Ramirez & Caballero-Mellado, 2006; Niranjan Raj et al., 2006).

2. Materials and Methods

2.1. Isolation and Stocking of Bacterial Isolates

Soil samples were weighed 1 gr and bean nodules were sterilized, cut, transferred to tubes containing 2 ml of sterile water and left to mix in a hematological shaker for about 2 hours. Serial dilutions were then prepared from the solution in the tube with a sterile pipette. Nutrient Agar (NA) medium was used as isolation medium. Cultures were incubated at 25-30 °C for 24-72 hours and transferred to new media from each colony with different characters, especially those with dense growth, as much as possible from the formed colonies (Klement et al., 1990).

By giving a separate code number to each isolate, information about the isolation (location of isolation, date, etc.) was recorded stored at -80 °C in stock media containing 30% glycerol and Lauria Broth (LB) to be used in diagnosis and characterization processes and other studies.

2.2. Determination of Phosphate Solubizing Potential of Bacteria

24-hour bacterial cultures grown on nutrient agar were suspended in sdH_2O and their density was adjusted to 10^8 CFU/ml. Tubes containing 5 ml of NBRIP-BPB (The National Botanical Research Institute's phosphate-bromophenol blue) in each suspension were inoculated. After a 15-day incubation period, the phosphate solubilization ability of bacteria that showed discoloration in the medium was evaluated as positive (Metha & Nautiyal, 2001). In addition, the potential of the isolates to dissolve Mazıdağı Rock Phosphate was tested by adding Mazıdağı Rock Phosphate to the $Ca_3(PO_4)_2$ medium contained in NBRIP (Nautiyal, 1997).

2.3. Detection of Nitrogen Fixation of Bacteria

Bacteria from stock culture were drawn onto nitrogen-free medium Burk's and Ashby's solid medium (N-Free Solid Malate Sucrose Medium) using the scatter plate method and allowed to grow in an incubator set at 27 °C for 7-10 days. Bacterial growth in the medium was evaluated as nitrogen fixation positive (Wilson & Knight, 1952).

2.4. Testing Different Combinations of PGPB Strains in Greenhouse Conditions

Different combinations of 2, 3 and 4 strains have been created from strains that have good plant growth promoting properties. Pea applications were made for bacterial strains whose biofertilizer properties will be determined. For each strain, 100 ml of liquid medium was prepared and bacteria were grown in this medium on a shaker for 24 hours. Then, the bacterial solutions were adjusted to 10^8 CFU/ml and 9 seeds were added into it. These solutions containing seeds were mixed in a shaker for 2 hours, and finally, after the seeds were filtered and dried, 3 seeds were planted in each pot. Dry and fresh weights of the planted seeds were determined. Sterile NB medium, used to dilute the bacterial solution, was used as a negative control (Angın & Dadaşoğlu, 2022).

2.5. Statistical Analyses

The results obtained from the experiments were analyzed in the JMP (Version 4.0) statistical program and their arithmetic means and standard deviations were calculated. Duncan ($p \leq 0.01$) test was performed to determine the significance level of the differences between the applications.

3. Results and Discussion

In the study, 57 different bacterial strains were isolated from soil samples and nodules of bean plants in different areas in Erzurum province, and among these bacteria, 12 strains with the best nitrogen-fixing and phosphate-dissolving properties were used Table 1. Double, triple and quadruple formulations were created among these bacteria and the experiments were designed with 3 replications. In the study, the effects of the treatments on the total dry weight and total fresh weight of peas are given in Figure 1 and Table 2. According to the results obtained, in terms of total wet weight, an increase of 51%, 48% and 32% was observed in the F2, F3 and F1 formulations, respectively, compared to the control applications. However, the effect of the F4 formulation on total wet weight compared to the control was found to be statistically insignificant. Similarly, in terms of total dry weight, F2, F3 and F1 formulations increased by 64%, 57% and 19%, respectively, when compared to the control applications. However, according to statistical evaluation, the effect of F1 and F4 formulation applications on dry weight was found to be insignificant. In the study, observations were also made in terms of stem fresh weight, root fresh weight, stem dry weight and root dry weight, and the results are given in Table 2. F2, F3 and F1 formulations were found to be statistically significant in terms of stem fresh weight and root fresh weight, but F4 formulation was found to be statistically insignificant when compared to the control treatments. F2 and F3 formulations were found to be statistically significant in terms of stem dry weight and root dry weight, but F1 and F4 formulations were found to be statistically insignificant when compared to the control applications.

There are many studies in the world on the development of vegetables by bacteria that promote plant growth that PGPB, i.e., potato, tomato, onion, pepper, beans, and lettuce (Zahran et al., 2020; Sun et al., 2022; de Andrade et al., 2023). Although previous researchers reported that plant growth-promoting bacteria vary in diverse agriculture crops and even in different varieties of the same crops (Ummara et al., 2022), only a few research papers have studied their effects on pea. Shabaan et al. (2021) found that treating pea seeds with PGP bacteria improves plant height, shoot and root dry weights, and seed weight under heavy metal stress. In a study conducted with *Rhizobium* sp. inoculation of seeds and leaf application, successful results were obtained in pea production, and similar results were obtained in this study by *Rhizobium* sp. bacteria used in the formulations. In a study investigating the effects of 42 different PGPR strains on the development of peas and chickpeas, it was determined that Pseudomonas fluorescens and Bacillus cereus species increased the yield of both plants (Uslu, 2006). In this study, the most effective formulation, F2, includes *Pseudomonas fluorescens* strain and is parallel to the studies conducted on this species.

According to all these results; It was observed that especially F2 and F3 formulations provided a significant increase in the development of pea plants compared to the control. For this reason, it is thought that both formulations have the potential to be used as alternative biofertilizers to chemical fertilizers in pea cultivation.

Table 1. Bacterial strains and some plant growth parameters used in the study.

Strain No	Strain name	Host	Nitrojen fixing	Phosphat Solubilizing
FDG-7	*Pseudomonas fluorescens* biotype F	Soil	+	+
FDG-72	*Arthrobacter oxydans*	Soil	+	K^{+}
FDG-116	*Acinetobacter genospecies* 9	Soil	K+	+
FDG-118	*Brevibacillus agri*	Soil	K+	+
FDG-121	*Pseudomonas alcaligenes*	Soil	K+	+
FDG-123	*Methylobacterium zatmanii*	Soil	K+	+
FDG-134	*Bacillus-megaterium*-GC subgroup B	Soil	K+	+
FDG-146	*Bacillus-megaterium*-GC subgroup B	Soil	+	+
FDG-153	*Bacillus-megaterium*-GC subgroup A	Soil	K+	+
FR-11	*Rhizobium* sp.	Nodules of bean	K+	K+
FR-13	*Rhizobium* sp.	Nodules of bean	K+	K+
FR-18	*Rhizobium* sp.	Nodules of bean	K+	K+

+: Positive K^{+}: Strong positive.

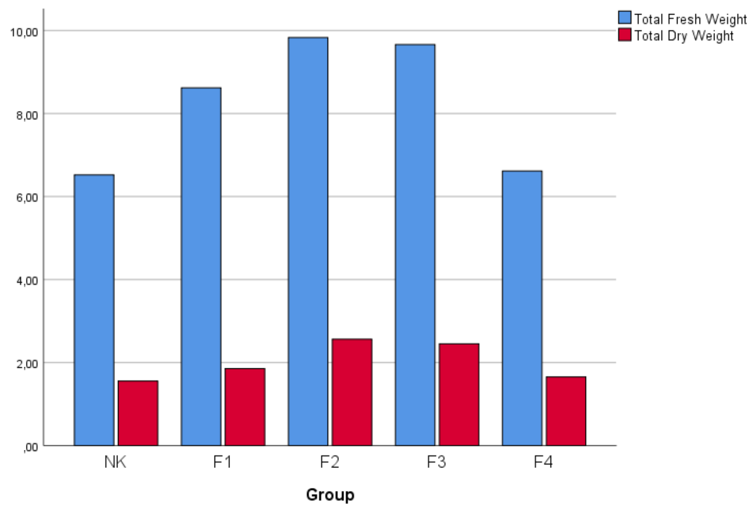

Figure 1. Total fresh weight and total dry weight indicators of the formulations compared to the negative control.

Table 2. Statistical results obtained from bacterial formulation applications.

					95% Confidence Interval for Mean			
		Mean	**Differences**	**Std. Error**	**Lower Bound**	**Upper Bound**	**Minimum**	**Maximum**
Total fresh weight	**NK**	6.521	B	0.436	4.643	8.399	5.833	7.330
	F1	8.618	A	0.439	6.731	10.506	7.833	9.350
	F2	9.876	A	0.212	8.962	10.790	9.488	10.220
	F3	9.662	A	0.585	7.143	12.181	8.735	10.745
	F4	6.614	B	0.311	5.277	7.952	6.107	7.179
Stem fresh weight	**NK**	5.324	B	0.294	4.058	6.589	5.000	5.911
	F1	6.790	A	0.239	5.763	7.818	6.313	7.045
	F2	7.033	A	0.135	6.453	7.613	6.788	7.253
	F3	7.322	A	0.429	5.478	9.166	6.736	8.157
	F4	5.331	B	0.116	4.834	5.828	5.119	5.517
Root fresh weight	**NK**	1.196	C	0.185	0.402	1.991	0.830	1.419
	F1	1.828	BC	0.256	0.725	2.931	1.520	2.337
	F2	2.843	A	0.078	2.509	3.177	2.700	2.967
	F3	2.135	AB	0.149	1.492	2.777	1.972	2.433
	F4	1.283	C	0.397	-0.426	2.993	0.750	2.060
Total dry weight	**NK**	1.555	C	0.291	0.301	2.809	1.238	2.137
	F1	1.854	ABC	0.200	0.992	2.716	1.477	2.160
	F2	2.563	A	0.189	1.752	3.374	2.296	2.927
	F3	2.453	AB	0.337	1.004	3.902	1.995	3.110
	F4	1.654	BC	0.175	0.902	2.405	1.441	2.000
Stem dry weight	**NK**	1.086	B	0.233	0.085	2.086	0.830	1.550
	F1	1.320	AB	0.167	0.603	2.037	1.000	1.560
	F2	1.761	A	0.157	1.084	2.439	1.537	2.065
	F3	1.650	AB	0.200	0.788	2.511	1.345	2.027
	F4	1.204	AB	0.098	0.782	1.626	1.100	1.400
Root dry weight	**NK**	0.469	B	0.059	0.216	0.723	0.408	0.587
	F1	0.534	B	0.036	0.380	0.688	0.477	0.600
	F2	0.802	A	0.031	0.668	0.935	0.759	0.862
	F3	0.804	A	0.140	0.202	1.406	0.650	1.083
	F4	0.450	B	0.078	0.116	0.784	0.341	0.600

Very significant at $p < 0.01$ level, insignificant at $p < 0.05$ level. Differences between numbers with the same letter in the same column are insignificant.

Conflict of Interest

The author has no conflict of interest to declare.

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REVIEW ARTICLE

Importance of Nano-Sized Feed Additives in Animal Nutrition

Büşra Dumlu

Muş Alparslan University, Faculty of Applied Sciences, Department of Animal Production and Technologies, Muş/Türkiye

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ABSTRACT

"Nano", which derives from the Latin word *nanus* and means dwarf, refers to a very small unit of measurement equal to one billionth of a meter. Nanotechnology, which deals with the manipulation of matter at the atomic and molecular level, has an application area in animal husbandry as well as in many fields. Nano-sized feed additives, which have come to the forefront in the livestock sector in recent years, have become an innovative application used to increase the nutritional value of feeds and optimize animal health and performance. Since these additives are nano-sized particles with increased specific surface area, they can have a positive effect on a number of factors such as digestibility, nutrient absorption, immune system, growth and development. Minerals in the form of nanoparticles used as feed additives can increase bioavailability by passing through the intestinal wall to body cells faster compared to larger particles. The nano level of the substance not only increases the productivity of animals, but also brings the potential to improve the functionality of feed molecules. Nano feed additives increase the digestion and absorption of feed, allowing animals to benefit from feed more effectively. However, there are several challenges associated with this approach. These include the potential for endotoxin production, reduced nutrient absorption due to interaction with natural nutrients, the possibility of nanoparticle accumulation in the animal body, health risks, ethical considerations, environmental concerns and some negative effects such as interference with natural nutrients that can be avoided by encapsulation. This article discusses recent studies on nano-sized feed additives that offer potential benefits in animal nutrition.

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1. Introduction

Consumer demand for high-quality, safe, diverse, functional, or nutritious food in animal production is increasing every day (Bölükbaşı et al., 2023). Increasing consumer demands and economic growth have brought animal production to a significant position (Latino et al., 2020). This is getting stronger due to urbanization and rising incomes. Increased demand triggering a higher global demand for animal feed, which requires large quantities of grain to feed livestock. This trend has important implications for the future sustainability of the livestock sector and resource management. Global feed demand is projected to almost double after 2050. It is estimated that 1.3 billion tons of grain will be needed to feed livestock alone (Valin et al., 2014; Rajendran et al., 2022). To meet these expectations, sustainable agricultural practices, feed processing, and animal nutrition techniques need to be developed. Biotechnological approaches are needed to obtain relatively cheap, high-quality products with reduced environmental impact to meet the nutritional needs of a global population expected to reach 9 billion by 2050 (Pirgozliev et al., 2019).

Following the ban on the use of ionophore group antibiotics in animal nutrition, interest in biotechnological methods for animal health and productivity has increased in recent years (Gilbert, 2012). As a result, increased awareness of feed additives has led to a trend towards sustainable practices in the

Correspondence
E-mail address: busradml@gmail.com

livestock sector (Pandey et al., 2019). Animal nutrition is a fundamental element of modern agriculture. It has a direct impact on healthy animal growth, productivity, and product quality. In recent years, there has been a shift away from antibiotics, traditionally used as growth promoters, towards natural feed additives (Özdemir et al., 2022). This shift both ensures the safety of products intended for human consumption and improves performance by minimizing the environmental impact of antibiotic additives. It is also positively changing the balance of the animal production industry (Schmidt, 2009). Feed additives play a very important role in animal nutrition. In particular, they increase the efficiency of nutrients in animal feed. These additives, which are used to promote animal growth by improving the quality and flavor of feed, exert their effects on the intestines or intestinal cell walls of the animal (Bai et al., 2022). Feed additives are also added to animal feed in non-therapeutic amounts to help protect the animal from various environmental stresses (Ban & Guan, 2021). These additives, which can include various substances such as crystalline amino acids, antioxidants, antifungals, etc. help to increase the production of animal protein for human consumption and reduce the cost of animal products. Conventional feed additives have long been used to provide animals with the nutrients they need. However, the use of these additives is sometimes limited in terms of efficiency, cost-effectiveness, and environmental impact. In this context, the use of biotechnology in agriculture has emerged as a revolutionary innovation in animal nutrition. One of these new technologies is the science of nanotechnology, which enables the use of materials at the atomic and molecular levels (Tona, 2017; Placha et al., 2022a, 2022b). Nanoparticles (NPs), which have the potential to provide innovative solutions to current problems in agriculture, can offer significant benefits to the sector (Usman et al., 2020). Manipulation of matter at the nanoscale offers the potential to enhance the functionality of feed molecules as well as increase animal productivity (Bunglavan et al., 2014; Nabi et al., 2020; Rajendran et al., 2022). Nano-sized feed additives such as NPs are extremely small particles that can provide various benefits in animal nutrition (Yadav et al., 2022). These additives can improve both the quantity and quality of safe, healthy and functional animal products. The results of the latest research show that incorporating nanomaterials into animal feed can lead to improved animal growth, better-feed utilization and more efficient nutrient conversion (Seven et al., 2018; Abdelnour et al., 2021). In addition, nanomaterials can help improve digestion and absorption of nutrients in animals, resulting in better overall metabolism and physiology (Abdelnour et al., 2021). The use of nano-sized feed additives in animal nutrition is an emerging field with the potential to revolutionize the livestock industry due to their potential benefits in improving animal health and performance (Dubey et al., 2017; Abdel-Rahman et al., 2022). Nano-sized feed additives, especially nanominerals, have superior bioavailability compared to conventional minerals. Nanominerals, with their low doses and enhanced bioavailability, are a viable substitute for antibiotics and can be incorporated into natural feed components (Schmidt, 2009; Gelaye, 2024). This means that animals require less of these additives to obtain the same nutritional benefits. Nano-sized feed additives have the effect of increasing growth, removing residues, reducing environmental pollutants, contributing to the production of non-polluting animal products, improving the immune function of animals, and reducing the risk of disease (Dei, 2021). This review article discusses the role, benefits and application challenges of nano-sized feed additives in animal nutrition and highlights future research directions. Firstly, the basic properties of nano materials and their potential effects on animal nutrition will be examined, and then the impact of this technology on animal health, feed efficiency, and environmental sustainability will be discussed in detail.

2. What is Nanotechnology?

The word "nano" is derived from "*nanus*", a Latin term meaning "dwarf", and represents a tiny unit of measurement that is one-billionth of a meter (Singh, 2016; Joudeh & Linke, 2022). It was introduced by the renowned physicist Richard Feynman in 1959 and was first employed as a concept of nanotechnology by Norio Taniguchi in 1974 (Feynman, 1959; Mulvaney, 2015; Singh, 2016; Ijaz et al., 2020). In principle, nanomaterials are defined as materials with a length of 1-1000 nm and a diameter of usually 1 to 100 nm. Today, there is various legislation in the European Union (EU) and the USA that contains specific references to nanomaterials. An internationally recognized definition for nanomaterials has not been universally agreed upon. Different organizations have different views on the definition of nanomaterials. NPs are substances composed of a minimum of 50% of components with dimensions in the range of 1-100 nanometers, occurring naturally or synthetically, as outlined by the EU Commission. A nanometer (nm) is a unit included in the International System of Units (Système International d'unités, SI), which represents a length of $1x10^{-9}$ meter (Jeevanandam et al., 2018). Nano-sized feed additives are based on the manipulation and use of materials at the nanometer scale (billionths of a meter). This scale can alter the physical, chemical and biological properties of materials, greatly enhancing the efficacy and functionality of feed additives. Thanks to the small size, high surface area and increased reactivity properties of NPs, such additives can be more effective on the digestive system of animals and maximize the absorption of nutrients (Peters et al., 2016). Nanotechnology, as a field focusing on the regulation and control of matter at the molecular level, involves the process of investigating physical, chemical and biological phenomena at nanometer dimensions (Tüylek, 2021). These studies aim to create impacts in a wide range of science and technology fields, from materials science to biology, electronics to medical applications. The main goal of nanotechnology is to control properties by manipulating nanometer-scale structures and to enable the design of new and advanced products by using these properties in the desired way.

The science of nanotechnology, which has a common application area with biotechnology, which brings together natural sciences and engineering disciplines and deals with the manipulation of matter at the atomic and molecular level, has found a field of study in the field of animal nutrition as well as in many fields with the development of technology.

3. Nano Size Feed Additives

Feed additives used in animal nutrition can be classified in two ways as feed additives that are nutrients such as amino acids, minerals and vitamins, and non-nutrients such as antibiotics, hormones, prebiotics, enzymes, pellet binder, yeast culture, antioxidants, etc. (Tasho & Cho, 2016). The main reasons for the use of feed additives are protecting animal health, increasing productivity, increasing the nutritional content of feeds, preserving the quality of nutrients and feed, as well as giving a certain form to feeds. Integrated into feed formulation and production processes, these additives are strategically used in the animal feed industry to contribute to the healthy growth and development processes of animals and to optimize production efficiency (Mantovani et al., 2022).

The difference between nanomaterials and larger materials is that due to the surface effects of nanomaterials, the energy required for atoms to combine is low, which causes the atoms to be less stable. For example, the melting point of gold NPs of a given size is lower than the melting point of the same element of a larger structure. Quantum effects, on the other hand, involve nanostructures at the nanometer scale behaving like a single atom (Singh, 2016). There are three approaches to the synthesis of NPs: physical methods (such as high-energy ball milling and vapor deposition), chemical methods (including colloid formation), and biological techniques. Two primary strategies are used for nanoparticle synthesis: the top-down approach, which includes methods such as thermal decomposition, ball milling, lithography, laser ablation, and sputtering, and the bottom-up approach, which includes techniques such as chemical vapor deposition (CVD), sol-gel processes, spinning, pyrolysis, and biological synthesis. In the top-down approach, starting materials are transformed into larger structures and then further reduced to nanoparticles. On the other hand, in bottom-up synthesis, nanoparticles are built at the molecular or atomic level and then assembled to achieve the desired properties. These methods require different synthesis conditions and control mechanisms, and they find applications in various fields such as nanotechnology and materials science (Ijaz et al., 2020). Apart from these, methods such as reactive precipitation, microemulsion, sono-chemical, and supercritical chemical processes are also included (Prakash et al., 2022). NPs can be prepared by a variety of methods. Among these methods, precipitation is made using nanotools used in nanotechnology such as emulsion crosslinking, spray drying, and emulsion-drop combination. NPs can be broadly classified into several types, including inorganic (such as nanominerals), organic (including nanomolecules of proteins, fats, and sugars), emulsions, dispersions, and nanopolymers such as nanoclay (Al-Beitawi et al., 2017). Inorganic NPs are preferred in packaging, treatment, storage, and antimicrobial applications for use in feeding and irrigation (Bunglavan et al., 2014). Inorganic NPs include substances such as titanium dioxide, which is used in the feed industry as a UV protection barrier. Organic NPs, on the other hand, have various applications, such as nanocapsules, which can be used to increase nutritional value. These NPs can transport vitamins or other nutrients without affecting the taste or appearance of the feed. Nanotechnology is used in feed processing to deliver nutrients directly to specific organs without altering taste or color, using various techniques such as encapsulation, chelation, packaging and the use of nanotubes (Gelaye, 2024). Overall, the use of nanomaterials in the feed industry includes a variety of applications, such as biosensors, diagnostic markers, shelf-life extenders, and antimicrobials (Ahmadi & Rahimi, 2011). NPs increase nutrient absorption by reducing the antagonistic effect of divalent cations, especially in small minerals. These properties make them useful in the nutrition of livestock and poultry and improve the use of feed and supplements (Marappan et al., 2017).

Nanometer-sized NPs have a larger specific surface area, which differs from conventional-sized particles, higher surface efficiency, high catalytic efficiency, and greater absorbing ability. In addition, nanoparticles have unique physical, chemical, and biological properties, such as enhanced material strength, improved solubility, increased conductivity, optical properties, thermal dynamics, and catalytic efficiency (Alhashmi Alamer & Beyari, 2022; Khan & Hossain, 2022). The increased surface area of nano-sized particles can have a positive effect on many factors such as digestibility, nutrient absorption, immune system, growth and development (Hatab et al., 2022; Hussain et al., 2023). These advantages are used to increase the bioavailability of feeds through the size effect and high surface reactivity. Minerals in NP form used as feed additives can increase absorption rates by penetrating the gastrointestinal barrier and entering the body's cells faster than larger particle sizes (Bunglavan et al., 2014; Jafari & McClements, 2017).

Feed additives are very important to improve the growth performance of the animals. However, it also presents challenges such as endotoxin production and the potential for reduced nutrient absorption due to interaction with natural nutrients. With this reduction in nutrient absorption, many macronutrients can be metabolized or converted by microbes in the rumen (Patra et al., 2019). However, the preservation of nutrients for absorption in the small intestine is very important in this regard. When feed additives are combined with encapsulation at the nano scale, they are better recognized and utilized by the animals' bodies (Albuquerque et al., 2020). Encapsulation involves coating or trapping one material (in this case, feed additives or nutrients) inside another material to protect it from the surrounding environment. This technology is already widely used in various scientific fields, especially in animal nutrition researches. These additives work to increase the feed's resistance to spoilage, heat and light, and protect it from degradation by proteases and various digestive enzymes (Wang et al., 2022a) by using encapsulation, nutrients can be protected against premature spoilage in the rumen and made available for absorption in the small intestine. This can increase the efficacy of feed additives and improve overall animal health and growth.

Nano-encapsulation technique ensures that feed additives are transported to specific target sites and released under control. This is aimed at ensuring that animals get the most out of the feed by preventing substances from entering into undesirable chemical reactions (Shah et al., 2016; Pateiro et al., 2021; Tolve et al., 2021). In short, encapsulation and nanotechnology can be used together to improve the efficacy and safety of feed additives used in animal nutrition. The integration of these approaches can improve the growth and health performance of animals and bring significant innovation to animal feeding strategies. As a result, it increases the bioavailability of feed additives.

4. Increasing Feed Efficiency with Nano-Sized Additives

The challenges facing modern agriculture are multifaceted and complex. One of the main problems is the low efficiency of converting plant products, which are primarily used as animal feed, into animal products. This inefficiency intensifies the pressure on agricultural systems, leading to an increase in demand for crop production. As the world grapples with this problem, there is a growing consensus that agriculture needs to develop in a more productive and diversified way. This evolution is forced not only by the growing global population but also by the changing climate and the declining availability of natural resources. The call for sustainable food production resonates around the world (Jararweh et al., 2023; Azlan et al., 2024). It's not just about increasing the amount of food produced; the point is to do this in a way that uses fewer resources, reduces environmental impact, and at the same time improves farmers' livelihoods. This balancing act presents a significant global challenge that requires a thoughtful and strategic approach to how we produce and consume food. Technology, on the other hand, is at the forefront of this agricultural revolution. Feed additives produced by preventing pollution and utilizing waste products are included in feed technology (Dumlu & Bölükbaşı, 2023). From precision farming techniques that optimize resource use and reduce waste, to biotechnology that can increase crop yields and resilience, technology offers a range of solutions for modernizing farming practices. The application of these technologies can lead to significant improvements in the efficiency of food production systems, enabling more food to be produced with a lower environmental footprint (Evcim et al., 2012; Qayyum et al., 2023).

There is evidence that nano-sized feed additives significantly improve feed efficiency in animals. These additives, which optimize nutrient absorption and utilization, help animals convert feed into energy more effectively, leading to improved growth rates and feed conversion rates (Kah et al., 2019). Incorporating nano-sized additives into animal feeds is a key approach to improving feed efficiency and in turn, the profitability of animal agriculture. Thanks to the use of nano feed additives to improve feed efficiency, it can both reduce feed costs and increase the quality and quantity of animal products (Poddar & Kishore, 2022).

Increasing feed efficiency means indirectly increasing the quantity and quality of the product. As a matter of fact, studies have shown that meat and egg quality in chickens and meat, milk and other yield parameters in cattle and sheep were mostly positively affected by the inclusion of nano feed additives in the ration through water and feed (Dong et al., 2022; Gelaye, 2024).

4.1. Advantages of Nano-Sized Feed Additives

Nanostructures developed with rational approaches are among the most impressive artificial materials and have unique chemical, physical, and/or biological properties (Khan et al., 2022). Nanominerals and nanoemulsion technologies offer low-cost, reduced additives, growth-promoting and immunomodulating properties in the production of cattle and poultry feed (El-Sayed & Kamel, 2020). Nanominerals can suppress harmful pathogens in feed, control the rumen fermentation process, and provide solutions to reproductive problems in cattle and sheep populations. At the same time, nano-zinc oxide used in treating animal diseases can improve the growth rate, immune response and reproductive efficiency of livestock and poultry; it can also reduce the incidence of diarrhea in piglets (Mishra et al., 2014; Hassan et al., 2021). Thanks to liquid vitamins prepared with nanotechnology, nutrients are mixed directly into the blood through the gastrointestinal tract, and their bioavailability increases (Shabani et al., 2019).

NPs also help to reduce the need for preservatives in feed and eliminate odours in feed that are unpleasant to animals (Reddy et al., 2020). Nanotechnology plays an important role in the treatment of animal diseases, including therapy, diagnostics, tissue engineering, vaccine development, and many other areas. The application areas include a wide range of fields such as animal health and production, animal breeding, reproduction, animal nutrition (Wang et al., 2022a).

Nano-sized feed additives offer several advantages in animals. Thanks to their very small size, these additives increase the nutritional value of the feeds and enable the animals to digest these nutrients more effectively. In addition, nanoadditives have positive effects on animal health and productivity by delivering targeted nutrients directly to specific organs or tissues. Nano-sized additives can increase nutrient bioavailability, thereby leading to improved growth, feed conversion, and overall animal health (Sharif et al., 2021). In addition, these additives can be more stable than traditional feed supplements and have a longer shelf life without deteriorating their quality due to their concentrated form (Mortensen et al., 2022). Firstly, their small size and large surface area in the intestinal lumen allow for easier absorption and can be utilized by animals, maximizing their nutritional benefits (Kumari & Chauhan, 2022). By leveraging advanced nanotechnology, farmers will now have a powerful impact on optimizing nutrient absorption and promoting sustainable livestock production. NPs have long been used in human and veterinary medicine for diagnostic and therapeutic purposes, although they are a new application in animal production. These applications encompass disease diagnosis, targeted drug delivery systems, vaccine administration, and nutrition management (Sertova, 2020).

While high doses of mineral feed additives as inorganic salts can improve animal growth performance, their low bioavailability may also pose environmental risks. This necessitates the addition of nano-sized additives to animals as a more effective alternative to mineral applications. Metal NPs such as zinc, silver, copper, gold, selenium and calcium are suggested to be effective alternatives due to their higher bioavailability and absorption (Michalak et al., 2022). Studies have shown that calcium NPs form denser bone in mice and turkeys compared to microcalcium (Jia et al., 2018; Wang et al., 2022b).

Nano enzymes have several advantages such as stability, biocompatibility, conductivity, and susceptibility, improving surface area to improve enzyme-based sensor performance (Song et al., 2019). In order to prevent denaturation and loss of function due to protein/enzyme binding of NPs, enzyme molecules are combined and cross-linked together to form NPs in a prearranged manner. This method has succeeded in unlocking the manufacturing potential of biosensors with increased analytical performance (Riley & Narayan, 2021; Liu et al., 2022; Thapa et al., 2022). In addition, recent research indicates that enzyme NPs exhibit high catalytic efficiency and thermal stability, which enhances both the temperature resistance and cellulose degradation capacity of these NPs (Khizar et al., 2021; Gu et al., 2022; Liu et al., 2022).

Another purpose of using NPs is to reduce the number of harmful bacteria and to be able to improve the growth performance of animals by promoting the growth of beneficial bacteria. These effects include a reduction in the production of bacterial toxins, an increase in the production of vitamins and growth factors, improved nutrient absorption through thinning of the intestinal epithelium, and a reduction in the turnover and motility of intestinal mucosal epithelial cells (Hill et al., 2017).

Giving a definitive answer about the impact of nano-sized feed additives on shelf life is challenging, as the May vary depending on the specific type of nanomaterial used, the composition of the feed, and the storage conditions. Research on the effects of nano-sized feed additives on safety, efficacy, and shelf life has reported that engineered and deliberately added NPs to feeds provide the food industry with innovative methods to improve the quality, shelf life, safety, and health of foods (McClements et al., 2017; Siemer et al., 2018).

Nano additives increase feed efficiency by enabling animals to use the nutrients they consume more effectively (Gopi et al., 2017). This means higher growth and more production with less feed. Another advantage is that nano additives can strengthen the immune system of animals and make them more resistant to diseases (Gelaye, 2024). Furthermore, improved feed efficiency helps reduce environmental impact because less feed production means less farmland and lower carbon emissions (Kah et al., 2019; Wang & White, 2022).

4.2. Potential Risks in Animal Nutrition

Although nano-sized feed additives have shown promising benefits in improving animal nutrition, it is crucial to consider the potential risks and safety implications. Because NPs are often used in end products, they typically do not interact with humans, animals or the environment (Hemathilake & Gunathilake, 2022). However, some concerns include the potential for undesirable accumulation in animal tissues, environmental impact, and the need for rigorous testing and regulation (Dupuis et al., 2022).

When incorporating these additives into animal diets, it is essential to prioritize the safety and welfare of animals and consumers.

In vitro and in vivo studies have been conducted on certain organisms on the limitations and risks of the use of nano-sized feed additives. NPs that have a particularly toxic effect can be listed as silver, gold, zinc oxide, titanium dioxide, silicon dioxide, copper oxide (Jamuna & Ravishankar, 2014). Studies on silver nanoparticle (AgNP), one of the NPs with common toxicity, have shown cytotoxicity and increased membrane permeability at the in vitro level, while at the in vivo level it has not shown any effect other than at high doses. In addition, effects such as DNA damage, functional degradation, cell death, apoptosis and cytotoxicity, cytotoxic effect due to ROS, and cell cycle diversity independent of ROS, mitochondrial damage have been reported as a result of some studies (Chairuangkitti et al., 2013; Ferdous & Nemmar, 2020; Li & Wang, 2021; Wang et al., 2021). Studies on gold NPs, on the other hand, have indicated that symptoms such as deterioration of the actin cytoskeleton, intracellular GSH depletion as a result of interaction with glutathione (GSH), mitochondrial membrane depolarization and apoptosis can be seen (Lopez-Chaves et al., 2018; Talarska et al., 2024). It has been reported that inflammation and bleeding in the lung, ROS accumulation and increased lipid peroxidation, decreased antioxidant capacity (Manzoor et al., 2024) can be seen in titanium dioxide NPs, and oxidative stress can be seen in silicon dioxide and copper oxide NPs (Alruwaili et al., 2022; Zhang et al., 2022). Dose and material size are among the main causes of all these adverse effects. The size of NPs can alter toxicity, and smaller-diameter NPs are more dangerous to cells than larger-diameter NPs, as smaller particles are more likely to reach intracellular sites such as mitochondria and nucleus (Kumar et al., 2017; Li & Lee, 2020). In addition, the cytotoxicity of NPs may be increased when they are mixed with different types of compounds. Therefore, attention should be paid to interactions in the selection of nanomaterials (Jurj et al., 2017).

The long-term health effects of nano-sized additives are not fully known. There are concerns that these substances may accumulate in the body or have side effects (Miller & Senjen, 2008). The effects of nano additives on the environment are not fully understood. Their introduction into soil and water resources can have negative effects on ecosystems (Boxall et al., 2007). Recent in vitro and animal studies have shown that excessive accumulation of NPs can damage cellular organs such as the liver, spleen, kidneys, and respiratory tract and cause toxic effects (Surendhiran et al., 2020). Further research is needed to assess the potential risks to human and animal health and the environment from exposure to NPs (Liu et al., 2022).

The dominant processes of nanoparticle-mediated toxicity include oxidative stress, inflammation, DNA damage and inhibition of cell division and death (Gatoo et al., 2014). While the toxicity of bulk copper is largely dependent on its salt composition, for Cu-NP, other physicochemical characteristics such as size, surface area, surface chemistry, surface texture, dispersion medium, and agglomeration capacity significantly influence the level of toxicity.

Although nano-sized feed additives have many benefits, there are challenges in their application to animal nutrition. In analyses on different species, Cu-NP used as a feed additive caused Cu accumulation in liver and lung tissues, decreased body weight, and dose-dependent lesions in the lung and liver. In addition, smaller Cu-NPs have been reported to be more toxic to zebrafish embryos than larger Cu-NPs. Cu-NP caused membrane damage, ROS generation and DNA strand breaks in lung cells and mammalian cell lines (Zhang et al., 2012; Hua et al., 2014; Song et al., 2014; Sadiq et al., 2015; Hedberg et al., 2016).

Nanotechnology faces many obstacles, including environmental hazards from the release of NPs into the environment, worker and consumer health and safety concerns, questions about the self-replication of nanomachines and their impact on human development, business challenges in commercializing nanotech-enabled products, and concerns about protecting intellectual property rights.

As with nano minerals, there are limitations in the use of nano enzymes in terms of animal nutrition. Enzymes used in animal feed can break down random components in feed products. Thus, it can potentially lead to a decrease in meat or egg production, low feed efficiency and digestive problems. In this respect, the use of nano is not preferred (Islam et al., 2023). It is important to ensure proper dosage and avoid over-supplementation to prevent any adverse effects. The cost of nano-sized additives can also be a limiting factor for some farmers. Furthermore, comprehensive research is required to fully grasp the long-term implications and possible hazards of incorporating these additives in animal feed (Seaton et al., 2010).

4.3. Nanotechnology in Animal Nutrition

As a novel approach to animal nutrition, nanotechnology offers the opportunity to improve nutrient bioavailability, production efficiency and the health and immune status of animals. Nanometer-sized feed additives have a stronger absorption capability thanks to their expanded specific surface area. In this way, it reveals its ability to increase the bioavailability of feeds (Budak, 2018).

By incorporating nano-sized additives into animal diets, positive effects on overall animal health and productivity can be achieved by optimizing nutrient intake. Various nano-sized feed additives can be used in animal nutrition. These include nanominerals, nano-vitamins, nano-enzymes, nano-antioxidants, nano-probiotics, and nano-herbs (Fesseha et al., 2020; Zaheer, 2021). Studies on nanotechnology applications in the field of animal nutrition have mainly focused on evaluating the effects of effects of mineral supplementation with NPs.

4.4. Activities Conducted

The addition of nano-sized feed additives has emerged as a game-changing development in the effort to increase the efficiency of animal nutrition. With the ability to deliver essential nutrients in precise doses, these additives play a critical role in improving animal health and performance.

Each nanoparticle has a different mechanism of action. Zinc oxide and selenium NPs increase the overall nutritional value and digestibility, while also increasing the production of volatile fatty acids. Zinc oxide, in particular, helps minimize environmental impact by reducing fecal mineral loss. It is also beneficial to the gut and has the potential to improve villus height, crypt depth and villus surface area. Magnesium oxide, along with silver and copper NPs, demonstrate strong antimicrobial effects against both gram-positive and gram-negative bacteria, offering effective microbial management. In addition, these NPs aid in microbial control by weakening the biofilm formation of microbial communities. Iron oxide and copper NPs support intestinal health by improving microbial growth in the digestive tract. The nanoform of calcium can be used effectively against periodontal disease and gastrointestinal parasites. (Singh, 2016; Adegbeye et al., 2019).

Zinc oxide nanoparticles have gained popularity as an alternative feed additive in poultry due to their impact on the metabolic activity and health status of birds, attributed to their antibacterial and immunostimulatory effects (Sagar et al. 2018; Akhavan-Salamat & Ghasemi, 2019).

Abedini et al. (2018) investigated the effects of zinc oxide NPs on egg quality, immune response, zinc retention, and blood metrics in laying hens during the later stages of production. As a result of this study with a total of 288 laying hens aged 64 weeks, it was reported that dietary supplementation with ZnONPs increased zinc absorption in laying hen intestines and improved performance. It was reported that NPs to be used in laying diets may be a more suitable zinc source than normal zinc oxide. According to this study, ZnONPs can be used as a substitute for antibiotic growth promoters in the diet of broilers due to their inclusion in the diet.

Hatab et al. (2022) investigated the effects of zinc oxide NPs (ZnONPs) synthesized by *Alternaria tenuissima* on reproductive performance, carcass characteristics and biochemical parameters of chickens. It was observed that ZnONPs supplementation resulted in higher body weight, improved feed intake and performance index compared to the control group. In addition, serum cholesterol, triglyceride, low-density lipoprotein, and uric acid concentrations were decreased, while high-density lipoprotein and liver enzyme concentrations were increased. Zinc accumulation in serum, liver and muscle showed a linear increase with the increase in zinc supplementation. In conclusion, it has been reported that supplementation with 40 or 60 mg/kg ZnONPs can improve productive performance and that low levels of Zn supplementation give positive results.

In another study conducted by Fawaz et al. (2019) on the yield performance of laying hens with zinc oxide NPs administered to 120 laying hens in four groups containing 0, 20, 40, or 60 mg/kg zinc oxide NPs. It was observed that the feed conversion ratio improved in correlation with the level of supplementation, resulting in a significant increase in both egg production and egg mass ($P<0.05$). The inclusion of ZnO NPs at concentrations of 20, 40 or 60 mg/kg in the diet of laying hens was found to improve several aspects of their productivity and health. These include improvements in overall production performance, egg quality as measured by Haugh units, shell strength, nutrient digestibility and reduction in cholesterol levels. In addition, these NP supplements have been shown to have a positive effect on liver and kidney function. In conclusion, ZnO NPs can be considered as an effective feed additive in diets used to optimize the health and productivity of laying hens.

Chromium picolinate (CrPic) NPs have been reported to reduce heat stress in broiler chickens, improve feed conversion, increase mineral concentrations including Cr, Ca, and P in the livers of the subjects, and also induce increased lymphocyte counts in broiler chickens (Sirirat et al., 2012). In research where chromium nanoparticles were added to pig diets, it was found to improve skeletal muscle mass and meat quality in pigs by reducing fatty acid synthase activity compared to the control group (Poddar & Kishore, 2022; Xiong et al., 2022).

As phosphorus (P) in poultry feces causes environmental problems, a study was conducted by Hassan et al. (2016) for the regulation of poultry diets to reduce phosphorus waste so that it does not negatively affect the performance of birds. The study investigated the effect of replacing conventional dicalcium phosphate (DCP) with nanodicalcium phosphate (NDCP) on the performance of broiler chickens and the reduction of calcium (Ca) and phosphorus excretion in their feces. Birds fed NDCP gained significantly more body weight and utilized feed more efficiently than the control group (1.75% CDCP). Body weight gain and feed consumption increased by approximately 25% and 10%, respectively, while feed conversion ratio improved by approximately 12% compared to chickens fed CDCP. Hassan et al. (2016) determined that the use of 0.44% NDCP in diets reduced excreted Ca and P by 50.74% and 46.24%, respectively, compared to the control group. In conclusion, the use of nanoparticle-sized dicalcium phosphate can mitigate the environmental impact of poultry farming by reducing excreted Ca and P by approximately 50%.

Another study was conducted in broilers to investigate the effect of nanodicalcium phosphate (NDCP) versus conventional dicalcium phosphate (CDCP) on carcass characteristics and bone measurements. The diets contained three different levels of either CDCP or NDCP: %1.75, %1.31, and %0.88, and a lower level of NDCP at %0.44. These levels were equivalent to providing 100%, 75%, 50%, or 25% of the recommended dietary phosphorus requirement. It was concluded that substituting NDCP for CDCP improved all measured bone parameters. Diets containing 25% NDCP can be successfully used instead of 100% CDCP, and when used in nanoparticle size, the level of dicalcium phosphate can be successfully reduced from 1.75% to 0.44%. Nanoparticle-sized dicalcium phosphate can be used approximately 400% more effectively than traditional dicalcium phosphate (Mohamed et al., 2016).

AgNPs, another element added to the diet of monogastric animals, have antibacterial activity (Fondevila et al., 2009; Pineda et al., 2012). Furthermore, AgNPs have been reported to potentially improve muscle morphology without affecting embryo growth in broiler performance (Sawosz et al., 2012). In another study conducted by the same researchers on quails, it was reported that 25 mg/kg Ag-nano supplementation in their water significantly increased the population of lactic acid bacteria (Sawosz et al., 2008). Nevertheless, it has been reported that a concentration of 50 mg/kg of AgNPs chelated with the amino acids threonine and cysteine could serve as potential agents to enhance the immune capacity in embryos and chickens (Bhanja et al., 2015).

A study on turkeys investigated the effects of reducing the amount of manganese (Mn) in their diet on their growth, antioxidant status and immune system. The researchers used two different sources of Mn: manganese oxide (MnO) and manganese NPs (NP-Mn2O3). They also tested three different levels of Mn (100, 50 and 10 mg/kg). According to the results of the study, reducing the amount of Mn did not have a negative impact on the health of the turkeys. Specifically, Mn in the form of NP-Mn2O3 allows young turkeys to reduce the amount of Mn in their diet without compromising their antioxidant defenses. Reducing Mn in the form of MnO can increase lipid oxidation, while replacing it with NP-Mn2O3 can increase apoptosis (cell death). In general, however, reducing the amount of Mn in the diet tends to reduce apoptosis, regardless of the form. This study shows that the form and amount of manganese used in turkey diets can have important effects (Jankowski et al., 2018).

Copper (Cu), zinc (Zn) and manganese (Mn) NPs were used to study their effect on aminopeptidase activity in turkey meat. 144 Hybrid Converter turkeys were fed standard and nanoparticle-supplemented diets containing 100%, 50% and 10% of the physiological requirement of these minerals, respectively. The study was conducted to investigate the effect of mineral supplementation on the activity of aminopeptidases (alanyl: AlaAP, leucyl: LeuAP, and arginyl: ArgAP) in both turkey breast and thigh muscles. The results showed that even at the lowest dose (2 mg/kg), nano-Cu had a significant effect on aminopeptidase activities in bud muscle. Moreover, at doses of 20 mg/kg (100% requirement) and 10 mg/kg (50% requirement) of nano-Cu, the activity of all aminopeptidases in the bud muscle was inhibited. Aminopeptidase activities indicated that the addition of nano-formed Cu and Zn to the diet, especially at doses meeting 10% of the requirement, is important for maintaining homeostasis in turkey muscle (Jozwik et al., 2018).

In a study conducted by Nguyen et al. (2015) Metal NPs added to chicken diets as metal nanoparticle additives in a feed premix consisting of Fe, Cu, ZnO, and Se, which replaced the inorganic mineral component due to the nanocrystalline metal content, reduced the inorganic mineral content by at least four times, reduced environmental pollution, and allowed animals to utilize these minerals more efficiently in the body.

Radi et al. (2021) investigated the comparative effects of zinc oxide ZnO and zinc oxide nanoparticles ZnO NPs as feed additives on growth, feed selection tests, tissue residues and histopathologic changes in broiler chickens. Observations showed that birds receiving a diet containing ZnO NPs at a dietary dose of 90 mg/kg from 2 to 20 days of age showed an increase in body weight compared to the zinc oxide treated group.

Hussan et al. (2022) conducted a study to evaluate the effect of supplementing zinc in the form of nano zinc oxide (nano ZnO) on broiler performance. In the study, nano ZnO was added to the basal diet at the ppm level and fed to a total of 6 groups. Results showed that 2.5 ppm nano ZnO supplementation led to a significantly higher increase in live weight, greater feed intake, and an improved feed conversion ratio (FCR) at 42 days of age compared to the control and other treatment groups.

Zinc NPs have a positive effect on growth, immunity, and reproduction in poultry and monogastric animals such as pigs, while improving feed efficiency (Swain et al., 2016; Milani et al., 2017). In a study involving 200 1-week-old Japanese quails, one group was fed a basal diet while the other groups were fed diets supplemented with nano-zinc (Zn-NPs) at increasing levels (0.1, 0.2, 0.3, and 0.4 g/kg diet). The study reported significant ($p \leq 0.0001$) improvements in body weight, weight gain, feed intake and feed conversion efficiency in quails fed diets supplemented with 0.2 g/kg Zn-NPs. In the groups where 0.1-0.3 g/kg was added to the diet, it was reported that ALT, AST, and LDH activities showed a positive result. Furthermore, within the same range (0.1-0.3 g/kg), Zn-NPs also showed a positive effect on parameters such as superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), immunoglobulin G (IgG), and immunoglobulin M (Reda et al., 2021).

In another study broilers were fed with zinc oxide NPs (ZnO NPs) added to the diets at specific ratios. At the end of the experiment, broilers fed diets containing ZnO NPs exhibited significantly higher zinc retention compared to those in the control group, which was statistically significant ($p < 0.05$). This increased zinc uptake subsequently led to a decrease in zinc excretion in their feces. This resulted in increased Zn absorption and bioavailability, decreased zinc excretion, and demonstrated potential antibacterial activity against the tested pathogens. It was also reported that the addition of different levels of ZnO-NP did not affect broiler growth performance ($p > 0.05$). However, the study observed a significant antibacterial effect when ZnO-NPs were included in the diet at a concentration of 100 mg per kg. This effect was reported by Yusof et al. (2023) which was characterized by a significant reduction in the number of *Enterococcus* species, while the population of beneficial partner bacteria such as *E. coli* lactic acid bacteria remained unaffected. In a similar study, Cu silicate NPs altered the intestinal microbiota in chickens, increasing the number of Lactobacillus species and decreasing *E. coli*. It has been reported that the inclusion of Cu silicate Nps can modulate the intestinal microflora, stimulate the growth of beneficial bacteria, suppress harmful bacteria, improve nitrogen metabolism, and reduce fecal ammonia emissions (Minglei et al., 2013).

Studies on the addition of Cu-NPs to the diets of marine organisms have yielded very successful results. It has been reported that administration of Cu-NPs to fish resulted in increased body weight, improved feed efficiency, increased protein retention, enhanced immune response and antioxidant defence system compared to the control group. In another study on shrimp, Cu-NPs were reported to improve growth, digestive enzyme activity, biochemical component concentrations and hemocyte counts (El Basuini et al., 2016; Muralisankar et al., 2016). In a separate study, El Basuini et al. (2017) reported that a mixture of Cu-NPs and vitamin C added to the red sea bass diet increased feed intake and consequently increased body weight. It was also reported to have a significant effect on body protein and lipid content, protease and bactericidal activity, and tolerance to stress compared to the control group.

Tomaszewska et al. (2017) compared the effects of administering two different chemical forms of copper, CuCO3 and Cu-NP (carbonate and NPs), through dietary mixtures on geometric and structural parameters of bones in young rats. Although there were no changes in body weight and bone morphology depending on Cu-NP dose, significant changes in geometric and mechanical parameters were found. It was reported that Cu-NP administered at low dose increased mechanical strength by increasing bone tissue density and ash content in bones, without changes in strain and stress compared to a low dose of Cu administered in conventional form.

Gao et al. (2014) supplemented culture media containing Caco-2 cells with different concentrations of copper sulphate (CuSO4), micron-sized copper oxide (micron-CuO), and nano-sized copper oxide (nano-CuO) and then examined their effects on the expression of Ctr1, ATP7A/7B, MT, and DMT1 genes and proteins. The results showed that nano-CuO significantly increased Ctr1 mRNA expression in Caco-2 cells compared to micron-CuO and CuSO4. In addition, at the same concentration, nano-CuO was found to be more effective than CuSO4 and micron-CuO in enhancing Ctr1 protein expression.

Studies have shown that in ovo administration of nano copper (Cu-NP) in broiler chicken eggs increases body weight, improves feed conversion ratio, and improves breast and leg muscles by promoting blood vessel development during embryogenesis (Mroczek-Sosnowska et al., 2015a, 2015b). Furthermore, it has been reported that in ovo injection of Cu-NPs stimulated proliferating cell nuclear antigen (PCNA) positive cells in the long bones of broiler chickens, demonstrating a stimulating effect during embryogenesis. (Mroczek-Sosnowska et al., 2017). Miroshnikov et al. (2015) reported that intramuscular CuNp administration in chickens supported development and increased hemoglobin levels, especially in red blood cells. In addition, serum Cu and protein levels increased, and the arginine content of the liver also increased.

The effect of highly dispersed copper particles on the metabolism of broiler chicks was studied after a single intramuscular injection. Preparations containing copper NPs, agglomerates, and microparticles were used in the study. It was observed that copper NPs triggered rapid growth and metabolic changes, while agglomerates and microparticles provided a similar effect but over a longer period. These results provide important findings that can be used to improve microelement preparations (Miroshnikov et al., 2015).

El-Nile et al. (2023) used nanosized and natural zeolite as a feed additive in a study on rumen fermentation, blood metabolites, milk yield, feed intake and fatty acid profile in dairy goats. Damascus goats in late pregnancy were used in this study and both forms of zeolite increased blood serum albumin and calcium levels. In contrast to natural zeolite, the nano zeolite-added group increased milk production and short-chain fatty acids, decreased ruminal ammonia-N concentration, and somatic cell count. It was also reported to have a positive effect on kidney function.

Elemental nano-selenium (NSe) was added to the diet as a feed additive in a study to investigate its effect on feed digestion, rumen fermentation and urinary purine speciation in sheep. Sheep were fed a basic diet and supplemented with 0 (control), 0.3, 3, and 6 g nano-Se/kg per dry matter (DM). It has been reported that supplementation of nanoselenium in the basal diet improves rumen fermentation and feed utilization, possibly by stimulating rumen microbial activity, digestive microorganisms, or enzyme activity. This supplementation resulted in a significant decrease in rumen pH (ranging from 6.68 to 6.80) and ammonia concentration (ranging from 9.95 to 12.49 mg/100 ml), along with an increase in total volatile fatty acid (VFA) concentration. The optimal dose of nanoselenium was determined to be approximately 3.0 g per dry matter of the diet in sheep (Shi et al., 2011).

In a study on broiler chickens, the selenium uptake of nano-elemental selenium (Nano-Se) and sodium selenite was compared. Nano-Se was retained more efficiently in the body, providing a wider optimal-toxic dietary range than sodium selenite. Nano-Se was able to increase growth performance and selenium concentration better than sodium selenite (Hu et al., 2012).

Lee et al. (2020) conducted a study to evaluate the effects of dietary selenium (Se) concentration and source on performance, nutrient digestibility, plasma Se levels, glutathione peroxidase (GPx) activity, and thiobarbituric acid reactive substances (TBARS) in broiler chickens. The experimental diets were administered in two phases (phase 1, days 0 to 14, and phase 2, days 15 to 32) for a total of 32 days. Treatments included a control group (no Se supplementation), sodium selenite (SeS; at 0.15, 0.30, or 0.45 ppm), and hot-melt-extruded sodium selenite (SeHME; at 0.15, 0.30, or 0.45 ppm). Significant linear responses ($P < 0.01$) were observed for the expression of SelW, GPx1, GPx3, and GPx4 genes in the liver and spleen in response to SeS and SeHME treatments. In conclusion, it has been reported that SeHME may enhance antioxidant activity and Se absorption, thus potentially serving as a more suitable source of Se compared to standard sodium selenite.

In a study on the effects of dietary nano-selenium on tissue selenium accumulation, antioxidant status, and immune functions in laying hens, inorganic sodium selenite and nano-Se were administered to diets for comparison. The study reported that Nano-Se was nutritionally superior. In another study in broiler chickens, Nano-Se supplementation was found to have a positive effect on body weight gain (BWG) and feed conversion ratio (FCR). A supplementation of 0.3-0.4 mg nano-Se kg-1 was reported to be the optimal level for inclusion in broiler diets (Ahmadi et al., 2020).

Overall, the usage of NPs as feed supplements has the potential to improve growth performance and feed efficiency, increase total protein and albumin levels in serum, and significantly enhance total antioxidant capacity. Furthermore, this approach can contribute to optimizing many biological processes such as digestion, metabolism, and nutrient uptake, increasing the efficiency of production processes and reducing environmental contamination.

5. Conclusion and Recommendations

Research on nano-sized feed additives in animal nutrition offers a transformative approach toward enhancing agricultural productivity and sustainability. In this research, the unique properties of nanoparticles are exploited to highlight their potential to significantly improve nutrient delivery and absorption, facilitate feed efficiency, and reduce the environmental footprint of livestock production with lower feed inputs. These small but powerful substances are critical for improving animal health and producing high-quality animal products. By striking the critical balance between technological innovation and sustainable, environmentally friendly food production systems, it is crucial to meet the growing global demand for food and contribute to future food security.

However, these advantages, as well as the potential risks and environmental concerns associated with nanomaterials, require the need for careful application. Rigorous scientific research, strict regulations and ethical considerations are vital to ensure the safety and effectiveness of nano-sized feed additives for both animals and the wider ecosystem.

As we move forward at the dawn of a nanotechnological revolution in animal nutrition, it is imperative to embrace innovation with a balanced perspective, ensuring that technological advances are compatible with environmental and ethical standards. This effort aims not only to harness the vast potential of nano-sized feed additives to transform livestock management, but also to combine these innovations with an unwavering commitment to ecosystem and public health. The promise of transforming livestock management and food production systems with nano-additives is significant. But they require scientific rigor, sustainability, efficiency and a commitment to health. Future research should look more closely at the effects nano-sized feed additives on different animal species, their specific applications, economic aspects of their use, and sustainability implications. Such a review will open new avenues of research in the field of animal nutrition and nanotechnology and enrich the achieved results. As research progresses, nano-sized feed additives will continue to revolutionize animal nutrition and herald a new era by enabling stakeholders to adopt these innovations with confidence.

Conflict of Interest

The author has no conflict of interest to declare.

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Kasumyan, A. O., & Døving, K. B. (2003). Taste preferences in fishes. *Fish and Fisheries*, *4*(4), 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x

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Taştan, Y. (2018). *Tatlısu kerevitindeki (Astacus leptodactylus) siyah solungaç hastalığı etkeni mantar Fusarium oxysporum'un PCR yöntemi ile teşhisi* (Master's thesis, Akdeniz University).

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Institution Publication:

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