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DOI: 10.17097/agricultureatauni.1445748

Comparison of Essential Oil Components and Yield Parameters of *Artemisia dracunculus* and *Artemisia dracunculoides*

Artemisia dracunculus ve *Artemisia dracunculoides*'in Uçucu Yağ Bileşenleri ve Verim Parametrelerinin Karşılaştırılması

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ABSTRACT

Medicinal and aromatic plants have attracted attention recently for their essential oil content. This study aims to compare, for the first time, essential oil of *Artemisia dracunculus* L. and *Artemisia dracunculoides* L., which are closely related species. Yield values of this *Artemisia* spp. were determined, and the highest drug-herb yield (119.01 kg/ha) was found for *Artemisia dracunculus* L. The essential oil components of these species grown under organic production conditions in Bayburt, Türkiye, were determined by the microwave hydrodistillation method for the first time and gas chromatography-mass spectrometry (GC-MS). The essential oil ratio for *A. dracunculus* was 1.40 %, whereas it was 1.21 % for *A. dracunculoides*; 42 components were found in *A. dracunculus* essential oil, while 38 were found in *A. dracunculoides* essential oil. Estragole was the most abundant essential oil component in *A. dracunculus* L. (69.34%) and *A. dracunculoides* L. (67.51%). The GC-MS results, showed that *A. dracunculus* L. is more suitable for use in perfumery and food industries than *A. dracunculoides*.

Keywords: Medicinal plants, estragole, linalool, tarragon

ÖZ

Tıbbi ve aromatik bitkiler son zamanlarda içerdikleri uçucu yağ nedeniyle ilgi çekmektedir. Bu çalışma, yakın akraba türler olan *Artemisia dracunculus* L. ve *Artemisia dracunculoides* L.'nin uçucu yağlarını ilk kez karşılaştırmayı amaçlamaktadır. *Artemisia* spp.'nin verim değerleri belirlenmiş ve en yüksek drog herba verimi (119,01 kg/ha) A. *dracunculus* L.'da bulunmuştur. Bayburt (Türkiye) ilinde organik üretim koşullarında yetiştirilen bu türlerin uçucu yağ bileşenleri mikrodalga hidrodistilasyon yöntemi ile ve gaz kromatografisi-kütle spektrometrisi (GC-MS) ilk kez belirlenmiştir. *A. dracunculus*'un uçucu yağ oranı %1,40, *A. dracunculoides*'in ise %1,21; *A. dracunculus* esansiyel yağında 42 bileşen bulunurken, *A. dracunculoides* esansiyel yağında 38 bileşen belirlenmiştir. Estragole bileşeninin *A. dracunculus* L.'de (%69,34) ve *A. dracunculoides* L.'de (%67,51) en fazla bulunan uçucu yağ bileşeni olduğu görülmüştür. Elde edilen sonuçlar, parfümeri ve gıda endüstrisinde kullanım için *A. dracunculus*'un *A. dracunculoides*'ten daha uygun olduğunu göstermektedir.

Anahtar Kelimeler: Tıbbi bitkiler, estragol, linalool, tarhun

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Introduction

It is known that medicinal aromatic plants have been involved in people's daily lives from the past to the present. In addition to traditional treatment methods, many medicinal aromatic plants are consumed as food. Generally, their delicious taste and pleasant smell increase the use of these plants. Many herbs that emit pleasant odors with the essential oils secreted from their leaves are used in different ways, such as tea and spices.

Asteraceae is a family with 1,535 genera and between 23,000 to 32,000 species, with a wide distribution worldwide (Zappi et al., 2015). Tarragon is a fragrant, medicinal, and aromatic plant. The two most commonly cultivated tarragon species are French (*Artemisia dracunculus* L.) and Russian tarragon (*Artemisia dracunculoides* L.) in Türkiye. When comparing *A. dracunculus* L. with *A. dracunculoides* L., it has been discovered that *A. dracunculus* has a thinner stem structure, while *A. dracunculoides* has a thicker stem structure with flower and seed production (Trendafilova et al., 2021). Especially, its anti-bacterial and antifungal properties support the industrial usage of tarragon (Azizkhani et al., 2021; Davis, 1982; Karimi et al., 2015). It is also known that, tarragon has muscle relaxant, anesthetic, and sedative properties (Sayyah et al., 2004). In addition, according to recent studies, some tarragon products are used to extend the shelf life and prevent mold formation by increasing the consistency of dairy products such as yogurt (Zedan et al., 2021). Some studies in the literature about this plant have various digestive, antioxidant, and pharmacological activities, including carminative, antiinflammatory, antipyretic, antiseptic, antispasmodic, antiparasitic, and anthelmintic antimicrobial, and fungicidal effects (Can et al., 2023; Mumivand et al., 2017; Obolskiy et al., 2011; Pelarti et al., 2021). The essential oil of tarragon, whose green leaves are used in salads and dried leaves in soups and pastries, gives flavor and aroma to the food industry (Ayoughi et al., 2011; Jazani et al., 2011). Also, this essential oil is used in perfumery with its pleasant smell. Golubkina et al., (2020), reported that tarragon plants contain 0.68% to 0.70% essential oil. On the other hand, Çil and Kara (2015) reported that tarragon essential oil ratio varied between 0.146% and 1.346%. In some studies, where the essential oil components of tarragon were determined, α-pinene 1.3%-5.1%, limonene 3.32%-12.40%, methyl eugenol 2.20-2.59% (Mumivand et al., 2021; Sayyah et al., 2004).

There is some research about essential oil components of *Artemisia* spp. but there isn't any study essential oil of *Artemisia dracunculoides* L. This study aims to determine the essential oil ratios and essential oil components of *A. dracunculus* L. and *A. dracunculoides* L. Especially in this study, the essential oil ratio of *A. dracunculus* and *A. dracunculoides* were determined for the first time by using the microwave hydrodistillation method. While this study can be a reference to new studies about essential oil of *A. dracunculus* and *A. dracunculoides*, the possible potential of these plants are elucidated in usage in the food and perfume industry.

Methods

Plant Material

This study used seedlings of *A. dracunculus* L. and *A. dracunculoides* L. as plant materials. Plant materials were obtained from local populations in the fields from Bayburt Province of Turkey.

The species identification of the plant specimens was carried out by Assoc. Prof. Dr. Abdurrahman Sefalı of Bayburt University, Department of Basic Education. Flora of Turkey and the East Aegean Islands (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000) were used as the main source for the identification of these samples. Plants were chosen randomly from the farmer fields and, they were grown in experimental plots. The experimantal area is located at the coordinates of 40º 12' North parallel and 40º 15' East meridian. Plants were grown in the research and application area of Bayburt University Faculty of Applied Sciences, in 60x60 cm row spacing and in-row spacing, in three replications according to the Random Blocks Trial Design. The general view of the grown plants is shown in Figure 1.

Figure 1.
Artemisia dracunculus L. and Artemisia dracunculoides L. grown in the experimental area.

Climatic and soil characteristics of the trial field

The plant material was grown in the central district of Bayburt, Turkey. The General Directorate of Meteorology provided climate data for the cultivation area for several years until 2020 (GDM 2021). Climate data is shown in Table 1.

Soil samples from 0-30 cm depth from the experimental field where the plant material was grown were analyzed in the laboratories of the Republic of Türkiye Ministry of Agriculture and Forestry, Black Sea Agricultural Research Institute. The pH value was 7.90, the lime rate was 17.31%, the organic matter rate was 0.99%, the potassium quantity was 43.80 kg/ha, the phosphorus amount was 1.33 kg/ha, and the total salt rate was 0.01% when the soil samples from the tribal territory were studied. Analysis results show that, according to the soil criteria analyzed by Gedikoglu (1990) and Ulgen and Yurtsever (1995), it is slightly alkaline, deficient in organic matter, low in phosphorus, moderately calcareous, and moderate in potassium. The locations where the plants are grown on the map of Turkey is shown in Figure 2. Tarragons that reached sufficient maturity were harvested and dried for 20 days in a dry, calm, and sun-free environment. The dried samples were stored at +4˚C for analysis.

Determination of yield values

It was determined the plant height in cm by measuring five plants randomly selected from each plot among the plants that reached harvest maturity, from just above the soil to the tip of the plant. After the plants were harvested by cutting five cm above the soil surface, they were weighed using a precision scale. After each plot's weighing process, the data obtained were converted to hectares, and the green herb yield in kg/ha was calculated. Tarragon plants were harvested from 50 m^2 and separated their leaves were from their stems. Determined the green leaf yield and green stems yield of the plant in kg/ha was by weighing the leaves and stems on precision scales.

Tarragon plant samples, harvested from 50 m^2, and the leaves were separated from their stems, were dried in a shaded and cool place. The leaf and stem parts of the dried plant were weighed separately on sensitive scales to determine the drug leaf yield and drug stem yield in kg/ha. After the plants were harvested, each plot was dried, they were weighed using the precision scale, the data obtained were converted to hectares, and the drug herb yield in kg/ha was calculated.

Figure 2.
The locations where Artemisia dracunculus L. and Artemisia dracunculoides L. plants are grown on the map of Türkiye.

Table 1.
Climate data, for long years up to 2020 of the land where A. dracunculus L. and A. dracunculoides L. plants were grown (GDM 2021).

Years	Months											
	January	February	March	April	May	June	July	August	September	October	November	December
Monthly Total Precipitation (mm)												
1959-2020	27.0	28.0	40.7	62.5	73.2	51.6	21.3	15.5	21.8	43.1	33.0	29.4
2020	19.4	52.3	65.7	87.0	113.8	51.9	61.0	14.4	8.5	16.4	24.6	22.2
Monthly Average Temperature (˚C)												
1959-2020	-6.3	-5.0	0.4	6.9	11.6	15.2	18.8	18.7	14.8	9.3	2.7	-3.2
2020	-4.5	-3.1	4.9	7.0	12.1	17.2	20.5	18.7	18.5	13.1	3.2	-1.4
Monthly Average Relative Humidity (%)												
1959-2020	71.7	65.5	58.2	42.5	64.8	52.1	49.7	45.4	50.9	54.9	67.1	77.0
2020	68.2	67.1	60.4	57.1	55.0	49.4	48.9	44.5	39.4	27.6	62.4	71.8
Monthly Average Wind Speed (m÷sn)												
1959-2020	1.3	1.2	1.4	1.3	1.3	1.4	1.4	1.4	1.2	0.9	0.8	1.0
2020	1.0	1.3	1.3	1.3	1.6	1.6	1.5	1.5	1.3	1.1	1.1	0.9

Extraction and analysis of essential oil

Microwave hydrodistillation

Microwave hydrodistillation was carried out on a Milestone brand Ethos X model microwave extractor. Wetted with 200 milliliters of water, 150 grams of dried tarragon samples were placed in the extraction tank. The power of the microwave digestion was set to 1000 Watt, and the program was set as follows: The temperature rose to 100˚C in 10 minutes and continued for 30 minutes. Moisture in the sample was removed with anhydrous sodium sulfate, and then the dehydrated sample was placed in a sealed glass vial and stored at +4˚C until analysis. The advantage of the microwave hydrodistillation method is that the entire plant sample is heated at the same time, unlike conventional methods of heat conduction by contact, which breaks down the oxidized weak hydrogen bonds at the poles of the molecules. This method was preferred because more reliable results were obtained.

Methylation of Samples

It puts a weighted 100 mg oil sample in a 20 mL screw capped tube or reaction vial. Oil sample, dissolved in 10 mL of hexane. Added 100 µL of 2N potassium hydroxide in methanol. The tube was closed and vortexed for 30 s. Centrifuged and transferred the clear supernatant into a 2 mL autosampler vial (David et al., 2005; IUPAC, 1992; Regulation, 1991).

Determination of essential oil components by GC-MS

A mass selection detector equipped Agilent series GC (model 7890A) and MS (model 5975C) were used for the GC-MS analysis of the plant sample. An HP-5MS capillary column (30 m x 0.25 mm i.d., 0.25 m) was used for the separation during the analysis. Injector and detector temperatures were 210°C. The column temperature was first set to 40°C for 3 min, then increased to 90°C at 3 min/degrees and held for 4 min, then to 115°C at 3 min/degrees and held for 10 min, then to 140°C at 2 min/degrees and held for 8 min, and lastly to 210°C at 3 min/degrees and held for 5 min. The carrier gas used was helium. The scan period was 0.3 seconds, the ionization energy was 70 eV, and the AMU mass range was 45–500. Using Agilent GC-MS solution software, the GC-MS system was managed, the GC and mass spectrometry parameters were established, and data was received and processed. According to the NIST Chemistry WebBook (https://webbook.nist.gov/chemistry), identification by GC-MS was based on comparing mass spectra with WILEY-NIST data libraries.

Statistical Analysis

Statistical analysis of this study was performed by using the SPSS (IBM SPSS Corp., Armonk, NY, USA) 25.0 software program. In this study, a One-Way ANOVA analysis of variance was made to determine the differences between Tarragon species in yield values.

Results

Plant height, green herb yield, green leaf yield, green stems yield, drug leaf yield, drug stems yield, and drug herb yield measure the yield for *Artemisia* spp. as in other green leafy plants. Tarragon is a plant that uses the leaf part, drug, or green. According to the results of yield measurement, although *A. dracuncculoides* L. has higher values for plant height, green herb yield, green stem yield, *A. dracunculus* L. has higher values for green leaf yield, drug leaf yield, drug stems yield, and drug herb yield (Table 2.).

Table 2.

Average yield values of Artemisia dracunculus L. and Artemisia dracunculoides L.

	Species	
	A. dracunculus L.	*A. dracunculoides* L.
Plant height (cm)	42.10	66.27
Green herb yield (kg/ha)	477.82	531.98
Green leaf yield (kg/ha)	358.11	311.14
Green stem yield (kg/ha)	121.11	238.89
Drug leaf yield (kg/ha)	82.28	64.94
Drug stem yield (kg/ha)	36.72	33.56
Drug herb yield (kg/ha)	119.01	98.50

Tarragon is a medicinal plant cultivated for its leaf, so the values of green herb yield, green leaf yield, and drug leaf yield have importance. When the differences between these values are examined, it is seen that *A. dracunculus* is more advantageous than *A. dracunculoides*. Although *A. dracunculoides* seem to be more efficient than *A. dracunculus* in terms of green herb yield, its superior properties in terms of drug herb, drug leaf, and green leaf yield make *A. dracunculus* more preferred.

It was seen that the yield values of Tarragon species in the study were different from each other by examining the average values. One-Way ANOVA Analysis of Variance was performed to determine whether these differences were significant ($p < .05$) (Table 3.). Interspecies yield values were at a significant ($p < .05$) difference. There wasn't a significant ($p < .05$) difference for only drug stem yield. Especially the most significant difference ($p < 0.001$) was mainly seen in the drug leaves yield value of *Artemisia* spp.

Table 3.
One-Way ANOVA Analysis of Variance of A. dracunculus L. and A. dracunculoides L.

		Sum of Squares	df	Mean Square	F	Sig.
Plant height (cm)	Between Groups	876.042	1	876.042	26.239	**0.007***
	Within Groups	133.547	4	33.387		
	Total	1009.588	5			
Green Herb Yield (kg/da)	Between Groups	7822.870	1	7822.870	8.905	**0.041***
	Within Groups	3514.052	4	878.513		
	Total	11336.922	5			
Green Leaf Yield (kg/da)	Between Groups	3309.741	1	3309.741	19.007	**0.012***
	Within Groups	696.518	4	174.129		
	Total	4006.259	5			
Green Stems Yield (kg/da)	Between Groups	20808.193	1	20808.193	25.747	**0.007***
	Within Groups	3232.728	4	808.182		
	Total	24040.920	5			
Drug Leaves Yield (kg/da)	Between Groups	451.360	1	451.360	71.026	**0.001***
	Within Groups	25.419	4	6.355		
	Total	476.780	5			
Drug Stems Yield (kg/da)	Between Groups	15.010	1	15.010	3.597	0.131
	Within Groups	16.691	4	4.173		
	Total	31.701	5			
Drug Herb Yield (kg/da)	Between Groups	630.990	1	630.990	30.974	**0.005***
	Within Groups	81.486	4	20.372		
	Total	712.476	5			

*Significance level $p < .05$ n.d.: not detected, N: Number

Artemisia spp. is known as a fragrant perennial medicinal and aromatic plant. *A. dracunculus* and *A. dracunculoides*, which are among the essential oil plants, are grown in many different regions and are also consumed as food. It is known that essential oils have been used to treat diseases from ancient times to the present day because of having important components (Azizkhani et al*.,* 2021; Galoviˇcová et al*.,* 2021; Wi´nska et al*.,* 2019). It is important to determine the essential oil ratio and components of plants used in the food industry. In this study, the essential oil ratio and the components of the tarragon species were determined, most commonly used in the food industry, to draw attention to this reason. The essential oil ratio for *A. dracunculus* was 1.40 %, whereas it was 1.21 % for *A. dracunculoides*; 42 components were found in *A. dracunculus* essential oil, while 38 were found in *A. dracunculoides* essential oil. According to the GC analysis, estragole was the most abundant component in the *Artemisia* spp. that was used in this study. Linalool (4.68%) and cis-ocimene (3.92%) for *A. dracunculus*, cis-ocimene (4.52%), and β-ocimene (4.00%) for *A. dracunculoides* are the highest components after estragole. The estragole component was 69.34% in *A. dracunculus* and 67.51% in *A. dracunculoides* (Table 4).

After estragole, the most abundant essential oil components are cis-ocimene, β-cis-ocimene, and β-ocimene, in the group of regular hydrocarbons. In this study, cis-ocimene values were between 3.92%-4.52%, β-cis-ocimene was 3.45%, and β-ocimene was 4.00%. In addition, β-ocimene could not be detected in *A. dracunculus*, and β-cis-ocimene was not found in *A. dracunculoides.*

In addition to being a monoterpene used in the food industry due to its pungent odor and aroma, D-Limonene has a chemo-preventive effect on breast, skin, and liver cancers, according to studies con-ducted with rodents (Kim et al*.,* 2013; Saini et al*.,* 2022). This study determined D-Limonene at 2.76%-2.67% for *A. dracunculus* and *A. dracunculoides*, respectively. It is known that volatile linalool is used in the production of food flavors and in the perfumery and pharmaceutical industries to remove undesirable odors (Amazonas et al*.,* 2022). This study found the linalool component in *A. dracunculus* and *A. dracunculoides* at 4.68%-1.82%, respectively. The linalool component was found in *A. dracunculus* and *A. dracunculoides* at rates of 4.68%-1.82%, respectively, the linalool content of *A. dracunculus*, showed that, more suitable for use in perfumery and food industries than *A. dracunculoides.*

Table 4.
Essential oil components of A. dracunculus and A. dracunculoides

		A. dracunculus		*A. dracunculoides*				*A. dracunculus*		*A. dracunculoides*	
N	Essential Oil Components	R.T	%	R.T	%	N	Essential Oil Components	R.T	%	R.T	%
1	α-Pinene	7.59	0.88	7.59	0.87	27	Trans-Carveol	17.75	0.04	n.d.	n.d.
2	Camphene	8.04	0.20	8.04	0.12	28	Borne ol, Formate	18.03	0.09	n.d.	n.d.
3	Sabinene	8.86	0.10	8.86	0.09	29	Hexyl 2-Methylbutanoate	18.22	0.10	n.d.	n.d.
4	β-Pinene	8.95	0.16	8.95	0.13	30	Propanal, 2-Methyl-3-Phenyl	18.35	0.08	n.d.	n.d.
5	β-Myrcene	9.45	0.14	9.46	0.14	31	D-Carvone	18.47	0.05	n.d.	n.d.
6	α-Terpinolene	10.31	0.04	12.86	0.11	32	Bornyl Acetate	19.84	0.12	n.d.	n.d.
7	Cymene	10.61	0.17	n.d	n.d	33	Carvacrol	20.30	0.08	n.d.	n.d.
8	P-Cymene	n.d.	n.d.	10.61	0.08	34	Eugenol	22.16	0.48	22.18	0.67
9	D-Limonene	10.82	2.76	10.81	2.67	35	Cinnamic acid, Methyl Ester	22.96	0.13	22.98	0.46
10	1,8-Cineole	10.88	1.68	10.87	0.76	36	Methyleugenol	23.73	1.70	23.72	1.34
11	Cis-Ocimene	11.19	3.92	11.21	4.52	37	Trans-Caryophyllene	24.16	0.08	24.16	0.16
12	β-cis-Ocimene	11.55	3.45	n.d.	n.d.	38	Trans-β-Farnesene	25.27	0.09	n.d.	n.d.
13	β- Ocimene	n.d.	n.d.	11.58	4.00	39	Germacrene-d	26.06	0.11	26.06	0.07
14	Gamma-Terpinene	11.83	0.06	11.84	0.04	40	β-İonone	26.19	0.08	n.d.	n.d.
15	Linalool	13.45	4.68	13.36	1.82	41	Trans-Beta-İonone	n.d.	n.d.	26.19	0.07
16	Alloocimene	14.32	0.11	14.31	0.14	42	Bicyclo[3.1.1]hept-2-ene hyl-6-(4-Methyl-3-Pentenyl)	n.d.	n.d.	26.44	0.07
17	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	n.d.	n.d.	19.84	0.32	43	Methyl Eicosan-5(Z),8(Z),11(Z),14 Z)-Tetraen-19-Onoate	n.d.	n.d.	28.64	0.15
18	Borneol	15.71	2.65	15.62	0.72	44	α-Farnesene	26.44	0.06	n.d.	n.d.
19	Terpinene-4-ol	16.08	1.02	16.04	0.27	45	Bicyclogermacrene	26.53	0.09	26.53	0.11
20	Estragole	17.54	69.34	17.55	67.51	46	Spathulenol	28.94	0.48	28.94	0.54
21	Benzaldehyde,4-Methoxy	n.d.	n.d.	18.76	0.09	47	α-Bisabolol	32.08	0.15	n.d.	n.d.
22	Camphor	14.88	1.34	14.85	0.44	48	Herniarin	32.78	0.26	32.79	0.56
23	3,4,6-Trinitro-2-methylanisole	n.d.	n.d.	23.65	0.21	49	2-Pentadecanone, 6,10,14-Trimethyl	34.01	0.02	34.01	0.03
24	1-Acetoxyindole	n.d.	n.d.	22.13	0.37	50	N-İsobutyl Deca-2,4-Dienamide	34.63	0.04	34.63	0.09
25	Hexadecanoic Acid	n.d.	n.d.	34.77	0.04	51	Phytol	35.51	0.05	n.d.	n.d.
26	Tetracosane	39.05	0.03	39.05	0.16	52	Achillea amide	35.67	0.02	35.67	0.06

*Significance level $p < .05$ n.d.: not detected, N: Number

After estragole, the most abundant essential oil components are cis-ocimene, β-cis-ocimene, and β-ocimene, in the group of regular hydrocarbons. In this study, cis-ocimene values were between 3.92%-4.52%, β-cis-ocimene was 3.45%, and β-ocimene was 4.00%. In addition, β-ocimene could not be detected in *A. dracunculus*, and β-cis-ocimene was not found in *A. dracunculoides.*

In addition to being a monoterpene used in the food industry due to its pungent odor and aroma, D-Limonene has a chemo-preventive effect on breast, skin, and liver cancers, according to studies con-ducted with rodents (Kim et al.*,* 2013; Saini et al.*,* 2022). This study determined D-Limonene at 2.76%-2.67% for *A. dracunculus* and *A. dracunculoides*, respectively. It is known that volatile linalool is used in the production of food flavors and in the perfumery and pharmaceutical industries to remove undesirable odors (Amazonas et al.*,* 2022). This study found the linalool component in *A. dracunculus* and *A. dracunculoides* at 4.68%-1.82%, respectively. The linalool component was found in *A. dracunculus* and *A. dracunculoides* at rates of 4.68%-1.82%, respectively, the linalool content of *A. dracunculus*, showed that, more suitable for use in perfumery and food industries than *A. dracunculoides.*

Discussion

Green herb yield, green leaf yield, drug herb yield, and drug leaf yield were found 477.82-531.98 kg/ha, 311.14-358.11 kg/ha, 98.50-119.01 kg/ha, 64.94-82.28 kg/ha, respectively (Table 2). In their study, Cil and Kara (2015), determined the effect of different plant densities on the yield of *Artemisia dracunculus* L., and they showed higher results than the values we obtained. Basiri and Nadjafi (2019), were found lower values for tarragon. It is thought that these differences can be caused by the ecological characteristics of the cultivation area.

The essential oil ratios obtained in this study were 1.40% and 1.21% for *A. dracunculus* and *A. dracunculoides*, respectively. Çil and Kara (2015) stated that the essential oil ratio for *A. dracunculus* varied between 0.146% and 1.346% in their previous study and showed results close to the values we obtained. Golubkina et al., (2020) reported that tarragon contains 0.68% to 0.70% essential oil, which is lower than the value we obtained. It is thought that the differences observed between the studies may be due to the age of the seedlings and the climatic conditions in which the plant samples were grown.

Estragole was the major component of *Artemisia dracunculus* and *Artemisia dracunculoides*, used in this study (Table 4). Although some studies have reported that the estragole component has carcinogenic effects (De Vincenzi et al., 2000; Gori et al., 2012; Zeller et al., 2009). Smith et al. (2013), reported that some herbal products containing this component are used in the field of food as a flavor enhancer and that the important point in this regard is the consumption amount, as consumption in low amounts is not harmful. Bahmani et al. (2018), determined that essential oil components obtained from tarragon leaves contain estragole between 76.67% and 83.06%. In addition; there are also current studies that support this study by reporting 67.70% of estragole in *A. dracunculus* (Ulu & Aksu Kılıçle, 2020). In addition, linalool and cis-ocimene for *A. dracunculus*, cis-ocimene, and β-ocimene for *A. dracunculoides* are the highest components after estragole. Some previous studies reported that the essential oil components of β-cis-ocimene and β-ocimene in *A. dracunculus* were between 3.40% and 11.00%, and higher values were determined than the results obtained in this study (Haghi et al., 2010; Ulu & Aksu Kılıçle, 2020). It is thought that the differences between the results obtained may be due to the cultivation of the plant material used in different locations and under different production conditions.

The obtained by using GC analysis in this study, D-Limonene values (2.76%-2.67) show that tarragon can be useful in the production of drugs with its anticancer effect as well as its use in the food field. In some previous studies, D-Limonene component values were between 1.63% and 3-9%, supporting the results obtained in this study (Haghi et al., 2010; Ulu & Aksu Kılıçle, 2020). Sayyah et al. (2004), determined that 12.4% limonene was found in *A. dracunculus* and showed different results from this study. The differences between these values are thought to be due to the eco-geographical features of the plant's growing area. It is known that the linalool molecule has many biological activities such as antitumoral, antioxidant, antimicrobial, and anti-inflammatory properties and is found in the essential oils of many medicinal and economic plants (Mitic-Culafic et al., 2009; Tepe et al., 2004). The linalool component content of *Artemisia* spp., used in this study, was between 1.8% and 4.68%. In previous studies, it was seen that the linalool content of tarragon ranged between 0.19% and 5.09%, and the results obtained were supported by this study (Ayoughi et al., 2011; Karimi et al., 2015; Sahakyan et al., 2021). In addition, when compared in terms of linalool content, *A. dracunculus* was determined to contain a higher rate of linalool than *A. dracunculoides*. As a result, *A. dracunculus* is considered to be preferred to *A. dracunculoides* in the perfumery, food, and pharmaceutical industries.

Conclusions

Green and drug yield values of *Artemisia* spp. showed that *Artemisia dracunculus* L. has more advantages for drug-leaf yield and drug herb yield. Tarragon is a valuable plant for its green and dry leaves as well as its essential oil. The results obtained in this study showed that *A. dracunculus* contained a higher percentage of essential oil than *A. dracunculoides*. It was determined that *A. dracunculus* and *A. dracunculoides* plant materials, used in this study have valuable essential oil components. For the first time, essential oil components of *A. dracunculus* and *A. dracunculoides* were compared. Although there are negative opinions about estragole, studies also show this possibility can be eliminated by determining the amount of use. It has been seen that the tarragon plant has positive potential for use in the perfumery and pharmaceutical industries, as well as in the food industries. With its aromatic effect and linalool content, *A. dracunculus* can use in food and medication manufacturer, particularly perfume. Considering the increasing use of medicinal aromatic plants and the variety of products needed, it is seen that more research is needed in this field. A more comprehensive study can be done on this subject, including different *Artemisia* spp. Considering its economic importance and other uses, it suggests the idea that *A. dracunculus* and *A. dracunculoides* should be cultivated in larger areas.

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Field Evaluation of Ukrainian Potato Varieties for Resistance to Fungal and Bacterial Pathogens in the Polissya Area of Ukraine

Ukrayna'nın Polissya Bölgesinde Ukrayna Patates Çeşitlerinin Arazide Fungal ve Bakteriyel Patojenlere Karşı Direnç Açısından Değerlendirilmesi

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ABSTRACT

This study aimed to assess the field resistance of Ukrainian potato varieties to fungal and bacterial pathogens under natural infectious conditions in the Polissya area of Ukraine. Field experiments were conducted during 2020-2022 to examine the manifestation and spread of fungal and bacterial diseases on 20 Ukrainian potato varieties across different maturity groups. Varieties were evaluated for resistance to Alternaria blight, *Rhizoctonia solani*, Common scab (***Streptomyces*** **spp.**), and Fusarium dry rot using predefined scales. The Ukrainian potato varieties Aria, Khortytsia, Kniahynia, Myroslava, Shchedryk, and Slovianka displayed field resistance to Alternaria blight. The varieties Charunka, Feia, Khortytsia, Okolytsia, and Shchedryk showed field resistance to *R. solani*. The varieties Aria, Okolytsia, Skarbnytsia, Strumok, and Slovianka were highly resistant to Common scab in field conditions, while Anika, Aria, Charunka, Kimmeria, Letana, Slovianka, Shchedryk, and Tyras exhibited field resistance to Fusarium dry rot. Twelve out of 20 potato varieties displayed field resistance to multiple pathogens with five of them (Aria, Charunka, Khortytsia, Slovianka, and Shchedryk) being resistant to the majority of pathogens under investigation. These varieties hold promise for integration into cropping systems with reduced fungicide usage. Additionally, these varieties can be recommended for inclusion in breeding programs as valuable sources of resistance to these fungal and bacterial pathogens. Future research should focus on elucidating the genetic basis of resistance in these varieties and further exploring the nature of inheritance of the observed resistance from the parental forms, that include the varieties Bellarosa, Beloruskyi 3, Bahriana, Slovianka, Oberih, Lyu, Meve, Kondor, Tyras and Barylchykha, and the hybrids 86.281c12, KE 78.50.53, 77.583/16, and P.88.12-11.

Keywords: Potato, resistance, *Alternaria solani*, *Alternaria alternata*, *Rhizoctonia solani*, *Streptomyces* spp., *Fusarium* spp.

ÖZ

Bu çalışma, Ukrayna'nın Polissya bölgesindeki doğal bulaşıcı koşullar altında Ukrayna patates çeşitlerinin fungal ve bakteriyel patojenlere karşı tarla direncini değerlendirmeyi amaçlamıştır. Farklı 20 Ukrayna patates çeşidinde fungal ve bakteri hastalıklarının ortaya çıkışını ve yayılmasını incelemek için 2020-2022 yılları arasında arazi denemeleri yapılmıştır. Çeşitler, önceden tanımlanmış skalalar kullanılarak Alternaria yanıklığı, *Rhizoctonia solani*, Adi uyuz (*Streptomyces* spp.) ve Fusarium kuru çürüklüğüne karşı direnç açısından değerlendirilmiştir. Ukrayna patates çeşitleri Aria, Khortytsia, Kniahynia, Myroslava, Shchedryk ve Slovianka çeşitleri Alternaria yanıklığına karşı; Charunka, Feia, Khortytsia, Okolytsia ve Shchedryk çeşitleri *R. solani*'ye karşı; Aria, Okolytsia, Skarbnytsia, Strumok ve Slovianka çeşitleri *Streptomyces* spp. ve Anika, Aria, Charunka, Kimmeria, Letana, Slovianka, Shchedryk ile Tyras, çeşitleri ise Fusarium kuru çürüklüğüne karşı arazi şartlarında direnç göstermişlerdir. 20 patates çeşidinden 12'si birden fazla patojene karşı çoklu direnç sergilemiş, bunlardan beşi (Aria, Charunka, Khortytsia, Slovianka ve Shchedryk) de araştırılan patojenlerin çoğuna karşı direnç sağlamıştır. Bu çeşitler, azaltılmış fungisit kullanımıyla ürün yetiştirme sistemlerine entegrasyon konusunda umut vaat etmektedir. Ek olarak bu çeşitler, fungal ve bakteriyel patojenlere karşı direnç kaynağı olarak ıslah programlarına dahil edilmek üzere önerilebilir. Gelecekteki araştırmalar, bu çeşitlerdeki direncin genetik temelini aydınlatmaya ve Bellarosa, Beloruskyi 3, Bahriana, Slovianka, Oberih, Lyu, Meve, Kondor, Tyras ve Barylchykha çeşitleri ile 86.281c12, KE 78.50.53, 77.583/16 ve P.88.12-11 melezlerini içeren ebeveyn formlardan sağlanan direncin kalıtımının doğasını araştırmaya odaklanmalıdır.

Anahtar Kelimeler: Patates, dayanıklılık, *Alternaria solani*, *Alternaria alternata*, *Rhizoctonia solani*, *Streptomyces* spp, *Fusarium* spp.

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Introduction

Many pests and pathogens can significantly affect potato cultivation in Ukraine, causing average annual yield losses of 20-25%. Total losses could be even higher because some of them can affect the crop not only during the growing season but also in the postharvest period and during storage of tubers (Cherednychenko et al., 2019; Kononuchenko & Molotskyi, 2003).

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, stands out as one of the most pervasive and devastating diseases, both on a global scale and within Ukraine. It is known to cause severe yield losses, particularly affecting early potato varieties, especially during epiphytotic years (Cherednychenko et al., 2008; Dong & Zhou, 2022; Flier & Turkensteen, 1999; Fry & Goodwin, 1997; Goodwin, 1997). Crop losses are higher in regions with emerging economies, primarily due to the unavailability of cost-effective chemical control strategies. As climatic conditions become more suitable for disease development, the consequences become even more severe, culminating in the total obliteration of yields (Rakotonindraina et al., 2012).

Alternaria blight, caused by *Alternaria solani* Sorauer and *Alternaria alternata* (Fr.) Keissler can also cause serious damage to the potato crop. A yield reduction of 30-35% may take place when over 40% of potato plants are affected in the field. Medium-ripe and medium-late maturing potato varieties are particularly susceptible to these pathogens (Andersen et al., 2018; Cherednychenko et al., 2016).

Rhizoctonia solani Kühn is another significant threat to potato seed production. The absence of resistant varieties may result in a mortality of approximately 50% of seedlings before emergence, with "white leg" symptoms appearing on nearly all plants during the growing season and sclerotia formation on the majority of tubers. Under favourable conditions, disease not only compromises seed material quality, but also causes substantial losses, potentially reaching 49% of the harvest (Zhang et al., 2021).

In Ukraine, the occurrence of late blight outbreaks exhibits substantial variability due to the multifaceted nature of factors influencing pathogen development, including weather conditions, agricultural practices, and potato varieties (Polozhenets, 1997). On the other hand, Rhizoctonia is documented annually at damage levels of 30-60% to sprouts, 25-70% to stolons, and 10-25% to roots, thereby significantly compromising the quality of planting material and overall tuber yields, where losses up to 40-60% were recorded (Cherednychenko et al., 2008).

In Polissya - the primary potato growing region of the country, potatoes are persistently affected by various diseases such as bacterial ring rot (*Clavibacter sepedonicus* (Spieckermann & Kotthoff; Li et al.), Common scab (*Streptomyces* spp.), powdery scab (cercozoan *Spongospora subterranea* (Wallroth) Lagerheim), potato wart disease (*Synchytrium endobioticum* (Schilbersky) Percival), late blight (*Phytophthora infestans* (Montagne) de Bary), early blight (*Alternaria* spp.), and Fusarium dry rot (*Fusarium* spp.). This persistence can be attributed to the optimal moisture and temperature conditions conducive to the development of these pathogens and cultivation practices of growers. Conversely, in the southern regions of Ukraine, characterized by elevated air and soil temperatures and a dearth of moisture during the plant's growing season, plant pathogens causing wilt diseases primarily prevail (Kononuchenko & Molotskyi, 2003).

Effective safeguarding crops against the diverse pathogens mentioned above can only be reached in an integrated approach. This approach involves the utilization of agrotechnical, chemical, biological, and economic methods, tailored to the specific soil and climatic conditions of the potato cultivation zone.

Presently, considerable emphasis within potato protection systems is directed towards the adoption of modern cultivation technologies and the utilization of new varieties featuring enhanced economic and qualitative characteristics. Nevertheless, chemical methods of combating pathogens remain important, although they often cause increased production costs, environmental pollution, and the emergence of pesticide resistance in harmful organisms (Andersen et al., 2018; Kononuchenko & Molotskyi, 2003; Lisovyi & Trybel, 1998).

In the Netherlands, a country with an annual potato production exceeding 7 million tons (including 1 million tons of seed potatoes), more than 50% of all pesticides employed for crops protection are dedicated to safeguarding potato against late blight. This safeguarding lead to annual losses of more than 100m euro on a harvest value of more than 700m euro (Haverkort et al., 2016). Notably, the measures implemented to combat late blight at global scale contribute to more than 10% of the overall CO_2 emissions associated with potato production (Haverkort & Hillier, 2011), and the combined expenses associated with yield loss and management worldwide range from of 3-10 billion USD annually (Dong & Zhou, 2022).

Given this scenario, significant efforts are directed towards the exploration of environmentally sustainable methods for pathogen control. These efforts span from the development of biological preparations as an alternative to chemical pesticides, adaptation of cultivation methods and fertilisation schemes, to the breeding of potato varieties with resistance against the most harmful pathogens, preferably in an integrated way (Ilchuk et al., 2021; Russell, 1982; Trybel et al., 2006).

To date, Ukrainian potato breeders have succeeded in generating numerous potato varieties characterized by (partial) field resistance to pests and diseases distributed in the country. Nevertheless, in practical cultivation, protective measures are typically formulated without taking resistance of these varieties into account (Ilchuk et al., 2021; Osypchuk, 2011; Podhaietskyi et al., 2018; Trybel et al., 2006). This, while it is widely acknowledged that favouring resistant varieties over those susceptible to pests and diseases can safeguard 15-20% of the yield and substantially enhances the overall efficacy of potato protection measures and reduction in pesticide usage (Lisovyi & Trybel, 1998; Osypchuk, 2011; Trybel et al., 2006).

Over the course of several decades, the Institute of Potato Research at the National Academy of Agrarian Sciences of Ukraine has undertaken a series of projects investigating the manifestation and development of fungal and bacterial diseases in potatoes, both under natural and artificially induced infection. The primary aim has been to enhance the potato breeding program, particularly with regards to resistance against Late blight, Alternaria blight, and Fusarium wilt (Andriychuk & Homyak, 2013; Cherednychenko et al., 2008; Cherednychenko et al., 2016; Cherednychenko et al., 2019; Koval et al., 1978; Koval et al., 1979; Koval et al., 1983; Podhaietskyi et al., 1994). Additionally, research has focused on improving resistance to Rhizoctonia and Common scab (*Streptomyces* spp.) (Polozhenets, 1997), as well as addressing issues related to Phytophthora and Fusarium dry rot (Podhaietskyi et al., 1994; Podhaietskyi et al., 2018). Throughout the research, several varieties were identified as valuable donors, providing essential traits for developing disease-resistant potato varieties.

This study aimed to extend the ongoing investigations into potato diseases dissemination, incidence and intensity on varieties from various maturity groups within the Ukrainian potato breeding programs. Additionally, the target was to evaluate the field resistance of these varieties to the predominant potato pathogens under the natural infectious conditions prevalent in the Polissya area of Ukraine during the 2020-2022 growing seasons.

Methods

The research was conducted at the Laboratory of Immunity and Plant Protection within the Breeding Department of the Institute of Potato Research, NAAS. The experimental site featured sod-podzolic sandy loam soil, characteristic of the Polissya area of Ukraine (village Nemeshaevo, Bucha district, Kyiv region, Ukraine) with humus content at 1.4%, easily hydrolysed nitrogen at 98, mobile phosphorus at 72, exchangeable potassium at 100 ml/kg, and calcium and magnesium at 4.4 and 0.5 mg equiv. per 100 g of soil, respectively. The meteorological data for 2020-2022 growing season is provided in Table 1. The presence of the pathogens studied (based on symptoms in the field expressed as incidence and intensity) during the growing seasons in the study years 2020-2022 is presented in Table 2.

The typical agricultural technology for potato cultivation in the Polissya area was applied during the study period, it comprises the following steps: 1) soil preparation involving disking and sowing of green manure (cover) crops or siderates such as oil radish, mustard or vetch-oat mixture; 2) successive stages of soil preparation and additional sowing of green manure crops (cultivation); 3) plowing the (green) mass of the green manure crops into the soil; 4) preparation of potato tubers for planting; 5) plowing; 6) pre-planting soil preparation (cultivating); 7) planting potatoes with localized application of mineral fertilizers, plant protection agents and growth regulators; 8) formation of high-volume ridges; 9) herbicide application before potato seedling emergence; 10) herbicide application on potato seedlings, combined with growth regulators, fungicides, and trace elements; 11) pest control measures against the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)); 12) up to five fungicide treatments and foliar fertilization with complex water-soluble fertilizers at intervals of 7-10 days; 13) mowing potato crops; 14) further treatment of potato crops with contact fungicides; 16) harvesting of tubers; 17) post-harvest processing of potato tubers; and potato tuber storage at 2-4°C.

Twenty potato varieties were used in the study: A) *early maturity group*: Kimmeria, Shchedryk, Skarbnytsia, Slauta, and Tyras; B) mid-early maturity group: Aria, Fantazia, Strumok, and Zlahoda; C) medium-ripe maturity group: Anika, Charunka, Feia, Hurman, Ivankivska rannia, Kniahynia, Khortytsia, Letana, Myroslava, Okolytsia, and Slovianka (all originated from the Institute of Potato Research NAAS).

The field experiment was laid down in two variants: 1) a control variant subjected to three pesticide treatments, using the fungicide Acrobat 90/600 WG (active substances: dimethomorph 90 g/kg + mancozeb 600 g/kg; BASF, Germany) at a rate of 2 kg/ha and an insecticide for Colorado potato beetle control (Coragen 20, KC, chlorantraniliprole, 200 g/l; DuPont, USA) at a rate of 0.05-0.06 l/ha, and 2) an experimental variant without fungicide treatment, but maintaining the Colorado beetle insecticide treatment as mentioned above. Pesticide applications were made at budding phase, flowering phase and 10 days after that phase.

Plantings were done in two-row plots of 15 m^2 with 25 tubers per row. The experiment was laid down in randomized block design with three repeats.

Throughout the growing season, phenological observations were conducted, which included noting the features and start of key plant development phases and visually assessing crop conditions and disease symptoms. Records were also maintained for plant density (measured in thousands of plants per hectare) and yield (measured per plot and calculated as tons per hectare) (Bondarchuk & Koltunov, 2019).

Table 1

Meteorological data for the 2020-2022 growing seasons, 5 month period (Kyiv region, Ukraine)

	April			May			June			July			August		
	AT ^0C	ST ^0C	P mm	AT ^0C	ST ^0C	P mm	AT ^0C	ST ^0C	P mm	AT ^0C	ST ^0C	P mm	AT ^0C	ST ^0C	P mm
Season	2020														
I decade	7.5	2.7	14.0	11.1	10.5	28.1	18.9	21.7	15	21.4	19.2	13	23.2	19.5	0
II decade	8.4	5.6	15.0	14.6	14.3	36.9	20.8	22.2	13	21.2	20.8	20	24.1	23.0	7
III decade	10.2	7.9	12.0	20.5	20.6	45.0	22.7	24.0	12	20.6	18.0	42	22.8	20.1	0.3
Month average	8.7	5.4	82	15.4	15.1	110	20.8	22.6	40	21.1	19.3	70	23.4	20.9	7.3
10 years average	10		41	14.2		65	19.5		80	21.3		85	20.4		56
Deviation +/-	– 1.3		+ 41	+ 1.2		+45	+1.3		– 40	-0.2		– 15	+3.0		– 48.7
Season	2021														
I decade	8.9	3.7	12	10.4	10.5	15	17.8	21.0	13.6	20.8	25.3	16	23.2	20.8	6
II decade	9.7	5.9	17	14.5	14.3	13	20.7	22.5	12.4	21.2	24.7	21	24.1	25.0	5
III decade	10.9	8.7	13	17.5	20.6	9	22.5	23.6	11	22.6	19.1	40	22.9	23.5	10.2
Month average	8.5	6.1	83	14.5	15.1	37	20.3	22.4	37	21.5	23.0	77	23.4	23.1	21.2
10 years average	10		42	14.2		65	19.5		80	21.3		85	20.4		56
Deviation +/-	– 0.5		+ 41	+ 0.5		– 28	+0.8		– 43	+0.2		– 8	+3.0		– 34.8
Season	2022														
I decade	7.5	2.8	13	15.6	14.8	0	16.9	20.7	15	21.8	25.2	13	24.2	21.5	7
II decade	8.3	5.4	14	19.7	16.4	0	18.8	22.0	13	21.2	24.8	20	24.0	25.0	0
III decade	9.8	8.6	15	20.8	20.7	0	20.7	23.1	29	20.6	18.0	42	23.7	24.1	0.2
Month average	8.5	8.3	82	18.7	17.3	0	18.8	21.9	57	21.2	22.8	74	24.0	23.5	7.2
10 years average	10		42	14.2		65	19.5		80	21.3		85	20.4		56
Deviation +/-	– 1.5		+ 40	+4.5		– 65	+4.5		–23	–0.1		–11	+3.6		–48.8

* AT: Air temperature; ST: Soil temperature; P: Precipitation

During the vegetation season the incidence (the number of plant units that are (visibly) diseased, relative to the total number assessed) and severity (the area or volume of plant tissue that is (visibly) diseased, relative to the total plant tissue) of potato diseases were evaluated (Kumar et al., 2023; Trybel & Bondarchuk, 2013).

For assessment of disease severity, diseased leaves were categorized as per the scale:

Grade	Leaf area infected (%)
0	No disease symptoms
1	1-2.5
2	2.6-5
3	6-10
4	11-15
5	16-25
6	26-50
7	51-75
8	Total blight (death of all plants)

The incidence of Alternaria blight was noted from the first decade of June to the second decade of August three times. The indicator (R) for disease incidence was calculated using the formula 1:

R = (n/N) x 100% (1),

where

R – disease incidence (%)

n – number of infected plants,

N- total number of plants examined.

The total disease severity was determined as a percentage by the formula 2:

Disease severity= (∑ab/NK) x 100% (2),

where

∑ summation,

a - number of leaves in each category

b - grade of disease severity

N – total number of leaves observed

K - Maximum numerical value/grade of disease severity

Table 2.

Severity and incidence of Alternaria *blight* (*A. solani*, *A. alternata*) *on potato varieties under cultivation conditions of the Polissya region in Ukraine, 2020-2022*

Potato variety	Severity, %						Incidence, %					
	I assessment		II assessment		III assessment		I assessment		II assessment		III assessment	
	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
Early maturity group												
Kimmeria	0	2.9*	2.0	7.6*	4.0	14.6*	0	7.5*	8.4	21.0*	12.0	34.0*
Skarbnytsia	0	3.7*	1.0	7.7*	3.5	14.7*	0	9.8*	8.0	30.0*	17.8	37.0*
Shchedryk	0	0.5*	0.5	3.2*	2.0	7.7*	0	2.5*	1.6	4.0*	5.6	14.0*
Slauta	0	2.1*	3.5	4.6	6.0	17.6*	0	4.9*	6.8	17.0*	10.4	26.0*
Tyras	0	3.7*	3.0	6.2*	9.5	15.6*	0	7.6*	8.8	22.0*	12.0	31.0*
Mid-early maturity group												
Aria	0	0.7*	0	3.0*	2.5	8.6*	0	2.0*	0	6.0*	8.0	14.0*
Zlahoda	0	4.7*	6.0	14.1*	11.5	20.9*	0	14.0*	13.7	41.0*	22.9	59.0*
Strumok	0	4.8*	2.9	11.5*	4.5	13.6*	0	12.0*	11.9	35.0*	16.7	48.5*
Fantazia	0	4.7*	2.7	10.7*	4.0	15.6*	0	10.5*	12.1	30.0*	15.7	41.5*
Medium-ripe maturity group												
Hurman	0	2.7*	2.3	9.1*	4.2	16.9*	0	6.8*	8.0	20.0*	14.4	36.5*
Slovianka	0	1.7*	1.8	7.2*	2.4	9.7*	0	4.9*	5.6	14.0*	8.0	30.0*
Anika	0	3.0*	1.4	5.5*	3.1	12.3*	0	7.1*	8.5	22.0*	15.4	38.5*
Okolytsia	0	8.1*	3.5	14.3*	4.7	18.7*	0	14.7*	14.2	44.0*	18.6	53.5*
Myroslava	0	2.3*	1.4	5.7*	4.6	10.0*	0	6.3*	7.6	19.0*	11.4	28.5*
Ivankivska rannia	0	2.5*	1.8	7.1*	3.1	12.6*	0	6.7*	8.0	20.0*	15.0	37.5*
Kniahynia	0	3.7*	2.1	8.3*	3.4	9.6*	0	9.7*	11.8	30.0*	15.4	35.5*
Charunka	0	3.3*	2.2	8.8*	3.2	13.0*	0	7.6*	9.2	23.0*	11.4	28.5*
Letana	0	2.0*	1.5	6.1*	3.0	12.1*	0	5.4*	6.4	16.0*	10.6	26.5*
Feia	0	1.7*	1.4	5.6*	3.8	15.4*	0	4.6*	5.5	14.0*	11.5	28.5*
Khortytsia	0	2.6*	1.6	6.5*	2.6	10.4*	0	4.1*	5.2	13.0*	9.8	24.5*

* the difference with the control is significant at $p \leq .05$

Table 3.
Varietal differences to potato tuber damage by fungal and bacterial pathogens, 2020-2022

No	Potato variety	Disease severity (percentage of potato tubers affected), %											
		Rhizoctonia solani				Common scab (*Streptomyces* spp.)				*Fusarium* dry rot (*Fusarium* spp.)			
		2020	2021	2022	Medium	2020	2021	2022	Medium	2020	2021	2022	Medium
Experimental group													
Early maturity group													
1.	Kimmeria	0	42.0*	24.0*	22.0	10.0*	13.0*	6.0*	9.7	8.0*	4.0*	17.0*	9.7
2.	Skarbnytsia	0	43.0*	24.0*	22.3	6.0*	5.0*	2.7*	4.6	16.0*	7.0*	17.8*	13.6
3.	Shchedryk	0	21.0*	9.0*	10.0	14.0*	23.0*	7.0*	14.7	6.0*	5.0*	12.0*	7.7
4.	Slauta	2.0*	49.0*	26.9*	26.0	6.0*	8.0*	4.4*	6.1	29.0*	10.0*	15.5*	18.2
5.	Tyras	0	42.0*	23.1*	21.7	24.0*	5.0*	2.8*	10.6	20.0*	3.0*	4.6*	9.2
Mid-early maturity group													
6.	Aria	4.0*	62.0*	2.2*	29.7	9.0*	9.0*	5.0*	7.7	19.0*	1.0*	1.5*	7.2
7.	Zlahoda	10.0*	21.0*	11.5*	14.2	7.0*	15.0*	8.2*	10.1	32.0*	19.0*	29.4*	26.8
8.	Strumok	8.0*	41.0*	22.5*	23.8	15.0*	7.0*	3.8*	8.6	21.0*	9.0*	14.0*	14.7
9.	Fantazia	3.0*	45.0*	24.7*	24.2	5.0*	19.0*	10.4*	11.5	17.0*	10.0*	15.0*	14.0
Medium-ripe maturity group													
10.	Hurman	0	49.0*	26.8*	25.3	11.0*	4.0*	2.0*	5.7	17.0*	10.0*	14.9*	14.0
11.	Slovianka	3.0*	56.0*	30.8*	29.9	4.0*	7.0*	3.8*	4.9	4.0*	5.0*	7.0*	5.3
12.	Anika	16.0*	55.0*	30.2*	33.7	1.0*	21.0*	12.0*	11.3	10.0*	8.0*	12.4*	10.1
13.	Okolytsia	0	20.0*	9.0*	9.7	6.0*	2.0*	1.2*	3.1	23.0*	8.0*	12.5*	14.5
14.	Myroslava	7.0*	42.0*	23.0*	24.0	17.0*	5.0*	2.8*	8.3	15.0*	9.0*	14.0*	12.7
15.	Ivankivska rannia	1.0*	57.0*	31.3*	29.8	22.0*	8.0*	4.5*	11.5	31.0*	5.0*	8.1*	14.7
16.	Kniahynia	2.0*	25.1*	14.5*	13.8	17.0*	4.0*	2.1*	7.7	12.1*	11.0*	13.2*	12.1
17.	Charunka	4.0*	26.0*	14.3*	14.8	7.0*	21.0*	11.8*	13.3	22.0*	4.0*	6.3*	10.8
18.	Letana	0	48.0*	26.4*	24.8	54.0*	21.0*	11.9*	29.0	15.0*	3.0*	4.6*	7.5
19.	Feia	4.0*	16.0*	10.0*	10.0	12.0*	7.0*	3.5*	7.5	15.0*	10.0*	16.0*	13.7
20.	Khortytsia	0	16.0*	8.8*	8.3	20.0*	12.0*	6.6*	12.9	23.0*	11.0*	17.1*	17.0
Control group													
Early maturity group													
1.	Kimmeria	0	8.4	4.8	4.4	2.0	2.6	1.2	4.3	1.5	0.8	3.4	1.9
2.	Skarbnytsia	0	8.6	4.4	4.3	1.2	1.0	1.4	1.2	3.2	1.4	3.5	2.7
3.	Shchedryk	0	4.2	1.5	1.9	2.8	1.6	1.3	1.6	1.2	1.0	2.4	1.5
4.	Slauta	0.5	9.8	5.0	5.1	1.1	1.6	0.8	1.2	5.8	2.0	3.1	3.6
5.	Tyras	0	8.2	4.2	4.1	4.6	1.0	0.6	2.1	4.0	0.6	0.9	1.8
Mid-early maturity group													
6.	Aria	1.0	8.5	0.5	3.3	2.1	2.2	1.2	1.8	4.7	0.2	0.4	1.8
7.	Zlahoda	2.5	5.0	2.9	3.5	1.7	3.7	2.0	2.5	8.0	4.5	7.3	6.6
8.	Strumok	2.0	10.0	5.6	5.9	3.5	1.5	1.0	2.0	5.2	2.5	3.5	3.7
9.	Fantazia	0.7	11.2	6.0	6.0	1.5	5.0	2.5	3.0	4.0	2.5	4.0	3.5
Medium-ripe maturity group													
10.	Hurman	0	9.8	5.5	5.1	2.2	1.0	0.5	1.2	3.4	2.0	1.0	2.1
11.	Slovianka	0.5	11.2	6.0	5.9	0.8	1.4	0.8	1.0	0.8	1.0	1.4	1.1
12.	Anika	3.2	11.0	6.0	6.7	1.0	4.2	2.4	2.5	2.0	1.6	2.5	2.0
13.	Okolytsia	0	4.0	1.8	1.9	2.0	0.5	0.2	0.9	4.6	1.6	2.5	2.9
14.	Myroslava	1.4	8.0	4.5	4.6	2.5	1.0	0.5	1.3	3.0	1.8	2.8	2.5
15.	Ivankivska rannia	0.2	11.4	6.3	6.0	3.5	1.6	1.0	2.0	6.2	1.0	1.6	2.9
16.	Kniahynia	0.4	9.0	4.9	4.8	3.0	0.8	0.4	1.4	5.6	3.0	4.6	4.4
17.	Charunka	0.8	5.0	2.9	2.9	1.5	4.0	3.0	2.6	4.4	0.8	1.3	2.2
18.	Letana	0	9.0	5.4	4.8	10.8	4.5	2.4	5.9	3.0	0.6	1.0	1.5
19.	Feia	1.0	3.0	2.0	2.0	2.0	1.4	0.7	1.4	3.0	2.0	3.2	2.7
20.	Khortytsia	0	3.2	1.8	1.7	4.0	2.4	1.3	2.6	4.6	2.2	3.4	3.4

* the difference with the control is significant at $p < 0.05$

Potato varieties were considered resistant to Alternaria blight, for which the disease incidence level should not exceed 30% of plants, while the level of disease severity had to be in the range of 3-10% (Trybel & Bondarchuk, 2013).

Varieties were considered resistant to Rhizoctonia (*R. solani*) for which 5 to 10% of the tubers were affected, for Common scab (*Streptomyces* spp.) – 5-20% of the tubers, and Fusarium dry rot (*Fusarium* spp.) – 5-10% of the tubers, respectively (Trybel & Bondarchuk, 2013).

The statistical processing of experimental data was conducted using disperse analysis (ANOVA) and software packages of Microsoft Office Excel 2010 and STATISTICA 10. The least significant difference (LSD) test was used to test for significant differences in multiple comparisons at the 0.05 significance level.

Results

It is known, that the incidence and severity of potato diseases can be influenced by weather conditions. Thus, a comprehensive analysis of the climatic factors during the research period was performed in objectively evaluating the growth and development of both potato plants and pathogens.

In the spring of 2020, the climatic conditions proved favourable for commencing field operations (Table 1). Soil preparation and potato planting took place between April 17 and April 19. The following month May was marked by higher temperatures (15.4°C as opposed to the long-term average of 14.2°C) and increased precipitation (exceeding the long-term average by 45 mm), which had a positive impact on the growth and development of potato seedlings. June, however, experienced a relatively drier period, with only half of the normal precipitation recorded. Nonetheless, early July brought rain, aiding plant development and facilitating the further formation of the crop following flowering.

The spring of 2021 witnessed favourable weather conditions for initiating field work, with soil preparation and potato planting conducted at the optimal period from April 5 to April 7, ensuring the speedy emergence of seedlings. May continued to feature elevated temperatures (14.5°C in contrast to the long-term average of 14.2°C) but was notably drier, with 28 mm less precipitation compared to the long-term average. This relatively dry condition had an initial adverse impact on the growth and development of potato plants. In June the dry spell continued, with only 37 mm of precipitation compared to the annual average of 80 mm. However, July brought more rainfall, mitigating the situation and creating more favourable growth conditions.

In 2022, during the start of field operations and the early stages of planting and the growth of early-ripening group plants, meteorological conditions had the most pronounced negative influence. In 2022, May and June experienced exceptionally high temperatures, surpassing the long-term averages by 4.5°C, and were marked by limited precipitation, with only 57 mm recorded compared to the long-term average of 80 mm. These conditions initially hindered the growth and development of potato plants. Nevertheless, July brought relief with 74 mm of rainfall (compared to the annual average of 85 mm), generally improving the conditions for plant growth and development. In 2022, the harvest yield was slightly higher when compared to 2020 (by 1.1-3.5 t/ha) and 2021 (by 1.4-3.8 t/ha), despite the earlier restraining meteorological conditions.

The relatively dry weather conditions during the 2020 - 2022 growing seasons significantly influenced the distribution of pathogens and the severity of potato diseases, particularly late blight (*Phytophthora infestans* (Mont.) de Bary) and Alternaria blight. These conditions, while unfavourable for late blight development, were rather optimal for the manifestation and harmfulness of Alternaria blight.

The initial signs of Alternaria blight on potato plants became evident during the formation of flower buds and at the onset of flowering. The disease exhibited a moderate progression during this phase, gradually extending its presence to encompass a larger portion of the plant. The disease severity levels were as follows: in the control, the range was from 2.0 to 11.5%, while in the experiment, it ranged from 7.7 to 20.9% at the third assessment. Furthermore, distinctions in lesion development were observed among potato varieties of different maturity groups. For early varieties, the severity of Alternaria disease ranged from 2.0 to 9.5% (control) and 7.7 to 17.6% (experiment), with the disease incidence from 5.6 to 17.8% (control) and 14.0-37.0% (experiment) of plants, respectively. Among mid-early varieties, the severity of Alternaria disease ranged from 2.5 to 11.5% (control) and from 8.6 to 20.9% (experiment), with the disease incidence of 8.0 and 22.9% (control) and 14.0-59.0% (experiment) of plants, respectively. In the medium-ripe variety group, the severity of Alternaria disease ranged from 2.4 to 4.7% (control) and 9.7 to 18.7% (experiment), with the disease incidence from 8.0 to 18.6% (control) and 24.5-53.5% (experiment) of plants, respectively. On average, over the course of three years of research, the following varieties demonstrated (partial) field resistance to Alternaria blight damage: Shchedryk among the early varieties (with disease severity at the end of the growing season at 7.7% and affecting 14.0% of the plants); Aria – among the mid-early varieties (with 8.6% of severity and 14.0% of incidence); and among the medium-ripe varieties, Kniahynia (9.6% and 35.5% respectively), Slovianka (9.7% and 30.0% respectively), Myroslava (10.0% and 28.5% respectively), and Khortytsia (10.4% and 24.5% respectively) (Table 2).

For Common scab development in 2020 – 2022, favourable conditions prevailed, as affirmed by the occurrence of the disease symptoms on the tubers of all studied varieties and significant disease severity observed on the majority of them. The manifestation of scab was facilitated by elevated temperatures and reduced precipitation levels during the latter part of the growth cycle of plants (Table 1). However, the year 2022 presented the least favourable conditions for this pathogen, resulting in a disease incidence on the majority of varieties that was 2-2.5 times lower than in the preceding years (Table 3).

In 2020, a decrease in the prevalence of *R*. *solani* was observed: the disease manifested only on a few studied varieties, with disease incidence generally remaining low (0-16%). The most conducive conditions for *R. solani* development were observed in 2021, notably characterized by low soil temperatures and early spring frosts with 16-62% disease incidence as a consequence.

Regarding Fusarium dry rot, a decline in disease incidence was in 2021 and 2022 compared to 2020, when up to 32% of the tubers were affected in the majority of the studied varieties (14 out of 20).

In the category of early potato varieties, observations of the development of pathogens showed that from 2020 to 2022 the extent of *R. solani* disease incidence on tubers ranged from 10.0 to 26.0% (1.9-5.1% in control); Common scab ranged from 4.6 to 14.7% (1.2-4.3% in control); and Fusarium dry rot ranged from 7.7 to 18.2% (1.5-3.6% in control). The Shchedryk variety displayed the highest field resistance to *R. solani*, with only 10.0% of affected tubers. Among the early varieties, the Skarbnytsia variety exhibited high field resistance to Common scab (4.6% affected tubers), while the other varieties were relatively resistant with disease incidence levels ranging from 6.1 to 14.7%. Notably, the Shchedryk variety also displayed field resistance to Fusarium dry rot (7.7%), with the Tyras (9.2%) and Kimmeria (9.7%) varieties showing adequate field resistance as well.

In the mid-early potato variety category, the percentage of tubers affected by *R. solani* ranged from 14.2 to 29.7%, indicating that none of the studied varieties displayed field resistance to the pathogen. Disease incidence for Common scab ranged from 7.7 to 11.5%, therefore, all varieties within this group demonstrated field resistance to the pathogen. Percentage of tubers affected by Fusarium dry rot ranged from 7.2 to 26.8%, where only the variety Aria demonstrated field resistance with the lowest degree of disease incidence (7.2%), while the other varieties were susceptible to the pathogen, with damage levels ranging from 14.0 to 26.8%.

Table 4.
Resistance and susceptibility rating of Ukrainian potato varieties to pathogens in the Polissya area of Ukraine, 2020-2022

No	Potato variety	Alternaria blight (*A. solani*, *A. alternata*)	*Rhizoctonia solani*	Common scab (*Streptomyces* spp.)	Fusarium dry rot (*Fusarium* spp.)
			Early maturity group		
1.	Kimmeria	S	S	R	R
2.	Skarbnytsia	S	S	HR	S
3.	Shchedryk	HR	R	R	HR
4.	Slauta	S	S	R	S
5.	Tyras	S	S	R	R
			Mid-early maturity group		
6.	Aria	HR	S	HR	R
7.	Zlahoda	S	R	R	S
8.	Strumok	S	S	HR	S
9.	Fantazia	S	S	R	S
			Medium-ripe maturity group		
10.	Hurman	S	S	R	S
11.	Slovianka	HR	S	HR	HR
12.	Anika	S	S	R	R
13.	Okolytsia	S	R	HR	S
14.	Myroslava	HR	S	R	S
15.	Ivankivska rannia	S	S	R	S
16.	Kniahynia	R	S	R	S
17.	Charunka	S	R	R	R
18.	Letana	S	S	S	HR
19.	Feia	S	R	R	S
20.	Khortytsia	R	HR	R	S

Note: HR - highly resistant, R - resistant, S - susceptible.

In the category of medium-ripe potato varieties, the percentage of tubers affected by *R. solani* ranged from 8.3 to 33.7%, by Common scab from 3.1 to 29.0%, and by Fusarium dry rot from 5.3 to 17.0%. Varieties Khortytsia, Okolytsia, and Feia exhibited a low level of *R. solani* disease incidence (8.3, 9.7 and 10.0% respectively) proving their field resistance to the pathogen. Varieties Okolytsia (3.1%) and Slovianka (4.9%) were highly resistant to Common scab, while the other varieties within this group, including Hurman, Feia, Kniahynia, and Myroslava, demonstrated relative field resistance to this pathogen. Slovianka (5.3%) and Letana (7.5%) were field resistant to Fusarium dry rot, while the majority of varieties within this group displayed an average field resistance to this pathogen (Table 3).

The data presented demonstrates, that several potato varieties exhibited field resistance to multiple diseases (Table 4). The varieties Kniahynia and Myroslava demonstrated resistance to the causative agents of two diseases: Alternaria blight and Common scab; varieties Anika, Kimmeria, and Tyras – to Common carb and Fusarium dry rot; varieties Feia and Okolytsia - to *R. solani* and Common scab.

The varieties Aria and Slovianka displayed relatively high field resistance to the causative agents of three diseases: Alternaria blight, Common scab, and Fusarium dry rot; whereas the variety Khortytsia exhibited resistance to the causative agents of Alternaria blight, *R. solani*, and Common scab and the variety Charunka – to *R. solani*, Common scab, and Fusarium dry rot. Within each ripening group, the presence and resulting resistance/susceptibility level to potato pathogens showed variations. While certain varieties exhibited resistance only to specific diseases, overall resistance was more pronounced in the early and mid-early groups, particularly in the variety Shchedryk, which demonstrated field resistance to all investigated pathogens.

Comparative analysis of the varieties showed that when there was no resistance to pathogens, potato yields were on average reduced with 16.7% during the study period. The highest yield losses were observed in the Slauta variety (17.0%) of the early maturity group, in the Zlahoda variety (16.6%) of the mid-early maturity group, and the Hurman (16.9%), and Okolytsia (18.0%) varieties of the medium-ripe group (Table 5).

Table 5.
Yield of Ukrainian potato variety in the Polissya area of Ukraine (2020-2022, t/ha)

		2020				2021				2022				average			
				difference to the control				difference to the control				difference to the control				difference to the control	
No	Potato variety	control	experiment	t/ha	%	control	experiment	t/ha	%	control	experiment	t/ha	t/ha	control	experiment	t/ha	%
Early maturity group																	
1.	Kimmeria	34.2	29.8*	4.4	12.9	32.5	28.1*	4.4	13.5	35.0	30.8*	4.2	12.0	33.9	29.6*	4.3	12.8
2.	Skarbnytsia	37.3	34.5*	2.8	12.0	30.9	27.0*	3.9	12.6	33.9	29.9*	4.0	11.7	34.0	29.9*	4.1	12.1
3.	Shchedryk	40.7	37.0*	3.7	9.1	38.7	35.0*	3.7	9.5	41.6	39.1*	2.5	8.4	40.3	36.7*	3.6	9.0
4.	Slauta	28.1	23.3*	4.8	17.2	29.4	24.1*	5.3	18.1	28.4	23.9*	4.5	15.7	28.6	23.7*	4.9	17.0
5.	Tyras	29.0	24.4*	4.6	16.7	25.9	21.4*	4.5	17.5	23.3	19.7*	3.6	15.4	26.1	21.8*	4.3	16.5
Average for the group														**32.6**	**28.2***	**4.4**	**13.5**
Mid-early maturity group																	
6.	Aria	37.7	34.9*	2.8	7.5	32.0	29.1*	2.9	9.0	35.5	33.0*	2.5	7.0	35.1	32.7*	2.7	7.8
7.	Zlahoda	25.4	21.2*	4.2	16.4	29.8	24.3*	5.5	18.5	26.3	22.4*	3.9	14.8	27.2	22.7*	4.5	16.6
8.	Strumok	29.2	24.7*	3.7	12.7	28.7	23.9*	4.8	16.6	25.4	22.4*	3.0	11.9	27.8	24.0*	3.8	13.7
9.	Fantazia	28.8	24.4*	3.4	11.8	30.1	25.6*	4.5	14.8	23.2	20.7*	2.5	10.7	27.4	24.0*	3.4	12.4
Average for the group														**29.4**	**25.7***	**3.7**	**12.6**
Medium-ripe maturity group																	
10.	Hurman	32.5	27.0*	5.5	16.9	23.7	19.4*	4.3	18.2	26.5	22.1*	4.1	15.5	27.6	22.9*	4.7	16.9
11.	Slovianka	37.4	34.4*	3.0	8.1	32.0	29.3*	2.7	8.6	43.4	40.2*	3.2	7.4	37.6	34.6*	3.0	8.0
12.	Anika	32.5	28.4*	4.1	12.7	21.7	18.8*	2.9	13.5	20.4	18.0*	2.4	11.7	24.9	21.8*	3.1	12.6
13.	Okolytsia	31.0	25.4*	5.6	18.1	27.0	21.8*	5.2	19.2	41.6	34.7*	6.9	16.6	33.2	27.2*	6.0	18.0
14.	Myroslava	35.8	32.8*	3.0	8.5	35.9	32.6*	3.3	9.1	54.6	50.3*	4.3	7.8	42.1	38.5*	3.6	8.5
15.	Ivankivska rannia	34.9	30.1*	4.8	13.7	31.9	27.1*	4.8	15.2	38.4	33.6*	4.8	12.6	35.1	30.3*	4.8	13.8
16.	Kniahynia	37.3	33.0*	4.3	11.6	37.7	32.8 *	4.9	12.9	49.5	44.2*	5.3	10.7	41.5	36.7*	4.8	11.7
17.	Charunka	29.1	25.4*	3.7	12.8	35.3	30.7*	4.6	13.1	33.3	29.4*	3.9	11.7	32.6	28.5*	4.1	12.5
18.	Letana	31.7	27.9*	3.8	12.0	28.0	24.4*	3.6	12.8	34.6	30.8*	3.8	11.0	31.4	27.7*	3.7	11.9
19.	Feia	32.4	27.5*	4.9	15.1	32.1	26.9*	5.2	16.3	36.1	31.1*	5.0	13.9	33.5	28.4*	5.1	15.1
20.	Khortytsia	27.1	24.6*	2.5	9.3	21.2	19.1*	2.1	9.8	23.4	21.4*	2.0	8.5	23.9	21.7*	2.2	9.2
Average for the group														**33.0**	**28.8***	**4.2**	**12.7**

* the difference with the control is significant at $p \leq .05$

Discussion

Ongoing research and studies on potato diseases and resistance at the Institute of Potato Research of NAAS date back to the 1970s and 1980s. Over the years, these efforts have resulted in the selection of potato varieties with significant (partial) field resistance to a range of pathogens, including Alternaria blight, Phytophthora, *R. solani*, Common scab, and Fusarium dry rot. These selected varieties have been important contributors to the development of other potato varieties, serving as donors of essential disease-resistant traits (Andriychuk & Homyak, 2013; Cherednychenko et al., 2008; Cherednychenko et al., 2016; Cherednychenko et al., 2019; Koval et al., 1978; Koval et al., 1979; Koval et al., 1983; Podhaietskyi et al., 1994; Polozhenets, 1997; Podhaietskyi et al., 2018).

The present investigation in the Polissya area of Ukraine showed that during 2020-2022 growing seasons Alternaria disease severity on the studied potato varieties was not exceeding 20.9% in the experiment group, while for *R. solani* it reached 29.9 %, for Common scab 29.0%, and for Fusarium dry rot 26.8%.

Our study highlights the importance of accounting for the specific disease resistance profiles of each potato variety when designing crop protection strategies. Varieties that exhibit a higher degree of susceptibility to wide range of potato pathogens, such as Fantazia, Hurman, Ivankivska rannia, Skarbnytsia, Letana, Slauta, Strumok and Zlahoda require meticulous (additional) protection measures, to mitigate crop losses and enhance overall quality.

To the contrary, varieties with field resistance to diseases, including Shchedryk (created with the involvement of multispecies hybrids 79/534/61 / 77.583/16), Aria (Delikat/Tyras), Slovianka (Kondor/KE78.50.53), Myroslava (Oberih/Bellarosa), Kniahynia (Slovianka/Bellarosa), and Khortytsia (UMO101696/Bellarosa), demonstrate noteworthy resistance to the key pathogens studied. This was in line with our previous studies, which took place under different weather conditions during the 2016-2017 seasons (Taktaev & Podberezko, 2020).

An analysis of the genealogy of these varieties suggests that the inheritance of resistance traits to specific diseases may be linked to the following varieties: Alternaria blight it is possibly derived from the varieties Amulet, Bellarosa, Bahriana and Slovianka; Rhizoctonia from Bahriana, Bellarosa, Delikat, and the hybrid KE 78.50.53; Common scab from Kondor, Bellarosa, and the hybrid 77.583/16; and Fusarium dry rot from Slovianka and the hybrid UMO 101696. The resistance exhibited by these varieties can significantly reduce the frequency of fungicide treatments during cultivation, thereby improving economic efficiency and promoting more ecological technology of potato production. These specific traits of the varieties should be thoughtfully considered when developing varietal technologies for their production.

The insights gained from these studies will continue to guide future research efforts, as such information on variety resistance is vital for the development of an effective potato protection system against harmful organisms. This knowledge contributes not only to the sustainability and efficiency of potato cultivation but also to the broader agricultural sector's efforts to address key challenges in crop disease management.

Conclusion and Recommendations

In conclusion, 12 out of 20 potato varieties developed within the Ukrainian selection program, including Anika, Aria, Charunka, Feia, Khortytsia, Kimmeria, Kniahynia, Myroslava, Okolytsia, Shchedryk, Slovianka, and Tyras displayed multiple (partial) field resistance to the pathogens causing Alternaria blight (*A. solani, A. alternata*), Rhizoctonia (*R. solani*), Common scab (*Streptomyces* spp.), and Fusarium dry rot (*Fusarium* spp.) These varieties stand out as promising candidates for inclusion in plant protection systems, offering the potential to significantly reduce the reliance on fungicide treatments, ultimately lowering production costs and increasing potato farming profitability.

Moreover, they present valuable resources in breeding programs for the development of new potato varieties with enhanced resistance.

While these findings shed light on the existence of resistance in these varieties, a profound study of the genetic basis of this resistance and the inheritance patterns from their parental forms is essential for a more comprehensive understanding. Such studies will not only strengthen our knowledge of resistance mechanisms but also contribute to the development of innovative and sustainable strategies for potato protection and breeding.

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Bio-priming Treatment with PGPB Strains in Cowpea Production Increases Grain Yield and Net Income

PGPB Strainleri ile Biyo-priming Uygulaması Börülce Üretiminde Tane Verimi ve Net Kazancı Artırır

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ABSTRACT

In the 21st century, the use of beneficial microorganisms as biological fertilizers has become a notable phenomenon, driven by the ongoing search for sustainable solutions due to environmental issues associated with synthetic fertilizer use. This study aimed to investigate the effect of bio-priming with plant growth-promoting bacteria (PGPB) strains comparing them with synthetic fertilizer and rhizobium inoculation in Siirt ecological conditions. The field experiment was laid out according to a completely randomized design with four replications in the arable land of Siirt University (Siirt, Türkiye) during the 2019 summer season. Three synthetic fertilizer doses as diammonium phosphate (SF1: 100 kg ha^{-1}, SF2: 200 kg ha^{-1}, SF3: 300 kg ha^{-1}) and seven biological fertilizer treatments (B1: TV61C, B2: TV62C, B3: TV126C, B4: TV24C, B5: TV53D, BMIX: TV119E+TV126C, RZB: *Bradyrhizobium* sp.) were compared with control (no fertilization+hydro-priming) in the study. The research results indicated that 300 kg ha^{-1} DAP and PGPB consortia showed the best results on agronomic characteristics. However, particularly when applied in the form of a consortium, PGPB strains exhibited performance very close to synthetic fertilization. Moreover, it was determined that 300 kg ha^{-1} DAP and PGPB consortia increased grain yield over hydro-primed plants by 54.6% and 42.4%, while they provided a net income of $654 and $721.6, respectively. Thus, bio-priming with PGPB increased higher net income compared with synthetic fertilizer due to lower treatment costs. In conclusion, bio-priming with PGPB strains has the potential of useful, sustainable and cost-effective strategy in cowpea production.

Keywords: Biological fertilizer, food security, grain legumes, net gain, sustainable agriculture, *Vigna unguiculata*

ÖZ

21. yüzyılda, sentetik gübre kullanımına ilişkin çevresel sorunlar nedeniyle sürdürülebilir çözümlerin arayışıyla tetiklenen faydalı mikroorganizmaların biyolojik gübre olarak kullanımı dikkate değer bir fenomen haline gelmiştir. Bu çalışma, Siirt ekolojik koşullarında bitki gelişimini teşvik edici bakteri (PGPB) suşları ile biyo-priming uygulamasının etkisini sentetik gübre ve rizobium inokülasyonuna göre karşılaştırmayı amaçlamıştır. Tarla denemesi, 2019 yaz sezonunda Siirt Üniversitesi (Siirt, Türkiye) arazisinde tesadüf blokları deneme desenine göre dört tekerrürlü olarak gerçekleştirilmiştir. Çalışmada diamonyum fosfat olarak üç sentetik gübre dozu (SF1: 100 kg ha^{-1}, SF2: 200 kg ha^{-1}, SF3: 300 kg ha^{-1}) ve yedi biyolojik gübre uygulaması (B1: TV61C, B2: TV62C, B3: TV126C, B4: TV24C, B5: TV53D, BMIX: TV119E+TV126C, RZB: *Bradyrhizobium* sp.) kontrolle (kimyasal gübre yok+hidro-priming) karşılaştırılmıştır. Araştırma sonuçları, 300 kg ha^{-1} DAP ve PGPB konsorsiyumunun tarımsal özellikler üzerinde en iyi sonuçları verdiğini göstermiştir. Ancak özellikle konsorsiyum şeklinde uygulandığında, PGPB suşları sentetik gübreleme ile çok yakın performans göstermiştir. Dahası, 300 kg ha^{-1} DAP ve PGPB konsorsiyumunun hidro-priming uygulanan bitkiler üzerinde %54,6 ve %42,4 artış sağlayarak, sırasıyla $654 ve $721,6 net kazanç sağladığı belirlenmiştir. Dolayısıyla, üstün PGPB suşlarıyla biyo-priming uygulaması, düşük uygulama maliyetine bağlı olarak sentetik gübreye kıyasla daha yüksek net kazanç sağlamıştır. Sonuç olarak, PGPB suşlarıyla biyo-priming uygulaması, börülce üretiminde kullanılabilir, sürdürülebilir ve düşük maliyetli bir strateji potansiyeline sahiptir.

Anahtar Kelimeler: Biyolojik gübre, gıda güvenliği, yemeklik baklagiller, net kazanç, sürdürülebilir tarım, *Vigna unguiculata*

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Introduction

Cowpea (*Vigna unguiculata*), which is an edible grain legume of the family Papilionaceae, shows a high adaptation to warm and adequate-rainfall ecological conditions and mostly cultivated at Southeast Asia, Southern United States, Latin America and Africa. The origin of cowpea is considered as Africa and traditionally cultivated in Mediterranean countries (Domínguez-Perles et al., 2015). Cowpea contributes the food chain for billions of people, mostly in developing countries, with annual production of about 9 million metric tons on 14.5 million hectares area worldwide (FAO, 2023). According to recent estimates, inadequate nutrition is responsible for more than one-third of child deaths worldwide. World Health Organisation (WHO) reported in 2020 that around 45% of child deaths in developing countries are associated with malnutrition, primarily due to the consumption of high-energy but less nutritious cereal-based foods (Okoth et al., 2017). Cowpea contains of 23-32% protein, 50-60% carbohydrates, and approximately 1% fat (Jayathilake et al., 2018). The total protein content is about two to four times higher than that of cereals and tuber crops. Moreover, cowpea protein is a rich source of the essential amino acid lysine when compared to cereal grains (Trehan et al., 2015). Cowpea is also a vital source of antioxidants, beneficial micronutrients, vitamins, and minerals (Owade et al., 2020). Thus, factors restricting cowpea yield and quality such as adverse climatic conditions, disease, pest damage or scarcity of plant nutrients play a critical role on food chain in especially developing countries.

Unfortunately, when it comes to developing countries, the issue is not only limited to nutritional inadequacy; there are also many factors that constrain agricultural production. Production of cowpea in developing countries such as Ethiopia are hindered by lack of modern Technologies, scarcity of advanced cowpea cultivars and accompanying disease and pest management practices, inputs such as fertilizers (Kebede et al., 2020). Another potential disaster scenario arises from the widespread use of uncontrolled synthetic fertilizers, which has become a global issue. Despite achieving high yields and quality in the short term, the use of synthetic fertilizers leads to a decrease in the biological diversity of agricultural soils (Tripathi et al., 2020), results in the deterioration of soil aggregate structure and carbon sequestration (Gupta et al., 2020), ultimately reducing soil fertility (Cai et al., 2019) and contributing to desertification (Huebner, 2023). Therefore, the use of biological fertilizers become a phenomenon to mitigate the damage caused by synthetic fertilizers to soil and the environment, while also providing essential nutrients for plant growth (Fasusi et al., 2023). More importantly, biological fertilizers not only contribute to plant nutrition but also help protect plants against adverse environmental factors and biotic stress conditions (Abdelaziz et al., 2023; Chieb & Gachomo, 2023; Hoque et al., 2023). Researchers have demonstrated that biological fertilizers are applied through various methods, such as foliar application, irrigation water, or seed application, and have shown positive results. Among these methods, the seed priming technique stands out as effective, environmentally friendly, and cost-efficient in providing benefits for plant growth (Akbar et al., 2019).

Seed priming that describes the soaking of seed to low osmotic potential of solution at pre-sowing is used to improve seed germination, seedling growth and also protect plants against biotic and abiotic stresses (Kumar et al., 2020; Singh et al., 2020). Seed priming provides faster and more homogeneous seed germination, boosts seedling development via controlled water uptake, activates starch disruption and enzyme actions, promotes ATP synthesis and antioxidant defense systems, thereby, increasing stress tolerance to negative environmental conditions (Aswathi et al., 2022; Farooq et al., 2017; Imtiaz et al., 2023). Seed priming is a cost-effective, easy-applicable and sustainable strategy for plant nutrition and protection (Sheteiwy et al., 2021). Most common priming techniques are hydro-priming, halo-priming, osmo-priming, nano-priming, bio-priming, solid matrix priming and thermo-priming (Hasanuzzaman & Fotopoulos, 2019). Out of these, bio-priming with plant growth promoting bacteria (PGPB) has a pivotal position due to superior properties including N fixation, P-solubilizing, ACC deaminase activity, indole acetic acid (IAA) and siderophore production, phytohormones secreting.

Research indicates that PGPB may not fully replace synthetic fertilizers, however, they have shown to be highly effective in facilitating reduced fertilization. Some research results even suggest that superior PGPB strains can provide higher yield and quality compared to synthetic fertilization. In such cases, adverse environmental conditions (salinity, drought, high temperatures, nutrient deficiencies, etc.) often suppress plant growth, and PGPB applications come into play, offering additional advantages beyond biological fertilization. In a broader perspective, PGPB applications have several advantages over synthetic fertilizers and align significantly with the vision of sustainability. This study aims to investigate the performance of PGPB strains comparing them with plants subjected to synthetic fertilizer and rhizobium inoculation in Siirt ecological conditions. The novelty of this study lies in the originality of the used PGPB strains and the unexplored results of their performance in cowpea cultivation under Siirt conditions.

Methods

Cowpea and Bio-priming Materials

“Karagöz”, which is a cowpea (*Vigna unguiculata*) cultivar, was used in the experiment. Özçelebi and Erman (2021) reported in their study on the adaptation of different cowpea genotypes to Siirt ecological conditions in which the highest grain yield was obtained from the “Karagöz”, therefore the variety was used in this experiment. Rhizobum culture for cowpea seeds was obtained from The Soil, Fertilizer and Water Resources Central Research Institute (Ankara, Türkiye). PGPB strains were isolated from Van Lake Basin within the scope of a TÜBİTAK project (108O147) at 2008 and performance of them were investigated under field conditions (Çakmakçı et al., 2010). The PGPB strains for bio-priming process were selected due to nitrogen fixation and phosphate solubilizing abilities. Taxonomic and isolation informations with superior properties of PGPB strains were given in Table 1.

Experimental Site

The field experiment was laid out from May to September 2019 at the Agricultural areas of Siirt University, Siirt, Turkiye. The experiment area was located at 37° 57’ N and 41°51’ E, an altitude of 585 m above sea level.

Climatic Characterization of Experimental Area

Mean temperatures (MT) of experimental year were lower than long years average (LYA) during march and april while they were higher compared with LYA during other months. Monthly total precipitaion (MTP) was significantly higher at experimental season compared with LYA data. The lowest and highest MT of experimental season were 8.3 °C and 32.0 °C, respectively. Climatic characterization of experimental season and its alteration as LYA was schematized in Figure 1.

Soil Characteristic of Experimental Area

The soil sample was obtained by mixing samples taken from different points of the A horizon. According to the analyses conducted on the sample, the soil in the experimental area has a clay-loam texture, a slightly alkaline, and calcareous structure. It is characterized as having a low content of organic matter and available phosphorus, while being sufficient in terms of available potassium (Table 2).

Table 1.
Taxonomic and isolation informations with superior properties of PGPB strains

Abb	Code	Taxonomy	Location	N fixation	P solubilizing
B1	TV61C	*Bacillus megaterium*	Çakırbey Köyü/Van	+	-
B2	TV62C	*Acinetobacter baumanii*	Tendürek/Van	+	-
B3	TV126C	*Pseudoalteromonas tetraodonis*	Ulupamir Köyü/Van	S	L
B4	TV24C	*Pseudomonas agarici*	Ulupamir Köyü/Van	+	L
B5	TV53D	*Brevibacillus choshinensis*	Çakırbey Köyü/Van	S	S
-	TV119E	*Bacillus* sp.	Ulupamir Köyü/Van	L	+
BMIX	TV119E+TV126C				

Abb: Abbreviation, +: Positive, S: Strong, L: Low, -: Negative

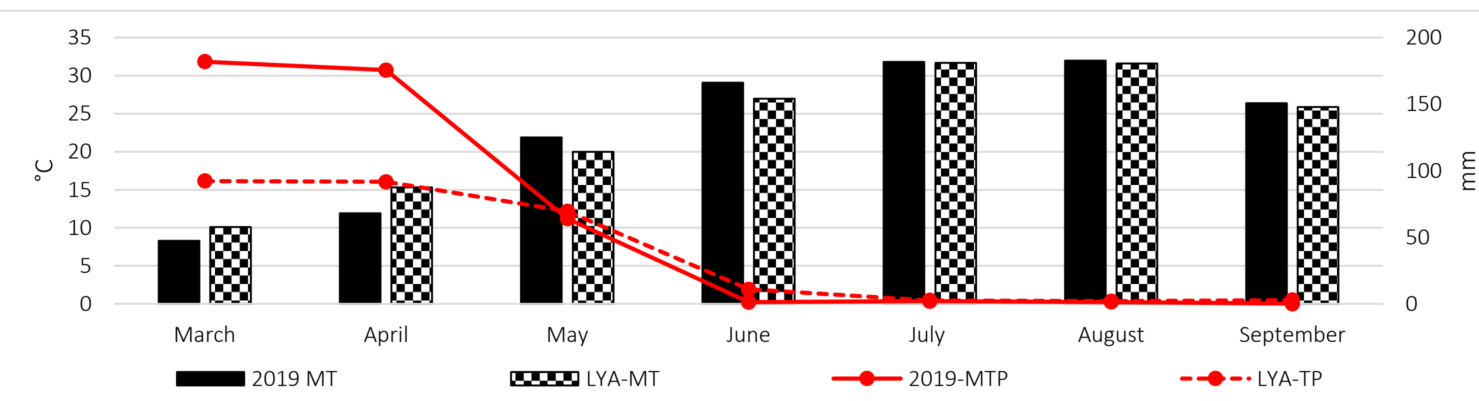

Figure 1.
Climatik properties of experimental area
MT: Mean temperature, LYA: Long years average, MTP: Monthly total precipitaion, TP: Total precipitation

Table 2.
Chemical composition of experimental soil

Sand (%)	Silt (%)	Clay (%)	Texture	pH	EC (S/cm)	Lime (%)	OM (%)	P (kg/da)	K (kg/da)
39.1	11.4	49.5	CL	7.78	685	10.1	1.2	2.02	147

CL: Clay-loam, OM: Organic matter

Laying out of bio-priming process

The process of preparing suspensions of strains preserved at -86 °C involved the use of solid and then liquid nutrient media. Samples obtained with the help of a loop from bacterial strains preserved for 72 hours on Nutrient Agar (NA) were inoculated into 100 ml of Nutrient Broth (NB) and incubated horizontally at 30 °C with constant shaking at 150 rpm overnight. The concentration of the obtained bacterial suspensions was adjusted to approximately ~108 CFU ml- using a turbidimeter by diluting them with sterile distilled water (Sonkurt & Çığ, 2019). Seeds to be used in the research were first weighed and subjected to surface sterilization by immersing them in 70% ethanol for 1 minute, followed by a 5-minute immersion in 10% NaOCl. After sterilization, they were washed three times with distilled water, placed on a sterile filter paper, and dried at room temperature. The prepared bacterial suspensions were added to autoclaved capped flasks at a seed:suspension ratio of 1:5 g/ml and sterilized at 121 °C for 20 minutes in an autoclave (HIRAYAMA, HV-110L, Japan) (Ceritoglu & Erman, 2021). Seeds were placed in the flasks for each bacterial suspension. The capped flasks were placed on a shaker (WiseShake, SHR-2D, Germany) and incubated at 120 rpm and room temperature for 2 hours. Hydro-primed seeds underwent hydro-priming, and distilled water was used as the priming solution. Hydro-priming was conducted as described in bio-priming, with seeds soaked in the solution for 12 hours (Farooq et al., 2019). After hydro-priming, seeds were air-dried between filter papers for 10-12 hours and stored in a refrigerator at 4 °C until sowing.

Experimental design

The field experiment was laid out according to a completely randomized design with four replications in erable land of Siirt University, Siirt, Türkiye during 2019 summer season. Three synthetic fertilizer doses as diammonium phosphate (SF1: 100 kg/ha, SF2: 200 kg/ha, SF3: 300 kg/ha), six PGPB Strains (B1: TV61C, B2: TV62C, B3: TV126C, B4: TV24C, B5: TV53D, BMIX: TV119E+TV126C) and 1 *Bradyrhizobium* inoculation were compared with hydro-primed plants. All treatment was accepted as a single factor in the experiment, i.e., did not cross each other.

Each plot was formed with 6 rows which was determined with 60 cm of inter-row distance (Çulha & Bozoğlu, 2017) and 5 m length, i.e., plot size was 15 m2. Seeds were sown with 10 cm intervals on rows. Before and after flowering, weed control has been carried out twice with hoeing. Throughout the study, due to high temperatures and lack of rainfall, to prevent drought stress in plants during the necessary periods, a total of 4 times irrigation has been applied through the drip irrigation system. No signs of diseases or harmful agents have been observed in the experimental area. After throwing one row each from the edges of each plot and 50 cm from the beginnings of the plots as edge effects, the remaining plants were harvested. Harvesting was done manually, and after drying the plants in the open field, the threshing process was performed.

Experimental observations

In the study, flowering period and pod-setting period were determined for the examination of phenological characteristics. For the determination of both characteristics, the periods when 50% of the plants in the plot flowered and 50% set pods were considered. Prior to harvest, on randomly selected 10 plant samples from each plot, plant fresh weight, plant dry weight, seedling length, node count, first pod height, pod length, seed count in the pod, and pod count on the plant were determined. For determining the dry weight of seedlings, plant samples were preserved in an oven set at 68 °C for 72 hours, then removed, and their weights were immediately determined. After the threshing process, the per-hectare grain yield was determined based on the obtained grains.

Economic Analysis

The cost of variable inputs and total income from grain yield was taken into consideration to calculate the economic analysis of treatments. Net income and benefit-cost ratio were calculated by the formulae of CIMMYT (1988).

Statistical analysis

The data obtained within the scope of the research were subjected to analysis of variance using a randomized complete block design. Tukey's Honestly Significant Difference (HSD) test was employed for grouping the means. Statistical calculations were performed using the JMP program.

Results

In the conducted research investigating the effects of different fertilizer methods on cowpea cultivation, it has been observed that, in almost all examined characteristics, the lowest values were obtained from hydro-priming. Additionally, it has been noted that increasing doses of synthetic fertilizers parallelly led to an increase in plant growth and yield components, with different reactions emerging depending on the species in the application of PGPB. While Rhizobium inoculation showed positive results compared to the hydro-priming, it was determined to be a less satisfactory solution compared to synthetic fertilizer. In the case of bio-priming application, competitive results with synthetic fertilizer were obtained in the group where a consortium was applied compared to individual PGPB applications.

Analysis of variance for the data indicated that there was no statistically significant difference in flowering time and plant fresh weight. However, it was recorded that statistically significant differences ($p < .01$) occurred in all other parameters depending on the fertilization systems. When examining the coefficients of variation for the characteristics, all parameters, except for plant dry weight (%24.3), were found to be below 20% (Table 3).

Table 3.
Analysis of variance on investigated characteristics

Trait	DF	VC	Mean of square	F prob.
Flowering time		4.36	7.660	nsd
Plant fresh weight		5.87	15426.500	nsd
Plant dry weight		24.3	105.800	**
Plant height		5.47	956.700	**
Number of nods	10	10.9	319.000	**
Pod length		13.3	22.600	**
Number of seeds per pod		7.36	15.790	**
Number of pods per plant		14.7	8.999	**
Grain yield		2.18	2187.200	**

DF: Degree of freedom, VC: Variation of coefficiency, nsd: No significant difference, **: $p < .01$

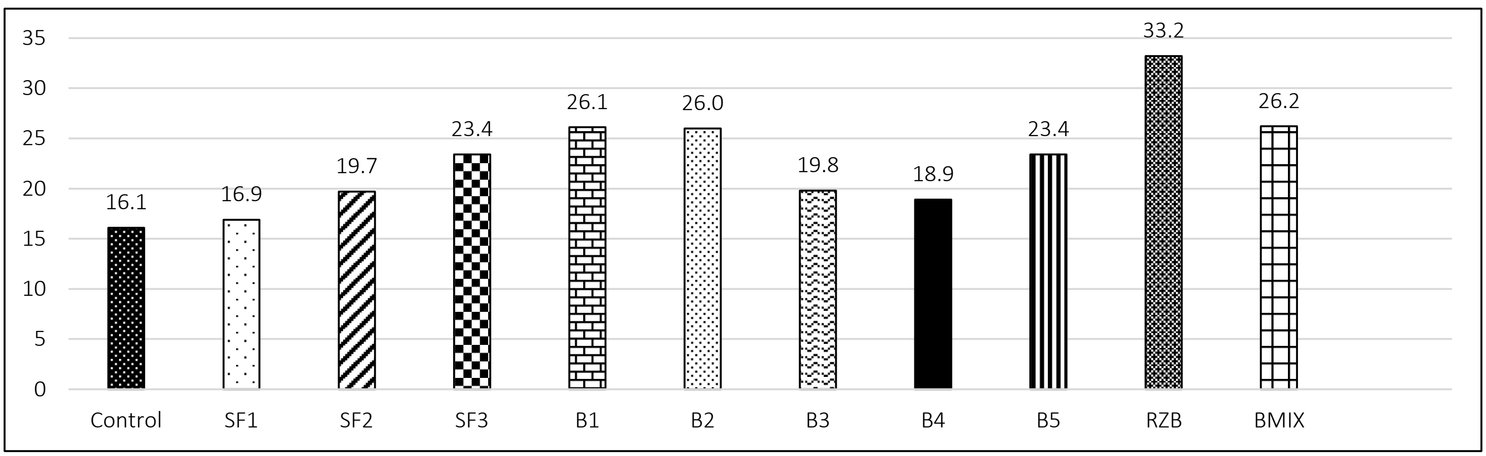

Figure 2.
Dry matter accumulation in cowpea plants depending on treatments
SF1: 100 kg ha^{-1}, SF2: 200 kg ha^{-1}, SF3: 300 kg ha^{-1}, B1: TV61C, B2: TV62C, B3: TV126C, B4: TV24C, B5: TV53D, BMIX: TV119E+TV126C, RZB: Bradyrhizobium sp.

The flowering time and plant fresh weight varied between 55.3-60.0 days and 37.4-67.4 g, respectively, in the study, with no statistically significant differences observed in the averages. The plant dry weight ranged from 16.1 to 33.2 g. The lowest dry matter accumulation was observed in the hydro-priming, while the highest value was detected in plants inoculated with rhizobium (Figure 2). Depending on the treatments, the lowest plant height (42.7 cm) was observed in the hydro-primed plants, while the tallest plant height (88.9 cm) was obtained from the 300 kg/ha DAP application, followed by the group treated with BMIX priming. The lowest and highest number of nodes (9.8-19.3) were observed in the B1 application and the 300 kg/ha DAP fertilization, respectively. Similarly, the shortest pod length (6.32 cm) was observed in the hydro-primed plants, while the longest pod length (13.92 cm) was observed in the 300 kg/ha DAP fertilization. The number of seeds in the pod varied between 3.74 and 10.88, with the lowest and highest values recorded in the B2 application and the 300 kg/ha DAP fertilization, respectively. While the lowest number of pods per plant was determined in the B4 application, the highest values were observed in the same statistical group, including the 300 kg/ha DAP, B5, and BMIX applications. Although the lowest grain yield (1456 kg/ha) was obtained in the hydro-priming, the results obtained from the B4-treated plants (1505 kg/ha) were statistically in the same group. The highest grain yield was obtained from the 300 kg/ha DAP application at 2251 kg/ha, followed by BMIX at 2074 kg/ha (Table 4).

Table 4.
Means of agronomic and yield attributes of cowpea plants under different fertilizations

Treatment	FT (day)	SFW (g)	SDW (g)	PH (cm)	NN	PL (cm)	NSP	NPP	GY (kg/ha)
Control	60.0	48.7	16.1^{b}	42.7^{f}	10.1^{d}	6.32^{e}	5.95^{ef}	3.75^{cd}	1456^{f}
SF1	58.8	37.4	16.9^{b}	50.9^{de}	11.9^{cd}	7.42^{de}	6.88^{c}-^{e}	5.45^{abc}	1749de
SF2	55.3	43.8	19.7^{ab}	59.1^{bc}	14.5^{bc}	8.59^{cde}	7.43^{cd}	6.33^{ab}	1952^{c}
SF3	57.8	49.5	23.4^{ab}	88.9^{a}	19.3^{a}	13.92^{a}	10.88^{a}	7.33^{a}	2251^{a}
B1	57.8	52.0	26.1^{ab}	52.2^{cde}	9.8^{d}	7.89^{cde}	4.61gh	4.60^{bcd}	1734de
B2	57.0	48.4	26.0^{ab}	51.9^{cde}	11.8^{cd}	7.09^{de}	3.74h	4.97^{bc}	1786^{d}
B3	58.3	37.6	19.8^{ab}	44.7^{ef}	11.9^{cd}	7.73^{cde}	7.73^{b}-^{d}	3.95^{cd}	1654^{e}
B4	56.5	39.9	18.9^{b}	53.2^{cd}	11.7^{cd}	9.71^{bcd}	6.53^{d}-^{f}	2.87^{d}	1505^{f}
B5	57.6	64.1	23.4^{ab}	64.5^{b}	13.7^{bc}	10.68^{bc}	7.95^{bc}	7.09^{a}	1891^{c}
RZB	59.0	52.3	33.2^{a}	57.4^{bcd}	12.2^{cd}	8.91^{cde}	5.60^{f}g	4.68^{bcd}	1741de
BMIX	56.0	67.4	26.2^{ab}	87.3^{a}	16.4^{ab}	12.61^{ab}	8.83^{b}	7.07^{a}	2074^{b}
Mean	57.7	49.2	22.7	59.3	13.0	9.17	6.92	5.28	1799

SF1: 100 kg ha^{-1}, SF2: 200 kg ha^{-1}, SF3: 300 kg ha^{-1}, B1: TV61C, B2: TV62C, B3: TV126C, B4: TV24C, B5: TV53D, RZB: *Bradyrhizobium* sp., BMIX: TV119E+TV126C, FT: Flowering time, PFW: Plant fresh weight, PDW: Plant dry weight, PH: Plant height, NN: Number of nods, PL: Plant length, NSP: Number of seeds per pod, NPP: Number of pods per plant, GY: Grain yield

The effects of different applications on grain yield and the economic returns of these variations are presented in Table 5. According to the table, the applications that most significantly increase grain yield in comparison to hydro-priming are determined as 300 kg ha^{-1} DAP (54.6%), BMIX (42.4%), and 200 kg ha^{-1} DAP (34.1%). Additionally, calculations were conducted by taking into account the additional gain and expenses compared to the hydro-priming, resulting in the determination of the net gain arising from the applications. According to net income data, the highest net gain per ha area over hydro-priming is observed in the following order: BMIX ($721.6), 300 kg ha^{-1} DAP ($654), and B5 ($502). When evaluated in terms of benefit:cost ratio, the highest value of 36.1 was achieved in the BMIX application, while the lowest ones were obtained from synthetic fertilizer applications (2.0-2.5) and B5 (1.9).

Discussion

The gradual increase in all statistically significant features due to the increase in synthetic fertilizer doses is evident. When the soil characteristics of the experimental area are examined, it is observed that the organic matter content and the available phosphorus amount are very low (Table 2). Therefore, due to the already low levels of nitrogen and phosphorus sources in the soil, plants have shown a significant response to chemical fertilization, exhibiting increasing development and productivity up to the highest DAP level. Numerous scientific studies since the Green Revolution have indicated that synthetic fertilization provides a rapid solution for plant development and yield, and these responses are more visibly manifested in deficiency conditions (Ishikawa et al., 2022), however, in terms of long-term effects, it is pointed out that this leads to the deterioration of soil and underground resources, ultimately threatening living health (Tripathi et al., 2020).

Among the characters investigated in the study, the highest results were obtained in the application with the highest DAP level of 300 kg/ha in all features except plant age and dry matter weight. In terms of seedling fresh weight and seedling dry weight, the best results were observed in the RZB and BMIX applications, respectively. Biological nitrogen fixation provides nitrogen support to plants from a few weeks after seed germination until the grain-filling period. However, compared to chemical fertilization, it offers a slower and more sustained nitrogen support. Studies suggest that a portion of the nitrogen applied to the soil through chemical fertilization undergoes leaching, while another part undergoes denitrification due to evaporation (Geng et al., 2022; Zhang et al., 2023). Nitrogen obtained through biological means directly binds to the plant root zone without any losses (Guo et al., 2023). Therefore, plants receiving nitrogen support until the late stages are believed to produce higher biomass in post-harvest plant samples and consequently accumulate more dry matter. Zhu et al. (1988) reported that biological nitrogen fixation led to an increase in dry matter accumulation in annual clover plants. Rodrigues et al. (2013) observed an increase in total biomass and dry matter accumulation in cowpea seeds inoculated with Bradyrhizobium, and when applied in conjunction with PGPB, it showed a synergistic effect, further enhancing the accumulation.

Table 5.
Economic analysis of grain yield depending on fertilization strategies

Fertilizer treatment	Increase in seed yield (%)	Difference over control (kg ha^{-1})	Difference in gain over control ($)	Cost of fertilizer ($)	Net gain over control ($)	Benefit:Cost ratio
Control	-	-		-	-	-
SF1	20.1	293	351.6	100	251.6	2.5
SF2	34.1	496	595.2	200	395.2	2.0
SF3	54.6	795	954.0	300	654.0	2.2
B1	19.1	278	333.6	20	313.6	15.7
B2	22.7	330	396.0	20	376.0	18.8
B3	13.6	198	237.6	20	217.6	10.9
B4	3.4	49	58.8	20	38.8	1.9
B5	29.9	435	522.0	20	502.0	25.1
RZB	19.6	285	342.0	20	322.0	16.1
BMIX	42.4	618	741.6	20	721.6	36.1

SF1: 100 kg ha^{-1}, SF2: 200 kg ha^{-1}, SF3: 300 kg ha^{-1}, B1: TV61C, B2: TV62C, B3: TV126C, B4: TV24C, B5: TV53D, RZB: *Bradyrhizobium* sp., BMIX: TV119E+TV126C, FT: Flowering time, PFW: Plant fresh weight, PDW: Plant dry weight, PH: Plant height, NN: Number of nods, PL: Plant length, NSP: Number of seeds per pod, NPP: Number of pods per plant, GY: Grain yield

Out of PGPB strains, the B5 has exhibited a performance so effective that it falls into the same statistical group as the consortium and synthetic fertilizer in terms of the number of pods in the plant. When the superior characteristics of PGPB strains are examined, the B5 strain (TV53D) shows a higher capability in N-fixation and P-solubilization compared to other isolates (Table 1). Therefore, it is considered more effective due to its ability to meet the plant's N requirements and the dissolution of P sources in the insoluble form in the rhizosphere, which can then be taken up by plant roots (Erman & Ceritoglu, 2022). Furthermore, when the characteristic features of the bacterial strains forming the consortium (TV119E and TV126C) are examined, it is predicted that they are strong in N fixation and P solubilization and exhibit a synergistic effect on each other. The applications that exhibit superior performance in terms of grain yield (300 kg ha^{-1} DAP and BMIX) are noteworthy for significantly improving not only plant development but also factors influencing yield. Ceritoglu & Erman (2020) reported statistically significant differences among agronomic traits symbolizing grain yield, pod number per plant, seed number per pod, and plant development in chickpea. Various researchers have reported that consortia consisting of PGPB strains can yield superior results compared to the individual performances of the strains (Çığ et al., 2021; Timofeeva et al., 2023). Many researchers denoted that PGPB increases grain yield (Galindo et al., 2022; Ma et al., 2019) in cowpea. Thus, the results of the experiment are in agreement with the fundings of previous researchers.

The BMIX application proves to be highly effective, nearly reaching the maximum synthetic fertilizer dosage, thereby influencing grain yield significantly. However, when considering application costs and the potential for extra gains, another advantage of PGPB (Plant Growth-Promoting Bacteria) applications emerges. The results indicate that PGPB applications allow for higher net gains compared to synthetic fertilization due to their lower application costs. Furthermore, it is found that, apart from the consortium used, the B5 application also outperforms synthetic fertilization in terms of economic gains. This finding makes bio-priming applications more attractive for agricultural production and creates a convincing scenario with potential benefits.

Conclusion and Recommendations

In this study, the effects of synthetic and biological fertilizer applications on the growth and yield of cowpea under Siirt ecology and field conditions were investigated. The research results indicate that, in terms of agronomic characteristics, the application of 300 kg ha^{-1} DAP showed the best results. However, particularly when applied in the form of a consortium, Plant Growth-Promoting Bacteria (PGPB) strains exhibited performance very close to synthetic fertilization. However, economic analysis of obtained crop indicated a novel scenerio in which 300 kg ha^{-1} DAP and PGPB consortia increased grain yield over hydro-priming 54.6% and 42.4%), while they provided net gain as $654 and $721.6, respectively. Thus, bio-priming with PGPB increased higher net gain compared with sythetic fertilizer due to lower treatment cost. In conclusion, bio-priming with superior PGPB strains might be a usefull, sustainable and cost-effective strategy in cowpea production.

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Walnut Seed Coat (*Juglans regia* L.), a Plant Effective in Human Health: Antioxidant Activity and in Rats Nephroprotective Effect

İnsan Sağlığında Etkili Bir Bitki Ceviz Tohumu Kabuğu (*Juglans regia* L.): Antioksidan Aktivitesi ve Sıçanlarda Nefroprotektif Etkisi

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ABSTRACT

In the study, the seed coat (WSC) of Posof (Ardahan/Türkiye) walnuts was extracted to determine their phytochemical components and antioxidant capacities. The effects of bioactive components in the ethanol extract of WSC (E-WSC) on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors were investigated. Additionally, antioxidant enzyme activity parameters were measured in the kidney tissues of Triton WR-1339-induced hyperlipidemic rats. Bioactive compounds in WSC were identified by GC-MS system. The antioxidant properties of WSC were measured using Fe^{+3}, Cu^{+2} and Fe^{+3}-2,4,6-tripyridyl-s-triazine (TPTZ) reducing agent, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'- azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activities. In this analysis, using 30 male Wistar rats (300 ± 30 g) randomly divided into five groups were treated as follows; K1: Healthy control group, K2: E-WSC (150 mg) o.d., K3: E-WSC (300 mg) o.d., K4: Hyperlipidemic group i.p., K5: Hyperlipidemic group i.p. + E-WSC (300 mg) o.d. Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) analyzes were performed in kidney tissues. Based on these results, it was clearly determined that E-WSC has significant antioxidant activity due to its bioactive components, has an inhibitory effect on AChE and BChE enzymes, and has a protective effect against oxidative stress by improving hyperlipidemia-related kidney damage.

Keywords: Antioxidant activity, kidney, oxidative stress, Triton WR-1339; walnut seed coat

ÖZ

Çalışmada, Posof (Ardahan/Türkiye) cevizlerinin tohum kabuğu (WSC), fitokimyasal bileşenlerinin ve antioksidan kapasitelerinin belirlenmesi için ekstrakte edilmiştir. WSC'nin (E-WSC) etanol ekstraktındaki biyoaktif bileşenlerin asetilkolinesteraz (AChE) ve butirilkolinesteraz (BChE) inhibitörleri üzerindeki etkileri araştırılmıştır. Ayrıca Triton WR-1339 ile indüklenen hiperlipidemik sıçanların böbrek dokularında antioksidan enzim aktivite parametreleri ölçülmüştür. WSC'deki biyoaktif bileşikler GC-MS sistemi ile tanımlanmıştır. WSC'nin antioksidan özellikleri, Fe^{+3}, Cu^{+2} ve Fe^{+3}-2,4,6-tripiridil-s-triazin (TPTZ) indirgeyici ajan, 1,1-difenil-2-pikrilhidrazil (DPPH) ve 2,2'- azino-bis (3-etilbenzotiyazolin-6-sülfonik asit) (ABTS) temizleme aktiviteleri kullanılarak ölçülmüştür. 30 adet erkek Wistar sıçanı (300 ± 30 g) kullanılarak yapılan bu analizde, rastgele beş gruba ayrılan sıçanlara aşağıdaki gibi uygulama yapılmıştır; K1: Sağlıklı kontrol grubu, K2: E-WSC (150 mg) o.d., K3: E-WSC (300 mg) o.d., K4: Hiperlipidemik grup i.p., K5: Hiperlipidemik grup i.p. + E-WSC (300 mg) o.d. Böbrek dokularında Süperoksit dismutaz (SOD), katalaz (CAT) ve malondialdehit (MDA) analizleri yapılmıştır. Bu sonuçlara göre E-WSC'nin biyoaktif bileşenlerine bağlı olarak önemli antioksidan aktiviteye sahip olduğu, AChE ve BChE enzimleri üzerinde inhibitör etkiye sahip olduğu ve hiperlipidemiye bağlı böbrek hasarını iyileştirerek oksidatif strese karşı koruyucu etkiye sahip olduğu açıkça belirlenmiştir.

Anahtar Kelimeler: Antioksidan aktivite, böbrek, oksidatif stres, Triton WR-1339, ceviz tohumu kabuğu

Introduction

Studies carried out to provide valuable products in terms of bioactive components against health problems that may occur due to the consumption of products with high amounts of harmful chemical substances have become the focus of attention recently. (Soccol et al., 2017; Zhang et al., 2016). Therefore, metabolites obtained from plants emerge as natural products of great importance of developing most of the existing drugs (Xu et al., 2019). The codeine alkaloid found spontaneously in Papaver spp. (Dicpinigaitis et al., 2014), quinine, a component of the Cinchona genus (Achan et al., 2011), and aspirin derived from the Salix genus are examples of these metabolites (Oketch-Rabah et al., 2019).

Clinical and epidemiological studies have revealed that consuming foodstuffs, especially those with herbal ingredients, protects by producing solutions against some problems, especially cardiovascular diseases and immune system problems (Aune et al., 2016). Phytochemicals such as carotenoids and polyphenols, when used alone or in combination, act as an effective defense mechanism in stopping disease formation and progression as they prevent the inflammatory state and possible oxidation (Hever & Cronise, 2017). Polyphenols, a wide-ranging secondary metabolite group containing important contributors such as phenolic acid and flavonoids, provide a great advantage to human health with important activities such as cytotoxic, anti-inflammatory and, cardioprotective, with many studies (Di Mauro et al., 2019; Fraga et al., 2019). In addition to these, it also eliminates the risk of many diseases with its antioxidant properties (Shahidi & Ambigaipalan, 2015; Silinsin and Bursal 2018). Plant polyphenols, which have radical scavenging, hydrogen donor functions, reducing and metal chelating activity and antioxidant properties against reactive nitrogen and oxygen species in the system can also balance energy in enzymatic pathways. (Bjørklund & Chirumbolo, 2017; Limmongkon et al., 2017). Many studies have confirmed that some macro and micronutrients of shell foods have positive effects on health through various mechanisms (Pei & Lu 2011; Rusu et al., 2019). To determine the antioxidant compounds in the plant, the extraction process is performed using a solvent. Ethanol, water, methanol, ethyl acetate (Hazli et al., 2019; Rasera et al., 2019), chloroform (Fernández-Agulló et al., 2013), and N-butanol are among the most commonly used solvents. Depending on the solvent used, the antioxidant activity and yield of the plant vary (Lateef et al., 2012).

Hyperlipidemia, which develops as a result of variable lipid metabolism, is defined as elevated serum triglyceride (TC), total cholesterol (TG), and low-density lipoprotein-cholesterol (LDL-C) levels (Nie et al., 2018). This disorder is also the main risk factor for the development of cardiovascular diseases including atherosclerosis and coronary heart disease among the most important health and economic problems (Cicero & Colletti, 2016). Additionally, hyperlipidemia can cause solid organ damage, including kidney damage (Zong-liang et al., 2006). Experimental and epidemiological studies have shown that high-density lipoprotein-cholesterol (HDL-C) acts as an anti-atherogenic in the prevention of atherogenic diseases triggered by atherosclerotic plaques formed by the accumulation of excess LDL-C on the arterial wall (Alissa & Ferns 2017). Oxidative stress disorders and disorders in the antioxidant defense system, which play a key role in the pathogenesis of hyperlipidemia and these critical diseases, are associated with excessive increase in reactive oxygen species in the biological system (Nijhawan et al., 2019) as these radicals cause modifications in LDL-C molecules (Yang et al., 2017). While oxidation of lipids causes the formation of many toxic products such as oxidized LDL-C and malondialdehydes (MDA) (Alissa & Ferns 2017), it also inhibits antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT).

Triton WR-1339, a nonionic detergent, is frequently used to establish an acute hyperlipidemia model in animals to evaluate potential hyperlipidemic drugs (Bertges et al., 2011). It inhibits the uptake of circulating lipoproteins by extrahepatic tissues, resulting in a high plasma lipid profile and in increased blood lipoprotein levels (Da Rocha et al., 2009). In other words, by inhibiting lipoprotein lipase (LPL) activity, it causes the accumulation of very low-density lipoprotein (VLDL) and TGs and reduces HDL-C production (Zarzecki et al., 2014). Studies have shown that a single intraperitoneal administration of Triton WR-1339 to adult rats produces hyperlipidemia within 24 hours (Palabiyik et al., 2022; Sheikha et al., 2018). Statins are a group of synthetic drugs used as a primary step in regulating lipid metabolism and reducing LDL-C levels (Nelson, 2013). The use of these traditional pharmacological drugs used in the treatment of hyperlipidemia has been limited due to the side effects they may cause (Chien et al., 2019; Chiu et al., 2019). Therefore, search for a cheap, safe, and easily available functional food in hyperlipidemia studies is an important and scientific area of interest (Chiu et al., 2019). Medicinal plants and natural components are considered excellent alternative medicine sources and may show anti-hyperlipidemic activity of treating experimentally induced diseases (Abdallah et al., 2020).

Walnut (*Juglans regia* L.), a very valuable product in terms of economic importance, belongs to the Juglandaceae family. It has a worldwide production, especially in mild climate conditions. It contains good nutritional and nutraceutical components (Acquaviva et al., 2021; Bernard et al., 2018) and is rich in unsaturated fatty acids (Linolenic, Oleic, Palmitic acid, etc.), proteins, tocopherols (g-tocopherol), phytocetrols, vitamin E and polyphenols (Ellagic, Tannins, Gallic acid) (Ros et al., 2018). It is considered as a nutraceutical in terms of this content. In addition to the antioxidant effect protecting the living body from reactive oxygen species and free radicals (Nunes et al., 2012), its antibacterial, anti-inflammatory, cytotoxic and prebiotic effects have paved the way for its use in various industries such as food, medicine and cosmetics, especially phytotherapy (Alasalvar & Bolling, 2015; Jahanban-Esfahlan et al., 2019). It has been observed that the results similar to these positive bioactivities of walnut on health and the results of the analysis made on other parts of the walnut (leaves, green peel, shell, skin etc.) (Cosmulescu et al., 2015; Vieira et al., 2020) support each other. In addition, it has been reported that the septum exhibits hypoglycemic, hematological regeneration and anti-aging activities (Rusu et al., 2020).

The main aim of this study is to determine the bioactive components and antioxidant activity of WSC with an experimental setup. While GC-MS analysis was used to detect phenolic bioactive components, different methods such as Fe^{3+}, Cu^{2+}, DPPH•, $ABTS^{•+}$ and FRAP were employed to evaluate antiradical and antioxidant activity. Besides, few studies have investigated the use of E-WSC to determine the protective effect in the hyperlipidemic state in Triton WR-1339-induced rats. Therefore, our study is significant as it is one of the few studies evaluating the effect of E-WSC on oxidative stress parameters in the kidney tissues of Triton WR-1339-induced hyperlipidemic Wistar (male) rats.

Methods

Reagents and Chemicals

Triton WR-1339 was purchased from Santa Cruz Biotechnology (California, USA). Kits used for oxidative stress (MDA) and antioxidant enzyme activity (SOD and CAT) measurements were obtained from SunRed Biological Technology Co., Ltd (Shanghai, China). Ethanol (C_2H_5OH) was obtained from isolab (Wertheim, Germany). Sevorane, AbbVie Medical Pharmaceuticals Industry, and Trade Ltd. Co. was obtained from İstanbul/Türkiye.

Plant materials

Walnut seed coats were extracted from walnuts obtained from Posof (Ardahan/Türkiye). The samples were dried at room temperature to avoid exposure to sunlight. The powdered substance was stored at -18 °C.

Extraction of WSC with Ethanol

50 g of WSC dissolved in 1000 mL of ethanol was stirred in a magnetic stirrer (Heidolph MeiR H-Standard, Schwabach, Germany) for 3 days and then extracted at 60-80°C for 4 hours. After separating the waste material with a sieve, the solvent was evaporated (Heidolph, Schwabach, Germany) at 155 rpm and 50 °C. The extract was preserved at +4°C by drying in an oven (Binder, Tuttlingen, Germany).

Determination of Chemical Compound Content of WSC

Chemical content of WSC was determined with ChemStation (Agilent Technologies, Palo Alto, CA, USA) software after using Agilent 7820A gas chromatography-mass spectrometry (GC-MS) instrument, 5977 mass spectroscopy detector and 7673 series autosampler. Compounds were separated with a 0.25 µm, 30m x 0.25 mm diameter HP-5 MS column. Both the injection temperature and the detector temperature were set to 250°C. The injection capacity was determined as 1µl indivisible injection mode, the transporter gas was helium, the flow rate was 1ml/min, and the ionization energy was 70 eV. The oven temperature was programmed to increase 50°C for 1 minute, increasing 20°C per minute for 1 minute at 100°C, increasing by 10°C per minute for 1 minute at 180°C, increasing by 5°C per minute for 5 minutes at 220°C by 10°C for 5.5 min at 300°C. Extract components chromatograms and mass spectra were evaluated with reference standard substance values. In addition, the chemical content was defined by comparing it with the NIST MS (National Institute of Standards and Technology) library values.

Reducing Power Protocols

Fe^{3+}–Fe^{2+} Reducing Power Analysis

Reducing capacity was studied by rearranging the Oyaizu process (Oyaizu, 1986). Compounds with antioxidant properties, which can negatively affect oxidants and render them dysfunctional, have reducing power (Li et al., 2020; Gulcin,2020). The Prussian blue color of Perl, which appears with the addition of Fe^{3+} ions to the experimental medium, forms the $Fe_4(Fe(CN)_6)_3$ complex with an effective absorbance by measuring 700 nm in the spectrophotometer (Göçer & Gülçin, 2011; Gulcin et al., 2010). A raised absorbance value is an indicator of reducing power.

CUPRAC Reducing Capacity

The CUPRAC method, that is copper ion reduction analysis, shows the Cu^{2+} reducing power of phenolic compounds (Apak et al., 2006). A strong absorbance at 450 nm is defined as an indication of an increase in reducing capacity (Gülçin & Daştan, 2007).

FRAP Analysis

It is a method based on the reduction of iron ions (Fe^{3+}) to iron ions (Fe^{2+}) under acidic conditions (Göçer & Gülçin, 2011). It is a blue-colored complex (Fe^{3+}-TPTZ complex) formed by iron ions (Fe^{3+}) and trippyridyl triazine (TPTZ), showing max absorbance at 593 nm (Köse et al., 2015).

Radical Scavenging Protocols

DPPH• Scavenging Activity

Radical scavenging is one of the most effective antioxidant methods for removing free radicals. Since the situations triggered by these radicals are dangerous for biological systems, it is very important to remove the radicals. DPPH• analysis, which is the most common method in which the electron or hydrogen donating abilities of the extracts are determined and accurate and reliable results are obtained, was carried out based on the Blois principle (Bursal et al., 2019; Blois, 1958). The radical scavenging power gives spectrophotometric absorbance at 517 nm. The decrease in absorbance seen in the extracts meant that the DPPH• radical scavenging activity was strong (Gülçin et al., 2007).

ABTS$^{•+}$ Scavenging Assay

ABTS$^{•+}$ scavenging capacity is widely used to measure the radical scavenging activities of various extracts or pure substances. DPPH• is a very effective method like radical scavenging activity (Re et al., 1999). Percent inhibition of ABTS$^{•+}$ was measured at 734 nm (Sujayev et al., 2016). The decrease in absorbance with the formation of discoloration was calculated. ABTS$^{•}$ radical scavenging activity was performed by the procedure specified by Re et al (1999).

AChE and BChE Enzymes Inhibition Assays

AChE and BChE enzyme inhibition analyses were performed by modifying the Ellman's method. Three replications of the analyses were taken, IC_{50} values were calculated and standard deviations were added to the obtained findings (Ellman et al., 1961).

Supply and Adaptation of Experimental Animals

Male Wistar rats (300 ± 30 g), obtained through the decision of Atatürk University Medical Experimental Application and Research Center ethics committee (ATADEM) dated 28.12.2020 and numbered 236643897-000-EBYS-1, were kept at a controlled temperature (22-24°C) and dark-light cycle (12:12 hours). After a seven-day adaptation period with a regular diet and free access to ad-libitum water, the animals were randomly divided into 5 groups and placed in (n=30) marked polycarbonate cages. The entire application and care process was carried out by the national animal care directive (Cinar et al., 2019).

Preparation of Substances to be Applied

1200 g of E-WSC extract was made up to 20 cc with distilled water, and 1 cc was applied to the animals in the relevant group. Triton WR-1339 (400 mg/kg) was dissolved in normal saline (pH 7.4) and injected once i.p. to rats fasted for 12 hours. After 24 hours, hyperlipidemia was induced (Baldissera et al., 2017).

Experimental Design

The applications to the rats divided into five groups were followed for five days. The application doses and the forms of administration of the substances are as follows: K1: Healthy control group. Physiological water only (2.5 mL/kg, intraperitoneally (i.p) administered group (Kumar et al., 2013). K2: E-WSC (150 mg). The group was given E-WSC (150 mg/kg, oral dose (o.d)) 30 minutes before physiological water (2.5 mL/kg, i.p) administration (Beigh et al., 2017). K3: E-WSC (300 mg). The group was given E-WSC (300 mg/kg, o.d) 30 minutes before physiological water (2.5 mL/kg, i.p) administration (Cintesun et al., 2023). K4: HL - Hyperlipidemic group. The group that received physiological water 30 minutes before Triton WR-1339 (400 mg/kg, 2.5 mL/kg, i.p) administration (Baldissera et al., 2017). K5: HL + E-WSC (300 mg). Group that received E-WSC (300 mg/kg, o.d) 30 minutes prior to administration of Triton WR-1339 (400 mg/kg, 2.5 mL/kg, i.p).

The experimental animals were anesthetized with sevoflurane (sevorane) one day after the basic inductions were made and the experimental period was completed. Using sterile materials, the abdominal region was carefully opened with a vertical incision, and the kidney tissue was removed with a surgical procedure. The excised tissues were washed in salt water and purified, then dried and treated with liquid nitrogen, consequently stored at -80 °C until analysis.

Oxidative Stress Indicators

The 0.2 g was taken from the kidney tissues heated to +4°C before analysis. Ceramic beads of different sizes and 1 mL homogenization buffer (0.1 M $K_2H_2PO_4$ – 10 mM EDTA) were added to the tissues taken into the screw tube and placed in the homogenizer device (4000 rpm, 5 cycles, 1 min shaking and 10 sec holding). Then, the supernatants were taken by centrifugation (4°C, 13000 rpm, 30 min), and the homogenate was ready.

SOD, CAT, and MDA activities were determined using freshly prepared kidney tissue homogenates. SOD analysis was conducted using the Rat (SOD) ELISA Kit (Catalog No: 201-11-0169, SunRed), CAT analysis was performed with the Rat (CAT) ELISA Kit (Catalog No: 201-11-5106, SunRed), and MDA analysis was carried out according to the protocol outlined in the Rat (MDA) ELISA Kit (Catalog No: 201-11-057, SunRed).

Statistical Analysis

The data from the treatment groups were analyzed using Analysis of Variance (ANOVA). Differences in the means of these groups were further assessed using Duncan's Test, with p-values calculated via the Unpaired T-Test. A significance level of $p < .05$ was used for all mean comparisons.

Results

GC-MS Analysis Results

In the GC-MS analysis performed on the ethanol extract of walnut seed coats; it was determined that there are many bioactive compounds such as phenolic compounds, mono and polyunsaturated fatty acids, alcoholic compounds, phytosterols and esters (Table 1).

Table 1.
Identification of phytocomponents isolated from the ethanolic extract of WSC.

Ret. time	Compound name	Peak area (%)
21.044	Lineoleic acid	0.564
32.523	1- Heptatriacotanol	1.067
33.199	Oleic acid amide	21.211
34.707	Ethyl iso-allocholate	0.614
35.525	Hexadecanoic acid	4.065
35.667	Cinnamic acid	3.225
37.675	Brassicasterol acetate	4.717
38.860	B- Sitosterol	61.071

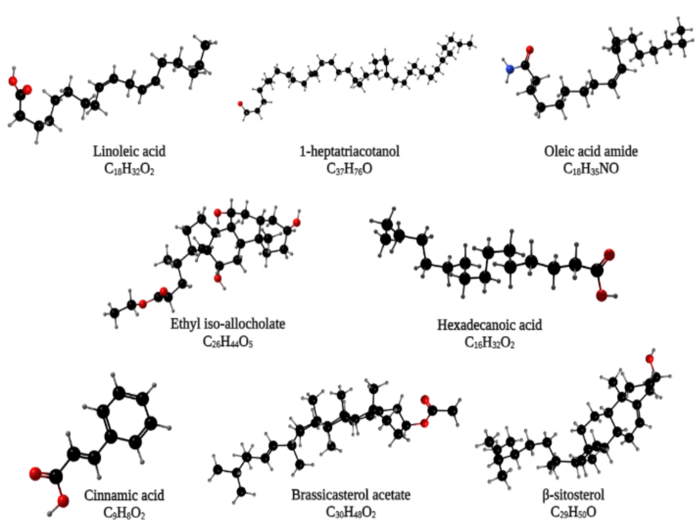

Figure 1.
Structures of bioactive compounds determined by GC-MS analysis (KingDraw).

The structure of the compounds identified by GC-MS is given in Figure 1. Among these compounds, β-Sitosterol (61.071%) has the highest percentage, while Linoleic acid (0.564%) has the low WSC percentage.

It is known that herbal extracts have many beneficial effects on health and is actively used in the prevention and cure of many diseases. In general, around 25% of medicines worldwide are derived from plants. This is largely due to the richness of phenolic compounds, which serve as secondary metabolites in these plants. Consuming foods rich in phenolic compounds is very important in terms of providing biological benefits (Mutlu et al., 2023). Of the eight identified compounds, Cinnamic acid is antibacterial, antifungal, anti-inflammatory (de Almeida Lima et al., 2018), neuroprotective (Lan et al., 2017), anticancer (Gießel et al., 2019) and antidiabetic (Adisakwattana et al., 2013), Linoleic acid is antihyperlipidemic (Mozaffarian et al., 2010; Mensink & WHO, 2016) and antidiabetic (Imamura et al., 2018), Oleic acid amidine antimicrobial (Ahmad et al., 2021), Hexadecanoic acid is anticytotoxicity, apoptotic (Harada et al., 2002) and anticancer (breast-colorectal) (Nazarudin et al., 2020), 1-Heptatriacotanol's antimicrobial, antioxidantve anti-inflammatory (Hadi et al., 2016), Ethyl isoallocholat's anti-inflammatory (Johnson et al., 2020), β-Sitosterol's anti-inflammatory (Kmiecik et al., 2011; von Holtz et al., 1998), anticancer activity (breast, liver, colon, stomach) (Sanches et al., 2005; Zhao et al., 2017) and antihyperlipidemic (Luo et al., 2015), and Brassicasterol acetate's immunomodulatory and antioxidant (Çakmakçı et al., 2015) effects have been reported in studies.

Antioxidant Results

Antioxidant activity of WSC was tested using DPPH• scavenging and $ABTS^{\cdot+}$ scavenging activities. Three separate analysis methods were used to determine the reduction ability of WSC: CUPRAC, FRAP and Fe^{3+} (Figure 3 and Table 2). Reduction potential affects the capacity of plant extracts to exhibit biological activity. These extracts neutralize reactive oxygen species, oxidants and reducing agents (Çakmakçı et al., 2015). The reduction capacity of WSC is measured by the Fe^{3+} reduction test system. Fe^{3+} ions addition to WSC occur blue colored complex of $Fe_4(Fe(CN\text{-})_6)_3$ (Tohma et al., 2016). The absorbance value of the $Fe_4(Fe(CN\text{-})_6)_3$ complex was determined as 700 nm (Cakmak & Gülçin, 2019). As a result of the formation of the complex, a yellow color appears in the sample. However, the color may vary from green to blue, which depends on the effect of the test compounds (Gülçin et al., 2012). Accordingly, it was determined that WSC, which uses the reduction properties of $Fe(Fe(CN\text{-})_6)_3$, $Cu^{2+,}$ and Fe^{3+}-TPTZ, has a very effective and valuable reduction potential. The reduction potential of WSC was determined by the Oyaizu method. For this measurement, Fe^{3+}- Fe^{2+} reduction was achieved (Oyaizu, 1986). The Fe^{3+} reducing capacity of WSC is shown in Table 2 and Figure 3A. The radical scavenging and reduction properties of WSC will not be detailed in the discussion section as the structure-activity relationship is relatively low. The Fe^{3+} reducing effects of 30 µg/mL of WSC and standards declined as following orders: BHA (1.471, r^2: 0.9699) > WSC (1.894, r^2: 0.9839) > Trolox (1.826, r^2: 0.9746) > BHT (1.173, r^2: 0.9601) > α-Tocopherol (1.004, r^2: 0.9749). Various antioxidant compounds serve as standards for determining the antioxidant capacity of samples. These standards are chosen based on important features such as stability in the solvent environment, price, and solubility (Han et al., 2018). The formation of complexes and the increase in the reducing effect are associated with the rise in absorbance values. The results clearly demonstrate that WSC has the ability to effectively reduce Fe^{3+} and neutralize free radicals and ROS. Therefore, WSC may serve as a potential agent against oxidative stress-induced damage in biochemical and biological systems.

Table 2.
Fe^{3+}, Cu^{2+}, and Fe^{3+}-TPTZ reducing ability of WSC and standards (30 µg/mL)

Antioxidants	Fe^{3+} reducing		Cu^{2+} reducing		Fe^{3+}-TPTZ reducing	
	λ_{700}	r^2	λ_{450}	r^2	λ_{593}	r^2
BHA	2.471	0.9699	2.598	0.8705	1.501	0.8576
BHT	1.173	0.9601	1.519	0.8807	0.772	0.8044
α-Tocopherol	1.004	0.9749	1.324	0.8710	0.949	0.9417
Trolox	1.826	0.9746	1.523	0.7630	1.619	0.9110
WSC	1.894	0.9839	1.245	0.8624	1.247	0.8857

Table 2 and Figure 3B show the Cu^{2+} reduction values of WSC. A positive correlation was observed between the Cu^{2+} reducing and WSC as concentration-dependently (10-30 µg/mL). At the concentration of 30 µg/mL, Cu^{2+} reducing capability of WSC and standards were declined as following orders (Table 2 and Figure 3B): The Fe^{3+} reducing effects of WSC and standards declined as following orders: BHA (2.598, r^2: 0.8705) > Trolox (1.523, r^2: 0.7630) > BHT (1.519, r^2: 0.8807) > α-Tocopherol (1.324, r^2: 0.8710) > WSC (1.245, r^2: 0.8624). Cuprac analysis is capable of testing both hydrophilic and lipophilic antioxidants. Since the optimal pH of the method is close to physiological pH, it will not carry the risk of underestimation (under acidic conditions) or overestimation (under basic conditions) of the total antioxidant capacity due to the protonation of antioxidants or proton dissociation of phenolic compounds, respectively. In addition, it is a low-cost, fast and stable test (Taslimi et al., 2020).

WSC determined to have Fe^{3+} and Cu^{2+} reduction potential was also observed to have an effective reduction capacity in FRAP analysis (Figure 3C and Table 2). Reducing ability of WSC was found to be in descending order of Trolox (1.619, r^2: 0.9110) > BHA (1.501, r^2: 0.8576) > WSC (1.247, r^2: 0.8857) > α-Tocopherol (0.949, r^2: 0.9417) > BHT (0.772, r^2: 0.8044). As similar Cu^{2+} reducing of WSC, a positive correlation was observed between the Cu^{2+} reducing and WSC as concentration-dependently (10-30 µg/mL). As in the previous reduction analysis, it was revealed that the strong reducing absorbance value is related to the effective reducing ability in the complex. The FRAP method is performed in acidic environment to maintain the solubility of iron ions (Sehitoglu et al., 2015).

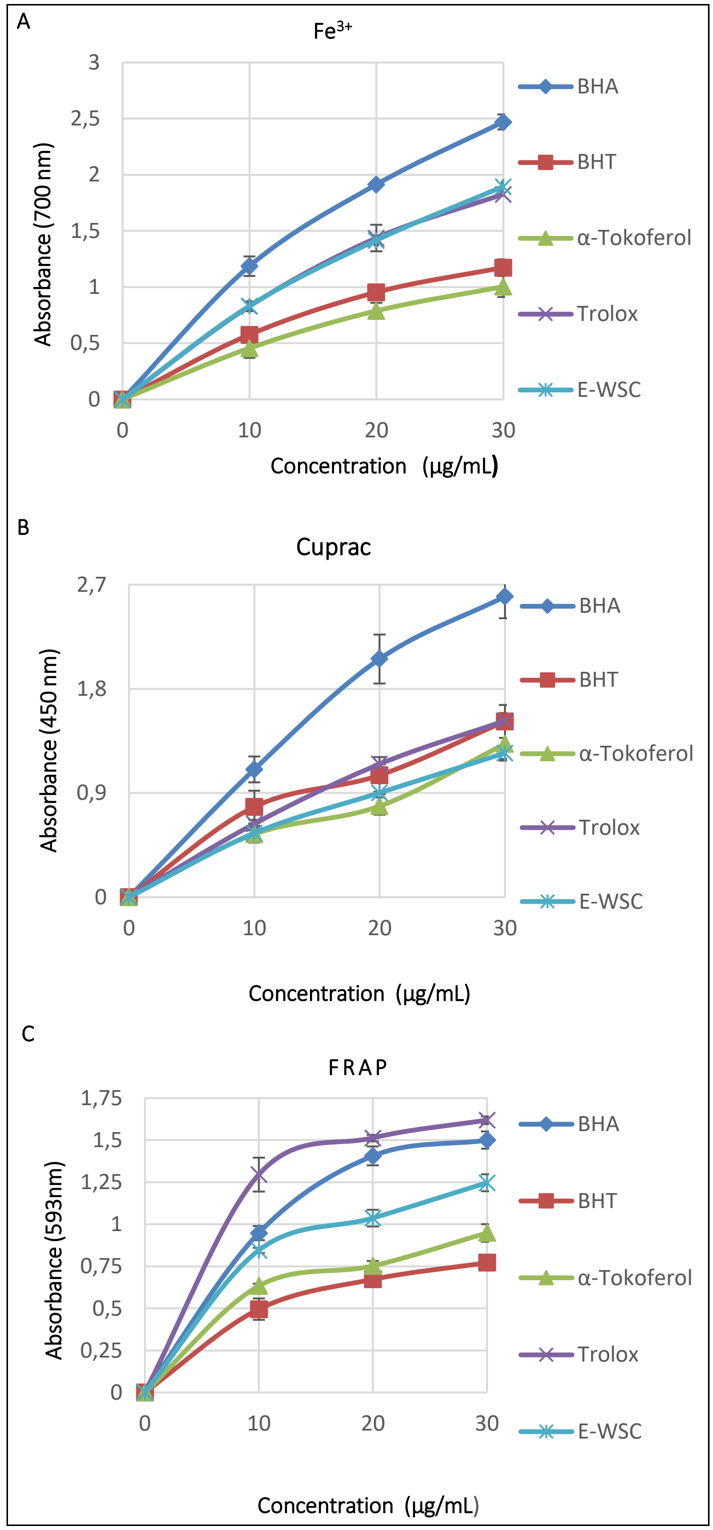

Figure 3.
*Different antioxidant assay for WSC: **A.** Fe^{3+} reducing method, **B.** Cu^{2+} reducing method, **C.** Fe^{3+}-TPTZ reducing method.*

DPPH• and $ABTS^{•+}$ removal analyzes are most commonly used as spectrophotometric reduction methods. These two tests used to measure antioxidant and radical scavenging potential in plant sample extracts were also used in this study (Gülçin et al., 2020). Some parameters stand out for inhibition percentage measurements., which are concentrations of antioxidants and radicals, solvent and reagent ratios, incubation time and temperature. In addition, the presence of hydrogen, water, and metal in antioxidant test systems is another important parameter used for measurement (Gulcin et al., 2019). Another element used to evaluate antioxidant properties is the IC50 (Half-maximum scavenging concentration) value. This value is defined as the concentration that plays an effective role in removing 50% of oxidant agents (Türkan et al., 2020). For DPPH radical scavenging activities of WSC and standards were found to be in the following order: Trolox (IC_{50}: 8.59 µg/mL, r^2: 0.9704) < BHA (IC_{50}: 11.59 µg/mL, r^2: 0.9451) < WSC (IC_{50}: 13.39 µg/mL, r^2: 0.9704) < α-Tocopherol (IC_{50}: 13.78 µg/mL, r^2: 0.9221) < BHT (IC_{50}: 26.55 µg/mL, r^2: 0.9794) (Table 3 and Figure 4A). A lower EC_{50} value demonstrates a higher DPPH• scavenging ability (Tohma et al., 2019).

Table 3.
DPPH• and $ABTS^{•+}$ IC50 (µg/mL) values of WSC and standards

Compounds	DPPH• scavenging IC_{50}	r^2	$ABTS^{•+}$ scavenging IC_{50}	r^2
BHA	11.59	0.9451	4.50	0.9334
BHT	26.55	0.9794	5.94	0.9033
α-Tocopherol	13.78	0.9221	8.43	0.9985
Trolox	8.59	0.9214	5.13	0.9557
WSC	13.39	0.9704	8.18	0.9687

Besides these, WSC showed a strong $ABTS^{•+}$ scavenging potential. As given in Table 3 and Figure 4B, WSC effectively scavenged ABTS radicals as concentration-dependently (10-30 µg/mL, $p < .001$). EC_{50} values of WSC in $ABTS^{·+}$ scavenging assay were found to be in descending order of BHA (IC_{50}: 4.50 µg/mL, r^2: 0.9451) < Trolox (IC_{50}: 5.13 µg/mL, r^2: 0.9214) < BHT (IC_{50}: 5.94 µg/mL, r^2: 0.9794) < WSC (IC_{50}: 8.18 µg/mL, r^2: 0.9704) < α-Tocopherol (IC_{50}: 8.43 µg/mL, r^2: 0.9221). The EC50 value indicates a high $ABTS^{·+}$ scavenging potential, as seen in radical scavenging tests. As previously reported, the ABTS radical scavenging capacity of antioxidants has been associated with the H-donating effect (Artunc et al., 2020; Balaydın et al., 2010).

Enzyme Inhibition Results

This study revealed the relationship between AChE/BChE enzymes and E-WSC. This extract effectively inhibited AChE and BChE with IC50 values of 5.75 µg/mL (r2: 0.9783) and 30.26 µg/mL (r2: 0.9191), respectively. On the other hand, tacrine was used as a positive control for AChE and BChE inhibition. The IC50 value of tacrine was found to be 0.044 µM for AChE and 0.0102 µM for BChE. When the results were examined, E-WSC inhibited the AChE enzyme 5.26 times more than the BChE enzyme (Table 4, Figures 5 and 6).

Table 4.
IC_{50} values of E-WSC for AChE and BChE enzymes

	AChE IC_{50}	AChE R^2	BChE IC_{50}	BChE R^2
E-WSC	5.75103 µg/mL	0.9783	30.262 µg/mL	0.9191
Tacrine	0.0441 µM	0.9805	0.0102 µM	0.9830

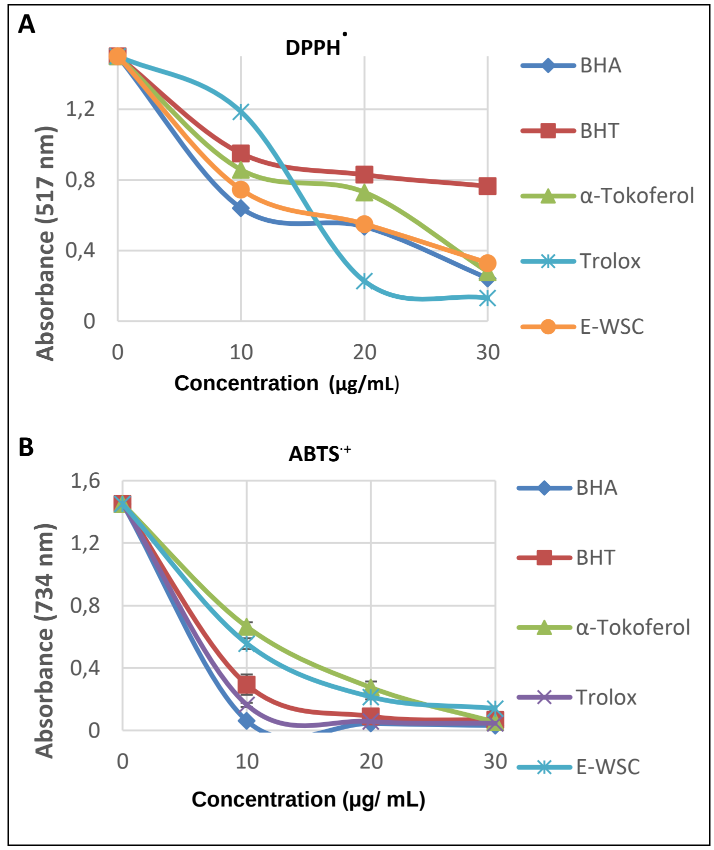

Figure 4.
Radical scavenging assays for WSC. ***A.*** *DPPH• scavenging method,* ***B.*** *$ABTS^{•+}$ scavenging method*

Enzyme inhibition study is seen as an effective step in the treatment of neurodegenerative diseases, especially Alzheimer's (AD), Parkinson's (PD) and senile dementia (Tohma et al., 2019). These diseases include many issues such as oxidative stress, misfolded proteins, protein aggregation, excitotoxicity, neuroinflammation, and neuron loss (Ramsay et al., 2016). Here, especially the oxidative stress factor has a very important effect on the oxidative damage to occur in cellular components such as lipids, proteins and DNA in AD and PD (Lezi & Swerdlow, 2012). The importance of phenolic antioxidants in preventing or delaying the onset of these damages is well documented (Bayrak et al., 2020; Ceylan et al., 2019; Demir et al., 2019; Palabıyık et al., 2023). When looking at the neuropathological features of AD and PD, dysfunctions in the cholinergic and dopaminergic systems are striking. AChE and BChE function at this point, slowing down and terminating nerve impulse transmission by hydrolyzing acetylcholine (ACh) and butyrylcholine (BCh). Inhibition of these two enzymes has a great effect on the duration of disease mechanisms such as AD, PD and dementia (Artunc et al., 2020). Although the drugs currently used in treatment (Tacrine, Donepezil and Rivastigmine) work to repair this disorder, they may cause some side effects, including gastrointestinal disorders (Artunc et al., 2020; Mathew et al., 2019).

Therefore, it is inevitable that E-WSC, which is both a natural product and has a strong antioxidant effect, helps increase ACh and BCh levels in synapses by inhibiting ChEs and improving cholinergic function.

Lipid Peroxidation and Antioxidant System Modulation of E-WSC in Hyperlipidemic Rats

The results of SOD activity, involved in the removal of superoxide radicals from the kidney tissue of the study groups, CAT activity, an antioxidant enzyme influencing cell life, and MDA activity, a biomarker of oxidative stress, are presented in Table 5.

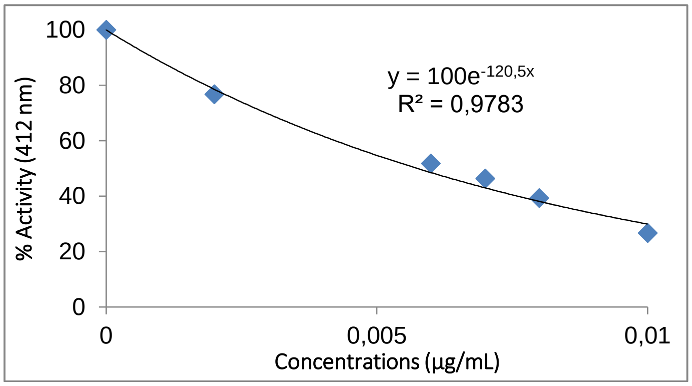

Figure 5.
AChE concentration-activity graph

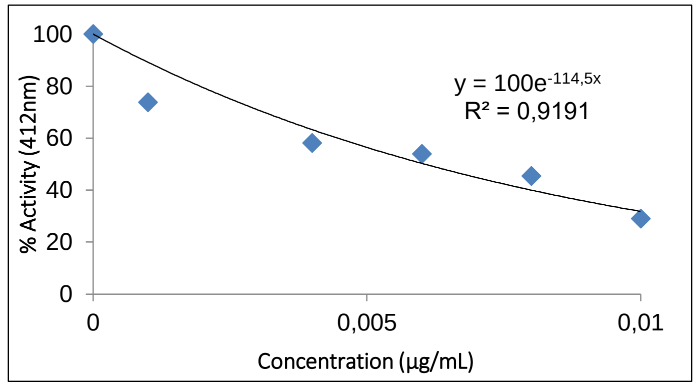

Figure 6.
BChE concentration-activity graph

Table 5.
SOD, CAT and MDA Level Values in Kidney Tissues (Mean ± SD, n=6)

Groups	Kidney Tissue		
	SOD (ng/mL)	CAT (ng/mL)	MDA (nmol/mg protein)
K1 (Control)	30.8553±0.95896[a,b]	57.046±4.46961[a,b]	2.33065±0.095218[b]
K2 (E-WSC-150mg)	28.0468±0.42904[a,b]	49.6262±2.54587[b]	2.06552±0.091717[b]
K3 (E-WSC-300mg)	27.1279±0.50183[b]	59.267±3.12407 [a,b]	2.03018±0.064749[b]
K4 (HL with Triton WR-1339)	27.4672±1.8076[b]	52.678±5.97322[a,b]	2.65356±0.256121[a]
K5 (E-WSC-300mg+ Triton WR-1339)	31.8495±4.37602[a]	62.075±10.2431[a]	2.72167±0.217631[a]

* While the difference between the group means with the same letter is not significant (p > .05), the difference between the group means with different letters is significant (*p* < .05).

There was a significantly decrease in SOD activities (27.4672±1.8076 ng/mL) compared to the control group (30.8553±0.95896) due to Triton WR-1339 applied to create hyperlipidemia in the kidney tissue. The dose-related decrease (28.0468±0.42904/27.1279±0.50183 ng/mL) and deterioration in SOD activity in the groups administered E-WSC-150 mg and E-WSC-300 mg alone, E-WSC-300 mg given with Triton WR-1339 eliminated with. These changes are shown in Figure 7. CAT activity decreased in the hyperlipidemia group (52.678±5.97322 ng/mL) compared to the control group (57.046±4.46961 ng/mL). In the group in which E-WSC-300 mg was administered alone (59.267±3.12407 ng/mL), an increase in activity was detected, but this increase was not statistically significant. E-WSC-300 mg (62.075±10.2431 ng/mL) given with Triton WR-1339 caused a critical increase in activity compared to the hyperlipidemia group. Renal tissues showed tremendous improvement in CAT activities, especially in the groups administered E-WSC 300 mg. (Figure 8) MDA levels were measured to determine lipid peroxidation as a result of in vivo induced hyperlipidemia. Accordingly, a statistically significant increase in MDA levels occurred in the hyperlipidemia group (2.65356±0.256121 nmol/mg protein) developed with Triton WR-1339 induction compared to the control group (2.33065±0.095218 nmol/mg protein). E-WSC-300 mg (2.72167±0.217631 nmol/mg protein) given with Triton WR-1339 statistically significantly reduced MDA levels (Figure 9). However, E-WSC-300 mg showed a significant protective effect against lipid oxidation in kidney tissue when administered alone (2.03018±0.064749 nmol/mg protein).

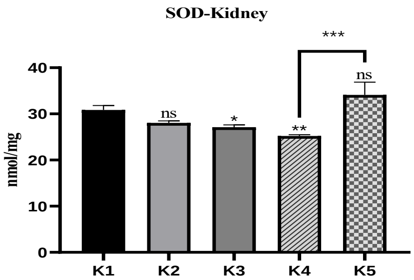

Figure 7.
*Effect of ethanol extraction (E-WSC) of Walnut Seed Coat on superoxide dismutase (SOD) activity in kidney tissue in hyperlipidemic rats induced by Triton X-1339. Results are expressed as Mean ± SD (n=6). The degree of importance compared to the control group was given as * p* < .05.

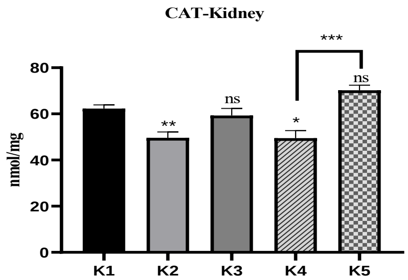

Figure 8.
*Effect of ethanol extraction (E-WSC) of Walnut Seed Coat on catalase (CAT) activity in kidney tissue in hyperlipidemic rats induced by Triton X-1339. Results are expressed as Mean ± SD (n=6). The degree of importance compared to the control group was given as * p* < .05.

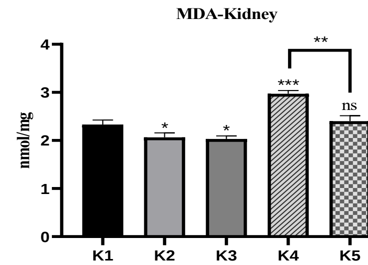

Figure 9.
*Effect of ethanol extraction (E-WSC) of Walnut Seed Coat on malondialdehydes (MDA) activity in kidney tissue in hyperlipidemic rats induced by Triton X-1339. Results are expressed as Mean ± SD (n=6). The degree of importance compared to the control group was given as * p < .05.*

Hyperlipidemia has occurred due to the deterioration of lipid and lipoprotein metabolism and has caused deaths in the world (Monguchi et al., 2017). Triton WR-1339, which is used to induce hyperlipidemia by changing the physicochemical properties of lipoproteins (Venkadeswaran et al., 2014), is an ionic group-free detergent used to prevent the uptake of plasma lipoproteins by peripheral tissues by inhibiting the activity of LPL (Surya et al., 2017). While this enzyme is involved in the lipolytic process, it plays a role in the reuptake of TG from chylomicrons and VLDL to peripheral tissues (Tooulia et al., 2015). A model of acute hyperlipidemia was created in rats within 24 hours with Triton WR-1339, which we used in our current study. Our model was supported by using this induction in the study in which the anti-hyperlipidemic properties of *Campomanesia xanthocarpa* leaves (De Sousa et al., 2019) and N-(Benzoylphenyl)-Carboxamide derivatives were determined (Sweidan et al., 2022). With the application of Triton WR-1339 by Attia et al. (2020), TGs, TC, LDL-C and lipid peroxidation (LPO) increased and HDL-C decreased (Attia et al., 2020). Experimental studies suggest that there is a relationship between the development of renal impairment and dyslipidemia. Increases in cholesterol and triglyceride levels are shown as independent risk factors for the development of kidney disease (Kovesdy et al., 2017).

Hyperlipidemic induction with Triton WR-1339 caused an increase in MDA level with inhibition of antioxidant enzymes (SOD and CAT) activities in rat kidney tissues. These resulting changes and disorders manifests in the kidney tissue with an increased production rate of reactive oxygen species (ROS) or a gap in the antioxidant system (Beyegue et al., 2012) as lipid peroxidation is a free radical that triggers protein oxidation, disrupts cell membrane structure and function, and produces MDA (Gaschler & Stockwell, 2017). The formation of the foam cell is the first step in the onset of the atherosclerotic state. The reason for this is LDL oxidation due to oxidative stress (Winklhofer-Roob et al., 2017). In other words, oxidative stress poses a problem for many related diseases such as hyperlipidemia, atherosclerosis and cardiovascular (Chiu et al., 2019).

The data obtained from our research revealed that E-WSC exhibited antihyperlipidemic activity in Triton WR-1339-induced hyperlipidemia. As expected, the SOD and CAT values, indicative of antioxidant activity, decreased in the hyperlipidemic group compared to the control group. However, it was observed that E-WSC at a dose of 300 mg, both alone and in combination with Triton WR-1339, stimulated activities, resulting in a significant increase in SOD and CAT values (Figures 1 and 2). In a similar study, it was found that the myocardial infarction group, induced by exposure to Isoproterenol (ISO) in rat hearts, exhibited significantly lower activity of SOD and CAT enzymes compared to control rats. Walnut kernel application, on the other hand, cleaned the superoxide and hydrogen peroxides produced by ISO and improved SOD and CAT activities (Sun et al., 2019). In other studies, it has been reported that walnut intake prevents the decrease in SOD and CAT activity. In rats, scopolamine-induced cognitive impairment, SOD and CAT activities were regulated by walnut consumption (Haider et al., 2018). Another significant finding in our study was the measurement of MDA, an indicator of lipid peroxidation. With the induction of hyperlipidemia and the subsequent disruption in lipid metabolism, high levels of MDA were observed. However, our measurements revealed a significant decrease in MDA content following the administration of E-WSC-300 mg (Fig. 3). Additionally, in line with our findings, walnut supplementation in a high-fat diet (HFD) significantly reduced hepatic levels of lipid peroxidation (LPO) and cytosolic MDA levels (Choi et al., 2016). Another study demonstrated that oxidative stress induced by abnormal lipid profiles in pregnant rats, attributed to the polyunsaturated fatty acid (PUFA) content of walnuts, was alleviated due to the reduction in MDA content (Sun et al., 2020).

Statins and their derivatives, anti-hyperlipidemia drugs, react by inhibiting the HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA reductase) reductase enzyme, which is responsible for cholesterol synthesis in the liver. HMG-CoA activates a mevalonate pathway, which is the rate-limiting step in hepatic cholesterol synthesis, and ensures the synthesis of cholesterol. However, inhibiting HMG-CoA reductase reduces cholesterol synthesis in the liver and reduces the level of LDL-C in the bloodstream (Mo et al., 2019). This drug group and its derivatives, which have an anti-hyperlipidemic effect by inhibiting cholesterol synthesis, can cause some toxic effects, although they are used as a therapeutic in the first step (Abu-Raghif et al., 2015; Hashem et al., 2021).

Although the safety of plant-based drugs is of concern due to the high risk of contamination (Kosalec et al., 2009), natural products may inhibit lipid biosynthesis by showing a similar mechanism of action to statins (Shattat, 2015). For this reason, the use of natural substances to regulate and control hyperlipidemia has recently attracted attention. Plants, especially rich in flavonoid content, are associated as anti-hyperlipidemic agents because they can deactivate HMG CoA reductase in the cholesterol formation pathway. In a previous in vivo study on Wistar rats, it was reported that walnut septum extracts did not show subacute or acute toxic effects at doses of 1000 mg/kg (body weight) (Ravanbakhsh et al., 2016). E-WSC, which is the active ingredient of our study and has a rich content, greatly relieves kidney damage due to hyperlipidemia (Palabiyik et al., 2022) and the total flavonoids extracted from the leaves of *Actinidia kolomikta* improve hyperlipidemia through this mechanism (Yu et al., 2017) supports this thesis.

Oxidative stress has a great impact on the progression of diseases such as Alzheimer's, Patkinson's and Dementia, as it damages cholinergic neurons and causes loss of function (Chen et al., 2012). Free radicals such as ROS play a critical role, especially in AD pathology (Cheignon et al., 2018). It affects important pathways such as oxidative stress, macromolecule peroxidation, Aβ metal ion redox potential, and mitochondrial dysfunction. This interaction, in turn, induces cell homeostasis, ROS production, and upregulation of Aβ and p-tau formation (Chen et al., 2012; Gella & Durany, 2009; Hawking, 2016). As a result, oxidative stress can cause disease progression and even death in patients suffering from neurogenerative diseases such as AD, PD and Dementia. However, no effectiveness has been achieved in the treatments accepted by the FDA (Food and Drug Administration). Therefore, new approaches should be developed to improve cholinergic neurons. Especially the use of antioxidant drugs is extremely important in terms of providing positive results. Antioxidants obtained from polyphenolic compounds found in medicinal plants and natural products found in nature are a more valuable alternative to synthetic compounds. Because they are more harmless, easily cross the blood-brain barrier and improve cognitive decline (Cassidy et al., 2020).

The phytochemical content and strong antioxidant ability of E-WSC may have the capacity to reduce oxidative stress in individuals with AD. However, this should be clarified in more detailed studies. In addition, the fact that our extract is cheap and easily accessible and has no toxic effects increases its potential for treatment.

Conclusion and Recommendations

New developments in pharmacotherapy are of great importance to ensure the effective continuity and development of the healthcare system. Drug potentials obtained from bioactive components and successfully completing the testing phases day by day should be taken into consideration. Because these potentials may have a percentage that will increase success in chronic diseases. Mitigation of chronic diseases, mutagenesis, DNA damage, carcinogenesis and inhibition of pathogenic bacterial growth are generally associated with the scavenging of ROS and propagation of free radicals in living systems. At the same time, the presence of compounds with antioxidant properties is also very important to eliminate the undesirable side effects of some drugs used in the treatment of AD and PD. Antioxidant plants and extracts are, therefore, widely used in different food and medicinal applications. In this study, it was observed that WSC had effective antioxidant ability compared to standard and powerful antioxidants BHA, α-Tocopherol, BHT, and Trolox. Considering that E-WSC is a powerful antioxidant, it was thought that it could be a potentially effective source to treat AD and PD as its anticholinesterase properties were reported for the first time, its ethnopharmacological use against neurodegenerative disorders was, in a sense, confirmed. In the acute hyperlipidemia model caused by Triton WR-1339, lipid peroxidation caused by the increase in reactive oxygen species caused an increase in MDA levels. When E-WSC was administered to the application group, it was observed that the damage was largely repaired and there was a significant increase in antioxidant enzyme activities. These inhibitory activities are thought to be due to β-sitosterol, a phytochemical found in significant amounts in the WSC ethanol extract.

Analyses in our study proved that WSC has a renal protective effect against hyperlipidemia and is also a therapeutic candidate for treatment. To be evaluated as a combined drug in future studies, analyses must be made to determine drug dosages and application procedures of these drugs, reduce undesirable effects, adapt to the patient and provide maximum benefit. Thus, we are hopeful that it will make a great contribution to the areas that need to be developed.

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Effect of soil nutrient management on soil weed seed bank dynamics across a soil fertility gradient in smallholder farms, Eastern Zimbabwe

Doğu Zimbabwe'deki küçük çiftliklerde toprak besin yönetiminin toprak verimliliği gradyanı boyunca toprak yabancı ot tohum bankası dinamikleri üzerindeki etkisi

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ABSTRACT

Weed seed banks are latent biotic stress contributors to low crop production among smallholder farms. The objective of this study was to investigate effect of repeated soil nutrient amendments on weed seed bank dynamics in eastern Zimbabwe. Soil samples were taken from three farms with low, medium and high soil organic carbon (SOC) along the catena. Sampled treatments included varying combinations of compound D (7%N, 14%P, 7% K) fertiliser, cattle manure and lime. Weeds that emerged per plot were counted by species and data was tested for normality before running a linear mixed-effects model and Restricted Maximum Likelihood. Principal Component Analysis (PCA) was performed to establish the relationship between seasons, SOC, soil nutrient amendment, and emerged weeds. Significant ($p < .05$) species richness (Margalef index), and Shannon Weiner index were recorded in the medium (6.4g kg-1soil), high (8 g kg-1 soil) and low (< 3.9 g kg-1soil) SOC. Weed species emergence was significantly ($p < .05$) influenced by the level of SOC along the catena positions, representing results from the source. Emergency and abundance of weeds such as *Richardia scabra*, was associated with low SOC acidic sandy soils from the upper catena. However, application of nutrients (NPK+lime treatment) reduced weed species counts from 1.96±0.12 to 1.4±0.12. Cattle manure and NPK+CM treatments recorded significantly higher weed emergence, and weed biomass; compared to the control. Clearly, cattle manure treatments significantly increase the soil weed seed bank; thus, weeding intensity is likely to increase in cattle manure treated fields.

Keywords: Germination, organic matter, soil nutrients, weed diversity, weed management

ÖZ

Yabancı ot tohum bankaları, küçük ölçekli çiftçilik işletmelerinde düşük ürün üretimine katkıda bulunan gizli biyotik stres faktörleridir. Bu çalışmanın amacı, tekrarlanan toprak besin değişikliklerinin Doğu Zimbabve'de yabancı ot tohum bankası dinamikleri üzerindeki etkisini araştırmaktır. Toprak örnekleri, eğim boyunca düşük, orta ve yüksek toprak organik karbon (TOC) içeren üç çiftlikten alınmıştır. Örneklenen işlemler arasında bileşik D (7% N, 14% P, 7% K) gübresinin farklı kombinasyonları, sığır gübresi ve kireç bulunmaktadır. Parsel başına çıkan yabancı otlar türlerine göre sayılmış ve veriler normal dağılıma sahip olmadan önce bir lineer karışık etkiler modeli ve Sınırlı Maksimum Olabilirlik çalıştırılmıştır. Başlık Bileşen Analizi (PCA), mevsimler arası ilişkiyi, TOC, toprak besin düzenlemesi ve çıkan yabancı otları belirlemek için yapılmıştır. Orta (6.4g kg-1 toprak), yüksek (8 g kg-1 toprak) ve düşük (<3.9 g kg-1 toprak) TOC'de anlamlı ($p < .05$) tür zenginliği (Margalef indeksi) ve Shannon Weiner indeksi kaydedilmiştir. Yabancı ot türlerinin ortaya çıkışı, eğim boyunca SOC seviyesinden etkilenmiş ve kaynaktan gelen sonuçları temsil etmiştir. *Richardia scabra* gibi yabancı otların asidik kumlu topraklarda düşük SOC'ye bağlı olarak çıkışı olmuştur. Bununla birlikte, besin maddelerinin uygulanması (NPK+kireç tedavisi), yabancı ot türü sayısını 1.96±0.12'den 1.4±0.12'ye düşürmüştür. Sığır gübresi ve NPK+SG işlemleri, kontrolle karşılaştırıldığında önemli ölçüde daha yüksek yabancı ot çıkışı ve yabancı ot biyokütlesi kaydedilmiştir. Açıkça, sığır gübresi işlemleri toprak yabancı ot tohum bankasını önemli ölçüde arttırmaktadır; bu nedenle, sığır gübresi ile tedavi edilen tarlalarda çapa yoğunluğunun artması muhtemeldir.

Anahtar Kelimeler: Çimlenme, organik madde, toprak besin maddeleri, yabancı ot çeşitliliği, yabancı ot yönetimi

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Introduction

Weeds are among the major constraints to crop production worldwide, especially among resource-constrained smallholders in sub-Saharan Africa (Mashingaidze et al., 2009). Weeds smother crops but more importantly, mature weed plants disperse and shed seeds into the soil, which ultimately accumulate and form soil weed seed banks (Schwartz-Lazaro & Copes, 2019). Weed seed banks consist of viable, dormant and non-dormant seeds; with potential to germinate and compete with crops, particularly at the delicate seedling stages (Lopes et al., 2020). Weed seed banks comprise of potentially germinable, and dormant weed seeds which are vertically, and horizontally distributed within the soil profile (Hussain et al., 2017). The number of weed seeds in the seed banks varies considerably depending on soil type and farmer's management practices *(Hussain et al.,* 2017); and knowledge of weed seed bank status is important for designing appropriate weed management strategies (Hussain et al., 2017).

Literature relating soil fertility to weed growth and proliferation is quite extensive, but overly contradictory (Moyo, 2005; Mtambanengwe et al., 2006; Nezomba et al., 2015). For instance, Mashingaidze et al. (2009) noted that high levels of soil fertility tend to reduce the competitive effect of weeds on crop growth and production. On the other hand, Schwartz-Lazaro & Copes (2019) concluded that low soil fertility affects the weeds as well as the crops. There are also varied reports that soil heterogeneity due to differences in inherent properties (organic matter, pH, aluminium toxicity, CEC, etc.) of the soil arising from different parent material, catena position and differences in the management practices within and across farms (Dessalegn et al., 2014; Nezomba et al., 2015). The catena influences micro-environment, the physical and chemical properties of this soil which also affect soil biological activities. This implies that soil fertility could be strategically leveraged in designing weed management programmes. However, the link between soil fertility and level and diversity of species in weed seed banks remains a subject for further investigation.

An estimated 70 % of smallholder farms in Zimbabwe are situated on low fertile soils (Chaumba et al., 2003; Moyo, 2005). In a bid to address low soil fertility challenges and improve maize productivity, resource-poor farmers use with limitations options available to them such as pit compost, anthill soils, livestock manures, crop residues and inorganic fertilisers (Mtambanengwe et al., 2006; Nyamangara et al., 2011). However, the use of livestock manure as a soil-improving organic amendment may increase weed density, and weed reproductive structures; for example, weed seed capsules thereby affecting the weed seed bank status. The objective of the study was to investigate short-term effect of SOC, and repeated soil nutrient amendment on weed seed bank emergence and biomass on smallholder farms across a soil fertility gradient on the catena.

Methods

Study site

Soil used in this study was obtained from three smallholder farms that were under short-term (six years) soil nutrient management in Wedza, (18°15' latitude, 32°22' longitude), Eastern Zimbabwe. The farms varied in SOC ((low= 3.9, medium= 6.4, high=8.9 g SOC kg^{-1} soil) and situated on the upper, middle and lower catena position, respectively. Soils in the three farms are generally infertility with low content of major nutrients N, P, K. The farms used in this study had similar previous land use practices (maize mono-cropping) under conventional tillage system. They were located in the natural region (NR) IIb, at an altitude of 1150 m above sea level, with a mean annual precipitation of >800 mm falling between November and March. The predominant soil type across the three farms was sandy textured soils derived from granitic parent material and classified as Alfisol (USDA soil taxonomy) or Lixisol (FAO soil classification).

Experimental design and sampling procedures

Soils for the weed seed bank studies were sampled from five treatments, namely, unfertilised control, 7%N, 14%P and 7%K (NPK), cattle manure (CM), NPK+CM and NPK+lime from each of the three farms (Zaka, Mwendamberi and Vengesai) along the catena. The smallholder farms had received fertiliser rates of 120 N, 26 P and 30 K kg ha^{-1}, while cattle manure applied treatments had received 5 t ha^{-1} in each season. Lime as a treatment was applied at 1.5 t ha^{-1} and this was done biannually; that is, 2011, 2013 and 2015. Treatments were laid out in a randomised complete block design.

From the three fields, six cores each with a diameter 15 cm were randomly collected each into a clean plastic bucket (10 litres), from a 0-20 cm plough depth. The sub-samples from each field were mixed into a composite sample. Seed bank sampling was done in 2014/15 and 2015/16 cropping seasons.

Weed diversity, emerged weeds and biomass

Soil samples of about 1000 g for each plot, were evenly spread (20 cm deep) in labelled germination trays and watered regularly (Forcella et al*.,* 2003). The weed emergence experiment was carried out in asbestos trays from mid-October in each season under green-house conditions.

Weed species were identified two weeks after weed seedling emergence and counted by specie (number kg^{-1}

soil). Biomass of emerged weeds in the entire plot were determined using a method described by Parwada et al. (2020). The emerged weed species were identified botanically, counted at approximately 2 cm in height. Then the weed species were cut at ground level, packed in khaki brown paper bags, and oven-dried for 48 hours at 70 °C for determination of emerged weed biomass (Forcella et al., 2003). This process was repeated once a week. In the seventh week after emergency, the soil in the trays was sun-dry for about five days. The soil on the trays was thoroughly and rewatered following the same routine, for an additional six weeks. At four weeks after emergency, diversity was determined using richness (Margalef index), evenness (Pie Lou index), (Pie Lou, 1966) and Shannon Weiner index (Shannon, 1948), *viz*:

$$\text{Shannon Wiener index (H)} = -\sum_{i-1}^{s} Pi\ In\ (Pi) \quad (1)$$

Where: H is Shannon Wiener diversity index, S is the number of individual species in the community (richness), Pi will be the proportion of S made up of the i-th species that is $p_i = N_i / N_{total}$, where N_i is the individuals of species i (plants m^{-2}) and N_{total} is the total number of individuals (plants m^{-2}).

Species Richness was determined based on the Margalef index:

$$R = \frac{S-1}{InN} \quad (2)$$

The community Evenness was determined using the Pie Lou's index:

$$J = \frac{H\prime}{InS} \quad (3)$$

Data analysis

Data on weed seed emergence and biomass of emerged weed seeds were tested for normality of variance using Ryne-Joiner and Bartlett's test, respectively. Analysis of variance was carried out by the Linear Mixed-Effects model Restricted Maximum Likelihood, in GenStat Discovery 14. Principal component analysis (PCA) was performed to establish relationships between season, SOC, soil nutrient amendment, and emerged weed seeds, using CANOCO 5).

Results

Weed diversity

Weed seed bank richness (Margalef index) and Shannon Weiner index were significantly influenced by levels of SOC (p < .001); but not evenness (Table 1). Margalef index increased in medium and high SOC content by 1.5 and 1.3 times, respectively; while the Shannon Weiner index increased by 1.3 and 1.1 times; compared to low SOC (Table 1). The farm nutrient application (NPK+CM) significantly (p < .001) increased Shannon Wiener index by 1.3 and Margalef index by 1.4 times compared to unfertilised control treatment (Table 1).

Table 1.

Effect of SOC and nutrient application on diversity indices (Margalef, Shannon Wiener and Pie Lou indexes) of weeds on-farm in Hwedza, Zimbabwe

	Weed diversity indices		
Parameter	Margalef index Richness	Shannon Wiener index	Pie Lou index Evenness
SOC content			
Low	1.44^{a}	1.47^{a}	0.62
Medium	2.10^{c}	1.84^{c}	0.65
High	1.87^{b}	1.68^{b}	0.60
F pr	<0.001	<0.001	Ns
SED	0.08	0.05	0.024
Soil nutrient amendments			
Control	1.54^{a}	1.52^{a}	0.69^{d}
NPK	1.64^{b}	1.60^{a}	0.62^{c}
NPK+LM	1.79^{c}	1.56^{ab}	0.60^{a}
CM	1.95^{d}	1.79^{c}	0.60^{a}
NPK+CM	2.11^{e}	1.86^{c}	0.61^{b}
F pr	<0.001	<0.001	0.011
SED	0.09	0.07	0.003
Interaction (SOC × soil nutrient amendment)			
F pr	0.014	0.003	Ns
SED	0.172	0.12	0.044

***Means followed by the same letter superscript in a column are no significantly different at p < .05.

The interaction of SOC level and soil nutrient application significantly (p < .05) affected species richness (Margalef) and Shannon Wiener Indices (Figures 1a and 1b); with the highest Margalef index recorded from the medium SOC compared to the other SOC contents. In the low and medium SOC levels, the lowest species richness was found in unfertilised control and NPK treatment. Co-application of NPK+CM and/or sole CM increased richness by 38.6 %, in the low SOC, and 45.5 % in the medium SOC levels. However, the Margalef index was not affected by the soil nutrient management in the high SOC content (Figure 1a).

Shannon Wiener index of less than 1.4 ± 0.12 was observed in unfertilised control, NPK, and NPK+LM; while significantly high Shannon indices (1.6 ± 0.12 and 1.7 ± 0.12) were recorded in treatments that received CM and NPK+CM, respectively under low SOC (Figure 1b). In contrast, in medium SOC, the Shannon index values increased 1.2 times in NPK, NPK+LM and 1.4 times in CM, NPK+CM. However, in high SOC, the Shannon index in NPK+LM was lower compared to other treatments (Figure 1b).

Weed seed bank numbers and biomass

Weed seedling emergence varied across the SOC statuses with values of 31.6 ± 0.73 and 29.2 ± 0.73 and 32.4 ± 0.73, corresponding to low, medium and high SOC, respectively (Figure 2a).

Total emerged weed seed bank numbers were significantly (p < .001) affected by soil nutrient management and SOC content along the catena (Fig. 2b).

Weeds seedling total biomass was also significantly (p < .001) affected by soil nutrient management and SOC levels (Table 1). The medium and high SOC contents had significantly higher weed biomass (5.97 ± 0.40 and 5.78 ± 0.40, respectively) compared to low SOC 4.14 ± 0.40 (Figure 2b).

Soil nutrient management markedly (p < .001) increased emerged total weed seed bank counts in NPK, NPK+CM, and CM by 3, 6 and 8 %, respectively compared to unfertilised control (Figure 3a). Weed seedling biomass from was also significantly (p < .001) increased by soil nutrient application; increasing by 67, 80, 166, and 200 % in NPK+LM, NPK, CM, and NPK+CM, respectively; compared to unfertilised control treatment (Figure 3b).

Soil Organic Carbon and weed seed bank species

The principal component analysis (PCA) recorded an association between SOC, season and weed seedling species composition (Figure 4). The PCA bi-plot accounted for 44.5 % of total variance in weed composition, with Axis 1 accounting for 28.1 % (eigenvalue of 0.281); while Axis 2 accounted for 16.4 % (eigenvalue =0.164). SOC content had a strong effect on weed seedling species composition. Weed seedling species were significantly (p < .05) linked to SOC levels according to position on the catena; thus, mimicking results from the original field experiment (Figure 4). However, the soil nutrient management was not significant (p < .05).

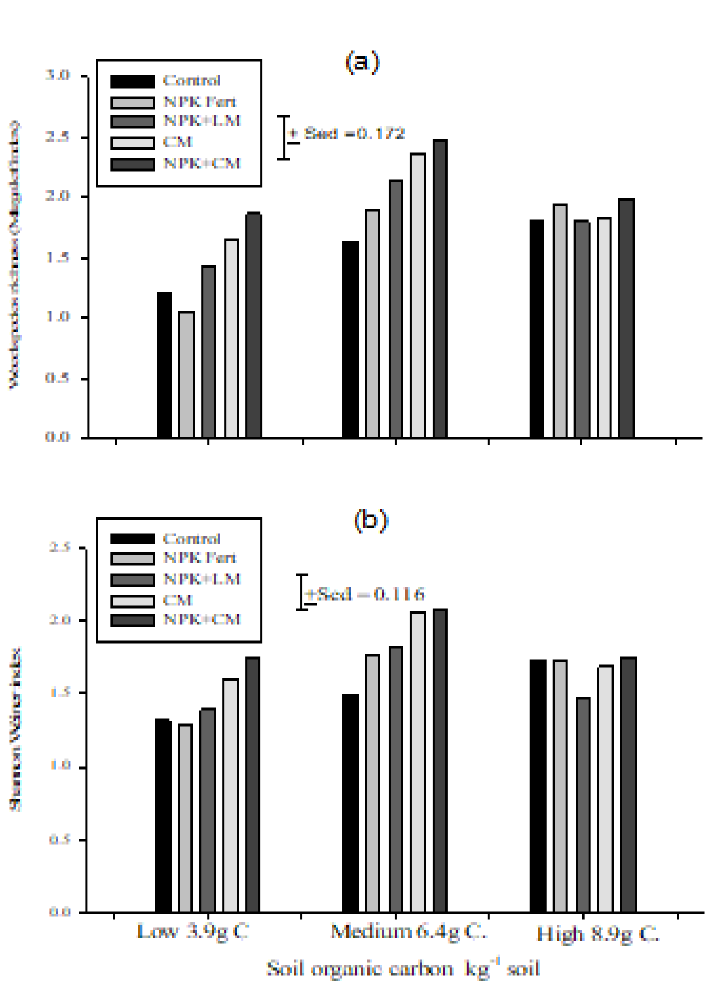

Figure. 1
Interaction of SOC × soil nutrient amendment on Margalef and Shannon Wiener indices.

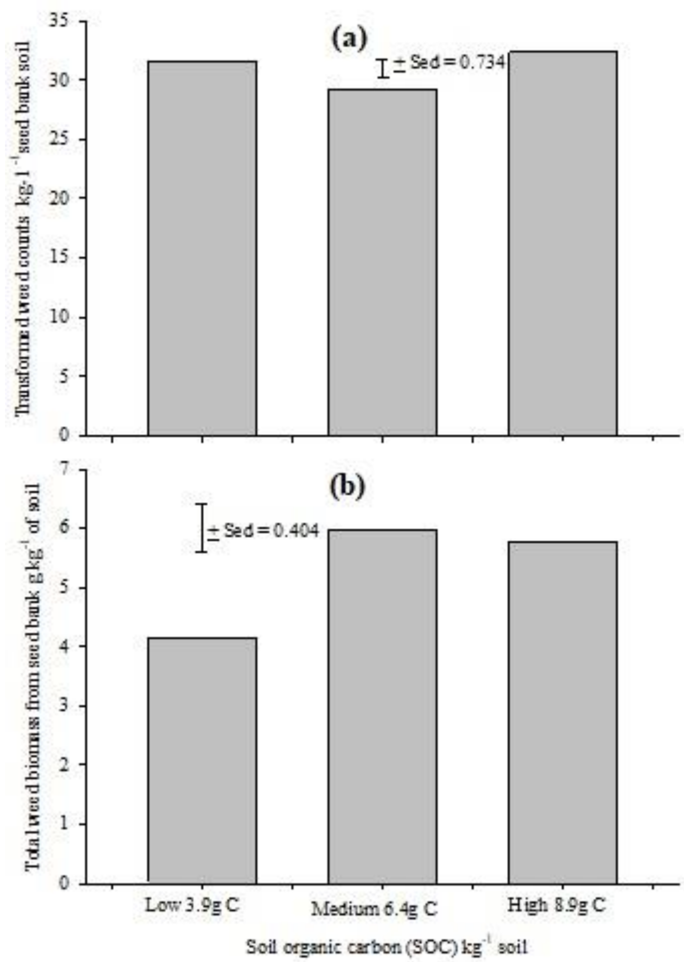

Figure 2.
Effect of SOC levels on weed seedling counts and biomass of during the 2014/15 and 2015/16 cropping seasons in Hwedza, Zimbabwe.

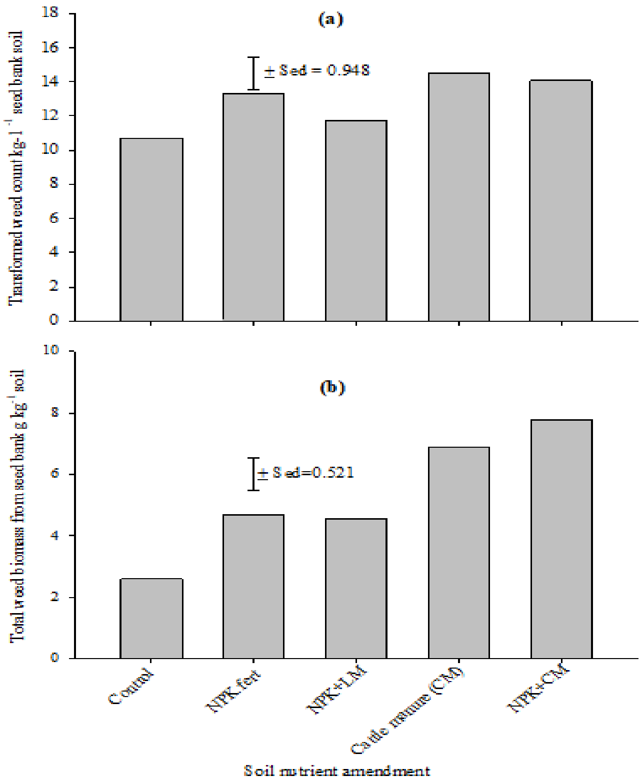

Figure 3.

Effect of soil nutrient management on weed seedling counts and biomass in 2014-2016) cropping seasons in Hwedza, Zimbabwe.

Weed seedling diversity and numbers varied markedly with season (Figure 5a). There was a spike in weed density in 2014 and 2015; then a decline in 2016 in high SOC content. In contrast, in the medium SOC levels, there was an increase in weed count in 2014; followed by a decline in 2015 and another increase in 2016 (Figure 5a).

The Principal Response Curves (PRCs) drawn from sampling season, SOC and their interactions was significant based on Monte Carlo permutation test (F= 30.2, *p*=.002). However, soil nutrient management was not significant ($p < .05$, Figure 5b). Weeds with positive weights on the PRC scale were *G. parviflora*, *D. stramonium*, *C. monophylla*, and *L. martinicensis;* while *R. scabra* and *S. pinnata* had negative weights (Figure 5b). The six species listed above were then further analysed using REML (Table 3). Seventy five percent of the weed species fell between -0.5 and 0.5 were not influenced by SOC content and were excluded from further analysis (Figure 5c).

The PRC showed that *C. monophylla*, *D. stramonium* and *G. parviflora* numbers increased with increase in SOC levels; while *R. scabra* and *S. pinnata* numbers decreased with increase in SOC (Table 2). Integration of NPK+CM increased the numbers of *D. stramonium* and *G. parviflora*; while sole NPK increased the counts of *R. scabra*. In contrast, *C. monophylla* and *S. pinnata* numbers were not affected by soil nutrient management (Table 2).

Table 2.

Principal Response Curve scale (PRC) of major seedbank weeds associated with affected by SOC content and nutrient amendments

	PRC weed species				
	C. monophylla	*D. stramonium*	*G. parviflora*	*R. scabra*	*S. pinnata*
SOC content (kg $^{-1}$ soil)					
Low	1.0^{a} (1)	1.9^{a} (6)	2.1^{a} (6)	5.5^{c} (28)	5.1^{c} (36)
Medium	2.3^{b} (9)	2.7^{b} (10)	2.3^{a} (6)	4.2^{b} (20)	3.0^{b} (17)
High	2.8^{c} (13)	4.1^{c} (21)	3.7^{b} (12)	2.7^{a} (7)	2.4^{a} (8)
F pr	<.001	<.001	<.001	<.001	<.001
SED	0.35	0.33	0.27	0.34	0.38
Soil nutrient amendments					
Control	2.1(9)	1.1^{a} (3)	1.6^{a} (3)	3.6^{b} (14)	2.8 (10)
NPK	1.8 (7)	2.1^{b} (9)	3.0^{c} (9)	5.2^{d} (26)	3.8 (25)
NPK+LM	1.9 (5)	1.6^{a} (3)	2.3^{b} (6)	2.1^{a} (7)	3.4 (21)
CM	2.4 (11)	4.7^{c} (21)	3.2^{cd} (10)	4.6^{c} (22)	3.5 (21)
NPK+CM	2.0 (5)	5.0^{c} (25)	3.4^{d} (12)	4.6^{c} (22)	4.0 (24)
F pr	Ns	<.001	<.001	<.001	Ns
SED	0.44	0.42	0.35	0.43	0.49

*****Means followed by the same letter superscript in a column are no significantly different at $p < .05$. †Numbers in brackets are back-transformed (actual) weed numbers m^{-2}.

Discussion

Diversity of weeds

Twenty-four weed species were identified from the soil seed bank study along a toposequence, indicating diverse weed species. An increase in Richness (Margalef index) and Shannon Weiner index was recorded in medium and high SOC content. The increase in weed diversity and weed community composition can be attributed to the effects of topography and nutrient management, on the across the toposequence. Topography steepness and orientation affect microclimate, determine water movement (infiltration, runoff, erosion, available moisture), deposition of nutrients, affect vegetation growth, radiation interception at different landscape positions (Boling et al., 2008; Kone et al., 2013). Boling et al. (2008) attributed the change in weed density along the catena to variation in soil texture, organic matter and macro-nutrient (NPK) content resulting from the above listed physical processes. The differences in texture, SOC and nutrient status along the catena also confirm the findings by Koné et al., (2013), who reported an increase in *Cyperus* density down the slope and attributed it to changes in soil physical and chemical properties along the catena.

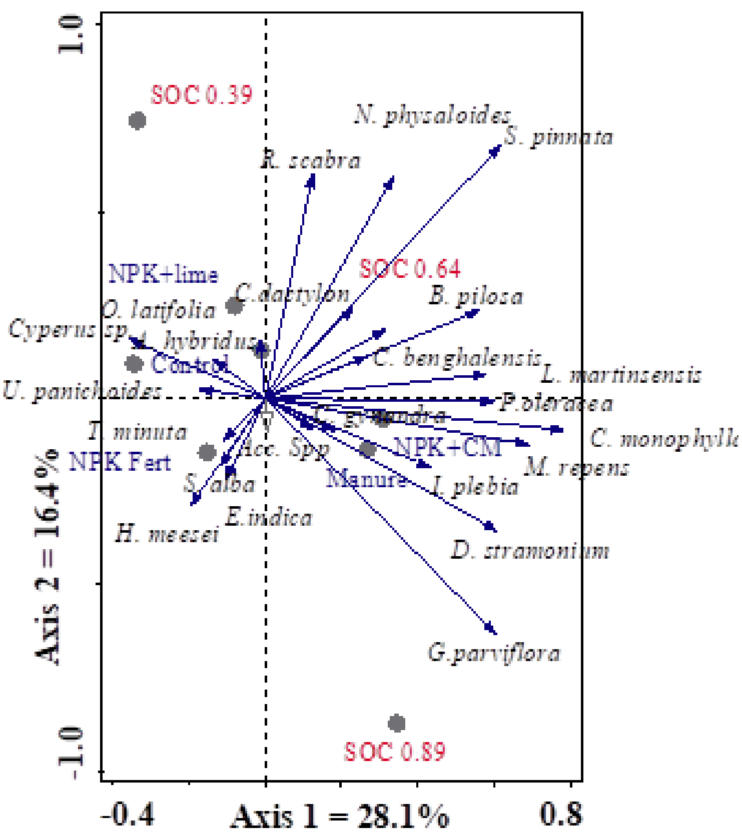

Figure 4.

Principal Component Analysis (PCA) Bi-plot projection of weed species for three seasons (2014-2016), affected by SOC content and soil nutrient management in Hwedza, Zimbabwe.

As depicted by soil characterisation of the experimental site, the upper catena consists of coarser soil texture with poor physical and chemical properties, compared to the lower catena (Boling et al., 2008). The sedimentation of fine-textured particles in the lower catena, according to Nezomba *et al*. (2015) increased clay content, iron oxides, pH and organic matter (OM), and may have resulted in a distinct fertility gradient from crest (upper) middle and lower (bottom) catena positions. The lower catena soil exhibits good physical and chemical properties for weeds establishment (Koné *et al*., 2013).

Weed species were strongly linked to SOC content along the soil catena (Fig. 6a). Weeds associated with sandy soils in the upper catena position appeared to be well adapted to infertile, low pH (acidophiles) and degraded soils (Mavunganidze *et al*., 2016). Weed species predominant in the middle and lower catena positions are known to have luxurious uptake of N and P and are referred to as nitrophilous and phosphophilous, respectively (Blackshaw *et al*., 2010) and pose a lot of competition in fertilised fields. The results confirm findings by Kone *et al*. (2013) and Touré et al. (2014) who reported physical spatial distribution of weeds along the catena as influenced by soil physical and chemical properties. Mavunganidze et al. (2016) also reported a strong association of weed species, soil texture and nutrient management.

Apart from variation in SOC content and location on the farm across the toposequence, nutrient management affected soil physical, chemical and biological properties and promoted nutrient uptake, water retention and improved soil health (Bera et al., 2018; Nezomba et al., 2015). Application of cattle manure (CM) and NPK+CM in low and medium SOC content increased species richness, Shannon Wiener, and Evenness. Soil fertility management, especially co-applying organic and in-organic fertilisers altered the composition and species diversity in farm fields (Table 2). The increased species diversity in NPK+CM suggested that balanced fertilisation improves or maintains soil stability and productivity. In contrast, Jiang et al. (2014) found that the application of organic fertilisers reduced the species number and richness index of the soil weed seed bank. These noted discrepancies could be due to difference in forage diets and mother plants content and diversity of weed species in each case.

Biomass of emerged weeds

Biomass of emerged weeds from soil samples increased by 7 % as the SOC content changed from 3.9 to 8.9 g kg^{-1} soil (Table 1). The inherently high SOC content, and optimum pH from medium and high SOC content could have stimulated the germination, and emergence of weed seeds (Tang et al., 2014). Moreover, the possible adequacy of nutrient and water supply to the emerged weeds, resulting from the treatments, could have caused rapid growth and therefore, boosted seedling biomass accumulation, which in turn could have translated into high weed reproductive capacity (Mavunganidze et al., 2009). Well-nourished weed plants tend to shed more weed seeds back into the seed bank, compared to their weak counterparts from low fertile upper catena (Koné et al., 2013).

The differences in weed biomass arising out of seed banks along the catena, have earlier been reported by several reserachers (Dessalegn et al., 2014; Koné et al., 2013; Malinowska and Szumacher, 2013; Touré et al., 2014). The increase in biomass from middle and lower catena is likely to be affected by the physical and chemical properties of the soils, which in turn affect nutrient and water uptake by the crop and weeds down the slope (Touré et al., 2014). Weeds tend to benefit more from inherent fertility, owing to their natural adaptation and greater ability to efficiently extract nutrient from such soils; these augment weed density and biomass (Wortman et al., 2011).

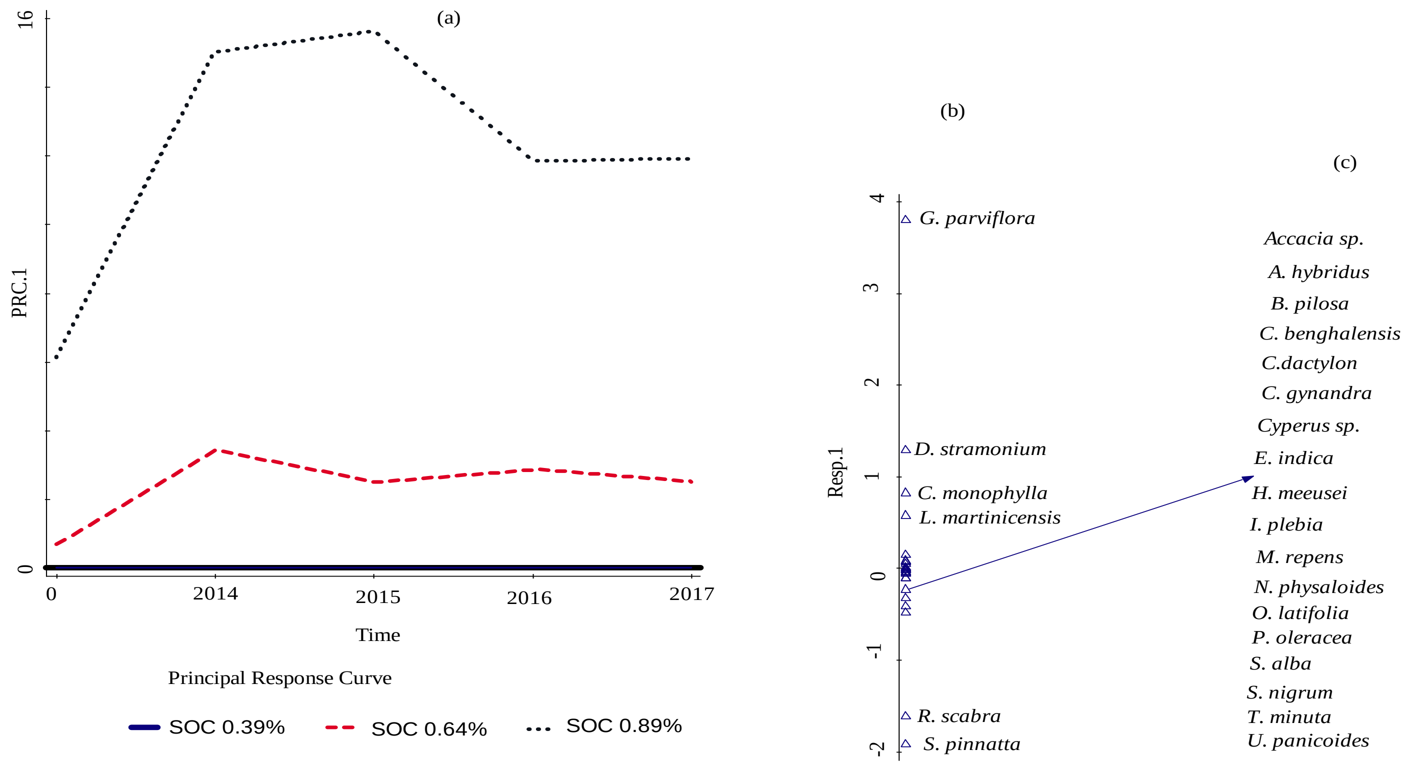

Figure 5

(a) Principal response curves (PRCs) on response of weed seed bank to SOC content. Medium and high SOC relative to standard low SOC over three sampling seasons (b) Principal response curve scale presentation on weed species strongly influenced by SOC status. (c)Weed species lying on –0.5 and 0.5 on the principal response curve scale and not considered for further analysis.

From the present study, NPK+CM and sole CM amended treatments increased weed seedling emergence from the soil by 6 and 8 %, respectively. *Datura stramonium, B. pilosa, A. hybridus*, *N. physaloides* and *G. parviflora* numbers were relatively high in these treatments and increased total biomass of emerged weeds as well. Application of livestock manure and fertiliser presumably benefit weeds through enhanced nutrition, breaking weed seed dormancy and initiation of weed seedling emergence (Karimmojeni et al. 2011), weed growth and competitiveness, weed biomass accumulation (Tang et al., 2014), and weed seed production (Blackshaw & Brandt, 2009).

Increased weed seedling numbers and biomass of treatments that received CM, NPK+CM was also attributed to cattle manure acting as a weed seed source or by providing possible stimulants for weed seed germination. This is a virgin area for future investigations. Otherwise, livestock manure is known to change physical (aggregation, aeration, infiltration, and water retention capacity) and chemical properties (cation exchange capacity, amelioration of soil pH) of the soil (Wortman *et al*., 2010; Kone et al., 2013). This has been found to benefit crops and also un-intentionally benefits weeds (Chipomho et al., 2018). Furthermore, cattle manure is known to carry enormous numbers of germinable weed seeds (Materechera & Modiakgotla, 2006) and this is associated with high density and biomass in field crops, especially when the soil has not been cured or incubated for less than five months. Rupende et al. (1998) observed that *A. hybridus*, *E. indica* and *N. physaloides* densities were associated with cattle manure which was treated for less than four weeks. Results from our study suggested that manure use in agro-ecosystems is a potential source weed seeds into the soil seed banks (Smith et al., 2008). Generally, the application of CM, NPK+CM, and NPK+lime ameliorates soil pH and increases nutrient uptake by weeds, thereby increasing weed numbers and biomass of emerged weeds from seedbank compared to the control treatment. Blackshaw et al. (2010) concluded that the use of organic and inorganic fertilisers does not only benefit the crop but weeds as well.

Conclusion and Recommendations

This study has demonstrated the effect of soil fertility management on weed species emergence from a weed seed bank, diversity, numbers and biomass across a SOC gradient of a cultivated catena in Zimbabwe. *Melinis repens*, *R. scabra*, *Cyperus* sp. and *S. pinnata* weed species were associated with low SOC content on upper catena position; while *B. pilosa*, *N. physaloides*, *L. martinicensis*, *C. monophylla*, *D. stramonium*, *H. meeusei* and *G. parviflora* were strongly linked to medium and high SOC content on middle and lower catena positions. Generally, livestock manure treatments were associated with the greatest seedling emergence, weed biomass and species diversity. Thus, manure can be external major source of weed seed, unless when pre-treated prior to its application. However, the mode of contribution of cattle manure to the weed seed bank, which may be by harbouring weed seeds, providing germination stimulants, enhancing weed nutrition or providing a more favourable growth environment, requires further investigation.

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Abundance and Dispersion Indices of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Tomato Plant in Nigeria

Nijerya'da Domates Bitkisinde *Helicoverpa armigera* (Lepidoptera: Noctuidae)'nın Popülasyon ve Dağılım İndeksleri

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ABSTRACT

Helicoverpa armigera is among the major insect pests that pose a threat to tomato production in Nigeria with an insufficient understanding of its population parameters, and available environmentally friendly control materials which are essential for the development of reliable and sustainable management strategies that can minimize the frequent use of synthetic pesticides; which are hazardous to the environment, non-target organism, and humans. The present study was conducted with the objectives of establishing population abundance and dispersion of *H. armigera.* Surveys were conducted in the savannah agroecological zone of Nigeria through fortnightly field visits to tomato farms from July to September 2019 (rainy season), and month of December 2019 to March 2020 (dry season), making a total of seven (7) and Nine (9) visits respectively. Ten plants were randomly selected and tagged from each farm, for observation of the presence of *H. armigera* larvae on the upper and lower parts early in the morning (6:00-7:30 am) on all sampling. Numbers found on each plant were counted and recorded with dates. Data obtained were subjected to analysis of variance and the least significant difference (LSD) at 0.05% level of probability was used to separate significant means. Data were further used in calculating the various dispersion indices. The result indicates that *H. armigera* was higher at 8 weeks after planting and aggregated on the upper part of the plant with a higher population in the dry season. Abundance and dispersion indices of *H. armigera* on tomato plants reported in this study will provide for appropriate decision-making in designing eco-friendly management of the pest.

Keywords: Tomato, *Helicoverpa armigera*, fruit worm, population, dispersion, Savannah

ÖZ

Helicoverpa armigera, popülasyon parametrelerinin yeterince anlaşılmaması ve sentetik pestisitlerin sık kullanımını en aza indirebilecek güvenilir ve sürdürülebilir yönetim stratejilerinin geliştirilmesi için gerekli olan mevcut çevre dostu kontrol materyallerinin yetersiz anlaşılmasıyla Nijerya'da domates üretimine müdahale eden başlıca zararlılar arasındadır. Tarım ilacı; çevre, hedef olmayan organizma ve insanlar için tehlikelidir. Bu çalışma, *H. armigera*' nın çalışma alanındaki popülasyon dağılımını belirlemek amacıyla yapılmıştır. Anketler, Temmuz-Eylül 2019 (yağmur mevsimi) ve Aralık 2019-Mart 2020 (kurak mevsim) arasında iki haftada bir domates çiftliklerine saha ziyareti yoluyla Nijerya'nın savan agro-ekolojik bölgesinde gerçekleştirildi. Tüm örnekleme günlerinde sabahın erken saatlerinde (06:00-7:30) üst ve alt kısımlarda *H. armigera* larvalarının varlığının gözlemlenmesi için her çiftlikten rastgele on (10) bitki seçildi ve etiketlendi. Her bitkide bulunan *H. armigera* sayıları sayıldı ve tarihlerle birlikte kaydedildi. Elde edilen veriler varyans analizine tabi tutulmuş ve anlamlı ortalamaları ayırmak için %0,05 olasılık düzeyinde en küçük anlamlı fark (LSD) kullanılmıştır. Veriler ayrıca çeşitli dağılım indekslerinin hesaplanmasında kullanıldı. Sonuç, *H. armigera* 'nın dikimden 8 hafta sonra daha yüksek olduğunu ve kurak mevsimde daha fazla nüfusa sahip bitkinin üst kısmında toplandığını göstermektedir. *H. armigera*'nın bu çalışmada bildirilen domates bitkisi üzerindeki dağılım ve dağılım indeksleri, zararlının çevre dostu yönetimini tasarlamada uygun karar verme için sağlam bir temel sağlayacaktır.

Anahtar Kelimeler: Domates, *Helicoverpa armigera*, meyve kurdu, popülasyon, dağılım, Savannah

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Introduction

Tomatoes are among the most important commodities and widely crop plants grown in Africa and the world (Ciceoi et al., 2021). They are considered culinary vegetables which are used in different types of recipes such as salads, sandwiches or soups (Olugbire et al., 2020). It can be sliced with a little onion and pepper and eaten with a local dish known as "danwake" or eaten with a little oil and chilies as spices. It can be processed into paste or puree, and used in cooking or the production of fruit drinks and ketchup (Chula et al., 2017; Ghaderi et al., 2017). In Nigeria, tomatoes are sundried and ground into powder (Olugbire et al., 2020) for use in preparations of different soups.

Nutritionally, tomatoes are filled with a variety of nutrients like fiber, potassium, and vitamins A and C. Medium, red, ripe tomatoes provide 22 calorie diet (Ghaderi et al., 2017; Yasser et al., 2019), and contain 8 percent of dietary potassium and 7 percent of recommended dietary allowance (RDA) of iron for women and 10 percent for men (Olugbire et al., 2020). Tomato contains high levels of lycopene which is a very powerful antioxidant that prevents the development of many forms of cancer (Hussaini et al., 2016). The leaves have also been used widely as a natural antiseptic agent because of their narcotic acid content (Ugonna et al., 2015).

Nigeria was ranked the second largest producer of tomatoes in Africa, and 16th largest producer in the world (Ghaderi et al., 2017), and about 1.8 million metric tonnes of tomatoes were produced in Nigeria, which accounts for about 68.4% of West Africa, 10.8% of Africa's total output and 1.28% of world output (Umar et al., 2015). Production in Nigeria spread all over the country, the total arable land under tomato production was estimated to be 1,000,000 ha with an estimated output of 1.8mm tones per annum (Umar et al., 2017). The major producing areas in Nigeria lies within the area with a temperature range of 25-34^{0}C (Ugonna et al., 2015), these areas include most states (Bauchi, Benue, Borno, Kaduna, Kano, Plateau, Sokoto, Jigawa and Yobe) in the Northern region.

Tomato production is affected negatively by numerous pests which result in poor yield, low quality, and financial loss. Ahmed et al. (2022) reported about 200 species of insect pests attacking tomatoes in Egypt. The major pests include aphids (*Aphis gossypii*), white flies (*Bemisia tabaci*), thrips (*Thrips tabaci*), leaf miners *(Lyriomyza spp* and *Tuta absoluta*), and the fruit worms (*Helicoverpa armigera*) (Kollie et al., 2014; Brévault et al., 2014).

Helicoverpa armigera is one of the most serious insect pests of tomatoes worldwide. It is widely distributed in Asia, Europe, Africa, and Australasia. The larvae are the destructive stage, they prefer to feed and develop on reproductive structures in general, feed on buds, flowers, and fruit which are rich in nitrogen (Liu et al., 2004); leading to severe yield loss in greenhouse and open-field tomato crops (Compolo et al., 2017). This moth has a high reproductive potential, completing up to 13 generations per year (Tropea Garzia et al., 2012).

In Nigeria, the use of synthetic pesticides has been reported to be the main control measure used for the management of fruit borer infestation on tomatoes and other crops (Olugbire et al., 2020). However, Nigerian farmers lack knowledge about safety and do not have access to appropriate training that could help them cope with pesticide hazards (Sule et al., 2020).

Lack of monitoring of insect pests may increase the unwarranted frequency of pesticide application by our local farmers, exacerbating the manifestation of the drawbacks of synthetic insecticides on the environment. There is a consensus among pest control specialists that insecticides should be applied as a last resort and should be based on the established threshold levels; which can only be economically estimated when there are reliable data on population dynamics, distribution, and dispersion of insect pests among others. There is currently some paucity of information in literature on most of the above insect population parameters particularly for *H. amigera* in this part of the world. Therefore, this study aims to determine the population distribution and dispersion indices of *H. armigera* on tomato plants in some selected tomato-producing areas of Kano and Yobe State in Nigeria to generate information on the same that can be harnessed in the development of IPM systems for its management.

Methods

Study location

The study was conducted in two states (Kano and Yobe) in Northern Nigeria. Two Local Governments were randomly selected from each State. Thereafter, six villages (Lamba-Burji, Takaraste, Doka, Gada, Gadana, and Ngalda) across the four LGs (Madobi, Tofa in Kano State, Nangere, and Fika in Yobe state) that are known for both dry and rainy season tomato cultivation were purposefully selected for the study. The mean annual rainfall in Kano and Yobe State during the sampling at rainy season is 178.33±6.20 and 210.15±3.20 mm respectively.

Survey and Sampling Methodology

Field visit to tomato farms in the study area was done from July - September 2019 (rainy season), December -2019 through March -2020 (dry season). The surveys were conducted each field starting from six weeks after transplanting up to harvest since the activities of *H. armigera* are high during this period in the tomato plant phenology. In total, there were seven (7) samplings in the rainy season and nine (9) in the dry seasons in each location.

In each of the selected villages, one farm was randomly selected from which ten plants were subsequently randomly selected and tagged for data collection. Each plant was divided into two equal parts (upper and lower parts). Observation for the presence of *H. armigera* larvae on the upper and lower parts of the tagged plants was made early in the morning (6:00-7:30 a.m.). Number of H. *armigera* found on each plant was collected, counted, recorded with dates and killed.

Statistical data analysis

To determine whether the mean population of *H. armigera* larvae differed significantly between the upper and lower parts of the tomato plant, the number of *H. armigera* larvae counted on the upper and lower parts of the tomato plants during each sampling visit were subjected to analysis of variance using computer software Genstat Discovery Edition for Windows. The least significant difference (LSD) at a 0.05% level of probability was used to separate significant means.

Also, based on *H. armigera* larvae counts from upper and lower parts of tomato plants, a mean number of *H. armigera* larvae per tomato plant over time was calculated, and used in calculating the various dispersion indices. Furthermore, the spatial distribution of *H. armigera* larvae was determined by different methods. The simplest method is the variance to mean ratio, S^2/m where the value of $S^2/m<$ 1 indicates a uniform dispersion, while $S^2/m=$ 1 indicates random dispersion and $S^2/m>$ 1 indicates an aggregated dispersion.

Lloyd's index of patchiness is described as the ratio of the mean of mean crowding (m^*) to mean density (m). The mean crowding was calculated using the formula as described by Queiroz-Santos et al. (2018) and Sule et al. (2012):

$$m^* = x + \left[\left(\frac{S^2}{x}\right)\right] - 1 \qquad (1)$$

Where
x is the mean density
S^2 is the variance,
Lloyd's (m^*) index =1 indicates a random dispersion; Lloyd's index > 1 indicates aggregated dispersion, and Lloyd's index < 1 indicates regular dispersion.

The degree of aggregation was determined by the most commonly used dispersion indices, i.e., the Greens coefficient (*Cx*). The Greens coefficient was calculated as described by (Sule et al., 2012; Wade et al., 2018) using the formula:

$$Cx = \frac{\left(S^2/m\right) - 1}{\Sigma x - 1} \qquad (2)$$

Where
S^2= variance of mean,
m= mean number of *H. armigera* per shoot and
Σx = total number of *H. armigera,* where
Cx= 1, the coefficients indicate a random dispersion; Cx>1, it indicates aggregated dispersion; where Cx<1, indicates regular dispersion

Table 1
Abundance of H. armigera Larvae on Tomato Plants during Dry and Rainy Seasons in Kano and Yobe States

	Mean number of larvae	
Season	Kano	Yobe
Dry	6.5^b	5.3^b
Rainy	0.8^a	0.8^a
SE±	1.85	4.10

Mean followed by different letters in the same column are significantly different at *p* = .05 level of probability according to the least significant difference (LSD) test.

Results

Abundance of *H. armigera* Larvae on Tomato Plants during Dry and Rainy Season in Kano and Yobe State

Table 1 shows the abundance of *H. armigera* larvae per plant in both dry and rainy season in the Kano and Yobe states. The result indicates that there is a significant difference ($p<$ 0.05) between the dry and rainy season for both states. Significantly high numbers of *H. armigera* larvae were recorded during the dry season compared to the rainy season in both States.

Table 2
Abundance of H. armigera Larvae on Upper and Lower Part of Tomato Plant at 6, 8, 10 and 12 weeks after planting in Kano State.

WAP	Upper	Lower
6	1.7^{ab}	0.7^a
8	1.7^{ab}	3.8^b
10	3.5^b	0.8^a
12	0.2^a	0.3^a
SE±	1.44	0.99

Mean followed by different letters in the same column are significantly different at p =.05 level of probability according to the least significant difference (LSD) test.

Abundance of *H. armigera* Larvae on Upper and Lower Parts of Tomato Plant at 6, 8, 10 and 12 Weeks After Planting (WAP) in Kano State

Table 2 shows the abundance of *H. armigera* larvae on the upper and lower part of tomato plants in Kano state at 6, 8,10 and 12 WAP. The result reveals a significant difference (p <.05) in the number of larvae obtained at different WAP in the upper and lower part of the tomato plant. On the upper part of the tomato plant, significantly high numbers of *H. armigera* larvae were recorded at 10 WAP, but this number was not statistically different from the number of larvae obtained at 6 and 8 WAP and the least number *H. armigera* larvae were obtained at 12 WAP, however, this number was not different statistically from the number of *H. armigera* larvae obtained at 6 and 8 WAP. When the lower part of the plant is been considered, a significantly high number of *H. armigera* larvae was obtained at 8 WAP compared to the remaining WAP, and the least number of *H. armigera* larvae was obtained at 12 WAP but was at par with the number of *H. armigera* larvae obtained at 6 and 10 WAP.

Table 3

Abundance of H. armigera Larvae on Upper and Lower Parts of Tomato Plant at 6, 8, 10 and 12 weeks after planting in Yobe state

WAP	Upper	Lower
6	3.7^{a}	1.0^{ab}
8	2.7^{a}	3.3^{b}
10	1.2^{a}	0.2^{a}
12	0.2^{a}	0.0^{a}
SE±	2.75	1.55

Mean followed by different letters in the same column are significantly different at p = .05 level of probability according to the least significant difference (LSD) test.

Abundance of *H. armigera* Larvae on Upper and Lower Parts of Tomato Plant at 6, 8, 10 and 12 Weeks After Planting (WAP) in Yobe State

Table 3 shows the abundance of *H. armigera* larvae on upper and lower parts of tomato plants in Yobe State at 6, 8,10 and 12 WAP. The result reveals that there is no significant difference (p <.05) in the number of *H. armigera* larvae at the different WAP on the upper part of a tomato plant. When the lower part of the plant is being considered significant difference was observed between the different WAP. A significantly high number of *H. armigera* larvae was recorded at 8WAP but was different statistically from the number of *H. armigera* larvae recorded at 6 WAP, and the least number of *H. armigera* larvae was obtained at 12 WAP but was at par with the number of *H. armigera* larvae obtained at 6 and 10 WAP.

Table 4

Distribution Statistics and Dispersion Indices of H. armigera Larvae on Tomato

S/No.	Mean (X)	Variance (S^2)	(S^2/ X)	Mean/ shoot	GC (C_x)	Lloyds (M*)
1	0.75	28	37.33	0.16	177.95	49.19
2	1.38	93.63	68.09	0.30	324.30	50.17
3	0.50	12.75	25.50	0.10	122.60	49.50
4	0.63	19.63	31.40	0.13	150.70	49.27
5	0.75	28.25	37.67	0.16	180.54	49.63
6	0.88	38.63	44.14	0.18	211.41	50.18
7	0.88	38.13	43.57	0.18	208.70	49.53
8	1.25	77.75	62.20	0.26	297.50	50.21
9	1.13	63.63	56.56	0.24	270.58	50.51
10	1.00	50.75	50.75	0.21	242.90	50.75
11	0.38	7.38	19.67	0.08	94.74	50.15
12	0.38	7.88	21.00	0.08	101.10	53.71
13	0.13	0.88	7.00	0.03	34.37	48.13
14	0.75	28.50	38.00	0.16	182.13	50.08
15	0.38	7.38	19.67	0.08	94.74	50.15
16	0.63	19.63	31.40	0.13	150.70	49.27
17	0.50	12.75	25.50	0.10	122.55	49.50
18	0.38	7.13	19.00	0.08	91.57	48.38
19	0.68	20.13	32.20	0.13	154.50	50.55
20	0.25	3.25	13.00	0.05	62.97	48.25
21	0.50	12.50	25.00	0.10	120.20	48.50
22	0.625	20.125	32.20	0.13	154.50	50.55
23	0.13	0.88	7.00	0.03	34.37	48.13
24	0.75	28.00	37.33	0.16	178.96	49.19
25	0.75	28.50	38.00	0.16	182.13	50.083
26	0.25	3.25	13.00	0.05	62.97	48.25
27	0.13	0.88	7.00	0.026	34.37	48.13
28	0.50	12.75	25.50	0.10	122.55	49.50
29	0.38	7.38	19.67	0.08	94.74	50.15
30	0.38	7.38	19.67	0.08	94.74	50.15

X=Mean, S^2 =Variance, S2/ X=Mean/shoot, C x=Greens coefficient and M*=Lloyds

Population Dispersion of *H. armigera* Larvae

The distribution patterns of *H. armigera* larvae on tomato plants were established by various indices of dispersion. Our result reveals the dispersion pattern of *H. armigera* larvae to be highly aggregated within tomato plants. All the tomato plants sampled, show variance to mean ratio (S^2/m) of greater than one with values ranging from 68.09 to 7.00 (Table 4). Furthermore, the Greens coefficient (C_x) values and the Lloyd's mean crowding (m^*)values of all the tree sampled were greater than one confirming the distribution of *H. armigera* larvae on tomato plant to be aggregative.

Discussion

The Abundance of *H. armigera* larvae on tomato plants depends on the season, cropping system, and developmental stage of the plant. A significantly high number of *H. armigera* larvae was observed during the dry season compared to the rainy season in both states. This may be due to the low quantity of eggs hatching as rainfall might have washed away the eggs. The high population of *H. armigera* larvae obtained at 8 and 10 WAP may be attributed to the presence of fully grown unripe tomato fruit, which coincides with 8 and 10 WAP. It was reported that *H. armigera* larvae prefer unripe fruits to leaves and ripe fruits (Czepak et al., 2013; Queiroz et al., 2018). Our findings agree with Murúa et al. (2014) who reported that *H. armigera* larvae prefer green fruits, as well as that of Mondal et al. (2019) *H. armigera* larvae reach their peak period during the fruiting stage. However, our findings contradict reports of Chula et al. (2017) who observed a high larval population of *H. armigera* during the entire crop period and the finding of Perkin et al. (2010) which reveals that *H. armigera* larvae prefer the vegetative part of plants over fruits.

The dispersion pattern of *H. armigera* larvae depends on the egg-laying habit of the female moths, the age of the larvae, variation in growth among the host plants, and the occurrence of natural enemies, among other biotic and abiotic factors (Khaing et al., 2002). The result shows that the dispersion pattern of *H. armigera* larvae on tomato plants is aggregated. This suggests their preference for the upper parts of the plant over the lower parts. This outcome conforms with Khaing et al. (2002), who reported an aggregate distribution pattern of *H. armigera* larvae on cotton plants. Mallampulli & Isaacs (2002) also reported the aggregate distribution of *H. armigera* on high-bush blueberries.

Abundance and dispersion of individuals are important characteristics of populations, affecting their spatial pattern of resource use and their effect on community and ecosystem attributes. Thus, the results of this study may come in handy for developing a successful management strategy for *H. armigera,* particularly if IPM is desired, where monitoring is an essential component to reduce unnecessary insecticide application. However, since this study was conducted in an open field in a savannah agro-ecological zone, its application to other agro-ecological zones needs to be investigated

Conclusion and Recommendations

The findings of this study showed that the population of *H. armigera* larvae is aggregated on the upper part of tomato plants at 8 WAP, exhibits higher densities during the dry season, and prefers developed fruits. The dispersion pattern of *H. armigera* larvae on tomato plants is aggregated. Based on the findings, it is recommended that when managing *H. armigera*, farmers should spray on a plant-by-plant basis and concentrate their efforts on the upper parts of the plant.

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