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Research Article

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Determination of the Effects of Heavy Metal Stress on Plant Development and Physiology in Feed Soybean (*Glycine Max* L.)

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HIGHLIGHTS

- With increasing doses of plants under cadmium and lead stress, a decrease was observed in stem diameter and fresh and dry weights of plants and roots.
- Seedling height and number of leaves per plant increase with the increase in metal applications. showed a decreasing trend. However, all of the applications are in the same statistical group and are not affected by the increase in metal. It was not affected as negatively as other parameters.
- It has been observed that as the concentration of heavy metals (Cd, Pb) increases, chlorophyll (SPAD) value and leaf area are suppressed.
- Increased cadmium and lead compared to the electrical conductivity control group has increased with its applications. It has been determined that metal applications have a negative effect on the RWC value. Enzyme activity is generally increased due to metal stress.

Abstract

This study was carried out to determine the tolerance level of cadmium and lead applications at different concentrations on physiological and morphological parameters in soybean (*Glycine max* L.) The experiment was carried out in Atatürk University Plant Production Application and Research Center greenhouses. Yeşilsoy variety of feed soybean (*Glycine max* L.) was used as plant material. Plant growth (seedling height, stem diameter, fresh and dry weight, etc.), physiological properties (tissue proportional water content, tissue electrical conductivity) and biochemical parameters (chlorophyll amount, superoxide distumase (SOD), catalase (CAT), heavy metal applications) peroxidase (POD) enzyme activities, etc.) were investigated. All treatments had negative effects on all parameters compared to the control group. Growth retardation was experienced at the highest concentrations applied, but plant deaths were not observed. Although it is known that cadmium metal is more toxic than lead metal, the wet weight values of the plants to which the highest dose of both metals were applied were included in the same statistical group.

Keywords: Soybean; Heavy Metal Stress; Cadmium; Lead; Phytoremediation

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1. Introduction

Environment; It is a physical, chemical, biological, social, economic and cultural environment in which all living things maintain their relationships and interact with each other throughout their lives. Environmental pollution basically exists in nature in the form of air, soil and water pollution, and as a result, it affects the entire ecosystem, including humans. While protecting the environment and natural resources from pollution is important in terms of preventing environmental pollution, the purification of polluted areas is also an important issue in the solution of existing environmental pollution (Özay & Mammadov 2013).

Industrialization and urbanization are the leading factors that cause environmental pollution (Bayçu 1997). The energy that emerged as a result of rapid population growth has led to rapid progress in industry and industrialization (Cihangir & Sağlam 1999). This situation results in the very rapid consumption of natural resources, and intensive agricultural practices cause heavy metal accumulation in the soil and in the environment (Çağlarrmak & Hepçimen 2010; Mikhailenko et al 2020). Earthquakes, floods and volcanic eruptions are the first ones that come to mind as sources of heavy metals in nature. Its spread to the environment occurs from human-based activities rather than natural resources (Kahvecioğlu et al 2010). Many factors such as the use of fossil fuels, mineral deposits, melting of metal ores, motor vehicles, urban wastes, wastewater, fertilizers, pesticides and sewage have paved the way for human-induced heavy metal pollution (Kabata-Pendias & Pendias 1999; Yerli et al 2020). It is stated that this situation has reached critical levels in many countries (Robinson et al 2001).

Heavy metals

Heavy metals are explained as metals with a density of more than 5 g/cm3, atomic number greater than 20, causing toxicity and pollution (Sönmez et al 2021). In plants, low stimulant concentrations of some heavy metals are necessary for normal and healthy plant growth (Bera et al. 2005). Metals such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), cobalt (Co) are micronutrients that play an active role in the growth and development of plants and animals. In addition, some heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) are elements that are not important for the development of living things (Niess 1999).

Heavy metals have a high relative density and even at low concentrations, they have a toxic effect on living things. All plants have the ability to collect the heavy metals necessary for them from soil and water. These metals are magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo) and nickel (Ni) (Langille and Maclean 1976). Some plants also have the ability to accumulate heavy metals whose biological functions are unknown. These heavy metals are cadmium (Cd), chromium (Cr), lead (Pb), cobalt (Co), silver (Ag), selenium (Se) and mercury (Hg) (Hanna & Grant 1962; Baker & Brooks 1989).

Reaching a certain level of heavy metal concentration in the atmosphere, water and soil also causes great problems for living things (Özay 2013). Filtering soil with its buffering feature can protect itself against various pollutants (Yerli et al 2020). However, high doses of heavy metals disrupt the physical, chemical and biological structure of the soil and cause a decrease in product yield and quality (Long et al 2002). Reaching a certain level of heavy metals directly affects the development and physiology of plants negatively and serious yield losses.

Lead (Pb)

It is the first metal of human origin to cause serious damage to the ecological system. Lead can enter the soil and atmosphere from a wide variety of sources. These sources include chimney and exhaust gases, wastes from different industries (paint, electricity, oil) and pesticides (Saygıdeğer 1995; Kalinowska 1984; Aksoy 1995). A large part of lead, which constitutes a large share in environmental pollution, is caused by the emission of gases into the environment by the combustion of gasoline used in motor vehicles (De Jonghe & Adams 1986). As a result of pollution, lead (Pb), copper (Cu), zinc (Zn) etc., which strengthens its place in the soil and atmosphere. heavy metals are becoming dangerous for plants, animals and humans. Lead has been reported to be the largest environmental pollutant. It is one of the heavy metals that cause worldwide concern

(Salt et al 1999) and the most severe toxic effect (Okçu et al 2009). It causes morphological, physiological and biochemical dysfunctions in plants. Although it is not essential for plants, it is rapidly absorbed by plants and accumulates in different parts of the plant (Fahr et al 2013). It has an inhibitory effect on activities such as seed germination, root growth, stem development, transpiration, chlorophyll synthesis and cell division in plants. However, these effects vary according to the amount of lead exposed, the duration of the effect, the developmental stage of the plant and the exposed plant tissue (Doğru 2020). Lead affects plants through their roots (Fahr et al 2013). The uptake of Pb by the roots of plants prevents its development by reducing nutrient intake (Öktüren & Sönmez 2006). Lead accumulation in plants causes stunted growth, chlorosis and darkening of the root system (Sharma & Dubey 2005). In plants under extreme lead stress, the number of leaves decreases, their length becomes smaller and their leaves become more fragile (Gupta et al 2009).

Cadmium (Cd)

Today, it is one of the heavy metals, which is an important factor in environmental pollution. Among the heavy metals, it is an element with high solubility in water. Since it is soluble in water, it is taken into biological systems by plants and sea creatures, and it accumulates and spreads rapidly in nature (Özkan 2009). Cadmium is found in phosphate fertilizers, detergents and refined petroleum derivatives in compound form, and cadmium pollution occurs with their widespread use. It is used industrially in nickel/cadmium batteries, coating of steel in the ship industry, paint industry, alloys and electronics industry due to its resistance to corrosion, especially in sea conditions (Kahvecioğlu et al 2007). Cadmium is an element that is toxic to plants (Çatak et al 2000). Some of the cadmium sources that affect the respiration of plants; water pipes, burning coal, fertilizers used in the seed stage and flue gases released from factories (Kahvecioğlu et al 2007).

There is no biological activity of cadmium, which is not an absolutely necessary metal in the nutrition of living things (Marschner 2008). Its mobility in the soil facilitates its inclusion in the food chain. Cadmium taken by plants causes disruption of many activities such as protein synthesis, nitrogen and carbohydrate metabolism, enzyme activation, photosynthesis and chlorophyll synthesis (Mengel 2001). Cadmium prevents the growth and development of plant roots, making it difficult to take water and ions, and also reduces the yield and quality of the plant because it inhibits the plant photosynthesis rate and enzyme activity (Asri & Sönmez 2007). In a study on legumes (alfalfa, soybean), it was reported that cadmium inhibits photosynthesis by respiration (Huang et al 1974).

Some of the plants can carry high levels of metal in their bodies. These types of plants, which can accumulate many metals in a hundred times more than other plants without any damage, are called hyperaccumulator (metal accumulator) plants (Özbek 2015). Plants take the heavy metal from the soil into their body at high rates through their roots, transmit it to the stem and store it in other tissues and organs of the plant (stem and leaf). Studies to investigate the usability of these tolerant plants in the remediation of heavy metal-containing soils or waters are increasing.

Phytoremediation is the use of hyperaccumulator plants (Dushenkov et al 1997) to bring the ecological pollution caused by heavy metals to a controllable level or to neutralize this pollution (Dushenkov et al 1997), a method of making nature cleaner and more useful through plants (Clemens et al 2002; Pulford & Watson 2003; Gardea-Torresdey et al 2005). In the phytoremediation technique, hyperaccumulator plants are selected that can absorb the heavy metals contained in the pollutants in the environment, have a high ability to accumulate in their tissues and passivate the heavy metals they have accumulated after different stages. Green breeding is a breeding method that achieves positive results by choosing genetically adjusted plants, especially selected, in the breeding of low and medium risk polluted environments. Compared with different breeding methods, it has many advantages such as low input cost, convenience in application and time saving (Glass et al 1999).

Soybean (*Glycine max* L.)

The first country where the soybean (*Glycine max* L.) plant, which is known to have emerged in the lands of East Asia about 5000 years ago, is accepted as China (Öner 2006). Soybean, which has been noticed in the Far East countries, has increased its importance in the region by creating an important food and livelihood for

the people living in these regions. Nutritionists underline that the use of soybean in food and animal feed use has increased rapidly in many countries in the last 30 years, and that it should increase even more in terms of good nutrition (Thuzar et al 2010).

Soybean is one of the plants with the largest cultivation area in the world (Turhan 2019). According to 2018 data, 34.68% of the world's oilseed production belongs to soybeans and ranks sixth among the most produced plants in the world (FAO 2019). According to 2022 data, world soybean cultivation area is 126.951.517 ha and production is 353.463.735 million tons. In our country, according to the agricultural statistics of 2021; soybean cultivation area was 438.917 da and soybean production was 182 thousand tons (TUIK 2021). Since our country cannot meet the demand for soybean, it is an importer country, and according to the data of 2021, it imported 3.0 million tons of soybean annually (Anonymous 2021). Soybean plant grows successfully in many parts of the world, as it adapts to various climatic conditions. The highest yield is obtained in climates where the temperature is 25°C between May and September. Temperatures lower than 18 °C and higher than 40 °C adversely affect the growth and development of soybean plant (Tüfekçi 2019).

80% of soybean (Glycine max L.) cultivation in Turkey is grown as a second crop in Çukurova (Metin and İlker 2016). Considering its place in human and animal nutrition (poultry, small cattle, dairy and beef cattle) and its workability in the industrial field, it is understood that the value of soybean has increased in our country (Nazlıcan 2010). Soybean, which is one of the few important plants in the world, takes its place as a protein source in the rations of poultry, sheep and cattle, dairy and beef cattle (Öner 2006). It is rich in linolenic acid, also known as omega-3 fatty acid. The amount of omega-3, which cannot be made by the body and is one of the essential fatty acids, varies by 5-11% in soybean (Arıoğlu 2007). Soybean, which has a wide place in the industry, is used as a variety of foodstuffs due to the excess protein content (in the production of flour, milk, yogurt and cheese in the production of soybean meat); It is used in the manufacture of many industrial products (paint, linoleum, glue, etc.). In summary, soybean, which is beneficial in all respects, is one of the most valuable industrial plants in the world (Turhan 2019).

Damage to the soybean plant during its developmental stages can create stress in the plant, which can negatively affect plant growth and development. The ability of soybean plant to compensate for these negativities is quite low. In the studies carried out to eliminate the damage caused by heavy metals in agricultural lands, it has been focused on preventing heavy metals from reaching people directly or indirectly. Studies conducted with many plant varieties also show differences in the reactions of plants to heavy metal stress. As a result of our literature research, it is seen that there are few studies of heavy metal stress in soybean plant. This study was carried out to determine the effects of soybean plant on some parameters against heavy metal stress, which has become an important agricultural problem in the world and in our country.

2. Materials and Methods

The study was carried out as pot work in the greenhouses of Atatürk University Plant Production Application and Research Center in 2021. In the study, 'Yeşilsoy' forage soybean variety, which was registered by the Eastern Mediterranean Agricultural Research Institute, was used as plant material. In pots with a volume of 2 liters; Garden soil, sand, peat mixture was filled and planted at a depth of 2-3 cm, with 5 seeds in each pot. The plants that reached the seedling stage were thinned so that three plants with homogeneous appearance were left in each pot.

Heavy Metal Application

Attempt; cadmium 3 doses ($CdSO_4.8H_2O$ (100, 200 and 300 mg/kg), lead 3 doses ($PbNO_3$ (1000, 2000 and 3000 mg/kg) and 1 control group (no application), 7 applications, 3 replications) and 5 pots from each replication, a total of 105 (7x3x5=105) pots were conducted in a randomized plot design. To pollute the soil, different concentrations of cadmium (Cd) (100, 200 and 300 mg/kg) and lead (Pb) (1000, 2000 and 3000 mg/kg) metals were mixed into the trial soil and watered in the amount of field capacity and the incubation period of 3 weeks was started. At the end of the incubation period, soybean seeds were planted. The pot trial was

completed in 50 days and at the end of the trial, the measurements, observations, weighing and analyzes stated below were performed while the plants were in or out of the pots. has been made.

Body Diameter (mm)

The stem (stalk) part of the above-ground parts of the plants at the harvest stage was measured in mm using a digital caliper (Güllap et al 2022).

Above Ground Fresh Weight (g/plant)

It was calculated by removing the above-ground part of the plant, weighing each plant on a sensitive scale and taking the average (Güllap et al 2022).

Above Ground Dry Weight (g/plant)

It was calculated by drying in an oven at 68°C for 48 hours (Güllap et al 2022).

Root Fresh Weight (g/plant)

The roots were removed from the soil, washed and cleaned of soil particles, and then the water was removed with blotting papers. Its weight was determined by weighing it on a precision scale (Güllap et al 2022).

Root Dry Weight (g/plant)

The measurement was made by drying in an oven at 68°C for 48 hours (Güllap et al 2022).

Seedling Length (cm)

It was measured in cm with a ruler (Güllap et al 2022).

Number of Leaves (piece/plant)

The number of leaves in each pot and each plant was taken as a number and the average was calculated (Güllap et al 2022).

Amount of Chlorophyll (as SPAD Value by Chlorophyll Meter)

Chlorophyll content of plant leaves (SPAD-502, Konica Minolta Sensing, Inc., Japan brand) was determined with the SPAD-502 meter device (Lichtenthaler & Wellburm 1983).

Leaf Area (cm^2 /plant)

Leaf areas of the plants in each heavy metal application were determined with a leaf area meter (LICOR, Model: LI-3100, Lincoln, USA) (Güllap et al 2022).

Tissue Electrical Conductivity

An indication of the damage caused by the stress on the leaf tissue and especially in the cell membrane is the electrical conductivity measurements taken from the wet leaf tissues. For this analysis, discs (1cm in diameter) taken from the last grown true leaves of 2 plants taken randomly from each of the replications were placed in glass bottles filled with 20 ml of distilled water, shaken for 24 hours in the shaker, and then the electrical conductivity of the soaking water was measured with a device, and the permeability of the cell membrane was determined. (damage rate) was determined (EC1). The samples were placed in an autoclave and kept at 121 °C for 20 minutes, and the second measurement was taken (EC2). Relative electrical conductivity values were determined by calculating the EC1/EC2 ratio (Kaya et al 2003).

Tissue Proportional Water Content (RWC)

The leaf discs (1 cm in diameter) taken from 2 randomly selected plants among the plants in the treatments were immediately weighed and their fresh weights were determined (TA). After weighing, the discs were taken into petri dishes containing 20 ml of distilled water and kept for 5 hours, then the discs were wiped with the help of blotting paper, the excess water was removed and weighed again. With this method, turgorous weights (TU) were determined. Afterwards, these discs were taken into petri dishes, dried in an oven at 72°C

for 48 hours, and their dry weights were determined (KA). Tissue water content (RWC) values were calculated according to the following formula (Kaya et al 2003).

(RWC) = [(TA – KA) / (TU – KA)] x 100

Determination of Catalase (CAT - EC: 1.11.1.6) Activity (EU/Gta)

The method used to determine catalase (CAT) activity is the method used by researchers named Havir & Mchale (1987) on the principles in the literature of Luck (1965). According to this method, the activity determination was made on the basis of monitoring the absorbance decrease at 240 nm while providing the conversion of H_2O_2 to O2 and H2O for catalase (CAT) activity measurement (Havir & Mchale 1987).

Determination of Peroxidase (POD - EC: 1.11.1.7) Activity (EU/gTA)

In order to determine the peroxidase (POD) activity assay, guaicol was made by monitoring the absorbance increase of the colored compound, which is the result of the reaction in which H_2O_2 is the substrate, at 470 nm (Angelini et al 1990).

Determination of Superoxide Dismutase (SOD – EC: 1.15.1.1) Activity (EU/Gta)

Superoxide dismutase (SOD) activity, inhibition of photochemical reduction of nitro blue tetrazolium (NBT) was determined on the basis of spectrophotometric detection (Agarwal and Pandey 2004; Yordanova et al 2004).

Hydrogen Peroxide (H_2O_2) Analysis (mmol/kg)

Hydrogen peroxide (H_2O_2) analysis, Velikova et al (2000) was carried out according to the method reported. (H_2O_2) contents were calculated using a pre-made standard calibration curve using different concentrations of (H_2O_2)

Malondialdehyde (MDA) Analysis(nmol/g)

Thiobarbituric acid ($C_4H_2N_2O_2S$)-reactive substances are formed as a byproduct of lipid peroxidation (ie degradation products of fats). Therefore, TBARS values were measured as mal ondialdehyde (MDA), which is a degraded product of lipid and determines lipid peroxidation. Lipid peroxidation analysis, Şahin et al. It is based on the transaction steps reported in (2018). MDA concentration was determined from the absorbance curve using an extinction coefficient of 155 mmol/L.

Statistical Evaluation

The experiment was set up in a randomized plot design with three replications. All the data obtained at the end of the study were subjected to the variance analysis test with the SPSS 18 package program, and the comparison of the averages was made according to the Duncan multiple comparison test (Yıldız & Bircan 1991).

Intervention ARY studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

3. Results

The differences in the mean stem diameter (mm), plant fresh and plant dry weight (g), root fresh and root dry weight (g) of lead (Pb) and cadmium (Cd) applications are presented in Table 1. It is known that heavy metal accumulation in the soil negatively affects plant production and reduces the yield and quality obtained from the unit area (Yerli 2020). In our study, the application without Pb and Cd pollution in all parameters (control application) had the highest values. With the increase of metal concentration, the amount of decrease in the values accelerated. For example, the difference between the lowest doses (Pb1000-Cd100) of the control application and Pb-Cd applications was 0.04-0.05 mm, while the difference between the highest doses (Pb3000-Cd300) was 0.36-0.28 mm. Similarly, in the plant fresh weight parameter, the control group has a higher weight than the plants in all treatments, but the decrease in the first applications (Pb1000-Cd100) was 0.52-0.83 g,

while in the highest concentration application (Pb3000-Cd300) it was 2.52-2 compared to the control. There was a weight loss of .39 g. The lowest value (1.45 g) in plant dry weight measurements was obtained from Cd 300 application. The negative effects of increasing doses of cadmium on plant weight have been supported by many studies (Bachir et al 2004; Tiryakioğlu et al 2006; John et al 2009; Safarzadeh et al 2013). The increase in metal concentration in root fresh and dry weights had a reducing effect on root mass. Researchers have reported that root inhibition is an important factor in determining metal toxicity (Lyu et al 2018). In our study, Pb (3000) and Cd (300) applications caused a 52-70% decrease in root fresh weight and 63-76% decrease in root dry weight compared to the control application (Table 2). According to Groppa et al (2008) determined as a result of his studies that heavy metals negatively affect root growth, development and new root formation in plants.

Table 1. Effects of Applications on Plant Growth in Soybean[1]

Treatments (mg kg^{-1})	Stem Diameter (mm)	Plant Fresh weight (g)	Plant Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Control	2.35 a	8.41 a	2.29 a	1.66 a	0.62 a
Pb 1000	2.31 a	7.89 a	1.96 ab	1.38 ab	0.35 b
Pb 2000	2.25 ab	7.64 a	1.87 abc	1.11 abc	0.28 bc
Pb 3000	1.99 c	5.89 b	1.61 bc	0.79 bc	0.23 bc
Cd 100	2.30 a	7.58 a	1.95 ab	1.02 abc	0.19 bc
Cd 200	2.19 ab	7.15 ab	1.91 ab	0.69 bc	0.16 c
Cd 300	2.07 bc	6.02 b	1.45 c	0.50 c	0.1 c

[1]Means marked with different letters are statistically different.

The average seedling length, which is one of the basic growth parameters, was determined as 20.06 cm in the control application (Table.2). Plant height averages of lead (Pb) and cadmium (Cd) metal applications were behind the average seedling height of the control group. Plant heights in lead applications were measured as 19.93 cm, 18.06 cm and 16.53 cm. A 17% height loss was observed between the seedling height of the control group and the highest concentration of lead application (3000 mg/kg). The average seedling height in Cd applications is 19.13 cm, 17.26 cm and 16.26 cm. The average height of the plants grown under the conditions of the highest Cd application (300 mg/kg) was determined as 19% shorter than the control group. In the stress studies on the subject, Siyah et al (2013) found that Cd applications in cotton plant, Aksu (2019) lettuce, Yılmaz & Kökten (2019) sorghum reduced plant height, root-stem dry and wet mass growth parameters compared to control.

Table 2. Effects of Applications on Seedling Height, Number of Leaves, Chlorophyll Value (SPAD) and Leaf Area in Soybean1

Treatments (mg kg^{-1})	Seedling height (cm)	Number of leaves (pcs/plant)	Chlorophyll SPAD	Leaf area (cm^2/plant)
Control	20.06 a	13.60 a	37.50 a	112.75 a
Pb 1000	19.93 a	11.13 b	37.10 ab	101.48 ab
Pb 2000	18.06 bc	11.06 b	35.46 bc	90.98 bcd
Pb 3000	16.53 cd	10.80 b	34.89 c	87.46 bcd
Cd 100	19.13 ab	11.66 b	35.96 abc	99.51 abc
Cd 200	17.26 cd	11.03 b	35.03 c	86.69 cd
Cd 300	16.26 d	11.53 b	34.66 c	83.99 d

[1]Means marked with different letters are statistically different.

In the study carried out under greenhouse conditions, it was determined that Cd and Pb applications caused a decrease in the number of leaves, leaf area and chlorophyll values in soybean (Table 2). While the lowest value (10.80 pieces/plant) was observed in the application of lead (3000 mg/kg), it was determined that all doses of Cd and Pb were in the same group statistically in terms of the number of leaves. Chlorophyll (SPAD) value decreased statistically in all treatments of Pb and Cd compared to the control treatment. One of the negative effects of cadmium toxicity on plants is that it affects the chlorophyll biosynthesis process (Sheoran et al. 1990). It has also been reported that all heavy metals increase chlorophyll destruction and inhibit its synthesis (Zengin & Munzuroğlu 2005). In terms of leaf area, the highest value was observed in the control application with 112.75 cm^2, while the lowest value was found in the Cd 300 application with 83.99 cm^2. In general, applied heavy metals decreased the leaf area compared to the control application (Table 2). It is known

that the essential nutrients of plants exposed to metal toxicity are less than plants under control conditions. Therefore, plant height, number of leaves and leaf area have lower values (Mengoni et al 2000; Jayakumar et al 2007)

The effects of different doses of heavy metal applications on tissue electrical conductivity (DEI) and tissue proportional water content (RWC) values in soybean are shown in Table 3. As Pb and Cd stress increased, it was observed that DEI value increased in soybean compared to the control application. The lowest value was seen in Cd 100 application as 16.25%. In RWC values, heavy metal applications showed a decreasing trend, although there was not much difference compared to the control, and the closest average value to the control was seen in Cd 100 application as 83.54%

Table 3. Effects of Applications on Electrical Conductivity and RWC in Soybean[1]

Treatments mg/kg	MP (%)	RWC (%)
Control	15.91 c	84.42 a
Pb 1000	16.3 bc	82.95 a
Pb 2000	18.7ab	80.8 bc
Pb 3000	19.9 a	78.81 d
Cd 100	16.5 c	83.54 a
Cd 200	17.3 bc	81.18 b
Cd 300	19.16 a	79.01 cd

[1] Means marked with different letters are statistically different.

Metal applications create toxicity in the plant, affect the plasma membrane permeability and cause a decrease in water content. Cadmium has been reported to interact specifically with water balance (Barceló et al 1986; Poschenrieder et al 1989; Costa & Morel 1994). The decrease in RWC values may be due to the decrease in hydraulic conductivity caused by metal applications (Ehlert et al 2009). Previous studies have shown that metal toxicity causes a decrease in RWC values in some plant species (Manousaki & Kalogerakis 2009; Ahmad et al 2011).

Table 4. Effects of treatments on antioxidant enzyme activities (CAT, POD and SOD) in Soybean[1]

Treatments (mg/kg)	CAT-(EU/Gta)	POD-(EU/gTA)	SOD-(EU/Gta)	H_2O_2-(mmol/kg)	MDA- (nmol/g)
Control	0.017 b	27.84 a	77.20 cd	241.66 e	157 d
Pb 1000	0.014 cd	21.52 bc	80.86 bc	310.04 c	2.04 c
Pb 2000	0.036 b	12.06 e	83.82 b	313.63 c	4.54 a
Pb 3000	0.077 a	18.29 cd	73.38 d	366.67 a	4.45 a
Cd 100	0.008 e	14.17 de	90.24 a	272.68 d	1.9 c
Cd 200	0.012 de	23.10 b	92.59a	266.04 d	2.35 b
Cd 300	0.013 cde	14.05 de	92.05 a	331.73 b	2.30 b

[1] Means marked with different letters are statistically different.

In the phytoromediation technique, antioxidant enzymes play a major role in the process of heavy metal uptake by plants or in the process of heavy metal stress (Bhaduri & Fulekar 2012). This role is to allow the plant to survive in case of heavy metal overload in plant organs. Numerous studies have reported that the working principle of enzyme activity depends on a combination of parameters such as the type of stress conditions, its functioning in the plant, and the type of plant. Table 4 shows the effects of heavy metal applications on antioxidant enzyme activities in soybean. It was observed that the activation of catalase enzyme increased as the cadmium concentration increased. In the lead application, it was concluded that the Pb1000 concentration decreased below the control group and increased in other applications. There was a decrease in POD value compared to the control application. The highest POD value was 23.10 in Cd 200 application. There was a regular increase in SOD values except for Pb 3000 application. It was observed that there was an increase in SOD enzyme activity in parallel with the increasing concentration in cadmium application. The highest increase was observed in the Cd 200 application as 92.59, followed by the statistically similar Cd 100 (90.24) and Cd 300 (92.05) applications. Cadmium-induced increases in enzyme activities in soybean are in agreement with other studies (Melo et al 2011; Alyemeni et al 2017; Finger-Teixeira et al 2010).

In Table 4, it was observed that hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) activity increased in the soybean plant treated with Pb and Cd compared to the control application. The highest peroxidase

(H2O2) values were observed in Pb 3000 and Cd 300 applications as 366.67 and 331.73 mmol/kg, respectively. Again, the highest malondialdehyde (MDA) value was determined as 4.54 nmol/g in the Pb 2000 dose, which increased compared to the control application, and it was found to be in the same group statistically with the subsequent Pb 3000 (4.46) application. Malondialdehyde (MDA) is a value that varies depending on the type and severity of the stress source (Tunçtürk et al 2021). In a study on quinoa, it was stated that root length, root fresh-dry weight and chlorophyll amount decreased due to the increase in drought stress, but the MDA value increased by 82% (Aslam et al 2020). In a thesis study examining the effects of cadmium applications on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) activities in cress, it was observed that the values increased due to the increase in metal application (Alım 2020).

Reducing the existing pollution through in situ plants has been one of the research topics of recent years. Knowing the metal uptake removal system of the plant to be used is a very important factor for success in removing pollution. In this study, it was tried to understand the reactions of soybean to heavy metal applications in greenhouse conditions through some parameters. It was observed that lead and cadmium metals significantly suppressed plant growth. However, since the results of the research are based on one-year data, it may be recommended to repeat the research by using more genotypes and metal concentrations, and to conduct similar studies in field conditions where soil depth and climate factors are important in order to reveal more reliable and precise results for the purpose of the research.

4. Discussion

Reducing existing pollution through plants has been one of the research topics of recent years. Knowing the metal uptake and removal system of the plant to be used in the environment, It is a very important factor for success in eliminating pollution. In this study conducted. The tolerance of soybean to heavy metal applications under greenhouse conditions was tried to be understood through some parameters. Lead and cadmium metals have important effects on plant growth. It was observed that it suppressed the research results consist of one year's data. Research using more genotypes and metal concentrations It may be recommended to repeat.

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Research Article

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Clonal Preselection in Grape (*Vitis vinifera* L.) Varieties of Ekşi Kara and Gök Üzüm

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HIGHLIGHTS

- Pre-selection of local grape varieties, Ekşi Kara and Gök Üzüm varieties, was carried out.
- 17 Ekşi Kara and 2 Gök Üzüm clones were selected, which were confirmed to be clean by repeated health selection and tests.
- Clone comparison vineyard was established with selected clones grafted onto 110R grapevine rootstock.
- In the vineyard facility, pollinator Gök Üzüm were planted next to the clone of the functional female Ekşi Kara variety.

Abstract

Ekşi Kara (functional female flowers) and Gök Üzüm (hermaphroditic flowers) are the two most important autochthonous varieties of middle Anatolia. This clone selection study started with mass-selection in producer vineyards consisting of approximately 5000 vines by The International Organization of Vine and Wine (OIV) clonal selection procedure. Two-years genetic and sanitation were examined visually in population and 220 clone candidates were ampelography and fertilization biology and bud fertility determined for Ekşi Kara variety. The clone candidates were ranked at the level of sums, with weighted grading of three-year yield, growth, and quality records. Sanitation analyses of the superior clones were made. 17 clones in the Ekşi Kara grape variety were selected according to their superior scores in genetic selection and sanitation analyses. Eleven clones were selected by mass selection from Gök Üzüm carried out in a single location, and 2 clones were selected with genetic selection scores and health tests. *Grapevine fleck virus* (GfKV) was the most common

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(≅ 29%) in the samples tested, *Grapevine leafroll associated virus* 1+3 (GLRaV-1 + 3, ≅ 26%), *Grapevine virus A* (GVA, 12%), *Grapevine leafroll associated virus-2* (GLRaV-2, ≅ 3%), *Arabis mosaic virus* (ArMV) / *Grapevine fanleaf virus* (GFLV) (≅ 1%) are fallowed with indicated percentage. Although virus and bacterial infections are common in the vineyards, enough healthy clones were selected. 17 Ekşi Kara and 2 Gök Üzüm clones selected as pollinators were grafted onto the 110R rootstock for clone comparison in homogeneous conditions, and a "Clone Comparison Vineyard" was established in Selçuk University.

Keywords: Clonal selection, clone comparison, native grape varieties, phytosanitary tests

1. Introduction

Vine (*Vitis vinifera* L.) is widely cultivated all over the world. Today, it is the most important fresh fruit in the world (Stanimirović et al. 2018). The health effects of grape and grape products contribute significantly to their economic value (Jackson 2008).

The clone is the vegetative generation of a grapevine whose identity is precisely determined with its phenotypic characteristics and health qualities and remains stable until a new mutation occurs (Aurand 2017; van Leeuwen et al. 2019). Clone selection in viticulture is one of the first steps in the development of grape varieties and viticulture, which is of great interest in all viticultural countries in terms of productivity, quality, and sustainability (Rühl et al. 2003). According to van Leeuwen et al. (2019) clonal variation is important in terms of grapevine health and reacting to changing environmental conditions. Grapevine breeders want to use plant material as close as possible to the original selected clone to ensure similarity and preserve the flavor characteristics of the grape. Clonal selection is a two-step process, genetic and health selection, that takes genetic diversity into account within the purity of the varieties. This method eliminates the negative effects of mutational changes in the vineyard areas of the future, as well as prevents the repropagation of plants infected with viruses and related diseases (Rühl et al. 2003). Intra-variety genetic diversity can be explained by their polyclonal origin and accumulation of genetic mutation over time (Vondras et al. 2019). The application includes estimation of clones in the field, examination of their agronomic and oenological performance, health tests and clone identification processes. Healthy and more interesting clones are selected to maintain as long a continuity as possible. Clones are compared under homogeneous conditions to determine their quality grape production capacity, to provide certification and distribution to producers (Loureiro et al. 2011).

Clonal selection in viticulture started in the nineteenth century in Germany and continued in other European countries such as France and Italy in the second half of the twentieth century. It started in Spain in the 1970s in La Rioja and Catalonia regions (Ibáñez et al. 2015). Since virus diseases, especially fanleaf, greatly affect the vine performance in the cold vineyard areas of Germany, the clone selection based on visual assessment and performance has done well there. Since viruses are accepted as the main factor in the reduction of vineyard areas, visual evaluation has been done by serological methods such as indexing of candidate clones since the 1970s, and enzyme-linked immunosorbent analysis in the mid-1980s. Since the early 1990s, all German clones have been subjected to virus tests and since 2013, all main blocks are managed according to the European Union legislation (European Union Council 14.02.2002, 23.06.2005 Commission Directive). This combined strategy has proven to be successful, and while many varieties were used in the mid-1950s, today vineyards are established almost entirely from clones (Eibach and Töpfer 2015).

Clonal selection is considered a crucial tool for genetic improvement. Improved overall performance of clones after purification has been confirmed by much evidence. In general, vigor of the plant increases all the time, but all other parameters are modified depending on the viruses. Healthy plants show higher physiological activity than those infected with GLRaV-3 and GLRaV-1 of the same clone. Grape quality was improved without any yield increase when purified from GLRaV-3, while yield and quality parameters increased without adversely affected when cleansed from GLRaV-1 (Mannini 1998).

Initially, the main purpose of clone selection was to obtain healthy plants and increase yield. Today, quality is considered as a goal to reduce yield in some cases (Martínez et al. 2006). The biggest bene-fit of using clones is to select the genotype within a particular variety, best adapted to a particular vine-yard region (soil, climate) and produce a product with a certain quality potential. Also, identical genotypes within a vineyard have the same behavior and growth stages, which facilitates the management and harvesting of the vineyard (Forneck et al. 2009).

The stable and uniform grape production character of modern viticulture requires virus-free planting material that can only be obtained through clonal selection process. Clone selection, which focuses on improving the characteristics of native grape varieties, improving planting material quality and health status, is carried out in three stages. 1) Selection of the first material from old vineyards and virus tests (ELISA); 2) Establishment of trial vineyards in the cultivar production site by vegetative generations of virus-free parent plants, 3) Final evaluation and registration of selected clones (Šikuten et al. 2018).

In this study, the selection of clone candidates made in the producer vineyards of the mass and individual clone selection (OIV procedure, Aurand 2017) studies of Ekşi Kara and Gök Üzüm varieties commonly grown in middle Anatolia Konya and Karaman provinces the genetic and health selection studies of the selected clones until the clone comparison are presented.

Within the scope of this study, "Clonal preselection in grape (*Vitis vinifera* L.) varieties Ekşi Kara" was carried out together with Selçuk University and Republic of Türkiye Ministry of Agriculture institutes with support 3 Selçuk University and 1 TAGEM projects.

2. Materials and Methods

The material of this study was generally identified (OIV 2009) and selected by mass-selection, Ekşi Kara and as pollinators of the variety Gök Üzüm variety populations, and clone candidates selected from these populations according to their phenotypic characteristics and sanitary status in the first phase of the mass-clone selection stage. Since the performance of a clone is determined by health conditions (Aurand 2017), the health status of the parent plants was monitored from the beginning of the vegetation in April and evaluated twice a year in June and September in wholesale selection studies.

At this stage, considering their exposure to abiotic (hail, frost, sunburn, nutritional disorders) or biotic (disease and pests) stress factors, approximately 5000 vines with superior performance from more than 30 years age vineyards were followed since they have a greater probability of carrying mutations.

The selection of the starting material is from 17 vineyards in total from Konya (14 vineyards) and Karaman (3 vineyards) belonging to producers with elevations varying between 800 m and 1500 m, taking into account the effects of environmental characteristics on the Ekşi Kara grape variety, as stated in The Organization Internationale de la Vigne et du Vin (OIV) Standard Protocol (Aurand 2017) done. The distribution of the selected clones according to the districts was Bozkır 2, Hadim 2, Güneysınır 6, Karaman Central district 7 clones. The Ekşi Kara variety requires an absolute pollinator, and this need is met most successfully with the Gök Üzüm variety in the region (Kara et al. 2016; Kara et al. 2017a). Both grape varieties are used for table, snack, dried and grape juice (Kara et al. 2016). Producer vineyards in Hadim district where all three features are used most intensively (Yağcı village, the vineyard area is 1000 m above sea level) was selected as the population for clone selection from Gök Üzüm variety.

The method for this clone selection study is mainly proposed by OIV (Aurand 2017). According-ly, clonal selection is most effective when the initial individuals constituting the starting population are preferably selected from vineyards established without the selected clones. Intra-variety variation in such vineyards is more likely, increasing the likelihood that seemingly superior individuals will be selected for the target traits of the clonal selection program. In addition, they must meet the desired requirements for other important

viticulture properties. In addition, selected individuals should be identified as the true type based on ampelographic and genetic studies. This first choice should be made with ampelographic and phenological considerations. Moreover, care should be taken to eliminate individuals affected by infectious diseases in selected clones. The second step of clone selection is the observation and protection of the vegetative lineage of the selected individuals. Selected clones that successfully completed the phytosanitary inspection may have come from various locations. Trial vineyards should be established for comparison with individually propagated clones, preferably in two area with different pedoclimatic properties. For comparison, this trial plot should contain one or more existing standard clones for reference. The test area should exhibit homogeneous soil and micro-climate conditions. The soil of the test area should not contain *Xiphinema* ssp, which acts as a vector for viral diseases. All clones of the experiment should be grafted onto the same clonal rootstock. The rootstock used for grafting should be suitable for local soil conditions and preferably one of the most frequently used rootstocks in this region. Each clone should have at least three replications and at least 5 vines per iteration. Evaluation should be done over a period of three to five years (Aurand 2017).

In the starting material, phytosanitary selection was visually performed at the stage of mass selection, negative traits were removed, diseased clone candidates were not selected (Loureiro et al. 2011) and a total of 220 clone candidates, apparently less susceptible to disease were selected as clone candidates.

Individual clone selection was carried out in two steps, genetic selection, and phytosanitary selection (Aurand 2017). In the genetic selection stage, in order to evaluate the genetic variations within the variety, clone quality and genetic characteristics, variants were monitored in their own environment in 17 different vineyards, their fruitfulness, yield, development and quality records were kept and their mathematical calculations (Stenkamp et al. 2009) were made according to the weighted grading method. In the weighted rating method Ibáñez et al. (2015), the criteria and relative scores used in the calculation of clone scores were determined based on birth rate (20%, OIV 153), yield (kg m-2, 40%, OIV 504), vegetative growth (g vine pruning weight-1, 10%), cluster weight (g cluster-1, 10%, OIV 502), berry weight (g 100 berry-1, 10%, OIV 503), The maturity index (°Brix, 5%, OIV 505 / total acidity (g L-1, OIV 506) values respectively (OIV, 2012).

Genetic potentials of selected clones were sorted by weighted grading method, and infections free were determined by sanitation tests, and a clone comparison vineyard was established by grafted clones onto the 110 R rootstock (Aurand 2017). In the next stage of the study, whether the genetic variability of the clones are spontaneous natural mutations fixed by vegetative propagation and their kin-ship relations will be examined.

2.1. Viral analyses

Ekşi Kara and Gök Üzüm clone samples were tested serologically with DAS-ELISA method in terms of ArMV / GFLV, GLRaV-1, -2, -3, GLRaV-4 strains -4 -5, -6, -9, -Ob, SLRSV, TBRV, RpRSV-ch, RpRSV-g, GVA and GFkV. DAS-ELISA tests were performed according to the "Double Antibody Sandwich" method (Clark and Adams 1977), that was used in accordance with the recommendations of the antibody and conjugate manufacturer company (Bioreba, Switzerland). The results were deter-mined by measuring the absorbance values of DAS-ELISA plates at 405 nm wavelength using Multiscan GO ELISA Reader (Thermo Scientific, USA). As a result of the measurement, samples reaching 2 times and above the negative control absorbance value were evaluated as positive for the tested virus / vi-ruses (Clark and Adams 1977).

2.2. Bacterial analysis

Dormant shoots of Ekşi Kara and Gök Üzüm clones were analyzed for the presence of *Rhizobium vitis*. Shoot washing method for extraction of bacteria from dormant shoots was made according to Benlioğlu and Özakman (1998). Extracts obtained by shoot washing were planted in R&S (Roy and Sasser 1983). After growth, bacterial colonies were purified into KB (King et al. 1954) broths. DNA extraction was performed from typical colony-growing bacterial isolates in the KB broth (Abolmaaty et al. 2000), then the PGF / PGR primer pair was tested for the presence of *Rhizobium vitis* by PCR method (Szegedi and Bottka 2002).

2.3. Fungal analysis

Fungal disease factors of *Phaeoacremonium* spp., *Phaeomoniella chlamydospora*, *Cylindrocarpon* spp., *Stereum hirsitum*, *Phellinus igniarius*, Eutypa dieback (*Eutypa lata*), dead arm (*Phomopsis viticola*) were analyzed in dormant shoots of Ekşi Kara and Gök Üzüm clones. For this purpose, sections of 5 mm from dormant shoots were planted in a medium containing potato dextrose agar (PDA) after surface sterilization and incubated in 20-25 °C dark environment for 14 days, after which the morphological diagnosis of the growing cultures was made (Poyraz and Onoğur 2013). These factors are wound parasites, they infect the plant by entering from wound sites. They can spread transversely and longitudinally in the plant wood tissue. Since their mycelial development is slow, symptoms in the plant may appear too late. The most suitable growth temperature of the agents is in the range of 20-30 °C. Signs of infection of fungal woody tissue disease agents are the appearance of pallor of green parts, growth retardation and even drying symptoms. These symptoms can be seen on the whole or only part of the vine. The disease has two types of symptoms. In the first, the disease is chronic and mani-fests itself with the symptoms on the leaves. The second has an acute course and the vine dies suddenly.

Small pieces, about 5 mm in size, were removed from the Dormant shoot specimens. These pieces were first kept in 70% ethyl alcohol for 30 seconds, then in 3% calcium hypochlorite for 15 seconds and were taken on sterile blotting papers. After isolation, samples were taken into petri dishes containing potato dextrose agar (PDA) and malt extract agar (MEA) and incubated at 20-25 °C in the dark for 14 days.

At the end of this period, the diagnosis of the isolates, taking into account the colony colors, conidia and conidiophore structures, was made by Halleen et al. (2004) and Alaniz et al. (2007). Selected cultures were transferred to Eppendorf tubes containing 40% glycerol and placed at -20 ° C for long-term storage (Akgül et al. 2014). DNA of fungi was obtained by following the extraction protocol of Cenis (1992) during the molecular identification of these factors. Molecular identification of the iso-lates was carried out by PCR amplifications per-formed specifically to three different protected gene regions of fungi. For this purpose, primer pairs of the ITS (White et al. 1990), β-tubulin (Glass and Donaldson 1995) and translation elongation factor 1-α (EF 1-α) gene regions were used. Sequences of ITS, β-tubulin and EF1-α oligonucleotides and Real-Time PCR cycles at 95 ˚C: 10 min (95 C: 20 sec, 58 ˚C: 20 sec, 72 ˚C: 35 sec) and 35 cycles was carried out. With the melting analysis performed after RT-PCR amplification, non-specific amplifications such as primer dimers were eliminated, and it was deter-mined whether the amplified region was the target region. Sequence data of PCR products obtained from ITS, β-tubulin and EF1-α gene regions were obtained by receiving bidirectional genome sequencing service from a Sanger sequencing laboratory. Chromatogram files of sequence data were analyzed with ChromasPro 1.7.6 chromatogram analysis program. The identification of the fungi was determined by blastn analysis using the Nation-al Center for Biotechnology Information (NCBI) GenBank database of the consensus sequences obtained for each gene region.

3. Results

The results of the research were presented under two subheadings as genetic selection studies and sanitation tests.

3.1. Genetic selection

After 2 years of observation in the population of the mass-selection, 220 healthy clone candidates were selected and their yield, quality and development characteristics were recorded for 3 years. Clone candidates were ranked at the level of vineyards according to the weighted grading scores based on the average values of the records kept for 3 years (Table 1). At the end of 3 years, sanitation tests were performed in duplicate in clone candidates without visible signs of virus, bacteria, or fungal disease infection. As a result of this evaluation, a total of 17 clones from 9 vineyards were selected with their superior scores in weighted grading

and negative sanitation tests. In the weighted rating, scores of clone candidates ranged from 380 to 780 (Table 1). The difference in scores was due to the care and cultural practices applied to the clone candidates.

Initially, 11 clone candidates were selected from the Gök Üzüm variety. Two clones with negative weighted rating and second sanitation tests were selected before the clone comparison stage.

Table 1. Scaled Rating Scores of Ekşi Kara Selected Clones *

Clone No	Place of vineyards	Birth rate	Berry weight	Cluster weight	Yield	Maturity index	Vegetative growth	Total
1	Hamzalar B	180	15	90	360	45	90	780
16	Yağcı H	140	135	70	200	35	30	610
63	Sarıhacı G	100	75	50	200	15	10	450
67	Sarıhacı G	60	105	30	200	15	90	490
72	Sarıhacı G	60	75	70	280	35	30	550
73	Sarıhacı G	140	75	50	200	25	70	560
103	Damlapınar K	180	15	90	280	45	70	680
106	Damlapınar K	100	15	50	200	15	30	410
114	Damlapınar K	100	105	50	120	25	10	410
127	Damlapınar K	100	105	70	280	25	70	650
136	Damlapınar K	60	75	70	280	35	50	570
138	Damlapınar K	100	135	30	200	15	90	570
148	Damlapınar K	180	105	90	360	15	10	760
153	Alanözü G	180	45	10	120	35	30	420
155	Alanözü G	140	15	30	200	45	30	460
182	Hamzalar B	60	75	90	280	45	10	560
197	Kalınağıl H	60	75	90	120	25	10	380

B: Bozkır, H: Hadim, G: Güneysınır, K: Karaman central district, *: The values given in the table are the average score values formed according to the class ranges created for the characteristics examined. The differences between the total scores were quite high as each vineyard was evaluated within itself. The vineyards in which the clone candidates which got a low total weight rating score were not watered. Training and other cultural practices also caused differences in the total score of the clone candidates, as they differed significantly according to vineyards.

The local producers take cuttings from the vineyards that they find better in terms of yield and development characteristics and establish their vineyards by rooting them or grafting them into vine rootstocks. With this method, we can talk about applying a rough positive mass-selection. In Ekşi Kara and Gök Üzüm varieties, the vines that constitute the vineyard population in which the clone selection study was carried out and the clone candidates selected among them do not come from the selected clones as origin. In other words, it is accepted that the Ekşi Kara and Gök Üzüm vineyard populations, which are the basis of clone selection, may be of polyclonal origin.

Since there is a mixture of varieties at different levels in each vineyard, and in the observations made in the near harvest period, it has been evaluated that the differences in the berry shape and parthenocarpic fruit set ratios may be intra-variety variations. Therefore, the ampelographic descriptions of the cultivars (Kara et al. 2016; Kara et al. 2018) and the fertilization biology of the Ekşi Kara variety (Kara et al. 2017a) and bud fertility (Kara et al. 2017b) were examined. In a similar study (Muganu et al., 2019), the morphological characteristics of the Romanesco variety in Italy were characterized in five growth periods. Ampelographic identification was analyzed using 50 OIV morphological descriptors.

It was understood that the flower type of the Ekşi Kara variety was functional female, the pollen vital-ity did not exceed 3% under the producer conditions, and foreign pollination was necessary to set seeded berry. The producers gave importance to weed cleaning to direct the honeybees to the vine during the flowering period, where honeybees were used effectively for pollen transportation.

It was understood that the differences observed in berry shape and size were due to the pollinator variety and therefore due to pollen, and it was not possible to seeded fruit set in all clone candidates when foreign pollination was prevented by closing the inflorescences. It was understood that the size of the cluster, as well as the berry size, and as a result, the yield changed directly depending on the fertilization biology (Kara et al. 2017a).

To determine whether the differences in the birth rate were clonal or not, the bud productivity of the selected clone candidates was examined. At the end of this study, the difference in the birth rate depends on the primary bud damage, in other words, the summer shoots developing on the canes may be from primary, secondary or tertiary growth cones, and their birth rates naturally differ according to the positions of the shoots and the location of the vine-yards, as a result, yield, maturity index and vegetative growth potency values (Kara et al. 2017a).

3.2. Sanitation analyses results

Sanitation analyses were performed in three stages as virus, bacteria, and fungi and two replications. Clone candidates selected in the first stage were tested for the viral diseases listed in Table 2. Health selection was performed by sanitation tests on 94 Ekşi Kara and 11 Gök Üzüm clone candidates, which were superior in weighted rating scores among 220 clones and had no visible signs of virus, bacteria, or fungal disease. All the dormant shoot samples of selected clones were tested for certification based ArMV / GFLV, GLRaV-1 + 3, GLRaV-2, GLRaV-4 strains, SLRSV, TBRV, RpRSC-ch, RpRSC-g, GVA and GfKV. Dormant shoot samples of the same selected clones were tested for the pres-ence of bacterial disease agent *Rhizobium vitis* and fungal disease factors *Phaeoacremonium* spp., *Paeomoniella chlamydospora*, *Cylindrocarpon* spp., *Stereum hirsitum*, *Phellinus igniarius*, *Eutypa lata*, *Phomopsis viticola* and *Rosellinia nealaria*. 51 clones were found healthy because of tests for viral, bacterial, and fungal diseases. Sanitation tests were repeated in 51 Ekşi Kara and 11 Gök Üzüm clone candidates before proceeding to the second stage of the clone selection study. According to highly weighted rating points and second sanitation tests results; 17 Ekşi Kara and 2 Gök Üzüm clones were selected.

Table 2. Virus test results in Ekşi Kara selected clones

Virus	Tested clone candidates	Infected clone candidates
ArMV/GFLV*	94	1
GLRaV-1+3**	94	24
GLRaV-2	94	3
GVA	94	11
GfKV	94	27
GLRaV-4,5,6,9 and Ob***	94	0
SLRSV	94	0
TBRV	94	0
RpRSC-ch	94	0
RpRSC-g	94	0
Total	94	43

*: Samples infected with ArMV and/or GFLV

**: Tables may have a footer. Samples infected with GLRaV-1 and/or GLRaV-3

***: Tables may have a footer. Samples infected with at least one of the GLRaV-4 strains -4, -5, -6, -9, -Ob.

In the 17 vineyards where clone selection was studied, no clean vineyards were found in the virus tests based on DAS-ELISA analyses. In the first stage, 43 (46%) of 94 clone candidates which had no symptoms of virus, bacteria or fungal diseases and had high scores in weighted grading were infected with at least one of the tested viruses. GfKV, one of the viral diseases, was found most common in the tested samples (27/94), followed by GLRaV-1 + 3 (24/94), GVA (11/94), GLRaV-2 (3/94), ArMV / GFLV (1/94). GLRaV-4 strains -4, -5, -6, -9, -Ob, SLRSV, TBRV, RpRSV-ch and RpRSV-g infections were not detected. In the region where we work, *Vitis rupestris* hybrid vine rootstocks, which form a lot of bottom shoots, were widely used. Producers

preferred to obtain saplings by grafting their bottom shoots with the green grafting method. This situation caused the spread of viral diseases in the region.

In a similar study, Çelik et al. (2019) reported the contamination rate of 80.5% and the most common viruses as GLRaV-1, GfKV and GLRaV-3 in the virus tests performed on selected clones of the Kalecik Karası variety. In another similar study, Vončina et al. (2019), by testing 9 viruses (ELISA) in 1116 vines in 14 autochthonous Croat grape varieties from 51 vineyards in the Dalmatian region (ArMV, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, GVA and GVB) had confirmed the existence of 8 viruses. Contamination rates were as GLRaV-3 (79.6%), GVA (61.4%), GLRaV-1 (40.8%), GFkV (19.9%), GFLV (19.6%), GLRaV-2 (4.1%), ArMV (3.2%) and GVB (3.1%) respectively, and total of 93 vines (8.3%) free of all viruses tested.

Vine is one of the plant species most susceptible to viral infections that cause many complex diseases. The effects of viruses on grapevine performance are generally considered to be potentially severe, but factors affecting the grapevine response such as mixed infections, viral species, environment, grape variety and rootstock, vineyard management, etc. are complex. However, diseases such as infectious degenerations caused by Nepoviruses are highly harmful and significantly affect plant viability and yield. More complex are the effects of members of the genus Ampelovirus, Closterovirus and Vitivirus, which are factors of leaf curl, leaf discoloration - spots and wrinkled woody tissue. Vines infected with these species usually produce sufficient crops, so growers are unaware of the true damage, especially in qualitative parameters. Grape vines generally offer better growth and increased yield; there-fore, cultural practices (green pruning, cluster thinning, wider spacing etc.) must be adjusted to cope with these improved performances. Vines are also affected by "small" virus diseases (e.g., speckle, vein mosaic, rupestris stem pitting, etc.), the effect of which is still uncertain. Their presence should not be overlooked, as the synergistic negative effects of these agents with other major viruses cannot be ruled out. Viruses are dangerous and difficult to eliminate pathogens whose presence in vines must be prevented using clean propagation material (Mannini and Digiaro 2017). Therefore, hygienic selection is the most economical strategy to reduce the presence of viruses in the propagation material and to limit their prevalence in newly established vineyards through the production of clean stocks from which high-quality planting material is obtained. Clean stock selection requires efficient therapy methodologies and careful screening of selected clones of scion and rootstock material for economically important viruses (Golino et al. 2017).

Šikuten et al. (2018) reported that due to the lack of clonal and sanitary selection in the past, native varieties in Croatia have a high level of intra-varietal variability and virus infections. Researchers were able to select enough virus-free clone candidates at the first stage of selection, despite the high level of virus infection they detected, as well as the high level of intra-varietal variability in native cultivar populations.

Lemos et al. (2020) reported that when they examined 30 "Tempranillo" clones in two regions for a period of two years, high variation was observed in terms of total phenols and antiradical activity, anthocyanin content was significantly affected by environmental conditions, and location tests enabled the recognition of elite grapevine clones. They also reported that the genetic variability exhibited by selected clones could be an important resource in the short / medium term to respond appropriately to the changing climate by selecting clones that best adapt to new conditions.

According to Gonçalves and Martins (2019), conserving intra-variety genetic diversity is a crucial strategy for preserving traditional viticulture and facing future challenges (Carbonell-Bejerano et al. 2019).

3.3. Establishing the clone comparison vineyard

Clone comparison vineyards established in the second stage of clone selection also form the field gene banks (FGB) of the selected clones. Although clonal repositories require less space, are easy to manage and cost-effective, FGB are needed to pre-serve genetic diversity. In the 1980s, procedures were developed for the maintenance of FGB germplasm collections (Rajasekaran and Mullins 1979).

To compare these selected clones together, all clones were grafted onto the virus-free 110R rootstock. The clone comparison vineyard was established in Selçuk University Vineyard research plot, where there were

three replicates of each clone and 6 vines for each repeat. The clone comparison vineyard was planned to be a Gök Üzüm clone next to each Ekşi Kara vine to allow pollination (Figure 1). The sanitary condition is usually initially assessed by visual inspection of the vineyards and the presence of various viruses by DAS-ELISA analyses. This test is generally considered definitive if one is positive, but when the result is negative it does not rule out an infection. Directive 2005/43 / EC of the European Union on the marketing of vine propagation material, GFLV, ArMV and GLRaV- 1 and GLRaV-3 to ensure that it is not included in the grapevine seedlings from each member country (Rizzo et al. 2015).

To protect the Ekşi Kara and Gök Üzüm clones, which were found to be free from viral, bacterial, and fungal diseases, cleanly and to prevent contamination, own rooted saplings were produced and planted one by one in the greenhouse for protection. At this stage, a study plan was prepared to determine the kinship relations of the selected clones. In a previous study, Roach et al. (2018) reported that many clones with differences in basic viticulture and oenological characteristics were formed in the Chardonnay cultivar with the accumulation of somatic mutations during the asexual reproduction process over centuries, the genetic diversity under-lying these differences was largely unknown, how-ever, Pinot noir and Gouais blanc. They determined that the Chardonnay genome exhibited features indicative of inbreeding.

Mannini et al. (2002) reported that serious and costly sanitation protocols were established world-wide to reproduce only clones free of harmful vi-ruses. In the study, virus-free clones performed best overall, whereas increased vegetative growth and/or yield associated with healthy vines may have ad-verse side effects on grape quality in cooler cli-mates, suggesting that cultural practices in the vineyard must be adapted to the changing abilities of the clones to cope with this. suggested.

In a similar previous study, Cirami et al. (1993) evaluated the field performance of selected clones of Cabernet Sauvignon for 30 years by examining yield and juice composition values with 9 reference clones. They suggested testing the clones in single vine plots with 10-20 replicates and in the vineyard areas where they will be planted for more precise statistical discrimination.

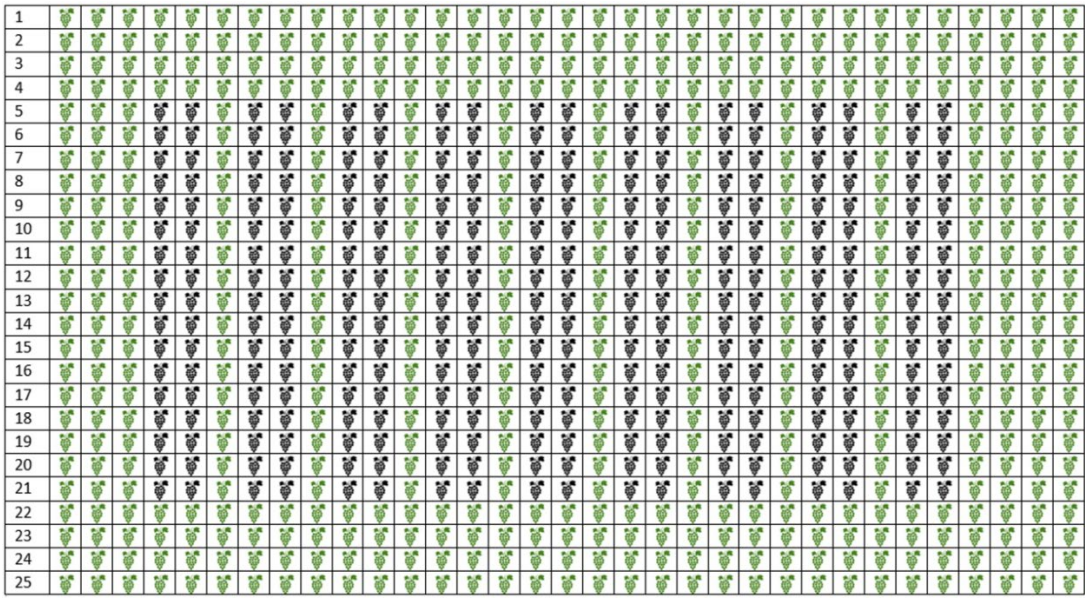

Figure 1. Ekşi Kara Clone Comparison vineyard planting plan. The green clusters indicate Gök Üzüm clones and the black clusters indicates Ekşi Kara clones. The Gök Üzüm clone was planted as an edge affect for all sides. In the middle area clones were placed in order of 18 vine from each clone

4. Conclusions

During the mass-selection stage of the Ekşi Kara grape variety, ampelographic description was made and the vineyard population of clone selection consisting of 5000 vines in 17 different vineyards varying between 800 m - 1500 m above sea level was determined. As a result of the 2-year yield, development and quality observations made in the population, 220 clones were selected, and the stage of single selection was initiated. Single clone selection was carried out in two stages, genetic selection, and health selection.

In the genetic selection phase, yield, quality, and growth values were determined in the clone candidates and the clone candidates were ranked separately according to their average weighted grading scores.

Considering the repeated sanitation tests in 51 Ekşi Kara and 11 Gök Üzüm clone candidates selected by genetic selection, 17 Ekşi Kara and 2 Gök Üzüm clones were selected for the third stage studies. Clone comparison vineyard was established in Selçuk University (38°03'50''N, 32°50'11''E) to compare the selected clones at the same location and on the same rootstock.

Author Contributions: at all stages except virus and bacterial analysis, ZK, AS, KY and OD; in field work MHÖ, MND, MB, AİO and AJKK; virus and bacterial analysis, SÖ and NGS. All authors have read and agreed to the published version of the manuscript.

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Research Article

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Selection of Superior Properties of Walnut Genotypes in Ereğli-Halkapınar (Konya) and Ayrancı (Karaman) Walnut Population

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HIGHLIGHTS

- In this selection study, 8 genotypes in total, including 5 genotypes in shelled walnuts and 4 genotypes in kernels were found to be promising.
- In selected genotypes showed homogamy, 1 showed protogeny and 1 showed protandry flowering properties.

Abstract

This selection study was conducted to determine the walnut genotypes with superior characteristics grown from seeds in Ereğli and Halkapınar districts of Konya province and Ayrancı district of Karaman province between 2019-2022. In 2021 on the fruits taken, 8 genotypes in total, including 5 genotypes in shelled walnuts and 4 genotypes in kernels (1 genotype received high scores in both shelled and kernel walnuts) were found to be promising. In 8 genotypes selected, the shelled weight is 10.66-26.31 g, the kernel weight is 5.32-9.82 g, the kernel percentage is 37.33-57.91%, the shell thickness is between 1.06-1.90 mm, the shell color is "light" in 1 genotype, "moderate" in 4 genotypes, "dark" in 3 genotypes, kernel color is in 1 genotype "extra light", "light" in 7 genotypes, remove of kernel from the shell was "easy" in all genotypes. Shell adhesion was "good" in all genotypes, cracking of the shell was detected "easy" in 7 genotypes, "moderate" in 1 genotype, shell texture was "smooth" in 3 genotypes, "moderate" in 4 genotypes, and "rough" in 1 genotype. Remove of kernel from the shell was determined "easy" in all genotypes. Kernel fill and healthy kernel percentage was determined as 100% in all genotypes. At the end of the research, 6 of the selected genotypes showed homogamy, 1 showed protogeny and 1 showed protandry flowering properties.

Keywords: Walnut; Selection; Ereğli; Halkapınar; Ayrancı

1. Introduction

Walnut belongs to the genus Juglans of the family Juglandacea of the order Juglandales. 21 genera are known, including the genus Juglans. Among these species, Juglans regia (Anatolian walnut), which gains importance for its fruit, grows naturally in Anatolia, Iran, Himalaya, and Southeast Europe (Şen, 1986). Juglans regia has a wide variety of genotypes, and when walnut is mentioned, it is the first species that comes to mind with its superior fruit quality.

Anatolia, whose fruit growing culture dates back to ancient times, is among the homelands of walnuts as well as many fruit species. Walnut is a species that is cultivated in many regions of Türkiye, except for extreme

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climatic conditions. In Türkiye, a significant part of walnut production is still made with plants grown from seeds. According to the data of 2020, walnut growing is conducted in an area of 1.417.899 decare in Türkiye. Türkiye production area is 10.9% of the world walnut production area and we are in the 3rd place in the ranking (Anonymous, 2022a).

While the export of shelled walnuts in the world was approximately 384 thousand tons in 2020, the USA ranks first with an export amount of 138 thousand tons. While the import of shelled walnuts in the world was 344 thousand tons in 2020, Türkiye ranked first with 63 thousand tons. While the world's kernel exports amounted to approximately 335 thousand tons, the USA ranked first with an export amount of 130 thousand tons. While the world's kernel import was 266 thousand tons in 2020, Germany ranked first with 45 thousand tons (TEPGE, 2022).

The most important reason, Türkiye is not at the point we want in walnut exports, and therefore Türkiye meets our production deficit through imports is that we still continue production with walnut trees grown from seed. Today, although the plants established with standard varieties is increasing, productivity is still not at the desired levels. One of the ways for our country to reach the desired yield and quality values in walnut cultivation is to determine the types with superior characteristics suitable for the climatic conditions of the regions, to register the variety and to continue production with these varieties.

Türkiye is a large source for selection studies due to its location among the homelands of walnuts. The first research in our country which started with the "Selection of Walnuts from the Marmara Region", was initiated by Olez (1971). Later, the research that Şen (1980) continued with his associate professorship thesis titled "Research on the Breeding of Walnuts in Northeast Anatolia and the Eastern Black Sea Region by Selection" and selection studies on the basis of provinces and districts in different regions until today. As a result of these selection studies, many promising genotypes were determined and some of them were registered as cultivars.

The fruit growing culture in Ereğli and Halkapınar districts of Konya province and Ayrancı districts of Karaman province, where the research was conducted, dates back to ancient times. Sweet Cherry, apple and walnut cultivation in the region is conducted both on the Ereğli plain and on the north-facing foothills of the Taurus Mountains. Most of the walnut trees in the study area are walnut genotypes grown from seed. This research was conducted for the selection of superior walnut types grown from seeds in the afore mentioned regions.

According to Turkish Statistical Institute data there are 100 hectares of walnut orchards in Ereğli district, The total number of fruit bearing walnut trees is 3,500 and the total number of fruitless trees is 7,630. The yield per tree is 35 kg and the production is 122.5 tons. There are 195 hectares of covered walnut orchards in Halkapınar district. The total number of fruit bearing walnut trees is 10.500 and the number of fruitless walnut trees is 20.700. Yield per tree is 28 kg and total production is 294 tons. There are 59.2 hectares of walnut orchards in Ayrancı district. The number of fruit bearing trees is 5.300 and the total production amount is 117 tons. In most of the old orchards, the trees are genotypes grown from seeds, and standard fruit production is not possible, and the yield is extremely low (Anonymous, 2022b).

This study conducted in 2 provinces, 3 districts and 19 neighborhoods 2019-2022.

2. Materials and Methods

This research was conducted in Ereğli and Halkapınar districts of Konya province and Ayrancı district of Karaman province between 2019 and 2022.

At the beginning of the research, 19 neighborhoods in 3 districts were visited in 2019, and a total of 67 trees with high yield and fruit quality were determined in the first stage by interviewing the District Directorates of Agriculture and Forestry, the headmen of the neighborhoods and the leading farmers. 50 fruit samples were taken from each tree in September-October period. The samples taken were separated from their hulls and dried in a cool place, then they were measured and weighed according to the modified ranked method and Interntional Union for the Protection of New Varieties of Plants (Anonymous, 2017). For nut fruit

characteristics in weighted ranked scores and their relative scores are, 1-fruit weight (g) has 25% (very high:10, high:8, moderate:6, low:4, very low:2), 2- kernel percentage (%), has 20% (very hig:10, high:8, moderate:6, low:4, very low:2) 3-shell color has 15% (light:10, moderate:6, dark:2), 4-fruit size (mm) has 10% (large:10, moderate:8, small:4, very small:2), 5-shell thickness (mm) has 5% (very thin:10, moderate:8, thin:4, thick:2), 6-shell adhesion has 5% (good:10, moderate:6, poor:2), 7-shell texture has 5% (smooth:10, moderate:6, rough:2), 8-cracking of shell has 5% (easy:10, moderate:6, difficult:2), 9-kernel fill has 5% (100%:10, 80-90%:6, 70%≥:2), 10- healthy kernel percentage 5%, (100%:10, 80-90%:6, 70%≥:2).

For kernel characteristics in weighted ranked scores and their relative scores are, 1- kernel weight (g) has 25% (very high:10, high:8, moderate:6, low:4, very low:2), 2-kernel percentage (%) has 25% (very high:10, high:8, moderate:6, low:4, very low:2),3- kernel color has 20% (extra light:10, light:6, dark:2), 4- remove of kernel from the shell has 15% (easy:10, moderate:6, difficult:2), 5- cracking of shell (easy:10, moderate:6, difficult:2), 6-kernel fill (%) has 5% (100%:10, 80-90%:6, 70%≥:2), 7-healty kernel percentage (%) has 5% (100%:10, 80-90%:6, 70%≥:2).

The total scores of the genotypes were calculated by multiplying the scores of the genotypes in terms of each feature with the importance level of that feature. These scores, which were calculated separately for each genotype in terms of shelled walnuts and kernels were evaluated by ranking from the highest to the lowest.

As a result of the scoring and grading, 24 genotypes were found to be superior. As a result of the evaluations made in the summer of 2020, 24 new genotypes with superior characteristics were included in the research and the number of genotypes was increased to 48. Samples from 48 genotypes were evaluated in 2020 and 33 genotypes received high scores. Samples from 33 genotypes were taken during the harvest period in 2021, and 5 genotypes were determined in shelled walnuts with the highest scores and 4 genotypes in kernel (one genotype scored high in both shelled and kernels).

Fruit weight, kernel weight, kernel percentage, shell thickness, fruit size, shell color, kernel color, shell texture, cracking of shell, shell adhesion, remove of kernel from the shell, kernel fill, healthy kernel percentage of genotypes, leafing and flowering dates and flowering characteristics were investigated. Evaluations were made using the modified ranked method. In the selection of genotypes, modified ranked method scores were taken as basis. 8 genotypes selected in 2021 according to the modified ranked method score were found to be promising.

The genotypes included in the evaluation were numbered starting from the license plate numbers of the provinces (42 and 70), the first two letters of the districts (Ereğli ER, Halkapınar HA and Ayrancı AY). For example: 42-ER-01.

3. Results and Discussion

The first fruit samples were taken from 67 genotypes in September and October 2019. Pomological measurements and weighing of dried fruits were made according to modified ranked method and UPOV (Anonymous, 2017) the scores of the selection genotypes for each feature were multiplied by the importance of that feature and written as the total score of the genotypes. These scores, which were calculated separately for shelled walnuts and kernels for each selection genotype, were evaluated by sorting from the highest to the lowest (Şen, 1980).

In 67 genotypes determined in 2019, the fruit weight 6.76-17.98 g, kernel weight 2.31-9.54 g, kernel percentage 29.60-66.31%, fruit size 25.59-43.14 mm, kernel color "extra light" in 40 genotypes, "light" in 22 genotypes, “dark” in 5 genotypes, shell color “light” in 15 genotypes, “moderate” in 26 genotypes, “dark” in 26 genotypes, shell thickness 0.92-2.02 mm, shell adhesion “good” in 34 genotypes, “moderate” in 29 genotypes, “poor” in 4 genotypes, remove of the shell from kernel “easy” in 47 genotypes, “moderate” in 16 genotypes, “difficult” in 4 genotypes, shell texture “smooth” in 24 genotypes, “moderate” in 27 genotypes, “rough” in 16 genotypes, cracking of shell “easy” in 53 genotypes, “moderate” in 6 genotypes, “difficult” in 8 genotypes, kernel fill and healthy kernel percentage 100% in all genotypes.

According to the modified ranked method, 24 genotypes with a score of 715 and above in shelled walnut and 17 genotypes with a score of 850 and above in kernel were selected from among 67 walnut genotypes sampled. 17 genotypes with high scores in kernel also scored high in shelled walnuts, and the total number of superior genotypes was 24.

Fruit weight was between 8.81-17.14 g in selected 24 genotypes, the lowest fruit weight was measured in 42-ER-24 genotype, and the highest fruit weight was measured in 70-AY-02 genotype, kernel weight 4.84 g (42-ER-07) to 8.85 g (42-ER-01), kernel percentage 37.16% (70-AY-02) to 66.31% (42-HA-01), fruit size 30.07 mm (42- ER-08) with 40.44 mm (42-HA-09), kernel color is "extra light" in 10 genotypes, "light" in 10 genotypes, "dark" in 4 genotypes, shell color "light" in 4 genotypes, "moderate" in 7 genotypes , "dark" in 13 genotypes, shell thickness 1.04 mm (42-HA-09) to 1.81 mm (70-AY-02), shell adhesion "good" in 13 genotypes, "moderate" in 11 genotypes, remove of kernel from the shell "easy" in 20 genotypes , "medium" in 2 genotypes, "difficult" in 2 genotypes, shell texture is "smooth" in 17 genotypes, "rough" in 7 genotypes, cracking of shell is "easy" in 10 genotypes, "moderate" in 6 genotypes, "difficult" in 8 genotypes, kernel fill and healthy kernel percentage rate was found to be 100% in all. It was determined that genotype 70-AY-02 (17.14 g), which had the highest fruit weight, had the highest shell thickness (1.81 mm) and the lowest kernel percentage (37.16%).

In the spring of 2020, phenological observations were made in 24 genotypes that were superior according to the modified ranked method result of 2019. While the leaf starting date was between 14-30 April in genotypes, male flowers were active between 25 April-20 May and female flowers were active between 25 April-20 May. The earliest leafing was observed in 42-HA-12 genotype, and the latest leafing was observed in 42-HA-01 and 42-HA-02 genotypes. The earliest male flower was seen on April 25 in 42-HA-02, 42-HA-11 and 42-HA-12 genotypes, and the earliest female flower was seen on April 15 in 42-HA-11 genotype. 15 genotypes homogamy, 7 genotypes protandry and 2 genotypes showed protogeny flowering properties.

It was decided to include 24 new genotypes that could not be detected in 2019, in line with the observations made during the 2020 harvest period and the information received from the regional farmers. 24 new genotypes were added to 24 genotypes from 2019, and fruits were taken from a total of 48 trees in September-October, dried in a cool place and measured.

Fruit weight was between 8.39 g (42-HA-13)-23.30 g (42-HA-07), kernel weight 3.67 g (42-HA-01)-9.59 g (42-HA-07), kernel percentage 30.07% (42-HA-15)-61.74% (42-HA-11), kernel color is " light" in 42 genotypes, "light" in 6 genotypes, shell color is "light" in 38 genotypes, "medium" in 9 genotypes, "dark" in 1 genotype, shell thickness 0.95 mm (42-HA-13)-2.24 mm (42-HA-05) determined in 48 genotypes.

According to the scoring a total of 33 genotypes were found to be superior, 25 genotypes with a score of 700 and above in shelled walnuts, 25 genotypes with a score of 800 and above in kernel (17 genotypes scored high in both shelled and kernels).

Fruit weight was between 9.17 g (42-HA-11)-23.30 g (42-HA-07), kernel weight 4.81 g (70-AY-02)-9.59 g (42-HA-07), kernel percentage 37.35% (42-HA-18)-61.74% (42-HA-11), kernel color is " light" in 28 genotypes, "light" in 5 genotypes, shell color is "light" in 28 genotypes, "moderate" in 5 genotypes, shell thickness 0.96 mm (70-AY-01)- 2.24 mm (42-HA-05) determined in 33 selected genotypes.

Phenological observations of 33 genotypes selected in the previous year were made in 2021. As a result of the observations, it was determined leaf starting date took place between 16-28 April, male flowering between 22 April-8 May and female flowering between 25 April-7 May. Due to the low temperatures in winter and late spring frosts (the lowest temperature in Ereğli district is -22.2°C in January, -5.1°C in March, -1.1°C in April, -15.8°C in January in Halkapınar district, -12.6°C in March and in April -1.8°C). No female flowers were observed in 2 genotypes (42-HA-11 and 42-HA-18) in Halkapınar district, and no male flowers in 1 genotype (42-ER-17) in Ereğli district. While 20 genotypes showed homogamy, 6 genotypes protandry and 4 genotypes showed protogeny flowering, 3 genotypes could not detect flowering properties.

Due to the average temperature of 10°C in Ereğli and Halkapınar districts in April 2021, 42-ER-17, 42-ER-18, 42-HA-12, 42-HA-16, 42-HA-19, 42-HA-20, and 42-HA-23 genotypes fruit dropping occurred during the

small fruit formation period after flowering (MGM, 2022). In the 42-HA-11 and 42-HA-18 genotypes, the female flowers were never seen. Thus, fruit samples from a total of 9 genotypes could not be taken. Samples could be taken from 24 genotypes during the harvest period.

As a result of pomological measurements and subsequent scoring in fruit samples taken from 24 genotypes in September-October 2021, 5 genotypes scored 715 and above in shelled walnuts, 4 genotypes with a score of 900 and above in kernel (1 genotype scored high in both shelled walnuts and kernel). A total of 8 genotypes were found to be superior at the figures (1-8).

In the genotypes superior in shelled walnuts, shell fruit weight is 12.99 g (70-AY-01) to 26.31 g (42-HA-07), kernel weight is 6.23 g (70-AY-06) to 9.82 g (42-HA-07), kernel percentage is 37.33% (42-HA-07) to 57.91% (42-ER-01), fruit size 34.71 mm (70-AY-06) to 50.95 mm (42-HA-07), kernel color "light" in all genotypes, shell color "light" in 1 genotype, "moderate" in 2 genotypes, "dark" in 2 genotypes, shell thickness 1.15 mm (70 AY 01) to 1.90 mm (70-AY-05), shell adhesion "good" in all genotypes, remove of kernel from the shell was "easy" in all genotypes, shell texture is "smooth" in 2 genotypes, "moderate" in 2 genotypes, "rough" in 1 genotype, cracking of shell status "easy" in 4 genotypes, "moderate" in 1 genotype, kernel fill and healthy kernel percentage is 100% found in all genotypes (Table 1).

In the genotypes superior in kernel, the shell fruit weight is 10.66 g (42-HA-04) to 26.31 g (42-HA-07), kernel weight is 5.32 g (42-HA-04) to 9.82 g (42-HA-07), kernel percentage is 37.33% (42-HA-07) to 53.08% (42-HA-03), fruit size 31.50 mm (42-HA-04) to 50.95 mm (42-HA-07), kernel color "extra light" in 1 genotype ", "light" in 3 genotypes, shell color "moderate" in 2 genotypes, "dark" in 2 genotypes, shell thickness 1.06 mm (42-HA-06) and 1.64 mm (42-HA-07), shell adhesion in all genotypes " good", remove of kernel from the shell is "easy" in all genotypes, shell texture is "smooth" in 1 genotype, "moderate" in 2 genotypes, "rough" in 1 genotype, cracking of shell status is "easy" in 3 genotypes, "moderate" in 1 genotype, kernel fill and healthy kernel percentage is found to be 100% in all genotypes (Table 2).

Table 1. The average values of selected superior genotypes in shelled walnuts

Genotype	Fruit weight (g)	Kernel weight (g)	Kernel percentage (%)	Fruit size (mm)	Kernel color	Shell color	Shell thickness (mm)	Shell adhesion	Remove of kernel from the shell	Shell texture	Cracking of shell
42-HA-07	26.31	9.82	37.33	50.95	Light	Dark	1.64	Good	Easy	Rough	Moderate
42-ER-01	16.83	9.75	57.91	43.17	Light	Dark	1.33	Good	Easy	Moderate	Easy
70-AY-06	13.82	6.23	51.37	34.71	Light	Moderate	1.52	Good	Easy	Smooth	Easy
70-AY-05	15.74	7.24	45.96	36.81	Light	Light	1.90	Good	Easy	Smooth	Easy
70-AY-01	12.99	7.06	54.54	35.39	Light	Moderate	1.15	Good	Easy	Moderate	Easy

Table 2. The average values of selected superior genotypes in kerne

Genotype	Fruit weight (g)	Kernel weight (g)	Kernel percentage (%)	Fruit size (mm)	Kernel color	Shell color	Shell thickness (mm)	Shell adhesion	Remove of kernel from the shell	Shell texture	Cracking of shell
42-HA-03	12.41	6.60	53.08	35.76	Light	Dark	1.32	Good	Easy	Moderate	Easy
42-HA-04	10.66	5.32	49.77	31.50	Extra Light	Moderate	1.44	Good	Easy	Moderate	Easy
42-HA-06	12.34	6.23	50.54	35.41	Light	Moderate	1.06	Good	Easy	Smooth	Easy
42-HA-07	26.31	9.82	37.33	50.95	Light	Dark	1.64	Good	Easy	Rough	Moderate

Table 3. Phenological observation of superior genotypes

Genotype	Leaf starting date	Male blooming date	Female blooming date	Type of blossom
42-HA-07	20 April	3 May-7 May	25 April - 30 April	Protogeny
42-ER-01	20 April	25 April - 30 April	1 May - 5 May	Protandry
70-AY-06	20 April	25 April - 30 April	25 April - 30 April	Homogamy
70-AY-05	20 April	29 April - 4 May	1 May - 5 May	Homogamy
70-AY-01	22 April	30 April - 4 May	26 April - 30 April	Homogamy
42-HA-03	22 April	30 April - 5 May	30 April - 5 May	Homogamy
42-HA-04	20 April	27 April - 3 May	27 April - 3 May	Homogamy
42-HA-06	20 April	25 April - 30 April	25 April - 30 April	Homogamy

At the end of the study, the phenological observations of a total of 8 genotypes in 2021 are presented below, 6 genotypes showed homogamy flowering, 1 genotype showed protandry and 1 genotype showed protogeny flowering status (Table 3).

4. Conclusions

This selection study was conducted to determine the walnut genotypes with superior characteristics grown from seeds in Ereğli and Halkapınar districts of Konya province and Ayrancı district of Karaman province between 2019-2022. At the end of the study, 8 genotypes in total, including 5 genotypes in shelled walnuts and 4 types in kernel (1 type received high scores in both shelled and kernel) were found to be promising.

In our research, the fruit weight was 10.66-26.31 g, the kernel weight was 5.32-9.82 g, the percentage of kernel was 37.33-57.91%, and the fruit size was 31.50-50.95 mm in 8 promising genotypes. Promising genotypes were found above the values obtained by Aslantaş (2006), Oğuz and Aşkın (2007), Serdar et al (2001), Sütyemez and Eti (2001), Kazankaya et al (2017), Akça (1993), Beyhan (1993), Cicek (2020), Oruc (2020), Güller (2020) and Mestav (2022) in terms of these characteristics.

In walnut selection, apart from fruit characteristics, another feature that is overemphasized is the thickness of the shell. The shell thicknesses of the selected genotypes were found to be between 1.06-1.90 mm. Promising genotypes were found above the values obtained by Yarılgaç et al (2005), Kahraman (2006), Yıldırım et al (2005), Abdis (2010), Kırısık (2017), Demir (2018), Ates (2018) and Demirhan (2021).

In the selected genotypes, adhesion to the shell was "good" in 7 genotypes (87.5%), "moderate" in 1 genotype (12.5%), and remove of the kernel from the shell was "easy" in all genotypes. Promising genotypes were found above the values obtained by Maden (2011), Aslansoy (2012), Orbay (2016), Goksuncukgil (2017), Cicek (2020) in terms of these characteristics.

In our study, at the end of 2021, the superior genotypes showed 6 homogamy (75%), 1 protandry (12.5%), and 1 protogeny flowering status. If there is no self-incompatibility in homogamy type flowering, it can be advantageous in breeding studies and breeding. Incompatibility and pollen viability should be evaluated separately, and it should be determined whether it requires a pollinator variety. Other researchers, Akça (2001), Unver and Celik (2005), Reis (2010), found fewer genotypes showing homogamous flowering status in their studies.

When the walnut genotypes examined in this study, which was conducted in Konya province Ereğli and Halkapınar districts and Karaman province Ayrancı district, are compared with the fruit characteristics of the genotypes obtained as a result of studies conducted both in our country and abroad, it is seen that they have important values. This situation shows that our region, like many regions of our country, has a rich genetic resource in terms of walnuts.

It is aimed that this study will contribute to the determination of genotypes with good fruit characteristics among the walnut genotypes grown from seed in our country and to prevent the extinction of our gene resources. This study will shed light on future studies on the protection, reproduction and standardization of genotypes determined as promising.

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Figure 1. 42 HA 07 genotype

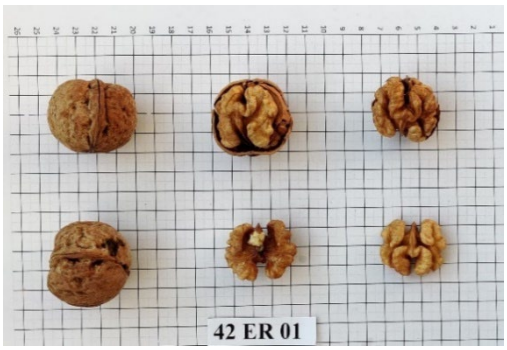

Figure 2. 42 ER 01 genotype

Figure 3. 70 AY 06 genotype

Figure 4. 70 AY 05 genotype

Figure 5. 70 AY 01 genotype

Figure 6. 42 HA 03 genotype

Figure 7. 42 HA 04 genotype

Figure 8. 42 HA 06 genotype

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Research Article

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The Effect of Some Plant Growth Regulators on Callus Culture of Different Pistachio Varieties

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HIGHLIGHTS

- Callus culture is an important method used for *in vitro* secondary metabolite production.
- Pistachio is a plant of high commercial importance that should be evaluated separately in terms of both micropropagation and secondary metabolites.
- It is critical to determine appropriate plant growth regulators in Callus Culture and micropropagation studies.

Abstract

Pistachio (*Pistacia vera* L) is one of the oldest cultivated plants in the world. Its fruits are rich in protein, minerals, carbohydrates, fiber, and vitamins. In addition, the demand for these plants is increasing due to the fact that they are very tasty and nutritious. On the other hand, pistachio cultivation is quite difficult. In addition, many problems are encountered in germination with seeds or reproduction with cuttings. These situations necessitate the development of different *in vitro* tissue culture protocols. In this study, callus culture optimization protocol was developed by using seeds of three different pistachio cultivars. Murashige and Skoog (MS) medium was supplemented with different concentrations of NAA, IAA, 2,4 D and BAP. When callus size (1,776 cm), callus weight (0.908 g) and embryogenic callus regenerations (27.94%) were considered, it was found that the best variety was Tekin. Again, in the evaluation made according to these factors, it was determined that the best improvement was in the MS medium containing 3 mg/L BAP and 1 mg/L 2,4D. The contamination rate detected throughout the studies ranged from 7.65% to 12.91%.

Keywords: Pistachioi; Callus culture; *Pistacia vera*; Plant Growth Regulators (PGRs); Secondary metabolites

1. Introduction

Known as *Pistacia vera* L., pistachio is an important member of the Anacardiacea family and is included in the genus Pistacia. There are 11 species belonging to the genus Pistacia and the only edible species is pistachio (Ferguson et al. 2005). It has also been reported that the genus Pistacia includes 20 species, including evergreen or deciduous species, resin-bearing shrubs, and xerophytic trees growing to a height of 5 to 15 m (Rauf et al. 2017; Bozorgi et al. 2013).

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The outer shell, which has a hard and whitish color, constitutes 50% of its weight. The seed has a thin rind and light green flesh color with a distinctive flavor. It has been reported that together with almonds, pistachios have a very important place in human consumption and even like fig and pomegranate fruits, which are known as objects of faith, pistachios are also mentioned in the Bible (Holland et al. 1992; Ergun and Bozkurt 2020; Mandalari et al. 2021; Bozkurt and Ergun 2021; Dreher 2012).

P. vera L. is resistant to harsh environmental conditions and is resistant to both heat and cold. Due to this feature, it grows both in semi-arid deserts and on dry slopes of low mountains and hills (Mir-Makhamad et al. 2022). It has been reported to be native to Northeast Iran, Southern Turkmenistan, and Afghanistan, and it is mentioned that there are still wild pistachio forests there. In addition, it has been reported that the commercial production of pistachios has spread far from its Eurasian origin to southwestern USA and southwestern Australia (Khezri et al. 2020). According to the data of FAO (2022), when the production amounts in 2020 are taken into account, the USA constitutes approximately 42% of the production in the whole world. This is followed by Turkey and Iran, respectively (Figure 1).

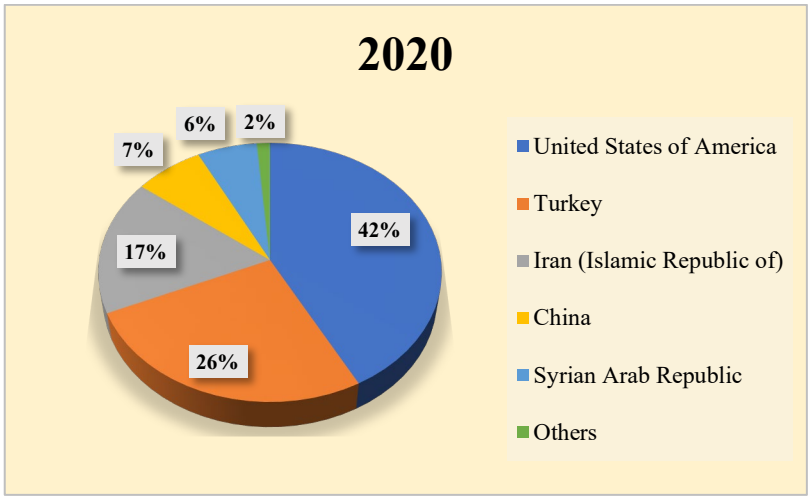

Figure 1. Distribution of pistachios production amount (%) in 2020

When the production of pistachios is evaluated, it is reported that it ranks fifth after cashew, walnut, almond and chestnut (Sheikhi et al. 2019). Although *Pistacia vera* L. is the most demanded species and commercially grown species in the Pistacia genus, other species are used as rootstocks for *P. vera* L. In addition, it has been reported that these species are used in the production of snacks or coffee-like beverages, and they are used as food coloring due to their anthocyanin content. It has been reported that the secondary metabolites of different parts of the pistachio plant are used for liver, heart, kidney, and respiratory system disorders (Longo et al. 2007) (Bozorgi 2013).

The production of pistachios is classically carried out by using the seeds by germinating or by using the cuttings by rooting. Propagation by seeds is not preferred since it will reveal genetic expansions as in other plants. On the other hand, rooting of cuttings is a very difficult and time-consuming process (Almehdi 2002; Benmahioul 2017). However, these methods are insufficient to produce the required number of seedlings for pistachio cultivation in the world. With *in vitro* tissue culture methods, it is possible to micropropagate disease-free pistachio rootstocks, thus enabling the production of economically important varieties in desired quantities.

The most important step in the production of plant species such as pistachios, which are very difficult to produce, by tissue culture will be the use of healthy protocols. In the selection of these protocols, many more parameters from the explant source used, the sterilization conditions applied, and the prepared nutrient media should be well evaluated. This study has been designed with the awareness that all kinds of experimental studies of tissue culture studies of pistachio are very important for sustainable production studies of this plant. The aim of this study is to determine the developmental responses of pistachio seed cells to different plant

growth regulators used *in vitro*. In this study, it was observed how the pistachio seed explant gave results to which plant growth regulator in terms of cellular growth rate and callus structure. It is aimed to be a scientific resource in terms of plant growth regulators and sterilization method for *in vitro* scientific studies to be carried out with pistachio from now on. In particular, it was predicted that this study would be beneficial for callus culture studies to be established for secondary metabolite production from pistachio.

2. Materials and Methods

Plant Material

Seeds of Tekin, Siirt and Pistachio varieties obtained from Pistachio Research Institute/Gaziantep were used as plant material.

Surface Sterilization

First of all, pistachio seeds were washed under a running fountain for 5 minutes. The washed seeds were taken into the laminar cabinet and the surface sterilization stage was started. At this stage, the seeds were first kept in 70% alcohol for 1 minute and then washed 4 times in sterile water. Then, it was kept in 20% (v/v) sodium hypochlorite and then cleaned from sodium hypochlorite with sterile distilled water 4 times.

Cultural Medium and Conditions

The content of the culture media is planned to form the most efficient callus for the pistachio varieties and their seeds to be used in this study, and the basic composition is MS (Murashige and Skoog 1962) medium. This medium was used as the basic nutrient medium and was supplemented with PGRs at different concentrations, and each was given a code (such as M1, M2, M3). The media combinations of the different PGRs clearly stated in Table 1 were determined by our own preliminary studies and research.

Table 1. Different PGRs and their concentrations in the media used in the study.

	NAA (mg/L)	IAA (mg/L)	2,4D (mg/L)	BAP (mg/L)
M1	1	1	1	1
M2			3	1
M3			1	1

The pH of the prepared media was adjusted to 5.8 using 1N NaOH and 1N HCl, and agar (8 g/L) was used as the solidifier. The prepared media were autoclaved at 15 psi at 121°C for 20 minutes and distributed in sterile plastic petri dishes. Endosperms were taken as explants (approximately 0.5-0.7 cm) from seeds whose surface sterilization was completed and placed in the solidified media in Petri dishes. Then, the petri dishes were cultured in a culture room with a temperature of 24°C, under 16 hours light - 8 hours dark conditions. Measurements were made every 2 weeks and the results of the observation of the developing calli were noted (Figure 2).

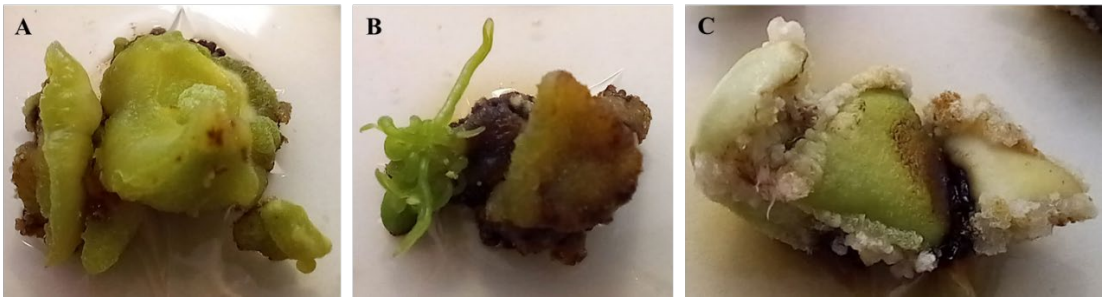

Figure 2. Calluses of pistachio varieties grown in different mediums. A: Antep variety growing in M1 medium, B: Tekin variety growing in M1 medium, C: Siirt variety growing in M2 medium.

Statistical Analysis

Experimental studies were set up in a randomized plot design with three recurrences. Calluses developed in different environments were evaluated in terms of weight, size, embryogenic status and contaminations developed in the environments. All data calculated as percentages were subjected to arcsine-transformed. The obtained data were analyzed using the SAS-JMP statistical program and the differences between them were compared with the LSD (least significant difference) multiple comparison test.

3. Results and Discussion

Considering the callus weights, Tekin (0.908 g) had the highest value, followed by Siirt (0.770 g) and Antep (0.755 g) varieties. There was no statistically significant difference between the varieties of Siirt and Antep. Considering the Plant Nutrient Media, M2 (0.952 g) and M1 (0.868 g) were found to be the highest and M3 (0.613 g) to be the lowest. When the medium and varieties are evaluated together; M2*Tekin (1.136 g) and M1*Tekin (1.104 g) were found to be the highest M1*Siirt (0.502 g) and M3*Tekin (0.484 g) the lowest (Table 2).

Considering the callus size, no statistical difference could be detected between the cultivars, and it was determined that Tekin (1,776 cm), Siirt (1,583 cm) and Antep (1,530 cm) cultivars were ranked from largest to smallest, respectively. When the difference between Plant Nutrient Environments is examined; M2 (1,783 cm) and M1 (1,739 cm) were the highest, while M3 (1,368 cm) was the lowest. When the medium and cultivars are evaluated together, Tekin*M1 (2,050 cm) has the highest value, Siirt*M1 (1,337 cm), Tekin*M3 (1,310 cm) and Antep*M3 (1,225 cm) has the lowest value (Table 2).

Table 2. Experimental results of pistachio cultivars (Tekin, Antep, Siirt)

Varieties	Medium	Weight (gr)	Size (cm)	Embryogenic callus Regeneration rate (%)	Contamination (%)
Tekin	M1	1,104 a	2,050 a	20 b (26,335)	2 b (8,087)
	M2	1,136 a	1,970 ab	40 a (39,147)	2 b (7,947)
	M3	0,484 d	1,310 d	10 c (18,344)	2 b (7,655)
Antep	M1	0,999 ab	1,830 abc	5 cde (12,837)	4 a (11,477)
	M2	0,755 c	1,537 cd	10 c (18,303)	4 a (11,522)
	M3	0,512 d	1,225 d	3 de (9,915)	4 a (11,399)
Siirt	M1	0,502 d	1,337 d	3 de (9,752)	5 a (12,910)
	M2	0,966 abc	1,843 abc	7 cd (15,180)	5 a (12,879)
	M3	0,843 bc	1,570 bcd	2 e (7,655)	5 a (12,824)

P<0.05*, P<0.01**, P<0.001***, LSD value was calculated according to the angle conversion value. Different letters (a–e) indicate significant differences according to the LSD test ($p \leq 0.05$). LSD_{Weight}: 0.218, LSD_{Size}: 0.425, $LSD_{Embryogenic\ callus\ regeneration\ rate}$: 5.904, $LSD_{Contamination}$: 3.180

After evaluating the embryogenic callus regenerations as %, angle transformation was performed and shown in Table 2 in parentheses. Accordingly, when the varieties are evaluated; In terms of percentage, the best regeneration was detected in Tekin variety (27.94%), followed by Antep (13.68%) and Siirt (10.86%). When the Plant Nutrient Environments are evaluated; While M2 had the highest value (24.21%), it was followed by M1 (16.30%) and M3 (11.97%), respectively. When the varieties and mediums are evaluated together, Tekin*M2 (39.14%), Tekin*M1 (26.33%) and Tekin*M3 (18.34%) were the highest, Antep*M3 (9.91%), Siirt*M1 (9.75%) and Siirt*M3 (7.65%) were found to have the lowest rates (Table 2).

In addition to all these studies, observations have been made about contamination, one of the most important issues for *in vitro* tissue culture studies. The angle of the data obtained as a result of these observations was made as angle trasformations and sorted from the highest to the lower according to the varieties (12.87%), Antep (11.46%) and Tekin (7, 89%). When the environments were evaluated, it was found that M1 (10.82%), M2 (10.78%) and M3 (10.62%) respectively. The highest Siirt*M1 (12.91%) and Siirt*M2 (12.87%) were found when evaluated together and the environments were found together, while the lowest Tekin*M2 (7.94%) and Tekin*M1 (7.65%) were found (Table 2).

Although many studies have been carried out in this area, it is striking that each of them gives different results. When some of them are examined;

For standardization of *in vitro* propagation techniques for an endangered palm species Areca concinna (Arecaceae), Veluru et al. (2022), conducted studies. Of the three explants, only somatic embryos were obtained from mature embryos, with the best response from M72 medium containing 25 mg/L 2,4D. In our study, on the other hand, when 2,4D was used with BAP, it gave more successful results. It is clear that it would be wrong to evaluate only on the medium, and the effect of plant varieties on callus formation is also important. Another factor to consider is the diversity of essential nutrient environments. In a study in this context, Nadalizadeh Ghannad et al. (2022) MS and Driver and Kuniyuki Walnut (DKW) investigated the effects of different concentrations of PGRs added to their media on callus induction and regeneration. The highest callus formation (96%) was observed in DKW medium containing 0.5 mg/L NAA and 1 mg/L Kin. On the other hand, the shoot regeneration rate gave the best results (41%-20%) in the same medium containing 1 mg/L BAP + 2 mg/L NAA, although it differed according to the varieties. In addition, significant differences were observed between explants in terms of callus induction and shoot induction.

Khande et al. (2017) investigated different media combinations for callus formation from leaf and shoot explants of Santalum album L. Maximum callus formation was observed in MS + 0.5 mg/L 2,4-D + 0.5 mg/L NAA + 0.5 mg/L BAP medium for both leaf (67%) and shoot (46.67%) explants. Callus weights were determined as 467.67 mg and 159.67 mg in the same environment, respectively. Although the plants and explants are different, callus formation was promoted by the optimization of the environment in our study like this study. Embryogenic callus regeneration, callus weight and size were considered rather than percent callus formation. In such studies, it would be useful to measure the callus size. In support of this idea, researchers who cultured callus on different explants of potato (Haque et al., 2009) included callus weight and callus length in their measurements.

Callus culture studies mostly focus on secondary metabolites and the studies should be supported by instrumental analysis. For example, Aghaei et al. (2013) investigated the effects of different concentrations of PGRs on callus formations of wild pistachio seedling stem explants. In addition, they compared essential oil analyzes in calli. While high callus formation was detected in the medium containing 1 mg/L 6-BAP (85%), the lowest callus formation was detected in the medium containing 2 mg/L BA+1 mg/L NAA. Although the main components of callus were Bornyl acetate (9.18%), Spathulenol (5.89%) and Ledol (5.37%), a total of 8 components were reported. In this study, both explant and PGRs were designed differently. In addition, no analysis of secondary metabolites was performed. Data that will enable the development of healthy calli for secondary metabolite studies were presented at the variety level.

Differently, Ceniza et al. (1992) who studied the *in vitro* callus culture of coconut endosperm examined the development of callus and fatty acids. Callus was developed by supplementing with modified Y-3 medium (Sugimura and Salvana 1989; Branton and Blake 1983), 20 ppm 2.4 D, 1 ppm BA, 1 ppm 2 ip, 0.25% activated carbon, and 0.2% gelrite. It has been determined that while approximately 82% of the total fatty acids are short chain fatty acids, long chain fatty acids are reduced to 16%.

It has been known and investigated for many years that one of the biggest problems encountered in plant tissue culture studies is contamination (Cassells et al. 2000; Leifert et al. 1994). Cobrado and Fernandez (2016) found that they encountered two fungal species as a source of contamination in their study and that they caused the death of the culture material. There have been researchers who mentioned that there are 3-15% losses in tissue culture laboratories because of contamination, and they mentioned the importance of the sterilization protocol (Tiwari et al. 2012; Leifert et al. 1989). In this study, contaminations were observed and calculated as % and it was found to vary between 7.65%-12.91% 2-5%. Predominantly bacterial contaminations were encountered, and no typing was done at the strain level. Observations were shared to provide preliminary information for future studies.

4. Conclusion

Pistachio, also known as green gold, is a plant grown in the world and mostly consumed as dried nuts. Pistachio has a very important place in human nutrition thanks to its carbohydrates, proteins, minerals, and vitamins. The reproduction of the pistachio plant, which is widely produced in the world, is mostly done with

seeds and cuttings. Production with these methods is very difficult and it is not possible to create production areas of the required size in a short time. Therefore, *in vitro* tissue culture techniques are preferred as alternative production methods. It is necessary to determine the environment and method according to the rootstock plant to be produced. In addition, it is possible to produce valuable secondary metabolites in plants with rich nutrient content such as pistachios with callus culture, which is one of the *in vitro* tissue culture methods. For this purpose, callus culture study was started for 3 different pistachio cultivars in this study and the effectiveness of MS medium supplemented with different PGRs was examined. It was determined that Tekin cultivar gave better results than Siirt and Antep cultivars in terms of embryogenic callus regeneration, callus weight and callus size. MS medium containing 3 mg/L BAP and 1 mg/L 2,4D was found to be more successful than other medium combinations. Observations were also made for contaminations that cause significant problems and product losses in tissue culture, and the contamination rate was determined as 7.65%-12.91%.

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Research Article

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Breeding Sunflower (*Helianthus annuus*) Assisted with Speed Breeding & Drough Tolerance Tests

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HIGHLIGHTS

- In the experiments, it was 4-5 generations could be achieved in one year.
- The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics.
- In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center.

Abstract

Sunflower production in the world is expending towards marginal areas, along with rapid changes in cultural practises like no-till planting and weed management. The frequency and severity of abiotic constraints also rise as a result of climate change. *Helianthus annuus* is well-known for its adaptability to a wide range of agronomic conditions, by its robust root system that is capable of absobing water from deeper soils. However, water stress lowers grain yields and fatty acid content with complex phenotypic, physiological and biochemical signs. In this study which was carried out to develop parental lines tolerant or high-tolerant to drought, physiological screenings were carried out on 8 sunflower genotypes. Genotypes were planted in pots in a greenhouse and grown at three different irrigation levels (I100, I66 and I33). The genotypes were watered together until they reached the 6-8 leaf stage. Then, each genotype was managed and irrigated solely. Number of days between sowing and floweing days, number of days between sowing and number of days between sowing and number of days to transfer the embryo to the nutrient medium, plant height, head diameter, number of seeds in the head was between 52-67 days; 65-80 days; 50-200 cm; 3.0-13.0 cm; 25-500 pieces, respectively. Plant weight, plant high, root length, number of leaf, nnmber of days from transplant to glasshouse, number of days from transplant to field was between 0.22-0.45 g; 2.09-4.62 cm; 1.70-5.27 cm; 3.60-5.87 pieces; 5 or 6 days; 10-12 days, respectively. In the experiments, it was found that two and a half generations could be achieved in one year. The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics. In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center.

Keywords: : Sunflower, *Helianthus annuus*, parental lines, drought tolerance, speed breeding, embryo culture

1. Introduction

The yield potential and stability of the sunflower (*Helianthus annuus*) have steadily increased as a result of conventional breeding. This improvement has been made possible by both the direct manipulation of a number of genes

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that control resistance to parasitic weeds, pests, and fungal diseases, as well as the indirect selection of quantitative trait loci that regulate heritable variability of the traits and physiological processes that control biomass production and its partitioning. Due to the distribution of sunflower production towards marginal areas, the rapid changes in cultural practises like no-till planting and weed management, and the rise in the frequency and severity of abiotic constraints as a result of climate change, this approach may no longer be sufficient because genetic progress has been slower in recent decades. Three key strategies were developed as a result of recent research to alter the goals and methods of sunflower breeding. In order to discover traits that are beneficial to increase selection efficiency, plant physiology has supplied new tools and models to analyse the complicated network of yield- and stress-related variables. Second, molecular genetics has helped identify numerous loci that influence yield under prospective and stressful situations or the development of characteristics connected to stress tolerance. Third, molecular biology has produced genes that can be used for transgenic methods or as candidate sequences to analyse QTL (Sala et al. 2012).

Sunflower is not an exception to the rule that drought stress is one of the major factors limiting agricultural yield in the twenty-first century, according to Salehi-Lisar & Bakhshayeshan-Agdam (2016). Drought stress significantly reduces sunflower productivity, which is a key barrier to worldwide sustainable crop production, especially in arid and semi-arid nations (Wasaya et al. 2021). *Helianthus annuus* L., the common sunflower, is well-known for its adaptability to a wide range of agronomic circumstances, particularly on soils with fluctuating water contents (Raineri et al. 2015).

Sunflower has a robust root system that is capable of taking up water from deeper soils (Hussain et al. 2013). Since the plant have stomata on both sides of its leaves, it also has a great capacity for photosynthetic growth. However, because it grows mostly in tropical and subtropical climates, it is more vulnerable to drought, which reduces the seeds and oil yields (Hussain et al. 2018). Additionally, water stress lowers grain yields and fatty acid content (Alberio et al. 2016, Howell et al. 2015).

The effects of drought on sunflowers are complex, ranging from phenotypic to physiological and biochemical signs. These changes include decreased plant height, leaf surface area, relative water content, closed stomata, and reduced levels of photosynthesis (Buriro et al. 2015); increased root length and the root-shoot ratio; shrinkage in cell volume; decreased water potential; and membrane stability disrupted the balance of various biochemical processes (Soleimanzadeh 2012).

Although sunflower is a crop that tolerates modest amounts of drought, severe drought episodes reduce the seed and oil yields. Therefore, it is essential to know how the physiological, biochemical, genetic, and agronomic bases of drought interact in order to control it sustainably and ensure sustainable production of sunflower achene and oil. Drought stress has a major impact on sunflower's achene yield, oil quality, morphological and growth variables, as well as physiological and biochemical traits (including photosynthesis, water relations, nutrient uptake, and oxidative damage). Exogenous hormone and osmoprotectant sprays, seed treatments, soil nutrient management, and traditional or biotechnological breeding for drought resistance are a few examples of management strategies. In reaction to water stress, sunflower modifies its osmotic balance, preserves its turgor, maintains its capacity to absorb carbon, and regulates its hormones. The improvement of sunflower achene yield and oil quality under drought stress necessitates in-depth investigation of the fusion of several management techniques, including agronomic management, conventional breeding, and modern technological breakthroughs (Hussain et al. 2018).

Drought-related sunflower yield reductions are substantial (Prasad et al. 2008). Pekcan et al. (2015) claim that exposure, specifically during the anthesis and dough stages, can result in crop losses of up to 80% (Seiler et al. 2017). Any water stress screening experiment must include genotype characterisation based on relative water content, leaf water potential, photosynthetic efficiency, and proline concentration (Darvishzadeh et al. 2011). The amino acid proline is necessary for both osmotic adjustments and free radical scavenging during drought stress. For the plants to experience a fewer change in relative water content even with a drop in water potential, osmotic adjustments are crucial. This mostly aids plants in continuing to develop and expand their cells while under the stress of drought (Cechin et al. 2006).

Early- and mid-flowering phases are particularly impacted by water shortage produced by reduced irrigation, as opposed to seed filling, where restricted irrigation is tolerated (Karam et al. 2007). Rauf (2008) asserts that lower photosynthesis brought on by early leaf senescence during drought conditions results in a decrease in the weight of 100 achenes.

Development of new crop varieties requires time because it is based on the crop's generation period. Speed breeding, often known as rapid plant breeding, is a quick growing method used by plant breeders to develop new cultivars. Here, the plants are grown in controlled growth chambers or greenhouses with the best light quantity and quality, as well as a specific day length and temperature, which accelerates a number of physiological processes in plants, most notably photosynthesis and flowering, and cuts down on the amount of time that the generation process needs. 4-6 generations can be produced annually using fast breeding, as opposed to 2-3 generations under standard glasshouse conditions. Speed breeding protocols and processes are well-established and standardised for major crop species like wheat, barley, and canola. This strategy is already in use, and protocols for standardisation are being developed for new crops. For the purpose of improving the traits of agricultural species, speed breeding may serve as the essential building block for integrating high-throughput phenotyping and genotyping techniques, marker-assisted/genomic selections, and gene editing (Abdul Fiyaz et al. 2020).

Rapid plant breeding, often known as speed breeding, is a method employed by plant breeders to accelerate the production of new cultivars. For speed breeding, new methods that accelerate flowering, seed germination, embryo development, and other processes are required. Over time, speed breeding has evolved and can broadly be divided into three types: The plants in the first category were grown under controlled growth chamber circumstances with speed breeding criteria; the plants in the second category were grown in a glasshouse with speed breeding specifications; and the plants in the third category were produced in a specially designed home-built growth room for low-cost speed breeding programmes (Watson et al. 2018). 22 hours of photoperiod, 70% humidity, 22 °C during the day and 17 °C at night, as well as strong light intensity (360 to 650 mol m2 s) are all requirements for fast breeding. These requirements change depending on the stage of vegetative and reproductive plant growth (Pandey et al. 2022).

Rapid breeding is facilitated by the in vitro tissue culture technique known as "embryo rescue," which accelerates plant embryo growth. This method, which requires harvesting immature seeds and germination in the culture media, may or may not use the plant growth regulator (PGR). Numerous crop species have successfully used this strategy (Zheng et al. 2013; Castello et al. 2016; Bermejo et al. 2016; Yao et al. 2017). The genotype of the plant, the age of the embryo, its preparation, age homogeneity, sterilisation method, the composition of the medium (sugar, hormone, vitamin, other nutritional additives), environmental adjustments (humidity, photoperiod, and temperature), culture time, the medium to which the seedlings are transferred after culture, and the trial pattern all play a role in how sunflower embryos respond to the embryo rescue method (Çil et al. 2021).

2. Materials and Methods

The Studies were carried out to develop parental lines tolerant or high-tolerant to drought. For this purpose, the responses of different selected sunflower parent genotypes to drought stress were compared. Physiological screenings were carried out on genotypes and the necessary plantings and emergence were achieved.

2.1.Tested oil type sunflower genotypes and their resistance levels

- MAS RYM 17-17 (1) - Resistant restorer
- RYM 13-97/2 (19) - Tolerant restorer
- RYM 13-152/2/2 (26) - Susceptible restorer
- HA 430 (105) - Control Public Group 1
- DA-VB 16-39 (78) - Tolerant

- DA-VB 17-29 (88) - Susceptible

- HA 429 (109) - Control Public Group 2

- DA-VB 16-41 (79) – Resistant

As a part of the greenhouse studies of the project, genotypes were planted in 30x50 sized pots and grown at different irrigation levels (I100, I66 and I33) between January and June 2023. The properties of the soil used in the pots were analyzed and plant water consumption amounts were calculated.

The physical properties of the soil used in the pots were performed at "Soil Analyzes Tarsus Soil and Water Resources Research Station". The results obtained are given in Table 1.

Table 1. Physical properties of the soil used in the experiment

Volume weight (g/cm³)	Clay (%)	Sil (%)	San (%)	Structure Class
1,41	39,52	37,78	22,69	Clay Loam

The pots werefully filled with soil. During filling, every 1/3 of it was compressed with the help of a tamper and this process was repeated three times. Then, irrigation water was applied, pots were left for free drainage for two days and the seeding process was started. Following plant emergences, five plants were left in each pot. The genotypes were planted in February. Each genotype were in 15 pots. Total 75 plants of each genotype were followed. There were 6 pots for each irrigation application group (I33, I66 and I100) with 5 plants in each pot. Plants were grown in the trial greenhouse to test genotypes under windless and sunny weather conditions.

2.2.Drought applicaqtion in greenhouse trials

Immediately after seed planting, all pots were irrigated to field capacity. Next irrigation practices were started when the plants were at 6-8 leaves stage when 50% of the available moisture in the soil was depleted. Irrigation was applied when wilting was observed on the plant leaves. Irrigation was repeated for five times during the entire growth season. On average, irrigation was applied every 7 days. The decreasing humidity level in the A-Pan evaporation container was used to determine the amount of irrigation water to be applied to cover every seven days. The lost moisture was delivered with each irrigation to reach field capacity.

The genotypes were watered together until they reached the 6-8 leaf stage. Then, each genotype was managed and irrigated solely. In this regard, plants with I100 irrigation criteria were checked daily and irrigation need of the genotypes were determined. Pots of the same genotype with I66 and I33 irrigation criteria were also irrigated with restriction. Restricted irrigation applied amounts were 6.5 liters for I100, 4.5 liters for I66 and 2.5 liters for I33 in each irrigation period.

2.3.Irrigation water amount calculations for pots

In the study, the amount of irrigation water applied every 7 days and evaporation (ETo) was determined by monitoring it from the evaporation container. For this purpose, a Class A evaporation pan was placed in the trial area. A-Pan evaporation vessel was made of galvanized sheet metal with a diameter of 121 cm, a height of 25 cm and a thickness of 2 mm. A 15 cm high wooden grill was placed under the container, allowing air flow. Water level changes in the container were measured. A wire cage has been placed over the container to prevent any animal from drinking water from the container. For measurements, water was applied up to 5 cm below the container edge height and the water level was not allowed to fall 7.5 cm below the container edge height. The water in the container was renewed at least every four days to prevent the water from becoming excessively dirty. All readings were conducted at 9:00 am each day. These measured values were used to calculate the amount of irrigation water to be applied every 7 days. The amount of irrigation water applied to the trial plots was calculated by taking into account the daily evaporation amount from Class A Pan.

Irrigation applications were made with measured beakers. Irrigation was not conducted with drip irrigation system due to the pressure drop towards the end of the line and reductions in amounts of applied water. In terms of full irrigation (I100), the water consumption of a plant in a pot was 30.6 liters.

Figure 1. Some photos from the experiment

The amount of irrigation water was calculated using open water surface evaporation and plant-pan coefficients, and determined according to the method of according to Gençoğlan et al. (2006). The amount of water given to parcels was calculated with the help of equations 1, 2 and 3.

$$I = Ep.\ kcp\ .\ P \quad (1)$$

$$V = A\ .\ I \quad (2)$$

I: amount of irrigation water (mm); Ep: evaporation from pan (mm); kcp: plant-pan coefficient (I33=0.33, I66=0.66 and I100=1.00); P: vegetation coverage percentage (%); A: parcel area (m2); V: water volume (L).

Equation 3 was used to determine the vegetation cover percentage (wetting factor) (Gençoğlan et al., 2006).

$$P = a / b \quad (3)$$

a: plant canopy diameter (cm); b: row spacing (cm).

Plant diameter (a) was measured from an average of five plant diameters before each irrigation. Irrigation applications were carried out in a controlled manner by passing three water meters to the irrigation areas. In the study, the water balance equation (method) was used to directly determine plant water consumption. The following water balance equation (equation 4) was used to calculate the plant's water consumption:

$$ETa = P + I - Rf - Dp \pm \Delta S \quad (4)$$

ETa: Evapotranspiration (mm); P: precipitation (mm); I: amount of irrigation water (mm); Rf: surface flux (mm); Dp: Deep infiltration (mm); ΔS (mm): Soil moisture change at the rhizosphere.

Since the drop flow rate preferred in the study was lower than the infiltration rate of the soil, surface runoff did not occur. Since no more water will be given to the soil than the field capacity during irrigation and the dripper flow rate is lower than the soil infiltration rate, deep percolation losses (Dp) are accepted as zero. Additionally, surface flow values (Rf) were considered unimportant and were not taken into calculation.

According to the water balance equation method in the early vegetative period, the weekly plant water consumption (ETa) value (per pot) in I100 irrigation, where the water need wasfully met, varied between 3.5 liters (per pot) week-1, while in the flowering period it was 9.0 liters (1.44 mm) week-1 and 36 liters month-1. Simply, it was 3.5 liters (2.16 mm) per week per pot in May and June, and 9 liters (2.16 mm) in July and August; as year 2022 was extremely hot. The ETa value decreased towards physiological maturity. According to the water balance equation method for sunflower lines during the growing season, ETa amount was determined as seasonal plant water consumption (ETa) as 128 liters (21 mm) pot-1.

2.4.Physiological measurements related to drought

Physiological screenings were carried out on drought tolerant and sensitive genotypes. Evaluations were made by taking physiological and morphological measurements in the pots before and after irrigation. The determination of excess water in pot irrigation was made by determining the amount of water filtered into the pot within 1 hour after irrigation. 5.5 liters of water was given to the pots, and the amount of water filtered under the pot was determined as 0.25 liters. Therefore, 4.5 liters of water was consumed.

According to the morphological observations, small leaves with narrow angles and a low rate of leaf wilting were determined to be more resistant. Physiological measurements also support each other. In infrared-meter measurements, high values showed sensitive and low values showed resistant genotypes, and in chlorophyll measurements, high values showed resistant and low values showed sensitive genotypes.

2.5.Embryo culture within the scope of speed breeding

With the "Growth Under LED Lights for Speed Breeding" system, required days to complete the vegetation period for the sunflower in embryo culture and the regeneration rate of the sunflower with limited water application were investigated by applying the LED light. In speed breeding, red light was applied as 343 lux, green as 69 lux, blue as 174 lux, a total of 586 lux until the flowering period. The temperature was 24 °C day and 18 °C night at 70% humidity for 22 hours light and 2 hours dark photoperiod. In the experiments, it was found that two and a half generations could be achieved in one year.

Application of Embryo Rescue Culture under LED Light

By combining the embryo culture method with CMS studies and restorer development studies, in previous studies conducted by (Çil et al. 2021), the embryo culture period was further accelerated and the "plant culture-field transfer" period was started. The period was reduced to 21-30 days. In this study, this period was reduced to 15 days under LED light with the same team. From the beginning of October to the flowering period, white light at 40 par, red light at 10 par, green light at 10 par, and blue light at 10 par were given. The temperature was 24°C during the day, 18°C at night, humidity was 70%, 22 hours during the day and 2 hours at night.

Embryo culture method

Seed surface sterilization process: Embryos in the fruits from the first five rows located at the outermost part of the table were used for embryo culture. The fruits separated from each head were subjected to surface sterilization with 70% alcohol for a very short time in a sterile cabinet in a glass container, then, alcohol was added and 20% bleach (5% Sodium hypochlorite, unperfumed) was added 3-5 drops. Sterilization solution containing Tween 80 was also added. The fruits were sterilized by shaking in the solution for 10 minutes (Dağüstü et al. 2010). Following this, surface sterilization was completed by rinsing the fruits with sterile distilled water by repeating 4-5 times.

Embryo isolation and culture of isolated embryos: The shells of the surface sterilized seeds were cut and removed. After the embryos were removed from the embryo sac and separated, five of them were placed in each petri dish of 60 x 15 mm before transferred to the embryo development medium.

Media and culture conditions: MS (Murashige and Skoog, 1962) medium containing 2% sucrose and 0.8% agar was used as the embryo medium in the experiments. The pH of the medium was adjusted to 5.8 using 1N KOH and 1N HCl. Sterilization of the medium was achieved by keeping it in an autoclave under 1.2 atmospheres of pressure and 121 oC for 15 minutes. All cultures were kept in growth cabinets providing a photoperiod of 22 hours of light and 2 hours of darkness and a temperature of 25 ± 2 oC.

Acclimatization to the external environment and transfer to the violet: The plantlets formed 6-7 days after the beginning of the culture were cleaned of agar residues with hot water. Well-developed plants were selected and transferred to glasses filled with soil. Plants developed in speedbreeding within 10-15 days were transferred to the outdoor environment.

Evaluation of data

Angle transformation was applied to the data obtained by the research and ANOVA (Oneway) test was applied with the JumpPro-13 statistical package program. Differences between means were compared

according to the Duncan test. In addition, comparison biplot analysis was performed in the Genstat 12th Edition package program to determine the ideal genotypes in terms of the examined characteristics in different environments. The relationship between features was interpreted with the scatterplot matrix produced by the JumpPro-13 package program.

3. Result and Discussion

3.1. Physiological results related to drought

Results of the examined properties of sunflower genotypes under greenhouse sowing are given in Table 1.

Table 2. ANOVA (Oneway) analysis of examined properties of sunflower genotypes under greenhouse sowing

Genotype	Sowing date	Number of flovering days	Embryo age	Number of days to transfer the embryo to the nutrient medium	Plant height (cm)	Head diameter (cm)	Number of seeds in the head (piece)
K26-100	21.03.2023	53	13	66	85.00±8.66 ıj	9.00±2.00 a-d	77.33±9.02 j
K26-66	21.03.2023	53	13	66	75.00±5.00 ıjk	8.00±2.00 a-d	69.00±9.00 jk
K26-33	21.03.2023	55	13	68	72.00±2.00 jk	8.00±2.00 a-d	63.00±3.00 jk
K78-100	21.03.2023	52	13	65	135.00±5.00 d	9.00±3.00 a-d	245.00±5.00 d
K78-66	21.03.2023	53	13	66	130.00±5.00 de	8.50±2.50 a-d	233.00±3.00 de
K78-33	21.03.2023	55	13	68	127.00±3.00 def	8.00±3.00 a-d	225.00±5.00 def
K19-100	21.03.2023	52	13	65	60.00±5.00 lm	5.00±1.00 cd	230.00±5.00 def
K19-66	21.03.2023	53	13	66	54.00±4.00 lm	4.50±1.50 d	214.00±4.00 ef
K19-33	21.03.2023	54	13	67	50.00±5.00 m	4.00±1.00 d	223.00±3.00 ef
K1-100	21.03.2023	52	13	65	80.00±5.00 ıj	4.00±2.00 d	67.00±2.00 jk
K1-66	21.03.2023	53	13	66	77.00±7.00 ıj	3.50±1.50 d	63.00±3.00 jk
K1-33	21.03.2023	55	13	68	69.00±4.00 jkl	3.00±1.00 d	58.00±3.00 k
K79-100	21.03.2023	58	13	71	170.00±5.00 bc	13.00±3.00 a	500.00±10.00 a
K79-66	21.03.2023	59	13	72	162.00±7.00 c	12.00±2.00 ab	456.00±6.00 b
K79-33	21.03.2023	60	13	73	157.00±7.00 c	11.50±1.50 ab	432.00±2.00 c
K88-100	21.03.2023	63	13	76	115.00±5.00 efg	8.00±1.00 a-d	118.33±2.89 h
K88-66	21.03.2023	64	13	77	111.00±6.00 fg	7.00±2.00a-d	116.00±6.00 h
K88-33	21.03.2023	66	13	79	108.00±5.00 g	7.00±1.00a-d	109.00±4.00 hı
K105-100	21.03.2023	64	13	77	200.00±5.00 a	11.00±2.00 abc	120.00±5.00 g
K105-66	21.03.2023	65	13	78	90.00±5.00 hı	6.00±1.00 bcd	195.00±5.00 f
K105-33	21.03.2023	67	13	80	85.00±5.00 ıj	5.00±1.00 c	53.00±3.00 k
K109-100	21.03.2023	64	13	77	180.00±5.00 b	11.00±3.00 abc	120.00±5.00 h
K109-66	21.03.2023	65	13	78	105.00±5.00 gh	8.00±3.00 a-d	96.00±6.00 i
K109-33	21.03.2023	67	13	80	90.00±5.00 hı	6.50±1.50 bcd	25.00±5.00 l
Mean		**58**	**13**	**71**	**107.77**	**7.52**	**171.15**
			Source	**DF**	**MS**	**MS**	**MS**
			Genotype	23	5268.26**	23.9660**	52204.3**
			Error	48	28.25	3.9688	27.0
			CV (%)	**4.92**	**26.42**	**3.03**	

**; significant at level 0.01, ±; Standard deviation. Levels not connected by same letter are significantly different.

Number of days between sowing and number of flovering days was between 52-67 days. Number of days between sowing and number of days to transfer the embryo to the nutrient medium was 65-80 days (Table 1). Plant height was between 50-200 cm with average of 107.8 cm. Plant height was lowest (between 50.0-60.0 cm) at K19-100, K19-66 and K19-33 genotypes, whereas highest (200.0 cm) at K105-100 genotype. Head diameter was between 3.0-13.0 cm. Number of seeds in the head was lowest (25 pieces) at K109-33 genotype whereas highest (500 pieces) at K79-100 genotype (Table 1). In comparison to other crops, *Helianthus annuus* is considered to be moderately drought resistant (Skoric, 2009). Additionally, there are numerous studies of genetic variation in drought responses as well as an array of genetic resources for sunflower (Poormohammad Kiani et al. 2007; Masalia et al. 2018).

Table 3. ANOVA (Oneway) analysis of examined properties of sunflower genotypes at *in-vitro*

Genotype	Plant weight (g)	Plant high (cm)	Root length (cm)	Number of leaf (piece)	Number of days from transplant to glasshouse	Number of days from transplant to field
K26-100	0.45±0.03 a	3.28±0.30 abc	4.10±0.44 a-f	5.07±0.23 abc	6	11
K26-66	0.33±0.03 a-d	2.90±0.26 abc	2.83±0.32 a-g	4.00±0.69 cd	6	11
K26-33	0.26±0.04 bcd	2.63±0.72 c	2.20±0.52 d-g	4.27±0.61 a-d	6	11
K78-100	0.36±0.06 d-d	3.57±0.65 abc	5.27±1.10 a	4.53±0.61 a-d	6	12
K78-66	0.23 ±0.01 cd	2.57±0.23 c	2.93±0.91 a-g	4.13±0.23 bcd	6	11
K78-33	0.29±0.05 a-d	2.73±0.47 c	3.70±1.21 a-g	3.67±0.12 cd	6	11
K19-100	0.28±0.03 a-d	3.63±1.16 abc	5.07±0.35 ab	4.13±0.23 bcd	6	12
K19-66	0.30±0.10 a-d	2.63±0.75 c	3.27±1.77 a-g	3.60±0.40 cd	6	11
K19-33	0.32±0.03 a-d	2.83±0.60 bc	2.30±0.70 c-g	3.73±1.01 cd	6	11
K1-100	0.40±0.04 abc	4.57±0.70 ab	4.87±0.92 abc	5.33±0.83 abc	6	12
K1-66	0.26±0.07 bcd	2.09±0.37 c	4.68±0.99 a-d	4.00±0.40 cd	6	11
K1-33	0.22±0.04 d	2.26±0.39 c	4.37±1.06 a-e	4.33±0.31 a-d	6	11
K79-100	0.43±0.02 ab	3.70±0.40 abc	4.33±0.75 a-f	5.07±0.46 abc	6	11
K79-66	0.32±0.02 a-d	2.30±0.36 c	2.50±0.36 b-g	3.87±0.61 cd	5	10
K79-33	0.36±0.18 a-d	2.43±0.51 c	2.30±1.32 c-g	4.13±0.46 bcd	5	10
K88-100	0.38±0.05 a-d	3.17±0.55 abc	3.50±0.75 a-g	4.80±1.06 a-d	5	10
K88-66	0.39±0.05 a-d	3.03±0.60 abc	3.27±1.31 a-g	5.20±0.80 abc	5	10
K88-33	0.28±0.01 a-d	2.27±0.15 c	1.70±0.10 fg	3.60±0.40 cd	5	10
K105-100	0.43±0.02 ab	4.62±1.05 a	4.80±0.60 a-d	5.87±0.61 ab	5	10
K105-66	0.38±0.06 a-d	3.50±0.40 abc	2.18±0.19 d-g	5.07±0.83 abc	5	10
K105-33	0.26±0.01 bcd	2.12±0.57 c	1.43±0.57 g	3.20±0.40 d	5	10
K109-100	0.42±0.03 ab	3.78±0.20 abc	4.23±0.98 a-f	6.00±0.40 a	5	10
K109-66	0.35±0.02 a-d	2.90±0.40 abc	2.00±0.10 efg	4.40±0.40 a-d	5	10
K109-33	0.28±0.01 a-d	2.08±0.13 c	1.34±0.24 g	3.73±0.61 cd	5	10
Mean	**0.33**	**2.98**	**3.29**	**4.41**	**5.5**	**10.7**
Source	**DF**	**MS**	**MS**	**MS**	**MS**	
Genotype	23	0.013845**	1.58756**	4.51102**	1.65903**	
Error	48	0.003052	0.31163	0.71786	0.34000	
CV (%)		**15.15**	**18.45**	**25.53**	**13.03**	

**; significant at level 0.01, ±; Standard deviation. Levels not connected by same letter are significantly different.

Plant weight was between 0.22-0.45 g (Table 2). Plant high was between 2.09-4.62 cm. Root length was between 1.70-5.27 cm. Number of leaf was between 3.60-5.87 pieces.

Number of days from transplant to glasshouse was 5 or 6 days. Number of days from transplant to field was between 10-12 days (Table 2).

Sunflower achene and oil output were discovered to be significantly influenced by the quantity and distribution of water (Krizmanic et al. 2003). It was demonstrated that irrigation at the flowering stage produced the highest achene yield. The effects of drought on plant growth have an impact on both the biological and economic benefits of the harvest. The main stem height, diameter, number of nodes or leaves, and leaf area are all decreased during vegetative development (Turhan and Baser 2004), whereas root length increases at the expense of above-ground dry matter. A larger root-to-shoot ratio acquired under drought stress has been used to confirm this. Lower plant surface area caused by the decline in vegetative biomass lowers photosynthesis and radiation usage efficiency (Germ et al. 2005). Finally, this limits photosynthetic assimilation during the reproductive phase, which decreases head diameter. Reduced head diameter also leads to a reduction in the number of rows and achenes per head and an association between yield components and the severity of the drought (Rauf and Sadaqat 2007a). On the other hand, stress during the flowering stage results in ovarian, embryonic, and pollen sterility abortions as well as a decline in the leaf area index. As a result, there are less achenes per head, less achenes per 100 grammes, and fewer fertile achenes per head. According to estimates, stress during the vegetative phase reduces production by 15–25%, and stress during the flowering stage might cause a yield drop of more than 50% (Reddy et al. 2003). If drought was applied during the achene filling stage, however, it was discovered that there would be minimal harm (Karam et al.

2007). As a result of the stress at this point, the plant responds by senescing its leaves prematurely and abruptly and mobilising stem reserves to support the growing achenes (Rauf and Sadaqat 2007b). However, an excessive amount of leaf loss at this stage could result in a reduction in the weight of 100 achenes due to lower photosynthate production (Rauf 2008).

Biplot analysis

In breeding studies, an ideal genotype should have both high average performance and high stability in different environments. The biplot analysis method is becoming frequently used in breeding studies in recent years (Yan and Tinker 2006; Tabrizi et al. 2011; Korkmaz et al. 2021; Koç and Güneş 2021). Figure 2 shows the ideal genotypes with the highest averages in terms of the examined characteristics (Yan and Tinker 2006).

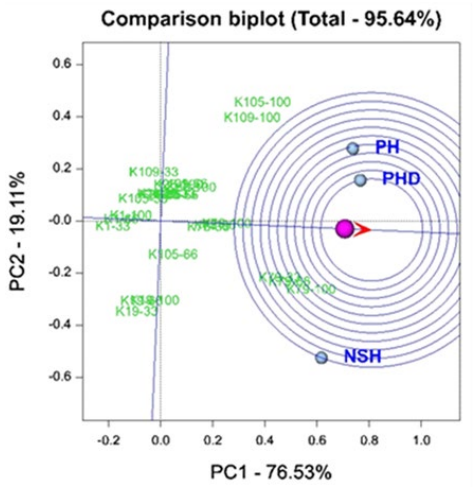

Figure 2. Relationship between the examined traits and genotypes by comparison biplot analysis under greenhouse. Abbreviations: PH: Plant high, PHD: Plant head diameter, NSH: Number of seed in head

In different studies conducted by other researchers, genotypes closer to the ideal genotype are preferred more than others. In the biplot model, based on the average data in our research, we obtained the variation rates as PC1: 76.53%, PC2: 19.11 and PC1+PC2: 95.64%. Highest variation was detected in the genotypes. The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics. Genotypes K79-100, K79-66 and K79-33 are also located close to the center of the diagram.

In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center. Other genotypes were positioned outside the circle and was not preferred (Figure 3).

Based on the relationship between the examined traits, under greenhouse conditions, there was a positive and very significant relationship between plant head diameter and plant height ($r = 0.8599$, $P \leq 0.01$), between the number of seeds per head and plant height ($r = 0.4808$, $P \leq 0.01$), between the number of seeds per head and head diameter ($r = 0.5810$, $P \leq 0.01$). In MS medium, there was a positive and a very significant relationship between plant root length and plant height ($r=0.5327$, $P \leq 0.01$), number of leaves and plant height ($r=0.7128$, $P \leq 0.01$), number of leaves and plant root length ($r=0.5250$, $P \leq 0.01$) (Figure 4).

In different researches, some have found negative and significant relationship between head diameter and head number and plant height (Koç and Güneş 2021). In their study, Tabrizi et al. (2011) reported a positive and significant relationship between root length and plant weight. The findings obtained in our research were different from results of Koç and Güneş (2021) but similar to Tabrizi et al. (2011).

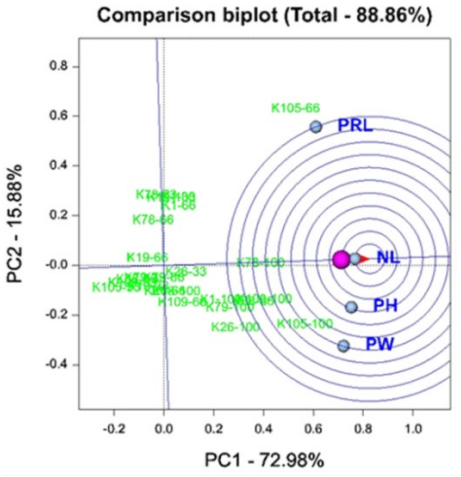

Figure 3. Performance of the genotypes for examined traits and genotypes by comparison biplot analysis at MS. Abbreviations: PRL: Plant root length, PH: Plant high, NL: number of leaf, PW: Plant weigh

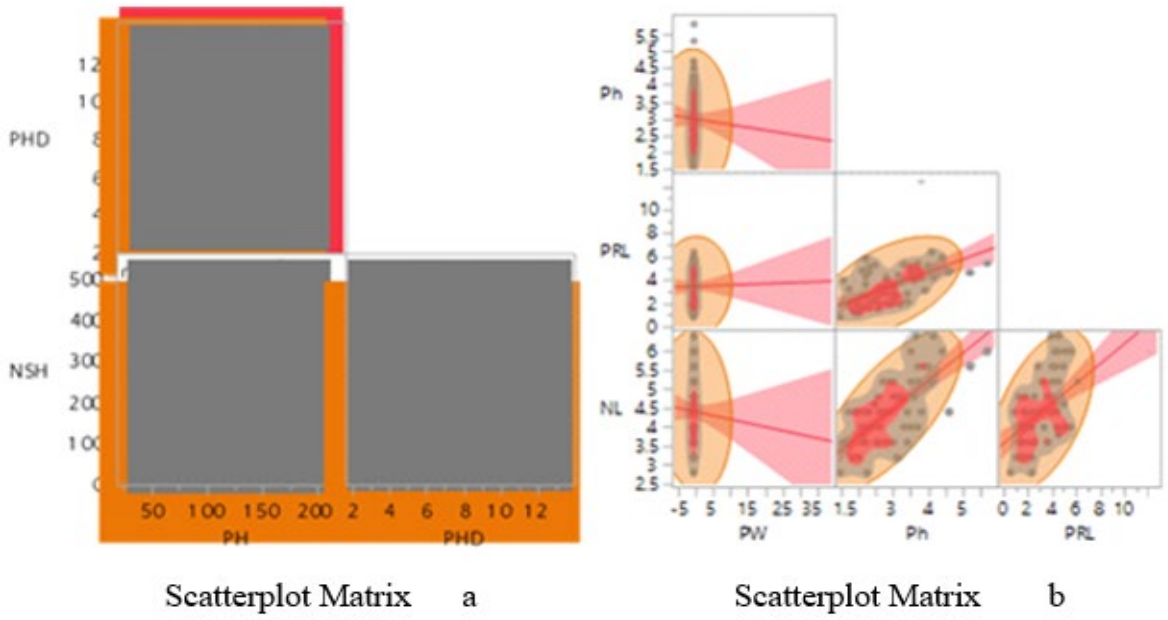

Figure 4. Correlation of traits under different conditions (a: under greenhouse, b: at MS) by scatterplot matrix. Abbreviations: PH: Plant high, PHD: Plant head diameter, NSH: Number of seed in head, PRL: Plant root length, PH: Plant high, NL: number of leaf, PW: Plant weigh.

4. Conclusions

Number of days between sowing and flovering days, embryo age and number of days to transfer the embryo to the nutrient medium, plant height, head diameter, number of seeds in the head was between 52-67 days; 65-80 days; 50-200 cm; 3.0-13.0 cm; 25-500 pieces, respectively.

In the experiments, it was found that 4-5 generations could be achieved in one year. The genotypes K26-33 and K78-100 are located closest to the center as the most ideal genotypes in terms of the examined characteristics. In the MS environment, in terms of the characteristics examined, the genotypes K105-100, K78-100, K105-66, K26-100 and K1-100 were the preferred genotypes as they are located close to the center.

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Research Article

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Forage Pea Pure Lines: Winter Hardiness, High Seed and Biological Yield

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HIGHLIGHTS

- Forage pea is an important forage crop used in animal nutrition with its rich content of protein, fibre and other nutrients and plays a fundamental role in sustainable agriculture and livestock systems.
- Winter forage pea is a valuable plant species that can grow in cold climates and is used in animal nutrition.
- Forage peas not only feed animals but also significantly increase soil fertility with *Rhizobium* bacteria.

Abstract

Winter forage pea is a plant that grows in cold climates and is a valuable source of protein for animal feed. In this study, 13 pure forage pea lines (PS3057M1, PS3057M2, PS3073G1, PS3073G2, PS3073G3, PS4028H1, PS4028H2, PS4028H3, PS4028H4, PS4053M1, PS4053M2, PS4053M3 and PS4053M4) and Emirbey and Şahin varieties were used as material. The research was conducted at Selçuk University Prof. Dr. Abdülkadir AKCİN Research and Application Station in the trial fields during the 2020-21 and 2021-22 plant growth periods without irrigation. The trials were established in both years according to randomized block design with 3 replications. Meteorological data indicated that despite variations in the lowest temperatures between the two years, all pea lines remained undamaged. This highlighted the adaptability of forage pea lines to the region's winter conditions, which is consistent with previous studies. Statistically significant differences were determined between the two years of the study in terms of all traits analyzed in the research. Higher values were recorded in the second year for all traits. The main reason for these differences is the precipitation and temperatures in the spring months, which is the period when the plants grow actively. Again, significant statistical differences were determined between the lines in all the traits analyzed in the study. This shows us that genetic traits and environmental conditions are important in determining the traits analyzed in this study. The forage pea lines used in the study performed better than the standard varieties in terms of seed and biological yield. In conclusion, this research shows that newly developed winter forage pea lines can be successfully grown in the Konya region and these lines have important genetic and environmental effects on various yield parameters.

Keywords: Seed yield; pea; biological yield; winter hardiness

1. Introduction

Forage pea is a valuable forage plant that plays an important role in animal nutrition. The nutritional value of this plant for animals is especially its high protein content. In addition, forage pea is an ideal protein source in feed mixtures used in animal production. The high-quality protein content supports the healthy development of animals, increases muscle mass and improves overall productivity. In addition, forage peas

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offer a balanced protein profile with essential amino acids, which helps animals meet their basic nutritional needs (Açıkgöz 2001, Ates 2012, Sözen et al. 2018).

Forage peas are also rich in fiber, vitamins and minerals. These nutrients support the digestive system, strengthen the immune system, and improve overall health. Another advantage of forage peas in animal nutrition is their ability to fix nitrogen. Thanks to this feature, forage peas provide nitrogen to the soil and stimulate the growth of other plants, thus increasing soil fertility (Sözen and Karadavut 2018, Karadavut and Sözen 2020). Forge pea is an important cool climate plant with its wide adaptation ability and its yield and quality characteristics, which are preferred in regional conditions, nitrogen fixation to the soil between 5-15 kg ha^{-1} and leaving a clean stubble for the following plant (Açıkgöz 2001, Ceyhan et al. 2005, Geren and Alan 2012, Uzun et al. 2012, Sözen and Peker 2023).

Winter sowing of forage peas is an important agricultural practice providing diversity and sustainability to farmers as an alternative between agricultural seasons. Forage pea is known for its resistance to cold weather conditions and this feature allows it to grow in winter (Ceyhan et al. 2005, Sözen and Karadavut 2017). Winter sowing of fodder peas is usually carried out in the autumn months and the rainfall in the spring months, when the plants actively grow vegetatively, is very well utilized. Winter sowing of forage peas is an important agricultural practice providing diversity and sustainability to farmers as an alternative between agricultural seasons. In addition, winter sowing of forage peas can contribute to preventing soil erosion and improving soil structure. In addition to diversifying farming practices, this practice has the potential to provide farmers with a more stable source of income by extending the production cycle. Winter sowing is a strategy that allows fodder peas to grow successfully in various climatic and soil conditions, thus offering farmers a more flexible production plan.

Forage pea is a plant suitable for winter sowing in the Central Anatolia region because it is a cool-season plant, its growing period is short, and its cold resistance is high compared to many forage plant species (Ceyhan et al. 2005). Winter forage pea is a plant that grows in cold climates and is a valuable source of protein for animal feed. This pea variety is usually sown in early autumn. Conducting a soil analysis and applying appropriate fertilization before sowing ensures that the plant grows healthily and yields a productive crop. Preparing the sowing area well; the soil should be cultivated and leveled. Peas are generally cold-resistant and resistant to frost events in winter. When the correct sowing and maintenance methods are applied, winter fodder peas grow efficiently and provide a quality protein source in animal nutrition. For this purpose, this study tried to determine the biological yield and some agronomic characteristics of newly developed winter fodder pea lines.

2. Materials and Methods

Within the scope of the research, pea genotypes with different characteristics were crossed according to the full diallel crossing method in 2015. The genotypes obtained from this crossing were selected according to winter-resistant high biological yield, seed yield, and upright growing or winding characteristics. In this study, 13 pure forage pea lines (PS3057M1, PS3057M2, PS3073G1, PS3073G2, PS3073G3, PS4028H1, PS4028H2, PS4028H3, PS4028H4, PS4053M1, PS4053M2, PS4053M3 and PS4053M4) with superior characteristics were used as material. In addition, Emirbey and Şahin varieties were used as control material in the study. The study used Emirbey and Şahin varieties as control materials. Emirbey and Şahin varieties were developed by Prof. Dr. Ercan CEYHAN and registered by the private sector.

This study was conducted in the winter growing season of 2020-21 and 2021-22 in the ecological conditions of Konya (1020 m above sea level), which has a continental climate. According to the long-term (2007-2019 year) average of the trial locations, the annual average temperature, relative humidity, and rainfall during the vegetation period (October-July) were 11.7 °C, 58.3% and 346.4 mm, respectively. However, the average temperature and total rainfall during the vegetative period in 2020-21 were 11.2 °C and 350.4 mm, while during the active vegetative growth stage (April-June) they were 18.3 °C and 55.4 mm, respectively. In the second year of the experiment, the average temperature and total rainfall were 11.8 °C and 304.2 mm, and 16.0 °C and 158.0 mm, respectively, during the active vegetative growth stage (April-June). In the second year of the

experiment, total rainfall was higher, and temperatures were lower in the active vegetative growth stage (April-June) compared to the first year (Table 1).

Table 1. Long years, 2020-21 and 2021-2022 average temperature, relative humidity, and rainfall data for Konya province.

Months	Average Temperature (°C)			Relative Humidity (%)			Rainfall (mm)		
	13-years	2020-21	2021-22	13-Years	2020-21	2021-22	13-years	2020-21	2021-22
October	14,3	14,1	14,5	56,9	49,2	60,6	33,2	0,0	43,4
November	8,1	10,1	9,7	67,2	63,1	62,7	35,1	25,6	14,2
December	3,2	3,7	6,3	77,8	73,9	72,5	54,5	76,2	5,4
January	1,6	-0,1	3,7	77,2	72,9	66,7	51,0	81,6	8,8
February	4,2	3,2	1,0	67,4	72,0	64,8	26,6	43,4	16,0
March	8,4	2,2	9,1	56,2	63,4	57,8	34,7	47,6	57,8
April	13,0	15,6	11,6	50,8	38,8	54,8	28,0	1,2	36,2
May	17,3	17,0	16,0	49,5	52,0	57,6	41,0	26,4	47,0
June	21,8	22,3	20,3	44,8	50,3	58,4	37,9	37,8	74,8
July	25,5	24,1	25,7	35,1	41,4	35,0	4,6	10,6	0,6
Mean/Total	11,7	11,2	11,8	58,3	57,7	59,1	346,4	350,4	304,2

[1] Meteorological data were obtained from the Konya Meteorological Regional Directorate.

The research was carried out at Selcuk University Prof. Dr. Abdülkadir AKCİN Research and Application Station in the experimental fields during the 2020-21 and 2021-22 vegetation periods without irrigation. Soil analysis results show that the soil texture of the experimental site is clay loam, slightly alkaline, no salt, extremely high lime content, low phosphorus content, sufficient potassium fertilizer, and low organic matter content (Sözen and Karadavut 2020, Küçük and Ceyhan 2022; Karadaş and Ceyhan 2023).

The experiments were established in both years according to the randomized block design with 3 replications. The plot area was set as 7.50 m^{-2} (5.0 m length x 1.50 m width) and each plot consisted of 6 rows. Sowing was carried out on 20 October 2020 in the first year and 14 October 2021 in the second year when the climatic conditions and soil temperature were suitable, and the soil was at the right temperature. The seeds were sown manually in the rows opened with 100 seeds per square meter. Considering the soil analyses; 150 kg DAP fertilizer was applied per hectare with sowing. Weed control was carried out from the emergence of fodder pea seedlings. Fodder peas were grown under dry conditions without irrigation. When the seeds of each plot matured and harvest time came, the plants were harvested by mowing. During the harvesting period, one row from the edges of the plots and 0.5 m from the heads were discarded as edge effect and the remaining part was measured.

In the study, the number of branches per plant (number), plant height (cm), number of pods per plant (number), number of seeds per pod (number), number of grains per plant (number), grain yield (t ha^{-1}), hundred-grain weight (g) and biological yield (t ha^{-1}) were measured according to the methods described by Ceyhan et al (2005), Sözen and Karadavut (2020) and TTSM (2023).

The data obtained from the study were analyzed using JMP (17.0.1) Statistical Package Programmed (JMP 17.0.1) in a yearly replicated random blocks experimental design. The significant means were grouped according to the LSD test.

3. Results and Discussion

According to the meteorological data of Konya province where the research was conducted, the lowest temperature was -9.0 °C in the first year of the experiment. In comparison, the lowest temperature was -18.0 °C in the second year of the research. This temperature did not damage all pea lines used in the study. The results of this research show that forage pea lines developed for this region can be easily grown for winter. Ceyhan (2004) reported pea genotypes resistant to -25 °C in this region. Ceyhan et al. (2005) also reported that forage pea lines can be easily grown for winter in this region.

Table 2. Mean squares of the characteristics analyzed in the research.

Source	DF	Number of Branches per Plant	Plant Height	Number of Pods per Plant	Number of Seeds per Pod
Total	89				
Year	1	20,544**	804,011**	409,600**	1,344*
Replication (Year)	4	0,311	466,156	4,033	0,844
Lines	14	1,487**	1354,043**	32,219**	1,373**
Year x Lines Int.	14	0,211	271,059**	5,171	0,725
Error	56	0,418	86,072	2,986	0,463
CV		21,47	12,03	8,55	10,84
Source	**DF**	**Number of Seeds per Plant**	**Seed Yield**	**Hundred Seed Weight**	**Biological Yield**
Total	89				
Year	1	21933,611**	12,305**	28,045**	30,750**
Replication (Year)	4	150,356	0,049	0,010	0,105
Lines	14	1428,952**	0,979**	42,346**	2,001**
Year x Lines Int.	14	285,254	0,079	0,090	0,168
Error	56	247,300	0,080	0,112	0,174
CV		12,43	11,98	2,15	11,90

* : $p < 0.05$, ** : $p < 0.05$.

3.1. Number of Branches per Plant

The difference between the years and lines was statistically significant at a 1% level, while the year x line interaction was insignificant (Table 2). The results of previous research also revealed significant differences among pea varieties in the number of branches per plant (Ceyhan 2003, Ceyhan et al. 2005, Temel et al. 2022).

In this study, the average number of branches per plant was 2.53 in the first year and 3.49 in the second year (Table 3). This may be due to the fact that there was less rainfall and higher temperature in the spring months (April and June), which is the growth period of the plant, in the first year compared to the second year. Lack of rainfall in pea plants decreases plant height and encourages branching (Ceyhan et al. 2005, Gençtan 2012).

According to the two-year averages, the number of branches of the lines varied between 2.17 (PS4053M2) and 4.17 (PS3057M2). PS3057M2 (4.17 number), PS3073G2 (3.50 pieces), and PS4028H2 (3.50 number) were the first three lines in terms of the number of branches. The number of branches in pea plants generally varies between 1.80 and 10.30, and the number of branches is greatly affected by environmental factors, especially plant density, soil moisture, and fertility, in addition to genetic factors (Ceyhan 2003). Ceyhan et al. (2005) reported that the number of pea branches varied between 3.8 - 7.8, Ateş and Tekeli (2017) 4-6, Temel et al. (2022) 1.2 -2.3. In this respect, the results obtained are largely consistent with the findings of the researchers.

3.2. Plant Height

The difference between the years in terms of plant height was statistically significant according to the 1% probability level (Table 2). As the average of the genotypes, plant height was 74.11 cm in the first year and 80.09 cm in the second year (Table 3). This may be because there was less rainfall and higher temperature in the spring months (April to June), the plants growth period in the first year compared to the second year. Ceyhan et al. (2005) and Gençtan (2012) reported that high rainfall in pea plants encouraged plant growth. In this study, high plant heights were obtained in the second year when rainfall was high during the growth period of the plants.

When pea lines and year x line interactions were analyzed, plant height differences were found significant at a 1% level (Table 2). It has been revealed by many researchers that plant height is affected by genetic structure (Önder and Ceyhan 2001, Ceyhan 2003, Ceyhan and Avcı 2005, Ceyhan et al. 2005, Kavut et al. 2016, Temel at al. 2022). According to the two-year averages, the plant height of the genotypes varied between 39.67 and 108.50 cm. The longest plant height was measured in PS3073G3, and the shortest was measured in PS4053M4 line. The plant height of other genotypes used in the experiment varied between these values. When the year x line interactions were analyzed, the lowest plant height was obtained from the PS4053M4 line in the first year of the experiment at 33.33 cm and the highest plant height was obtained from the PS3073G3 line in the first year with 109.33 cm. According to these results, it is seen that the lines produced different results when compared with each other in terms of plant height. This situation shows that plant height is affected by environmental conditions and is a genotype trait. Some researchers reported that plant height of pea

genotypes was between 35.4-56.3 cm (Önder and Ceyhan 2001), 36.6 - 75.8 cm (Ceyhan and Avcı 2005), 42.00-121.67 cm (Savur and Ceyhan 2011), 131.14-161.64 cm (Kavut et al. 2016), 120.4-135.3 cm (Ateş and Tekeli 2017), 78.9-91 cm (Temel at al. 2022). Although the results obtained from this study are similar to the findings of some researchers (Önder and Ceyhan 2001, Ceyhan and Avcı 2005, Savur and Ceyhan 2011, Temel at al. 2022), lower than the findings of Kavut et al. (2016) and Ateş and Tekeli (2017).

3.3. Number of Pods per Plant

The difference between years in terms of pod number was statistically significant at a 1% probability level (Table 2). The effect of years on the number of pods of peas was also reported in previous studies (Ceyhan et al. 2005, Uzun et al. 2012, Keskin et al. 2021). In the first year of the experiment, 18.07 pods per plant, and in the second year, 22.33 pods per plant were obtained in forage peas (Table 3). In the second year, the increased number of pods in peas can be attributed to higher rainfall between April and June and lower temperatures than the previous year. Similarly, in previous studies, it was determined that forage peas affected the number of pods in the plant depending on the years (Önder and Ceyhan 2001, Ceyhan et al. 2005, Geren and Alan 2012).

Table 3. Means and LSD groups of number of branches per plant, plant height, number of pods per plant and number of seeds per pod of forage pea varieties according to years.

Lines	2020-21	2021-22	Mean	2020-21	2021-22	Mean
	Number of Branches per Plant			Plant Height (cm)		
PS3057M1	2,00	3,33	2,67 bcd[1]	65,00 g-k	91,67 a-e	78,33 b-e
PS3057M2	3,33	5,00	4,17 a	66,33 g-j	75,33 d-j	70,83 de
PS3073G1	2,67	3,33	3,00 bcd	82,00 c-h	101,67 abc	91,83 b
PS3073G2	3,00	4,00	3,50 ab	88,33 b-f	80,33 d-ı	84,33 bcd
PS3073G3	2,67	3,67	3,17 bc	109,33 a	107,67 abc	108,50 a
PS4028H1	2,33	3,67	3,00 bcd	75,33 d-j	66,00 g-k	70,67 de
PS4028H2	3,33	3,67	3,50 ab	61,00 ıjk	81,33 d-h	71,17 de
PS4028H3	2,00	3,00	2,50 cd	68,33 f-j	62,00 h-k	65,17 e
PS4028H4	2,33	3,67	3,00 bcd	72,33 e-j	82,00 c-h	77,17 cde
PS4053M1	2,67	3,67	3,17 bc	81,00 d-ı	93,67 a-d	87,33 bc
PS4053M2	2,00	2,33	2,17 d	80,67 d-ı	67,67 g-j	74,17 cde
PS4053M3	2,33	3,33	2,83 bcd	58,33 jk	82,33 c-g	70,33 de
PS4053M4	2,00	3,00	2,50 cd	33,33 l	46,00 kl	39,67 f
Emirbey	3,00	3,67	3,33 abc	80,67 d-ı	84,33 c-g	82,50 bcd
Şahin	2,33	3,00	2,67 bcd	89,67 a-e	79,33 d-ı	84,50 bcd
Mean	2,53	3,49	3,01	74,11	80,09	77,10
		Lsd_{Lines}: 0.9953			$Lsd_{Year\ x\ Lines}$: 20.20, Lsd_{Lines}: 14.28	
Lines	**Number of Pods per Plant**			**Number of Seeds per Pod**		
PS3057M1	16,67	20,67	18,67 e-h	5,67	7,00	6,33 abc
PS3057M2	22,33	26,33	24,33 a	6,00	6,33	6,17 abc
PS3073G1	14,33	19,33	16,83 h	6,00	6,67	6,33 abc
PS3073G2	21,67	24,00	22,83 abc	5,33	6,00	5,67 c
PS3073G3	18,33	22,67	20,50 c-f	5,67	5,67	5,67 c
PS4028H1	21,67	23,00	22,33 a-d	5,67	6,67	6,17 abc
PS4028H2	17,33	22,33	19,83 d-g	6,33	6,33	6,33 abc
PS4028H3	16,67	18,33	17,50 gh	6,00	6,00	6,00 bc
PS4028H4	17,00	20,67	18,83 e-h	6,67	7,33	7,00 ab
PS4053M1	17,33	24,33	20,83 c-f	6,67	5,67	6,17 abc
PS4053M2	20,00	22,33	21,17 b-e	5,33	6,00	5,67 c
PS4053M3	13,67	21,67	17,67 gh	6,33	6,67	6,50 abc
PS4053M4	16,00	20,67	18,33 fgh	7,00	7,33	7,17 a
Emirbey	17,00	22,33	19,67 d-g	6,00	6,00	6,00 bc
Şahin	21,00	26,33	23,67 ab	7,67	6,33	7,00 ab
Mean	18,07	22,33	20,20	6,16	6,40	6,28
		Lsd_{Lines}: 2.660			Lsd_{Lines}: 1.048	

[1] The differences between the means denoted by the same letter were not statistically significant.

According to the analysis of variance, the difference between genotypes in terms of pod number was found to be statistically significant at a 1% probability level (Table 2). According to the average of the years, the highest number of pods was obtained from line PS3057M2 with 24.33 number/plant, and the lowest number of pods was obtained from line PS3073G1 with 16.83 number /plant. Other genotypes were within these values. Many researchers have reported that the number of pods varies depending on pea genotypes and climatic factors (Ceyhan et al. 2005, Geren and Alan 2012, Uzun et al. 2012, Keskin et al. 2021). The number of pods in peas was reported as 6.5 - 9.9 number/plant (Önder and Ceyhan 2001), 18.3 - 38.3 number/plant (Ceyhan et al. 2005), 12.3 - 24.0 number /plant (Ceyhan and Avcı 2005), 26.7-28.3 number /plant (Geren and Alan 2012), 10.4-15.5 number/plant (Tan et al. 2012), 8.7-11. 4 number /plant (Uzun et al. 2012), 8.2-9.2 number/plant (Kavut and Çelen 2017), 5.8-11.0 number/plant (Kadıoğlu and Tan 2018), and 14.6-17.3 number/plant (Keskin et al. 2021) and the varieties had different pod numbers. While the results obtained in terms of the number of pods of the forage pea lines used in the experiment are in agreement with the results reported by Ceyhan et al. (2005), Ceyhan and Avcı (2005) and Keskin et al. (2021), higher than the results reported by Önder and Ceyhan (2001), Tan et al. (2012), Uzun et al. (2012), Kavut and Çelen (2017), Kadıoğlu and Tan (2018) but lower than the results reported by Geren and Alan (2012). These differences are due to the genetic structure of these newly developed lines.

3.4. Number of Seeds per Pods

In the study, the difference between the years in terms of the number of seeds in pods was statistically significant at 1% probability (Table 2). As the average of the lines, the number of seeds in pods was determined as 6.16 number /plant in the first year of the experiment and 6.40 number/plant in the second year, and it was determined that the number of seeds in the pod was significantly affected by years (Önder and Ceyhan 2001, Geren and Alan 2012, Keskin et al. 2021).

In this study conducted in Konya ecological conditions, the difference between genotypes in terms of the number of seeds in pods was found significant at a 1% level (Table 2). According to the results of the research, the highest number of seeds in pods was obtained from the PS4053M4 line with 7.17 and the lowest number of grains in pods was obtained from the PS3073G2, PS3073G3 and PS4053M2 lines with 5.67. The number of seeds in pods of the other genotypes used in the study was between these values and the average was 6.28 (Table 3). In the studies conducted on this subject, the number of seeds per pod in forage pea was 5.8 - 7.4 seeds per pod (Önder and Ceyhan 2001), 5.93 - 8.27 seeds per pod (Savur and Ceyhan 2011), 5.4-6.6 seeds per pod (Geren and Alan 2012), 4. 3-5.0 seeds per pod (Uzun et al. 2012), 4.9-5.7 seeds per pod (Kavut and Çelen 2017), 4.8-7.6 seeds per pod (Kadıoğlu and Tan 2018) and 2.90-4.20 seeds per pod (Keskin et al. 2021). It is seen that the number of grains in pods was obtained differently in forage pea varieties in different ecologies. These results show that the number of grains in pods is highly affected by environmental conditions and genetic structure (Önder and Ceyhan 2001).

3.5. Number of Seeds per Plant

There were statistically significant differences at 1% level between the pea lines and years used in the study in terms of the number of seeds per plant (Table 2). As the average of the lines, the number of seeds per plant was 110.89 number/plant in the first year of the experiment and 142.11 number/plant in the second year. According to the two-year averages, the number of seeds per plant of the lines varied between 105.00 (PS4028H3) and 162.83 (Şahin).

The number of seeds per plant of the lines varies depending on genetic structure and environmental factors (Ceyhan 2003). Ceyhan et al. (2012), Ceyhan et al. (2013) and Karadaş and Ceyhan (2023) stated that seed number per plant is a very variable character, and this limit varies between 35.50 and 231.93 number. These results support our research results.

3.6. Seed Yield

Regarding the seed yield of pea lines, the years were statistically significant at a 1% level (Table 2). As the average of the genotypes, seed yield was 1.99 t ha-1 in the first year of the research, while 2.73 t ha-1 in the second year. This difference between the years is due to climatic conditions. Some differences were observed between the years of the study, especially in terms of rainfall (Table 1). In the first year of the research, the total vegetation rainfall was 350.4 mm and this value was higher than the value recorded in the second year (304.2 mm). However, there were great differences between the years in terms of the distribution of precipitation to the months, and especially the total precipitation values of March, April, May and June, which are the development period of the plants, were lower in the first year (123.6 mm) than in the second year (216.4 mm) (Table 1). In the same period, average temperatures were slightly higher in the first year. Therefore, the months in which fertilization and fruit setting took place were

drier in the experiment's second year than in the first year. Ceyhan et al. (2012) and Avcı and Ceyhan (2013) reported that high temperatures and insufficient rainfall during the full flowering period negatively affected seed yield. In our research, it can be said that the differences mentioned differences in terms of climatic factors, especially precipitation, were effective in the differences observed between the experimental years in terms of seed yield.

The differences among pea lines regarding seed yield were statistically significant at a 1% level (Table 1). The different results of the lines in terms of seed yield are due to their different genotypic structures (Önder and Ceyhan 2001, Ceyhan et al. 2012, Avcı Ceyhan 2013). The highest seed yield was the PS4053M4 line with 2.84 t ha^{-1} and the lowest seed yield was obtained from the PS4053M3 line with 1.71 t ha^{-1}. The seed yields of the other genotypes used in the experiment were between these values and the average yield was calculated as 2.36 t ha (Table 4). In terms of seed yield, PS4053M4 (2.84 t ha^{-1}), PS3057M2 (2.81 t ha^{-1}), PS4028H4 (2.76 t ha^{-1}), PS4028H3 (2.84 t ha^{-1}), and PS4028H2 (2.84 t ha^{-1}) pea lines had higher seed yield than the average seed yield of standard varieties (2.62 t ha^{-1}) (Table 2). Ceyhan and Önder (2001), 1.12- 1.61 t ha^{-1}, Ceyhan et al. (2005) 1.13 -2.43 t ha^{-1}, Geren and Alan (2012) 2.19-2.85 t ha^{-1}, Uzun et al. (2012) 257.4-362.0 2.19-2.85 t ha^{-1}, Kavut and Çelen (2017) 0.95-3.10 t ha^{-1}, Kadıoğlu and Tan (2018) 196.5-314.7 t ha^{-1} and Keskin et al. (2021) 1.82-2.86 t ha^{-1}. Our research results are in harmony with the researchers findings.

Table 4. Means and LSD groups of number of seeds per plant, seed yield, hundred seed weight and biological yield of forage pea varieties according to years.

Lines	2020-21	2021-22	Mean	2020-21	2021-22	Mean
	Number of Seeds per Plant			Seed Yield (t ha^{-1})		
PS3057M1	94,33	144,67	119,50 cde[1]	1,42	2,35	1,88 c
PS3057M2	134,00	167,33	150,67 ab	2,44	3,18	2,81 a
PS3073G1	86,00	128,33	107,17 de	1,60	2,60	2,10 c
PS3073G2	115,33	143,33	129,33 bcd	1,77	2,36	2,06 c
PS3073G3	102,67	128,33	115,50 cde	1,52	2,12	1,82 c
PS4028H1	122,33	153,33	137,83 bc	2,16	2,94	2,55 ab
PS4028H2	110,00	140,67	125,33 cde	2,31	3,10	2,71 a
PS4028H3	100,00	110,00	105,00 e	2,52	2,95	2,73 a
PS4028H4	113,67	150,33	132,00 bc	2,31	3,22	2,76 a
PS4053M1	115,67	137,67	126,67 b-e	1,76	2,29	2,02 c
PS4053M2	107,33	133,67	120,50 cde	1,79	2,49	2,14 bc
PS4053M3	87,00	144,00	115,50 cde	1,21	2,21	1,71 c
PS4053M4	111,67	151,67	131,67 bc	2,32	3,37	2,84 a
Emirbey	102,00	134,00	118,00 cde	2,18	3,01	2,60 a
Şahin	161,33	164,33	162,83 a	2,52	2,76	2,64 a
Mean	110,89	142,11	126,50	1,99	2,73	2,36
		Lsd_{Lines}: 24.21			Lsd_{Lines}: 0.44	
Lines	**Hundred Seed Weight (g)**			**Biological Yield (t ha^{-1})**		
PS3057M1	12,55	13,53	13,04 g	2,06	3,55	2,80 c
PS3057M2	15,19	15,86	15,52 e	3,61	4,73	4,17 a
PS3073G1	15,50	16,89	16,20 d	2,37	3,94	3,15 bc
PS3073G2	12,79	13,79	13,29 g	2,71	3,63	3,17 bc
PS3073G3	12,39	13,76	13,08 g	2,27	3,25	2,76 c
PS4028H1	14,73	15,98	15,35 e	3,10	4,33	3,71 ab
PS4028H2	17,50	18,37	17,94 b	3,35	4,62	3,99 a
PS4028H3	21,01	22,32	21,67 a	3,62	4,36	3,99 a
PS4028H4	16,95	17,83	17,39 c	3,45	4,84	4,15 a
PS4053M1	12,66	13,84	13,25 g	2,61	3,44	3,03 c
PS4053M2	13,95	15,53	14,74 f	2,61	3,66	3,13 bc
PS4053M3	11,61	12,75	12,18 h	1,78	3,36	2,57 c
PS4053M4	17,27	18,51	17,89 bc	3,31	4,90	4,10 a
Emirbey	17,81	18,71	18,26 b	3,31	4,59	3,95 a
Şahin	13,02	14,00	13,51 g	3,72	4,21	3,96 a
Mean	14,99	16,11	15,55	2,92	4,09	3,51
		Lsd_{Lines}: 0.52			Lsd_{Lines}: 0.64	

[1] The differences between the means denoted by the same letter were not statistically significant.

3.7. Hundred Seed Weight

There were statistically significant differences at a 1% level among the pea genotypes and years used in the study in terms of hundred seed weight (Table 2). As the average of the genotypes, the hundred seed weight was 14.99 g in the first year of the experiment and 16.11 g in the second year. According to the two-year averages, the highest hundred seed weight among the genotypes was obtained from the PS4028H3 line with 21.67 g, and the lowest hundred seed weight was obtained from the PS4053M3 line with 12.18 g. The hundred seed weights of the other genotypes used in the study were between these values, and the average hundred seed weight was calculated as 15.55 g (Table 4).

Hundred seed weight, one of the important yield components, is closely related to the genetic structure of genotypes and is affected by environmental conditions (Ceyhan et al. 2005). Önder and Ceyhan (2001) found 14.50 - 22.61 g, Ceyhan and Avcı (2005) 8.71-18.31 g, Ceyhan et al. (2005) 10.12-23.63 g, and Savur and Ceyhan (2011) 12.83-27.65 g. Our results are similar to the results of the researchers.

3.8. Biological Yield

The difference between the years in terms of biological yield was found statistically significant according to the 1% probability limit (Table 2). As the average of the genotypes, 2.92 t ha^{-1} biological yield was obtained in the first year of the experiment and 4.09 t ha^{-1} in the second year (Table 2). Many researchers have reported that the effect of years on the biological yield of forage peas is significant (Önder and Ceyhan 2001, Keskin et al. 2021).

The effect of genotypes on biological yield was statistically significant at a 1% probability level. According to the average of the years, the highest biological yield was obtained from the PS3057M2 line with 4.17 t ha^{-1} and the lowest value was obtained from the PS4053M3 line with 2.57 t ha^{-1}. Biological yields of 5 lines were higher than the average of the standard variety (3.96 t ha^{-1}). The results obtained in terms of biological yield of the genotypes used in the experiment are within the results of the research conducted on similar subjects (Önder and Ceyhan 2001, Geren and Alan 2012, Uzun et al. 2012, Tan et al. 2012, Keskin et al. 2021, Temel et al. 2022).

4. Conclusions

In this study in which newly developed fodder pea varieties were tested for two years, it was determined that years significantly affected the parameters examined in the research. In these results, the climatic characteristics (especially the amount of precipitation falling in April-June, when the plants actively show vegetative development) played an important role. These results show that winter sowing is very important for forage pea varieties to benefit from the rainfall in spring months, especially for Konya's ecological conditions.

The two-year results from the research revealed that the newly developed forage pea variety candidates are important in forage pea cultivation under Konya ecological conditions. As a result of this study, it was determined that PS3057M2, PS4028H4, PS4053M4, PS4028H2 and PS4028H2 forage pea lines with high biological yields according to two-year averages are promising variety candidates. It is undeniable that these newly developed forage pea lines will play an important role in closing the roughage deficit in the Central Anatolia region, especially in animal nutrition.

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Research Article

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In Vitro Vitamin C Equivalent Antioxidant Capacity, Cytotoxicity and Anti-Cancer Activity of *Methanolic Urtica dioica* L. Leaf Extract as a Food Supplement

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HIGHLIGHTS

- The average Vitamin C Equivalent Antioxidant Capacity was calculated as 42.3 µg/mg methanolic Urtica dioica L. leaf extract.
- Cytotoxic Concentration 50% was calculated as 15.71 mg/ml and 5.14 mg/ml for mammalian kidney cells.
- Inhibition Concentration 50% was calculated as 2.46 mg/ml for human hepatocellular carcinoma.
- This dose-response study presented the effects of methanolic Urtica dioica L. leaf extract on kidney cytotoxicity and the proliferation of liver cancer cells.

Abstract

Antioxidant capacity, cytotoxicity on two vital cell lines and the anti-cancer activity of methanolic *Urtica dioica* L. leaf extract (UDE) collected from Duzkoy, Giresun, Turkey were studied by determining safe concentration. The antioxidant capacity of the extract was expressed as vitamin C equivalency by spectrophotometric MTT assay. The cytotoxic concentration 50% was measured by the linearity between UDE concentrations (CC50) and the cell viability of non-cancer kidney cell lines (BHK-21, MDBK). The anti-cancer activity was conducted on human hepatocellular carcinoma cells (HepG2) by determining inhibition concentration (IC50) on cell proliferation. The vitamin C equivalence of UDE increased linearly by increasing the concentration. The cytotoxic and non-toxic concentrations of UDE were determined on BHK-21 and MDBK with 15.71 mg/ml and 5.14 mg/ml of CC50 respectively. The extract inhibited the proliferation of human hepatocellular carcinoma cells with a 2.46 mg/ml of IC50. In conclusion, the present study tried to explain in detail the dose-dependent activity of *Urtica dioica* L. leaf extract. The dose-response results showed that *Urtica dioica* L. leaf extract could have low cytotoxicity, but potential anti-cancer activity at safe concentrations.

Keywords: Anti-cancer; Cytotoxicity; Food additive; Nettle; *Urtica dioica* L.

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1. Introduction

Urtica dioica L. belongs to the Urticaceae family. It has been identified and widely distributed worldwide and is considered to be native to Europe. North Africa. Asia. and North America (Upton, 2013; Dhouibi et al., 2020; European Commission, 2022). It and its extracts are used in both pharmaceutical and food industries as a supplement and a food additive for extending shelf-life, ensuring microbial safety of foods and higher consumer acceptability (Alp and Aksu, 2010). Phytochemical studies have mainly focused on its bioactive compounds and activities related to antioxidant contents (Körpe et al., 2013; Dhouibi et al., 2020; Veiga et al., 2020). Therefore, a wide range of plants and their extracts were applied as food additives and preservatives during food production and service to improve safety and quality by presenting antioxidant activities at their safe concentrations (Alp and Aksu, 2010; Körpe et al., 2013; Dhouibi et al., 2020).

In addition to antioxidant capacity, several in vitro and in vivo studies of plants aimed to determine the non-toxic doses for preventing adverse effects before using them including Urtica dioica L. as a potential therapeutic agent in modern and traditional medicine (Özkol et al., 2012; Sayhan et al., 2012; Dhouibi et al., 2020; Veiga et al., 2020). In recent, the aerial and subsoil parts of Urtica dioica L. were experimentally used to treat many cancer types by inhibiting cell proliferation (Konrad et al., 2000; Gözüm et al., 2003). Also, the cancer treatment has a risk of toxicity in different tissues including kidney and liver by inducing oxidative stress and free radical generation (Özkol et al., 2012; Dhouibi et al., 2020). So, the present study aimed to assess dose-dependently the antioxidant capacity, cytotoxicity on non-cancer kidney cell culture lines and anti-cancer activity on liver cancer cells of methanolic U. dioica L. leaf extract (UDE).

2. Materials and Methods

The Materials and Methods should be described with sufficient details to allow others to replicate and build on the published results. Please note that the publication of your manuscript implies that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose any restrictions on the availability of materials or information at the submission stage. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

2.1. Extraction of U. dioica L.

The sampling and extraction methods used in this study were adapted from the thesis of Aydın (2022) (Aydin, 2022). Briefly, U. dioica L. leaf samples were collected from Duzkoy province (40°56'59.1"N. 38°36'06.5"E), the city of Giresun on the Black Sea coast of Turkey in May 2021. They were identified and authenticated as “Urtica dioica L." at the Department of Pharmaceutical Botany, Faculty of Pharmacy in Marmara University, and an herbarium record was created with the code "MARE 23334". The samples were dried in cool room conditions avoiding direct sunlight.

Solid/liquid extraction and evaporation methods were used for the extraction of dried samples. Dried samples were ground by a water-cooled miller. Fifty grams of ground leaves were macerated in 500 ml of methanol (reagent grade. ≥ 99.7%) on a magnetic stirrer for 24 hours. The alcoholic suspension was filtered twice through filter paper (FilterLab-50 g/m2). The solvent in the remaining filtrate was evaporated in a rotary evaporator (Heidolph. Germany) at 40-45°C. 150 mbar, and 135 rpm. The extract was completely concentrated in a vacuum oven (Nüve EV 018. Turkey) at 45°C and -1 bar pressure. A UDE stock solution of 40 mg/ml was prepared in ultra-distilled water and stored at +4 °C for further analysis.

2.2. Handling of Cell Lines

Baby hamster kidney fibroblast (BHK-21, CCL-10), bovine kidney epithelial (MDBK. CCL-22) and human hepatocellular carcinoma (HepG2. HB-8065) cell lines were from ATCC, USA. All cell lines were cultured with Eagle's Minimum Essential Medium (EMEM) in the incubator with the standard condition (SC) of 37 °C and 5% CO2. The medium contained foetal bovine serum (10%). L-alanyl-L-glutamine (200 mM) and 1% penicillin (10.000 unit/ml)-streptomycin (10 mg/ml)-amphotericin B (0.025 mg/ml). For the anti-cancer and cytotoxicity

assay, the stock viable cell suspension (3x105 cell/ml) (100 µl) was transferred in each well (3x104 cell/well) and kept in SC for 24 h to achieve the confluence of at least 90% in 96-well microplates.

2.3. Vitamin C equivalent antioxidant capacity

Vitamin C (l-ascorbic acid. HPLC grade) was two-fold diluted as 600. 300. 150. 75 and 37.5 µmol with ultra-distilled water. UDE was two-fold diluted as 0.62. 1.25. 2.5. 5. 10 and 20 mg/ml with ultra-distilled water. MTT (2.5-diphenyl-2H-tetrazolium bromide, 1 mg/ml) (380 µl) and each Vitamin C or extract dilution (20 µl) were mixed in an Eppendorf tube and incubated at 37 °C for 4 h. After incubation, DMSO (400 µl) was added to all tubes and mixed well to solve the blue formazan salt formed during the incubation. 100 µl of each mixture was added quadruplicated to the U-bottom 96-well microplate. The microplate was read at 570 nm.

2.4. Anti-cancer and cytotoxicity assays by MTT

The extract was two-fold serially diluted with the maintaining medium at the concentration of 0.62, 1.25, 2.5, 5, 10 and 20 mg/ml. Then, each dilution (100 µl) was added to the microplates with monolayer cell culture at six-replicated wells. The cell control wells contained only fresh medium and cells. The blank wells contained the medium without cells. The microplates were incubated in SC for 24 h. 10 µl MTT in PBS (5 mg/ml) was added to each well. After a 4 h incubation in SC. the supernatant was discarded and DMSO (100 µl) was added to wells. The microplate was gently shaken to solubilize the formazan crystals and read at a wavelength of 570 nm (Absorbance 96. Byonoy. Germany).

2.5. Data analysis

The percentage of cell inhibition was calculated using the equations as follows,

Cell viability (%) = (OD sample – OD blank) / (OD control – OD blank) x 100".

The 50% cytotoxic concentration (CC50) was calculated from concentration-based-curves after non-linear regression analysis (y = mebx). The vitamin C standard curve was generated with optic density (OD. nm) values of five vitamin C dilutions by linear regression analysis (vitamin C equivalence = m x OD570 + b. R^2). The equivalence to vitamin C of extract dilutions was calculated concerning the standard curve (Figure 1). Statistical analyses were conducted by using SPSS version 15 software (IBM. USA). The graphs were generated by using Office Excel 2016 (Microsoft. USA.

3. Results

3.1. Vitamin C equivalent antioxidant capacity of UDE

The natural antioxidants in herbal additives such as carotenoids, tocopherol (vitamin E), some phenolic compounds, and ascorbic acid inhibit oxidative damage by free-radical scavenging (Dini, 2019; El-Saber Batiha et al., 2021). Previous research suggested that fresh *U. dioica* L. leaves contained various amounts of total ascorbic acid content from 16 to 112.8 mg/100 g fresh weight at different harvest times (Skalozubova and Reshetova, 2013; Shonte et al., 2020). The highest vitamin C was measured in August while the lowest was in September (Paulauskienė et al., 2021). As the vegetation period and drying methods influenced the vitamin C content and antioxidant capacity of *U. dioica* L. leaves, it was not varied by meteorological conditions. The highest vitamin C content was determined with low-temperature drying compared to owen drying (Shonte et al., 2020; Garcìa et al., 2021; Paulauskienė et al., 2021). In this study, *U. dioica* L. leaves were harvested in May, dried under cool-dry room conditions and extracted by methanol. The vitamin C equivalence of UDE was calculated with regards to the standard curve by linear regression analysis (vitamin C equivalence. µmol = 1191.6 x OD of UDE - 1.4723. R^2 = 0.998). The vitamin C equivalence of UDE increased by increasing the concentrations of UDE with a linearity of R^2=0.690 (vitamin C equivalence, µmol = 29.578 x UDE mg/ml + 501.51) (Figure 1). In harmony with previous studies, the average vitamin C content was calculated as approximately 42.3 µg/mg in methanolic extract of *U. dioica* L. leaves collected from Giresun region in Turkiye.

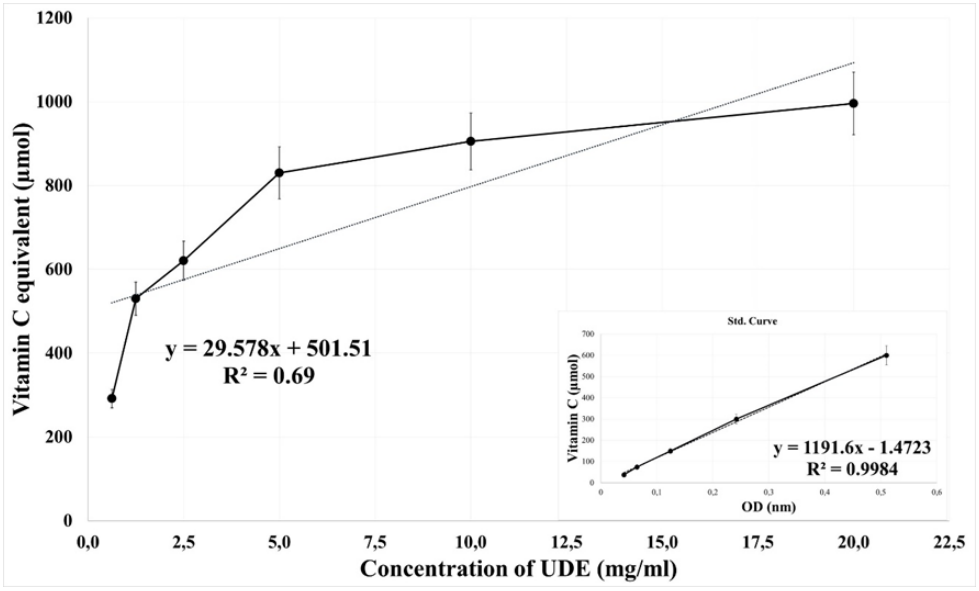

Figure 1. The vitamin C equivalency antioxidant capacity of UDE based on concentration.

3.2. Cytotoxicity of UDE

The cytotoxicity and safety levels of plant extracts vary related to the extraction method and are generally tested with cell lines of mammalian kidney and liver. A previous study suggested that methanolic extract (CC50:0.702-0.803 mg/ml) was safer than aqueous extract (CC50:0.37-0.49 mg/ml) of *U. dioica* L. on BHK-21 (Flores-Ocelotl et al., 2018). Also. the ethanolic extracts of *U. dioica* L. showed higher cytotoxicity than the aqueous extracts (Mannila et al., 2022). In this study, the cytotoxic effects of methanolic *U. dioica* L. leaf extract experimented on the viabilities of non-cancer (BHK-21 and MDBK) cell lines with concentration-response curves (Table 1). The relation between the cell viability and the concentration was the cell viability %=99.474e-0.044 x UDE (mg/ml) (R^2 = 0.936) for BHK-21 and the cell proliferation %=163.76$e^{-0.23}$ x UDE (mg/ml) (R^2 = 0.913) for MDBK (Figure 2). The CC50, was calculated as 15.71 mg/ml and 5.14 mg/ml for BHK-21 and MDBK respectively (Table 1). The Higher CC50 than the previous study indicated that the methanolic extract of *U. dioica* L. leaves might be a safer and potential food supplement and additive.

Table 1. The dose-response effects of UDE on cell viability %.

UDE Conc. (mg/ml)	Non-Cancer Cells		Cancer Cell
	BHK-21	MDBK	HepG2
	Mean±SD (%)	Mean±SD (%)	Mean±SD (%)
20	44.66±5.00c	1.13±4.44e	2.37±3.38d
10	56.36±6.50bc	43.21±4.30d	22.65±2.30c
5	73.59±9.15b	61.73±8.35c	34.49±5.25bc
2.5	93.45±4.25ab	79.42±4.01b	44.05±2.30b
1.25	99.82±8.35ab	101.54±2.02a	60.05±6.61a
0.62	99.95±5.51a	102.82±1.51a	60.13±5.45a
P value	<0.01	<0.01	<0.01
CC_{50} (mg/ml)	15.71	5.14	2.46

3.3. Anti-cancer activity of UDE

Various studies have recently demonstrated the cytotoxic and anti-cancer properties of *U. dioica* L. in particular against colon, gastric, lung, prostate and breast cancers (Esposito et al., 2019). Aqueous extract of *U. dioica* L. leaf cultured in Iran inhibited the proliferation of human breast cancer (MCF-7) at 2 mg/ml after 72h treatment (Fattahi et al., 2013, 2018). Its aqueous extract (from 5 to 30 µg/mL) harvested in Turkey has shown a dose-dependent inhibition effect on three breast cancer cell lines (MCF-7, MDA-MB-468, and MDA-MB-231) with IC50s of 14-18 µg/ml (Karakol et al., 2022). For Its dichloromethane extract against both mouse and human breast cancer cells, IC50 was determined between 31.37 and 38.14 mg/ml. Similarly, its anti-cancer

activity was measured by inhibiting the metastasis of breast cancer with 20 mg/kg daily injection treatment in vivo mouse models (Mansoori et al., 2017; Mohammadi et al., 2017) (Mohammadi 2017 Mansoori 2017). For its anti-cancer activities on human prostate cancer, the dichloromethane and aqueous extracts inhibited the proliferation of PC3 and LNCaP cells with 15.54 µg/ml and 42-50 µg/ml of IC50, respectively (Durak et al., 2004; Levy et al., 2013; Mohammadi et al., 2016). Meanwhile, no cytotoxicity was determined in non-cancer human prostate stromal cells (Konrad et al., 2000). *U. dioica* L. hydroalcoholic (50:50 v/v) extract inhibited 25.4% of human hepatocellular carcinoma (HepG2) cell viability at the concentration of 0.35 mg/ml (Carvalho et al., 2017). In this study. The anti-cancer effect of UDE on the proliferation of HepG2 cells was determined by spectrophotometric MTT assay with the concentration-response curve (cell proliferation % = $74.941e^{-0.163}$ x UDE (mg/ml), R^2 = 0.965) (Figure 2). The IC50 was calculated as 2.46 mg/ml for human hepatocellular carcinoma (Table 1). These IC50 values present the potential anti-cancer activity through hepatoprotective and reducing tumorigenesis activities of methanolic extract of *Urtica dioica* L. leaf used at safe concentrations.

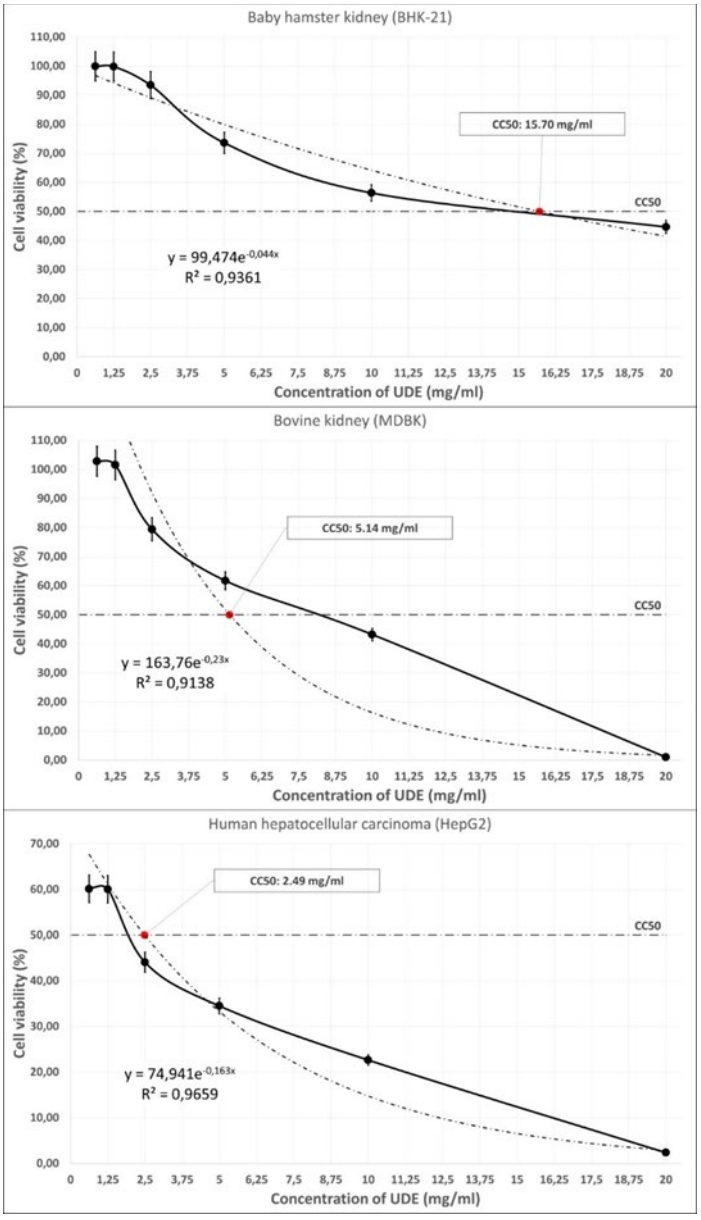

Figure 2. The cell viability and inhibition with CC50 (mg/ml) of UDE

4. Conclusions

In conclusion, the present study gives information about the bioactivity of U. dioica L. leaf harvested during the growing season in Giresun city of Turkey. The extract had dose-dependently antioxidant capacity expressed as vitamin C equivalency. Non-toxic and safe concentrations of the extract were determined in detail on vital cell lines originating from kidney tissue which are generally used in food and drug research for consumer safety. The dose-response results indicated that the extract was less toxic to kidney cells while inhibiting the proliferation of liver cancer cells at a lower concentration.

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Research Article

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Investigation of the Usability of Biodiesel Produced from Coriander *(Coriandrum Sativum L.)* Gürbüz Registered Variety Crude Oil in Diesel Engines

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HIGHLIGHTS

- Natural Growth in Anatolia: Although native to Mediterranean countries and the Middle East, coriander naturally grows in Anatolia, making it an accessible resource for biodiesel production.
- Extraction and Production: The study obtained coriander crude oil from the Gürbüz registered variety of coriander seeds using a screw press. Biodiesel production was achieved through a single-stage transesterification method, known as CGBD.
- Fuel Properties Assessment: Various fuel properties of the coriander biodiesel, including density, kinematic viscosity, flash point, water content, copper strip corrosion, calorific value, color, cloud point, cold filter plugging point, and pour point were examined.
- Conformance to Standards: The study assessed the conformity of coriander biodiesel to the EN 14214 standard, ensuring its suitability for use as a biodiesel fuel.

Abstract

In recent years, the use of biodiesel has become increasingly important. One of the potential plants that can be used in biodiesel production is coriander (*Coriandrum sativum L.*). Coriander *(Coriandrum sativum L.)*, a member of the Apiaceae family, is a medicinal, spice and essential oil plant. Although the homeland of coriander is the Mediterranean countries and the Middle East region, it grows naturally in Anatolia. In this study, coriander crude oil was obtained from coriander (*Coriandrum sativum L.)* (Gürbüz registered variety) seeds using a screw press. Biodiesel production was obtained from coriander crude oil by transesterification method in a single stage (CGBD). Fuel properties of coriander (Gürbüz registered variety) (CGBD); density (kg m^{-3}) (at 15 °C), kinematic viscosity (mm^2 s^{-1}) (at 40 °C), flash point (°C), water content (mg kg^{-1}), copper strip corrosion (3h at 50 °C), calorific value (MJ kg^{-1}), color (ASTM D1500), cloud point (°C), cold filter plugging point (°C), pour point (°C). The conformity of coriander biodiesel according to EN 14214 standard was examined.

Keywords: Biodiesel; *Coriandrum sativum L.*; Gürbüz registered variety; fuel properties

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1. Introduction

Coriander (*Coriandrum sativum L.,* 2=22), a member of the Apiaceae family, is a medicinal, spice and essential oil plant. Although the origin of coriander is the Mediterranean countries and the Middle East region, it grows naturally in Anatolia. In the Flora of Turkey, two species, C. sativum L. and Coriandrum tordylium (Fenzl) Bornm. have a natural distribution (Menemen, 2012). The plant, which is about 25-60 cm long, has an annual herbaceous structure, has small pinkish white flowers. Its fruits are schizocarp and rounded. According to the fruit size of the cultivated *C. sativum*, large fruit (*C. sativum var. macrocarpum*, 1000 fruit weight: more than 10 g, fruit diameter: 3-5 mm) and small fruit (*C. sativum var. microcarpum*, 1000 fruit weight: less than 10 g, fruit diameter: 1.5-3 mm) has two varieties (Tunçtürk, 2011). Fruit color turns from green to brown with ripening (Ulutaş Deniz et al., 2018). All parts of the plant can be used for food, but mostly fresh leaves and dried fruits are used as spices to add flavor and flavor to various food products (Singh and Verma, 2015; Kumar et al., 2017). Its fruits contain essential oil (0.15-1.40%) and fixed oil (18-22%). Linalool (69-91%) is the main component of essential oil (Farooq et al., 2011, Abou El-Nasr et al., 2013; Saxene et al., 2014; Beyzi et al., 2017; Gökduman and Telci,2018; Wahba et al., 2020). Essential oil is used in the food, beverage and perfumery industries (Yalçın, 2016). However, coriander essential oil is also used in folk medicine and pharmaceutical industry due to its pharmacological properties (antioxidant, hypoglycemic, anti-inflammatory, hypolipidemic, analgesic, sedative, antimutogenic, diuretic, antimicrobial, etc.) (Yalçın, 2016; Al-Khayri et al., 2023). Petrocelinic acid constitutes 72-75% of the fixed oil obtained from coriander fruits, while linoleic acid (13-14%) and oleic acid (5-6%) are other important fatty acids (Uitterhaegen et al., 2006; Sriti et al., 2009).

The primary objective of this research was to generate biodiesel from coriander seed oil, specifically sourced from the Gürbüz registered variety. Coriander, a plant of significant importance in both our nation and global trade, is renowned for its applications in medicinal and culinary contexts. The study sought to employ the transesterification method within a single stage, aiming to produce biodiesel efficiently. Additionally, the investigation aimed to assess the compliance of coriander crude oil (CGCO) and the resulting biodiesel (CGBD) with the EN 14214 standard, a critical benchmark for evaluating the suitability of biodiesel as a fuel. This evaluation was conducted through a comprehensive analysis of fuel properties, thereby offering insights into the potential of coriander-derived biodiesel for meeting established quality standards in accordance with EN 14214.

2. Materials and Methods

2.1. Obtaining the seed

Coriander (Gürbüz registered variety) seeds used in the study were obtained from Ankara University, Faculty of Agriculture (Figure 1.) and coriander (Gürbüz registered variety) crude oil was obtained with the help of a screw press in Oğuzhan Farm located in Gövdecili/Yozgat village. Methyl alcohol (CH_3OH) required for biodiesel production and sodium hydroxide (NaOH) as a catalyst were used as materials.

Figure 1. Coriander (*Coriandrum sativum L.)* Seeds

2.2. Obtaining the Crude Oil from Coriander (Coriandrum sativum L.) Seeds via Screw Press

Coriander (Gürbüz registered variety) seed oil was obtained from coriander seed crude oil with the help of an American KERN&KRAFT brand, 3.5 kW electric motor, screw press with a pulp outlet opening of 12 mm (Figure 2).

Figure 2. Extraction of oil from Coriander (*Coriandrum sativum L*). seeds via screw press

2.3. Biodiesel Production

Biodiesel production from coriander *(Coriandrum sativum L*). (Gürbüz registered variety) seeds crude oil was carried out by transesterification method in a single stage with a temperature regulated magnetic stirrer with probe heater according to the flow diagram given in Figure 3. Coriander (Gürbüz registered variety) seed crude oil was filtered to remove impurities before biodiesel production (Figure 4). To obtain methoxide, methyl alcohol (CH_3OH) was used as alcohol and NaOH as catalyst.

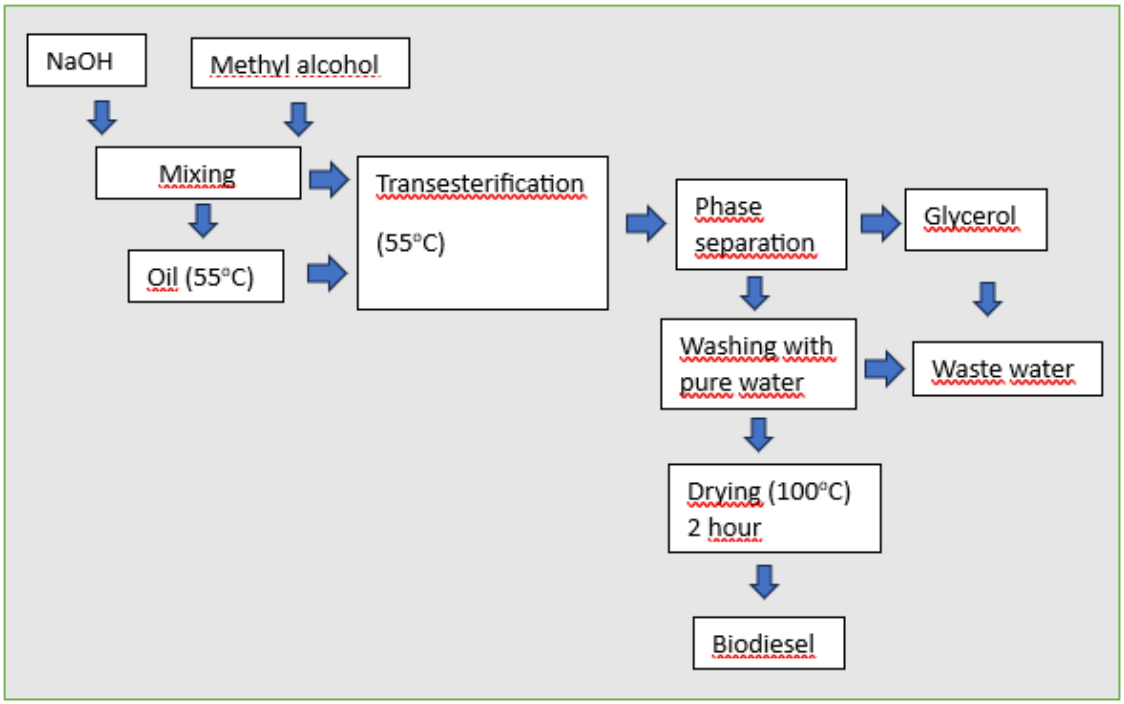

Figure 3. Process flow diagram for biodiesel production

Figure 4. Filtering of coriander *(Coriandrum sativum L).* (Gürbüz registered variety) crude oil

In the reaction, 20% (100 mL) methyl alcohol (CH_3OH) (Merck, d=0.791-0.793 kg m^{-3}) to be used for 500 mL coriander crude oil. 1.75g NaOH (Merck) to be used for each 500 mL oil was dissolved in a magnetic stirrer with heater and methoxide was obtained. This methoxide was added to the coriander crude oil which was heated at 55^{0}C. The stirrer speed was adjusted to 1000 min^{-1} and the mixture was reacted for 90 minutes. Then the mixer and heater were switched off. The temperature of the coriander crude biodiesel was raised to 75^{0}C and the methyl alcohol (CH_3OH) remaining in the crude biodiesel was removed. Glycerol was allowed to precipitate for 24 hours and glycerol was removed.

Then, the pH value of coriander oil biodiesel was checked, and since the reaction was basic, it was subjected to washing using pure water by misting method until it reached a neutral state (Eryılmaz et al., 2014; Cesur et al., 2021).

The purpose of washing is to remove unreacted alcohol, residual fatty acids, Na+, K+ ions, catalyzing agent and glycerol that may remain in the biodiesel during decomposition (Eryılmaz, 2009). During washing, the temperature of the biodiesel was 50^{0}C and the temperature of the pure water used in washing was 50^{o}C and the pH of the biodiesel was washed by using pure water by mist method until the pH of the biodiesel was neutralized. After the washing process, 12 hours were waited for the precipitation of the water and the precipitated waste water was taken. The crude biodiesel from which the precipitated water was removed was subjected to drying at 120^{0}C for 120 minutes and thus coriander (*Coriandrum sativum L*). (Gürbüz registered variety) crude oil biodiesel was produced (Özgün and Eryılmaz 2018; Cesur et al., 2021). Figure 5 shows the coriander (Gürbüz registered variety) biodiesel (CGBD) produced.

Figure 5. Coriander (*Coriandrum sativum L*). (Gürbüz registered variety) biodiesel

2.4. Fuel analysis

In this study, some fuel properties of coriander (*Coriandrum sativum L*) (Gürbüz registered variety) oil biodiesel; density, kinematic viscosity, flash point, water content, copper strip corrosion, calorific value, color, cloud point, cold filter plugging point, pour point) were determined according to the devices and working methods given in Table 1. Some fuel analyses of the obtained biodiesel were carried out in the Fuel Analysis Laboratory of Selçuk University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering.

Table 1. Properties of test devices

Fuel Property	Devices	Measurement Range	Unit	Measurement Accuracy	Manufacturer	Standard
Density	Kem Kyoto DA-130N	0.0000 - 2.0000	g cm^{-3}	±0.0001	Kem Kyoto Elektronik, Japonya	EN ISO 3675 EN ISO 12185
Kinematic viscosity	Koehler K23377	Ambient temperature – 150	^{0}C	±0.01	Koehler Instrument Company, US	EN ISO 3104
Flash point	Koehler K16270	Ambient temperature - 370	^{0}C	±0.01	Koehler Instrument Company, USA	EN ISO 2719 EN ISO 3679
Water content	Kem Kyoto MKC-501	10µg-100mg	µg	±0.01	Kem Kyoto Electronic, Japan	EN ISO 12937
Calorimeter	IKA C 200	0-40.000	J	±0.0001	IKA, UK	DIN 51900
Cold filter plugging point	Tanaka AFP-102	With a coolant down to -60^{0}C	^{0}C	±0.01	Tanaka Scientific Limited, Japan	ASTMD6379
Cloud and pour point	Koehler	-	^{0}C	-	-	ASTM D97
Copper strip corrosion	Koehler K 25330	0-190	^{0}C	±0.01	Koehler Instrument Company, USA	EN ISO 2160

3. Results

Coriander (Gürbüz registered variety) crude oil (CGCO), Coriander (Gürbüz registered variety) crude oil biodiesel (CGBD) analysis results are given in Table 5.

Table 5. Fuel properties of the crude oil of the registered coriander (*Coriandrum sativum L.)* variety and the biodiesel produced from this oil.

Features	CGCO	CGBD	EN 14214	
			Min.	Max.
Density (at 15°C) (kg m^{-3})	907,5	869,9	860	900
Kinematic Viscosity (at 40°C) (mm^2 s^{-1})	34,19	4,95	3,5	5,0
Flash Point, ºC	-	140	120	
Water Content, (mg kg^{-1})	368,2	75		500
Copper Strip Corrosion (3h/50°C)	1a	1a	1	
Calorific value, MJ kg^{-1}	39,910	39,322		
Colour (ASTM D1500)	3,2	1,9	-	-
Cloud Point, ^{0}C	4	-13,8	-	-
Cold Filter Plugging Point ^{0}C	0	-16		
Pour Point, ^{0}C	-5	-16,8	-	-

3.1. Kinematic Viscosity

First, it is important to note that the kinematic viscosity of CGBD, measured at 40°C, was found to be 4.95 mm^2 s^{-1}, a value that is indicative of its flow characteristics. The importance of kinematic viscosity in biodiesel lies in its direct correlation with the fluid's flow behavior. Lower kinematic viscosity values signify enhanced fluidity, indicating that the biodiesel is more adept at flowing through conduits and atomizing during combustion. This characteristic is especially significant in optimizing the performance of engines and fuel delivery systems. The fact that the result we obtained is within the standards plays an important role in the usability of biodiesel.

3.2. Density

Defined as the mass per unit volume, biodiesel density is a fundamental property that contributes to the overall understanding of the fuel's physical characteristics and behavior. The density at 15°C was recorded at 869.9 kg m^{-3}, which is significant for assessing the fuel's mass properties. The specified value aids in formulating biodiesel blends with conventional diesel fuel, ensuring that the resultant mixture maintains an appropriate density for seamless integration with existing distribution systems and combustion engines.

3.3. Water content

The water content, an important consideration in fuel quality, was measured at 75 mg kg^{-1}, indicating a low moisture level in the CGBD sample. In the realm of biodiesel, the water content assumes particular importance due to its potential implications for both the fuel's immediate performance and its long-term stability. The reported water content value of 75 mg kg^{-1} in the CGBD sample signifies a relatively low moisture level. This observation is noteworthy as excessive water content in biodiesel can have detrimental effects on combustion efficiency and engine performance. Water, when present in biodiesel, may lead to issues such as corrosion, microbial growth, and reduced lubricity, all of which can adversely impact the overall quality and functionality of the fuel.

3.4. Calorific value

One of the most critical parameters, the calorific value of CGBD, was measured at 39.32 MJ kg^{-1}, which is remarkably close to traditional diesel fuel. This high calorific value suggests that CGBD can provide a substantial amount of energy when combusted, making it a promising alternative. Calorific value, indicative of the energy content per unit mass, stands as a pivotal metric in determining the combustibility and overall utility of biodiesel. The measured calorific value of 39.32 MJ kg^{-1} for CGBD is noteworthy for its proximity to

that of traditional diesel fuel. This close resemblance suggests that CGBD possesses a comparable energy content to conventional diesel, a crucial factor in assessing its suitability as a substitute fuel. The high calorific value implies that CGBD can release a substantial amount of energy upon combustion, positioning it as a promising and energetically efficient alternative within the realm of renewable fuels.

3.5. Flash point

A flash point of 140°C indicates a relatively high resistance to ignition, suggesting that the biodiesel sample, in this case, Coriander Biodiesel (CGBD), requires elevated temperatures before vapor concentrations reach the flammable threshold. This property is particularly advantageous in terms of safety during storage and transportation, as it reduces the risk of inadvertent ignition under normal operating conditions.

3.6. Colour

In biodiesel analysis, the ASTM D1500 color measurement serves as an important quality control parameter. A color value of 1.9 indicates a relatively low degree of coloration, suggesting a pale or clear appearance. This is typically desirable in biodiesel, as it implies a cleaner and more refined product with fewer impurities or contaminants. The ASTM D1500 color measurement is particularly valuable in assessing the effectiveness of production processes and ensuring compliance with industry standards.

3.7. Flow characteristics in cold

The cloud point, cold filter plugging point, and pour point, -13.8°C, -16°C, and -16.8°C, respectively. These results imply that CGBD exhibits properties conducive to efficient combustion and operation even in cold weather conditions. The cloud point, cold filter plugging point, and pour point represent key indicators of the low-temperature operability of biodiesel. The recorded values for these cold flow properties, i.e., -13.8°C, -16°C, and -16.8°C, suggest that CGBD exhibits favorable characteristics for operation in cold weather conditions. The relatively low values indicate that the biodiesel remains in a liquid state at temperatures lower than those specified, mitigating concerns related to the formation of wax crystals and filter plugging. This is particularly crucial in regions or seasons where low temperatures are prevalent.

The copper strip corrosion test, which is indicative of the fuel's corrosive potential, showed a rating of 1a, indicating minimal corrosion risk. A rating of 1a in the copper strip corrosion test is indicative of a low corrosive impact, implying that Coriander Biodiesel (CGBD) poses minimal risk to copper surfaces. This result is noteworthy for several reasons, most notably in terms of engine and fuel system integrity. Corrosion can lead to the degradation of metallic components, negatively impacting the overall efficiency and lifespan of engines and associated infrastructure.

4. Discussion

In CGBD fuel; kinematic viscosity ($mm^2\ s^{-1}$) (at 40 ^{0}C), density ($kg\ m^{-3}$) (at 15 ^{0}C), water content ($mg\ kg^{-1}$), calorific value ($Mj\ kg^{-1}$), flash point (^{0}C), copper strip corrosion (3h at 50^{0}C) were within the standards (Table 5). These values were similar to those reported in other studies for B100 biodiesel (Moser and Steven, 2010; Kumar, M. et al., 2023; Tibesigwa, T. et al., 2023). The copper strip corrosion values were found to be 1a in KGBD fuel and were similar to Ciubota-Rosieet et al., 2013. The cold filter plugging point was determined at the dates specified in the standard (16 April to 30 September: 0^{0}C; 1 March to 15 April and 1 October to 15 November: -10^{0}C; from 16 November until the end of February: -20^{0}C). From 16 November to the end of February: -20^{0}C in CGBD fuel, but not in other dates (Table 5). In the study conducted by Moser and Steven (2010), a CFPP of -15 was reported, which was found to be close to the result obtained in our research (-16).

The results of this study provide valuable insights into the fuel quality of biodiesel (CGBD) derived from coriander (Gürbüz registered variety) crude oil and its potential adherence to the EN 14214 standard. A comprehensive analysis of various physicochemical properties was conducted, and the findings are crucial in evaluating the suitability of CGBD as a viable alternative fuel source for diesel engines.

Overall, the results of this study demonstrate that CGBD exhibits fuel quality characteristics remarkably close to conventional diesel fuel. The similarities in key parameters, such as calorific value and viscosity,

suggest that CGBD could serve as a suitable substitute for diesel fuel in various applications, particularly in diesel engines. These findings are promising and support the feasibility of utilizing CGBD as a renewable and environmentally friendly fuel source, contributing to the reduction of greenhouse gas emissions and promoting sustainable energy solutions. Further research and field testing may be necessary to fully validate the practical use of CGBD in diesel engines.

5. Conclusions

In this study, the parameters related to the fuel quality of biodiesel (CGBD) produced from coriander (Gürbüz registered variety) crude oil were investigated. CGBD biodiesel was examined whether it meets the requirements specified in (EN 14214) standard.

The physicochemical properties analyzed for the KGBD fuel sample were kinematic viscosity ($mm^2\ s^{-1}$) (at 40 ^{0}C) 4.95, density ($kg\ m^{-3}$) (at 15 ^{0}C) 869.9, water content ($mg\ kg^{-1}$) 75, calorific value ($Mj\ kg^{-1}$) 39. 32, flash point (^{0}C) 140, cloud point (^{0}C) (-13.8), cold filter plugging point (^{0}C) (-16), pour point (^{0}C) (-16.8), copper strip corrosion (3h at 50^{0}C) 1a and color (ASTM D1500) 1.9. According to the results obtained, the fuel quality of biodiesel produced from coriander (Gürbüz registered variety) crude oil showed values close to the fuel quality of diesel. According to these results, it can be said that CGBD fuel can be used in diesel engines.

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Research Article

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The Characterization of Volatile Compounds of Lupin Türkiye Genotype HS-SPME/GC-MS Method

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HIGHLIGHTS

- Volatile Compounds of Lupin Türkiye Genotype
- HS-SPME / GC-MS Method
- 65 µm PDMS/DVB fiber is the most preferable in terms of compound identification, with 97 compound.

Abstract

The aim of this study is to reveal the volatile and semi-volatile constituent of the local *Lupinus albus* L. genotype grown in Türkiye by using the HS-SPME technique. Local lupin is a legume plant with great potential due to its high seed yield and protein and oil content in the seeds. Powdered seeds of local lupin genotypes were analysed and compared with respect to types and contents of volatile semi-volatile compounds contents using four different SPME fiber with GC-MS system in W9N11, SWGDR4G4 and SWGDR4G5 libraries. Fiber with 50/30 µm DVB/CAR/PDMS identified 54 compounds, 65 µm PDMS/DVB fiber 97 compounds, 85 µm Carboxen/PDMS fiber 28 compounds, and 85 µm polyacrylate 12 compounds. As a result, 65 µm PDMS/DVB fiber is the most preferable in terms of compound identification, with 97 compound. In terms of content, Benzene, Methyl(1-Methylethyl) (39.92%), Gamma-Terpinene (12.26%), Cis-Ocimene (5.93%), 1,3,6-Octatriene,3,7-Dimethyl-(% 5.51), Beta-Myrcene (4.70%), Alpha-Pinene (4.39%), Alpha.-Thujene (4.21%), Alpha Terpinene (2.55%), Camphene (2.23%), 1,6-Octadien-3-Ol,3,7-Dimethyl (2.10%) were mostly detected in the analysis with 50/30 µm DVB/CAR/PDMS fiber. With the other fiber 65 µm PDMS/DVB; phospine oxide, triphenyl- (15.49 %), pulegone (5.03 %), L-linalool (3.85 %), cyclopentasiloxane, decamethyl (3.54 %), cyclohexasiloxane, dodecamethyl (3.13 %), cyclotrisiloxane, hexamethyl (2.32 %), oxime-,methoxy-phenyl (2.08 %), 1-pentanamine,N-pentyl (1.60 %), 3,5-octadıen-2-one (1.55 %), I-menthone (1.50 %). Fiber 85 µm Carboxen/PDMS; the most four components in the content are; Benzene, 2, 4-diisocyanato-1-methyl (54.27 %), Pyrrole-3-carbonitrile, 5-formyl-2, 4 (18.41 %), 1-Butanol, 4-(1-methylethoxy) (14.67 %), pyridine,4-(1-pyrrolidinyl)- (8.13 %). With 85 µm polyacrylate fibre the most found component is 1, 2-Benzenedicarboxylic acid, diethyl ester (98.90 %). In conclusion, as far as we know this study is the first study about the volatile content of local lupin genotype of Türkiye.

Keywords: Volatile compounds, Solid phase microextraction, Lupin, Local genotype, HS-SPME, GC-MS.

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1. Introduction

Lupinus is a large and diverse genus that belongs to the Fabaceae family and includes about 170 species (Gresta, 2017). *Lupinus albus* "white lupin" has a wide distribution in the Mediterranean region. It is planted over all the Mediterranean region and also in Egypt, Syria, Sudan, Ethiopia, Central and Western Europe, South America and USA, Tropical and Southern Africa, Ukraine, and Russia. Like legume seeds, lupin contains high amounts of protein, minerals and dietary fibre. The protein content of white lupin seed (33-47%) is higher than other legumes and close to the soy protein content (Dervas et al., 1999).

Lupins have a long date of for using both as ornamental plants in gardens and as an agricultural crop. There are four lupin species *L. albus*, L. *angustifolius*, *L. luteus* and *L. mutabilis* that have gained agricultural significance. Lupin seeds and flour are used in several cereal products such as pasta, crisp, bread, cookie, cake and breakfast cereal (Dervas et al., 1999; Erbaş et al., 2005; Yaver and Bilgiçli, 2021). Lupin seeds have admitted increasing international interest as an alternative source of human food ingredients due to their high-quality protein and dietary fibre. The definition of seed content is very important for breeding and crossing studies. According to the literature, headspace solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) has gained wide approval as effective extraction technique for various samples in the last twenty years (Cuevas-Glory et al., 2007; Panighel and Flamini, 2014; Xu et al., 2016; Royandazagh ans Pehlivan, 2016). Solid phase microextraction (SPME) involves the adsorption of analytes upon a fused silica fibre coated with proper stationary phases and their following desorption instantly before chromatographic analysis (Arthur and Pawliszyn, 1990; Bicchi et al., 2000; Kataoka et al., 2000; Krutz et al., 2003; Pawliszyn, 2012; Ulrich, 2000; Zhang and Pawliszyn, 1993). The target analytes can be adsorbed on the fibre by immersing it in the sample or by exposing it to the sample headspace (HS-SPME), in which case matrix interferences can be highly reduced. This properly reduces analysis time and improves least detection limits, while maintaining resolution and can be used in two main modes, that is direct-extraction and headspace configurations. Due to these benefits, HS-SPME is strongly used to sample the volatile components from aromatic and medicinal plants (Bicchi et al., 2004; Muselli et al., 2009; Ercan and Dogru, 2022). Simsek Sezer et al. (2023) was determined to volatile compounds belonging to some lupin genotypes by SPME GC-MS.

The aim of this study is to reveal the volatile and semi-volatile constituent of the local *Lupinus albus* variety grown in Türkiye by using the HS-SPME technique.

2. Materials and Methods

Lupinus albus seeds grown under Destigin/Doğanhisar-Konya/Türkiye conditions were used as material. Seeds are local population grown in that region.

2.1. Seed Propagation

Local lupin genotypes were studied to identify volatile and semi-volatile content during our analyses. Seeds are stored in the department of Field Crops of Agriculture Faculty at Selçuk University. Seeds were dried at ambient temperature without sunlight exposure. Dried seeds were ground by using a hand grinder. The ground samples (3gr) were diluted and sealed in a 10 ml vial. The HS-SPME fibres, 50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB, 85 μm Carboxen/PDMS and 85 μm polyacrylate were preferred for analysis. The SPME apparatus was directly injected into the upper space of the vial to adsorb volatile compounds and then directly injected into the Shimadzu QP2010ULTRA GC-MS apparatus using a Restek Rxi-5 MS capillary column.

2.2. *Analyses of GC-MS*

The volatile compounds of lupin were analysed by applying the method of (the injector temperature was 250 °C) using SPME-GC-MS. Compounds were isolated by a 15 min. SPME fibre exposure into a GC injector at 250 °C. The extracts from the SPME procedure were analysed on a Shimadzu QP2010 ULTRA FID GC–MS system. A 30 m length Restek Rxi-5 MS column (0.25 mm id, film thickness 0.25 μm) was used. Carrier gas was helium with a flow rate as 1.8 mL/min. The GC oven temperature was programmed to hold at 40 °C for 3 min and then to increase to 240 °C at 5 °C/min, finally holding at 240 °C for 3 min. The detector ion source temperature was 200 °C, and the interface temperature was set at 250 °C. Mass spectra were acquired in the electron impact mode at 70 eV, using m/z range of 50–350 and 2 s scan time.

2.3. *Data Analyses*

Chromatograms of SPME fibers all samples were subjected to noise reduction prior to peak area integration, and later, the peak areas of components in the chromatogram were integrated (Figure 1). Compounds were identified by comparing with three libraries, W9N11, SWGDR4G4 and SWGDR4G5. Compounds mostly matched in W9N11 library. Identification of components in the sample was based on the retention time (RT). The identification of the components present in the samples was calculated using Kovats retention index. The relative rate of the volatile oil compounds was obtained from peak areas. All analyses were performed in three replications, and all the numeric data are means of three independent analyses.

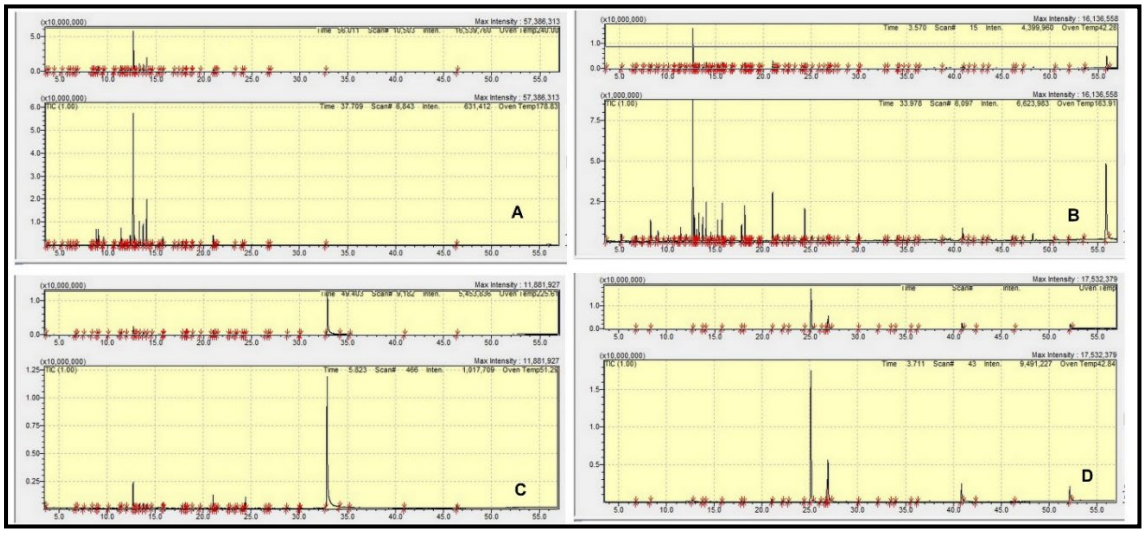

Figure 1. The total ion chromotograms of studied local lupin specimens via different SPME fibers (A: 50/30 μm DVB/CAR/PDMS, B: 65 μm PDMS/DVB, C: 85 μm Carboxen/PDMS and D: 85 μm polyacrylate)

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Totally 147 compounds were separated and identified from the studied local lupin sample with 50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB, 85 μm Carboxen/PDMS and 85 μm polyacrylate fibres respectively (Table 1). The total ion chromatograms (TIC) of studied local Lupin specimens were given Fig.1. With 50/30 μm DVB/CAR/PDMS fiber; the twelve components that are the most found in content; benzene methyl (1-methylethyl) (39.92 %), gamma-terpinene (12.26 %), cis-ocimene (5.93 %), 1,3,6-Octatriene,3,7-Dimethyl (5.51 %), beta-myrcene (4.70 %), alpha-pinene (4.39 %), alpha-thujene (4.21 %), D-Limonene (2.86 %), Pulegone (2.69 %), alpha terpinene (2.55 %), camphene (2.23 %), 1,6-octadien -3-ol,3,7-dimethyl (2.10 %). With the other fiber 65 μm PDMS/DVB; benzene methyl (1-methylethyl) (23.61 %), phospine oxide, triphenyl- (15.49 %), pulegone (5.03 %), L-linalool (3.85 %) and gamma-terpinene (3.79 %), cyclopentasiloxane, decamethyl (3.54 %), cyclohexasiloxane, dodecamethyl (3.13 %), cis-ocimene (2.58 %), D-Limonene (2.39 %), cyclotrisiloxane,

hexamethyl (2.32 %), 1,3,6-octatriene,3,7-dimethyl (2.24 %), oxime-,methoxy-phenyl (2.08 %), 1-pentanamine,N-pentyl (1.60 %), 3,5-octadıen-2-one (1.55 %), I-menthone 1.50 %). Fiber 85 µm Carboxen/PDMS; the most four components in the content are; Benzene, 2, 4-diisocyanato-1-methyl (54.27 %), Pyrrole-3-carbonitrile, 5-formyl-2, 4 (18.41 %), 1-Butanol, 4-(1-methylethoxy) (14.67 %), pyridine,4-(1-pyrrolidinyl)- (8.13 %). With 85 µm polyacrylate fibre the most found component is 1, 2-Benzenedicarboxylic acid, diethyl ester (98.90 %) (Figure 2).

In accordance with analyses, four compounds are common for all fibres with variable proportions. These compounds are benzene methyl (1-Methylethyl), gamma-terpinene, cyclopentasiloxane, decamethyl and pulegone respectively.

Table 1. The % peak area values of identified compounds of studied samples

Compound Name	% Area			
	50/30 µm DVB/CAR/PDMS	65 µm PDMS/DVB	85 µm Carboxen/PDMS	85 µm Polyacrylate
1-Butanol,3-Methyl-	-	0.13	-	-
Cyclotrisiloxane,Hexamethyl-	0.17	0.76	-	-
Benzene,Ethyl-	0.05	0.09	-	-
O-Xylene	-	0.54	-	-
1-Hexanol	0.39	0.52	-	-
Styrene	-	0.09	-	-
Benzene,1,2-Dimethyl-	-	0.24	-	-
Ethanol,2-Butoxy	-	0.05	-	-
Oxime-,Methoxy-Phenyl-	0.11	**2.08**	**-**	**-**
Propanamide,3-Amino-3-(Hydroxyimino)	-	0.43	-	-
Alpha-Pınene,	**4.39**	0.96	**-**	**-**
Bicyclo(2.1.1)Heptane,2,2-Dimethyl-3	-	0.23	-	-
Benzaldehyde	-	0.25	-	-
Chloroacetic Acid,Heptyl Ester	-	0.12	-	-
Bıcyclo(3.1.1)Heptane ,6,6-Dımethylethyl	-	0.15	-	-
Hexanoic Acid	-	0.11	-	-
3-Octanone	0.70	0.52	-	-
Beta-Myrcene	**4.70**	1.30	**-**	**-**
Decane	-	0.15	-	-
Cyclotetrasiloxane,Octamethyl-	0.15	0.74	0.04	-
Alpha-Terpinyl 3-Methylbutanoate	-	0.16	-	-
Benzene,Methyl(1-Methylethyl)-	**39.92**	**23.61**	0.62	0.25
D-Limonene	**2.86**	**2.39**	**-**	**-**
1,8-Cineole	0.72	0.26	-	-
1-Hexanol,2-Ethyl-	-	0.26	-	-
Benzyl Alcohol	-	1.15	-	-
Cis-Ocimene	**5.93**	2.58	**-**	**-**
1,3,6-Octatriene,3,7-Dimethyl-	**5.51**	2.24	0.05	
Gamma-Terpinene	**12.26**	3.79	0.08	0.04
Nonane,5-(2-Methylpropyl)-	-	0.30	-	-
Cıs-Sabınene Hydrate	0.07	0.17	-	-
3,5-Octadıen-2-One	0.63	**1.55**	**-**	**-**
3-Oxatricyclo(4.1.10(2.4))Octane,2,7,7	-	0.10	-	-
Benzene(2-Methyl-2-Propenyl)-	-	0.32	-	-
Cyclotrisiloxane,Hexamethyl-	**-**	**2.32**	**-**	**-**
L-Linalool	**-**	**3.85**	0.33	0.05
3-Ethyl-3-Methylheptane	-	0.48	-	-
Nonanal	-	0.40	-	0.03
Undecane,5-Methyl-	-	0.22	-	-
Benzaldehyde,2,5-Bis((Trimethylsily)Oxy)	-	0.42	-	-
Octanoic Acid,Methyl Ester	-	0.12	-	-
Limonene Oxide	-	0.19	-	-
I-Menthone	**-**	**1.50**	0.11	**-**
Cyclopentasiloxane,Decamethyl-	0.10	**3.54**	0.07	0.08
P-Menthone	-	0.45	-	-
1-Borneol	-	0.32	-	-
1-Nonanol	-	0.11	-	-
Isopulegone	-	0.17	-	-
Terpinen-4-Ol	-	0.10	-	-

Table 1. (Continue)

Naphthalene	0.08	0.40	-	-
Benzene,1-Methoxy-4-(2-Propenyl)-	-	0.15	-	-
Dodecane	0.07	0.29	-	-
Decanal	-	0.12	-	-
Pulegone	2.69	**5.03**	0.64	0.15
Benzene,1-Methoxy-4-Methyl-2-(1-Methyletyhl)	-	0.38	-	-
2,5-Cyclohexadiene-1,4-Dione,2-Methyl-	-	0.19	-	-
Benzaldehyde,4-Methoxy-	-	0.69	-	-
Lactose	-	0.15	-	-
Hexane,3,3-Dimethyl-	-	0.06	-	-
Anethole	-	0.64	-	-
Tridecane	0.05	0.23	-	-
Dodecane,2,2,11,11-Tetramethyl-	0.15	0.21	-	-
Tridecane,3-Methyl	-	0.27	-	-
Cyclohexasiloxane,Dodecamethyl-	**-**	**3.13**	0.11	0.12
3-Hexanol,2-Methyl-5-Nitro-,(R@,R@)-	-	0.26	-	-
Benzene,2,4-Diisocyanato-1-Methyl-	**-**	0.88	**54.27**	**-**
Propanoic Acid,2-Methyl-,3-Hydroxy-2,4	-	0.21	-	-
Cyclopentasiloxane,Decamethyl-	-	0.14	-	-
Tetradecane	-	0.44	-	-
Beta-Clovene	-	0.17	-	-
Undecanal	-	0.05	-	-
Hexasiloxane,Tetradecamethyl-	-	0.09	-	-
Beta-Copaene	-	0.06	-	-
1-Butanol,4-(1-Methylethoxy)-	**-**	0.17	**14.67**	**-**
Pentadecane	-	0.10	-	-
Cycloheptasiloxane,Tetradecamethyl-	-	0.69	0.05	
A Tetradecanol	0.14	0.15	-	-
Hexadecane	-	0.13	-	-
4,4,5,6-Tetramethyltetrahydro-1,3-Oxazin	-	0.52	-	-
Phenol,2,6-Bis(1,1-Dimethlethyl)-4-	-	0.16	-	-
Trans Methyl Dıhydrojasmonat	-	0.10	-	-
Cyclooctasiloxane,Hexadecamethyl-	-	0.20	-	-
Octadecane	-	0.11	-	-
Benzoic Acid,2-Ethylhexyl Ester	-	0.26	-	-
Nonadecane	-	0.09	-	-
1-Pentanamine,N-Pentyl-	**-**	**1.60**	**-**	**-**
Hexadecanyldimethylamine	-	0.13	-	-
Hexadecanoic Acid, Methyl Ester	-	0.14	-	-
1,2-Benzenedicarboxylic Acid,Dibutyl Ester	-	0.24	-	-
Eıcosamethylcyclodecasıloxan	-	0.42	-	-
Morpholine,4-Octadecyl-	-	0.46	-	-
Dodecanoic Acid,İsooctyl Ester	-	0.55	0.15	-
Cyclooctasiloxane,Hexadecamethyl-	-	0.50	-	-
Cyclononasiloxane,Octadecamethyl-	-	0.39	-	-
Bis(Di(Trimethylsiloxy)Phenylsiloxy)Trimethyl	-	0.34	-	-
1H-Purin-6-Amine((-2-Fluorophenyl	-	0.30	-	-
Phosphine Oxide,Triphenyl-	**-**	**15.49**	**-**	**-**
1-Pentanol (Cas)	0.31	-	-	-
Butanoic Acid,2- Methyl-Methyl Ester	0.55	-	-	-
Octane	0.07	-	-	-
2- Butenoic Acid, 3- Methyl-, Methyl Ester	0.04	-	-	-
Butanoic Acid,2- Methyl- Ethyl Ester	0.06	-	-	-
Benzene,1,4-Dimethyl-	0.34	-	-	-
(-)-Beta-Pinene	0.09	-	-	-
Tricyclene	0.12	-	-	-
Alpha-Thujene	**4.21**	**-**	**-**	**-**
Bicyclo (2.1.0)Hex-2-Ene,4-Methylene-1-	0.11	-	-	-
Camphene	**2.23**	**-**	**-**	**-**
Sabinene	0.20	-	-	-
Beta-Pinene	0.75	-	-	-
Ethyl Amyl Carbınol	0.21	-	-	-
Alpha-Phellandrene	0.48	-	-	-
Delta.3 Carene	0.41	-	-	-
Alpha Terpinene	**2.55**	**-**	**-**	**-**
Cis-Ocimene	**5.93**	**-**	**-**	**-**
Cyclohexene, 1- Methyl-4-(1-Methylethyl)	0.28	-	-	-
1,6-Octadien -3-Ol,3,7-Dimethyl	**2.10**	**-**	**-**	**-**
Alloocimene	0.38	-	-	-
2,4,6- Octatriene,3,4-Dimethyl-	0.25	-	-	-
Cyclohexanone,5- Methyl- 2-(1-Methylethyl)	0.86	-	-	-
Cyclohexanone, 5- Methyl-2-(1-Methyl)	0.23	-	-	-
Cıs- Isopulegone	0.04	-	-	-
Dodecane	0.09	-	-	-
Carvacrol Methyl Ether	0.19	-	-	-
2,5- Cyclohexadiene-1,4-Dione,2-Methyl	0.19	-	-	-
Tetradecane,2,2-Dimethyl-	0.16	-	-	-
Longifolene	0.07	-	-	-

Table 1. (Continue)

Laurınsaeure , 4- Octylester	0.57	-	-	0.05
Formic Acid,Hexyl Ester	-	-	0.03	-
4-Ethylbenzoic Acid,Cyclopentyl Ester	-	-	0.06	-
Beta-Ocimene	-	-	0.06	-
2-Ethoxyethyl Acrylate	-	-	0.61	-
Benzene,1-Methoxy-4-(2-Propenyl)-	-	-	0.12	-
1,3-Benzenediamine,4-Methyl-	-	-	0.24	-
7-Amino-1,3-Dihydro-İndol-2-One	-	-	0.26	-
Pyridine,4-(1-Pyrrolidinyl)-	**-**	**-**	**8.13**	**-**
Pyrrole-3-Carbonitrile,5-Formyl-2,4	**-**	**-**	**18.41**	**-**
3H-Imidazo(4,5-F)Quinoline,2-Amino-3	-	-	0.11	-
1,4-Dıbutoxybutane	-	-	0.08	-
Benzophenone	-	-	0.21	-
3H-Imidazo(4,5-F)Quinoline,2-Amino-3	-	-	0.06	-
1(2H)-Quinolinecarboxylic Acid,6-Amino	-	-	0.32	-
Formic Acid,2-Methylhex-3-Yl-Ester	-	-	0.19	-
Trans Beta-Ocimene	-	-	-	0.04
1,2-Benzenedicarboxylic Acid,Diethyl Ester	**-**	**-**	**-**	**98.90**
7,9-Di-Tert-Butyl-1-Oxaspirp(4,5)Deca-6,9	-	-	-	0.15
2-Ethylhexyl Methyl İsophthalate	-	-	-	0.05
The number of identified compound	**54**	**97**	**28**	**12**

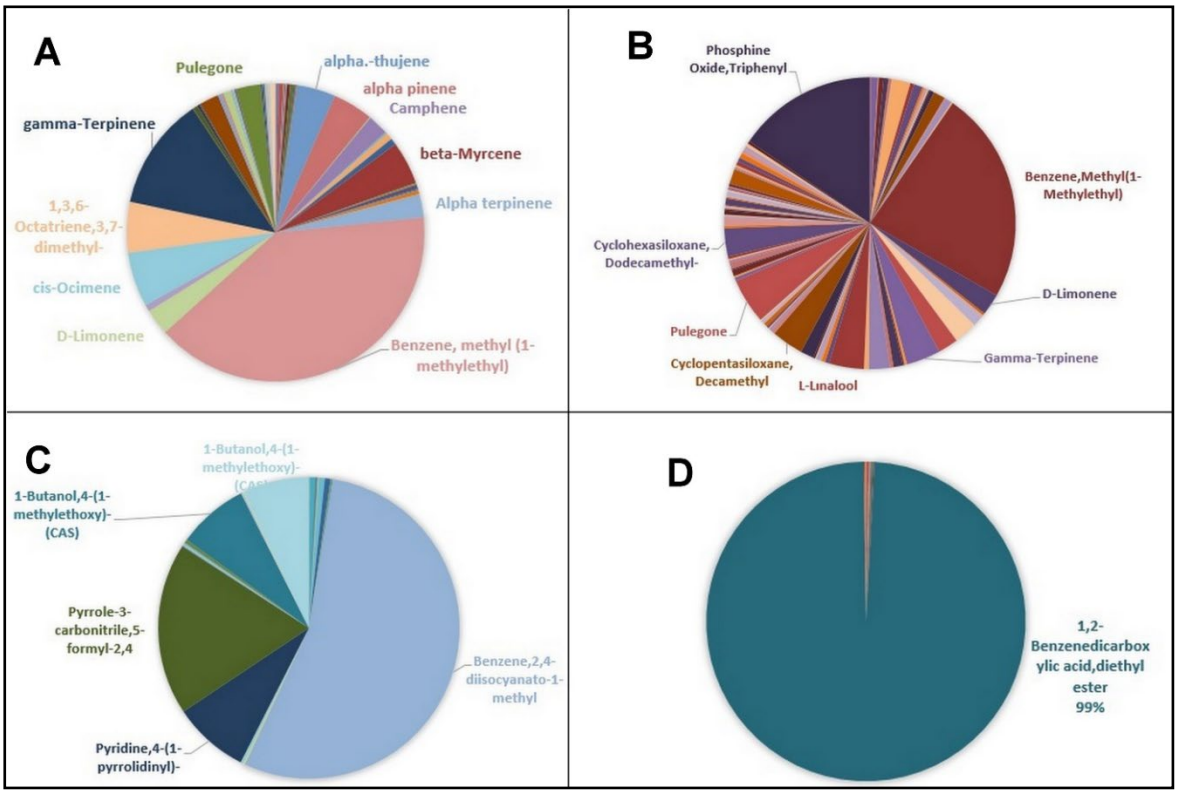

Figure 2. The pie charts of most found compounds in studied samples

4. Discussion

As far as we know, there are limited studies to reveal the content and the volatile composition of local lupin seeds. Some of the work on this subject is inadequate. The phenolic compound profiles and antioxidant capacities of wild and varieties *L. albus* L. seeds were determined before. The total phenolic content (TPC), antioxidant activity, radical scavenging activity and ferric-reducing antioxidant power (FRAP) in a beta-carotene-linoleic acid emulsion have been detected (Karamać et al., 2018). Also, the nutritional and chemical properties of white lupin (*L. albus* L.) were characterized via the HPLC system (Erbaş et al., 2005). Straková et al. (2006) the nutritional composition of the Lupinus genus seeds was reported in the study. They compared Lupin seeds and soybean and reported Lupin seeds significantly exceeded the content of crude protein in

soybeans. In the other study, the chemical composition of a new Lupin species from Spain; Lupinus mariae-josephi has been revealed. The chemical composition of this species is found similar to other lupin species in terms of total protein, oil, and alkaloid contents . Liquid chromatography-mass spectrometry was used to identify phenolic compounds (Múzquiz et al., 2011). Some physical properties and nutritional compositions of lupin (*L. albus* L.) seeds in Türkiye were reported (Yorgancılar et al., 2018). Similarly, the mineral content of debittered white lupin (*L. albus* L. local genotypes) seeds was determined (Yorgancilar et al., 2009). In addition, the nutritional and chemical changes of bitter and sweet lupin (*L. albus* L.) bulgur products were determined Yorgancilar and Bilgiçli (2014). In another study conducted in local lupin seeds (*L. albus* L.) the metabolite content was determined and the mite and insecticidal effects of the seed extract were investigated (Elma et al., 2021).

5. Conclusions

In this study, local lupin seeds from the Konya region were analyzed using four different fibers via SPME-GC/MS. According to the analysis with SPME fibers, we can clearly say that PDMS/DVB is the most preferable fiber in terms of compound identification. As far as we know this study is the first study about the volatile composition of local lupin genotype from Türkiye and characterization of seed content is very important for breeding and crossing studies.

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Research Article

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Effects of Supplementation Black Soldier Fly Larvae (*Hermetia illucens* L.) to The Diets of Breeder Japanese Quails on Performance, Egg Quality, and Incubation Parameters

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HIGHLIGHTS

- The nutritional composition of the black soldier fly, one of the edible insects, is quite rich.
- Black soldier fly has a high potential as an alternative feed source.
- In the experiment, the effects of black soldier fly larvae used in breeder quail diets were determined.

Abstract

This study was performed to assess the effect of different levels of addition (0, 4, and 8%) of dried and ground Black Soldier Fly Larvae *(Hermetia illucens* L.) to breeder Japanese quail (Coturnix coturnix japonica) diets on performance, egg quality and incubation parameters. In the experiment, 72 breeder Japanese quails (18 males, 54 females) at 8 weeks of age were randomly distributed into 3 treatment groups with 6 replications, each with 3 females and 1 male quail. Treatment groups were fed with diets supplemented with different levels of black soldier fly larvae (BSF) for 8 weeks.Feed intake was significantly higher in the control group than in the other treatment groups, and there were no significant differences between the groups in feed conversion ratio, egg production, egg weight, and egg mass. Similarly, no significant differences were observed among the groups in terms of egg quality parameters such as eggshell thickness, eggyolk index, eggshell breaking strength, and eggshell weight parameters. However, the shape index was significantly higher in the BSF 4% group than the control group. Albumen index was highest in the control group. While there was no significant difference among the groups in terms of egg yolk L* value, as the amount of BSF in the diet increased, there was a significant increase in the a* and b* values. Incubation parameters were not affected by the treatments. According to the results of the experiment, it was concluded that the addition of 4% and 8% BSF to breeder Japanese quail diets can be used at 8% level without any negative effects on performance, egg quality, or incubation parameters.

Keywords: Breeder quail, Black soldier Fly (*Hermetia illucens L.*) larvae, egg quality, incubation parameters, performance

1. Introduction

The Food and Agriculture Organization of the United Nations has recognized the potential of using edible insects for food and feed since 2003 and supports the production and sharing of knowledge through

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publications (Mishyna et al. 2020). Insects have the potential to be a possible alternative food source that can help meet the increasing demand and prices of traditional feed raw materials needed in the livestock industry in a more sustainable way(Cullere et al. 2016). Insects are rich in fat, amino acids, carbohydrates, amino acids ,trace elements, and vitamins(Skotnicka et al. 2021). In addition to meeting the required amino acid requirements due to the nutritional content they contain; most insects are also sufficient in terms of energy and protein values, and also rich in fatty acids. Trace elements (such as Cu, Fe, Mg, Mn, P, Se, and Zn) and vitamin contents such as riboflavin, pantothenic acid, biotin, and folic acid are also very high (Rumpold and Schlüter 2013). Oil is found in various forms in insects. Triacylglycerol makes up approximately 80% of the oil present in insects. Phospholipids are the second most important group and their proportion in insect oil is generally less than 20% (Kouřimská and Adámková 2016). Black soldier fly is rich in lauric acid and has been reported to be a suitable lipid source in poultry diets (Schiavone et al. 2017; Kierończyk et al. 2020). The amino acid profile of black soldier fly is similar to fishmeal (Barroso et al. 2014). This study was carried out to determine the effects of dried Black Soldier Fly larvae (BSF) on egg quality, performance, and incubation parameters of 4% and 8% supplementation to breeder quail diets grown with high yield expectation. The suitability of BSF as a feed raw material with high energy and protein values, especially rich in essential amino acids, which can positively affect the yield of animal products was investigated.

2. Materials and Methods

The trial was carried out at the Quail Unit of the Department of Animal Science, Faculty of Agriculture, Selcuk University (Konya, Turkey). The animal experiment was conducted according to the guidelines of the local ethics committee of Selçuk University that were arranged according to the "European Union 2010/63/EU is the European Union (EU) legislation." All processes in this experiment agree with the ethical rules of animal welfare. A total of 72 breeding Japanese quails (54 females and 18 males) at 8 weeks of age were used in the experiment. Quails were randomly allocated to 3 treatment groups with 18 subgroups of 3 females and 1 male each. Breeder quail feed was prepared to contain 2900 Kcal/kg ME and 20% crude protein during the eight-week experiment (NRCCouncil 1994). In the experiment, the basal diet (0%) was prepared based on corn and soybean meal containing dried and ground Black Soldier Fly larvae (BSF;*Hermetia illucens* L.) at 4% and 8% levels (Table 1).

Table 1. Nutrient content of experimental diets

Ingredients	Black Soldier Fly Larvae Meal %		
	0	4	8
Corn	50.1	52.60	54.60
Black soldier fly larvae meal	0	4.0	8.0
Soybean meal	36	31.2	27.00
Soyoil	6.2	4.5	2.7
Limestone	5.10	5.10	5.10
Dicalcium phosphate(DCP)	1.8	1.8	1.80
Salt	0.25	0.25	0.25
Premix[1]	0.25	0.25	0.25
L-Lysine	0.10	0.10	0.10
DL-Methionine	0.20	0.20	0.20
Calculated nutrients			
Crude protein, %	20.00	20.03	20.29
Metabolizable Energy, kcal/kg	2904	2922	2929
Calcium, %	2.50	2.49	2.48
Available phosphorus, %	0.35	0.35	0.34
Lysine, %	1.04	0.94	0.89
Methionine, %	0.47	0.45	0.44
Methionine-cysteine, %	0.70	0.70	0.70

[1]: Premix provided the following per kg of diet: vitamin D_3: 2.200 IU, vitamin E: 11 mg, vitamin A: 8.800 IU, nicotinic acid: 44 mg, Cal-D-pantothenate: 8.8 mg, thiamine: 2.5 mg, vitamin B_{12}: 6.6 mg, riboflavin: 4.4 mg, folic acid: 1 mg, D-biotin: 0.11 mg, choline: 220 mg, iron: 60 mg, zinc: 60 mg, manganese: 80 mg, copper: 5 mg, selenium: 0.15 mg, cobalt: 0.20 mg, iodine: 1 mg, BSF: dried black soldier fly larvae

Dried and ground black soldier fly larvae purchased from a commercial company were used.The duration of the experiment was 8 weeks. A lighting program of 16 hours light / 8 hours dark was applied in the experiment. Feed and water were given ad-libitum. Body weight (BW) of quails were determined at the beginning and the end of the experiment by weighing the quails in a group. Egg production (EP) and feed intake (FI) were recorded daily. Egg weight (EW) was calculated by averaging the weights of all eggs collected in each subgroup during the last 2 days of each four-week period. Egg mass (EM) was calculated using the formula egg production×egg weight/period. Feed conversion ratio (FCR) was calculated using the ratio of feed intake to egg mass. Eggshell breaking strength, shell thickness, shell weight, egg weight, shape index, yolk and albumen index, Haugh unit were determined in the eggs collected on the last 2 days of each 4-week period during the experiment. Measurements were made twice and the results are given as the average of these two measurements. Egg shell weight (%) was calculated by the formula egg shell weight (g)/egg weight x 100. Egg shell breaking strength was measured by applying pressure to the blunt part of the egg with an assisted system (Egg Force Reader, Orka Food Technology, Israel). Egg shell thickness was calculated by averaging the numbers obtained from three points of the egg using a micro meter (Mitutoyo, 0.01 mm, Japan). Egg yolk color was measured as L*, a* and b* values by colorimeter (KonicaMinolta CR410).

At the end of the experiment, the eggs collected for 7 days were placed in the incubator with incubation parameters. After the seventeenth day, the number of hatched chicks and after the twentieth day, the number of fertilized and unfertilized eggs were determined by breaking the unhatched eggs. Hatching efficiency, fertility rate and hatchability were calculated according to the data obtained(Erensayın 2000). Statistical analysis of the data obtained from the experiment was performed according to one-way analysis of variance (ANOVA) (Minitab 2000) and Duncan multiple comparisons test was used to determine the differences between the means.

3. Results

The results of the effects of dried and ground BSF at 0%, 4% and 8% levels on BWC, FI, FCR, EP and EM parameters of broiler Japanese quail diets are given in Table 2.

Table 2. The effect of dietary different levels of BSF on the performance parameters of breeder Japanese quails

Parameters	Control	BSF **4 %**	BSF **8 %**	P-Value
Initial body weight (g)	212.17 ± 3.34	208.71 ± 4.10	204.92 ± 4.43	0.456
Final body weight (g)	228.39 ± 2.76	226.50 ± 3.80	223.85 ± 4.53	0.699
Body weight change(g)	16.222 ± 3.16	17.792 ± 4.41	18.931 ± 3.38	0.874
Feed intake (g)	26.55 ± 0.32^{A}	24.90 ± 0.31^{B}	24.89 ± 0.31 ^{B}	0.003
Feed conversion ratio	2.64 ± 0.07	2.45 ± 0.05	2.45 ± 0.05	0.073
Egg production (%)	85.21 ± 1.48	83.53 ± 0.71	83.43 ± 0.49	0.382
Egg weight(g)	11.818 ± 0.288	12.179 ± 0.268	12.162 ± 0.209	0.548
Egg mass(g/quail/day)	10.06 ± 0.22	10.17 ± 0.26	10.14 ± 0.18	0.932

^{A,B}: The differences indicated by different letters on the same row are statistically significant, P<0.01

The effect of different levels of BSF supplementation to the diet on BWC, FCR, EP, EW and EM parameters was not statistically significant (P>0.05). The highest feed intake was in the control group. Feed intake in the control group was significantly higher than BSF 4% and BSF 8% groups (P<0.01). There are studies reporting that the addition of BSF in various forms (live, larvae, etc.) to broiler diets resulted in higher feed intake in the control group compared to the treatment groups(Murawska *et al.* 2021; Mat *et al.* 2022). However, there are studies with laying hens that report that feed intake is not affected(Maurer *et al.* 2016; Marono *et al.* 2017; Bellezza Oddon *et al.* 2021; Elangovan *et al.* 2021; Yan *et al.* 2023). In the experiment in which the effects of black soldier fly maggot substitution instead of fish meal in laying quails were investigated, it was reported that feed intake decreased in the treatment groups compared to the control group, but there was no significant effect on egg production(Widjastuti *et al.* 2014). Addition of defatted black soldier fly larvae meal up to 15% to laying quail diets did not cause any negative effects and it was concluded that it could be a potential substitute for soybean meal. No negative effects on hatching performance and egg quality were observed(Dalle Zotte *et al.* 2019). It has been reported that defatted black soldier fly (*Hermetia illucens*) larval

meal can be used as a protein source in laying hens without adverse effects on animal health and has positive effects on immunity(Marono *et al.* 2017). In the present study, the addition of dried BSF larvae at 4% and 8% levels to breeder Japanese quail diets had no negative effect on performance. The effects of addition of dried and ground BSF to breeder Japanese quail diets on egg quality parameters, yolk L*, a* and b* values are given in Table 3. The effect of BSF addition to breeder Japanese quail diets on egg shell breaking strength, shell thickness, shell weight, shell ratio, yolk index, Haugh Unit and L* values were not statistically significant (P>0.05). In terms of shape index, the statistical difference between BSF 4% group and BSF 8% group was not significant, while the difference between BSF 4% group and control group was significant (P<0.05). The difference between BSF 8% group and control group was insignificant (P>0.05). While the difference in albumen index between the control and BSF 8% group was insignificant (P>0.05), the difference between the control and BSF 4% group was significant (P<0.05). The difference between BSF 4% and BSF 8% groups was also insignificant (P>0.05). There was an increase in a* value with increasing BSF in the diet. The difference between BSF 8% group and BSF 4% group in a* value between the treatment groups was insignificant, while the difference between BSF 8% group and control group was significant (P<0.05). The difference between the BSF 4% group and the control group was insignificant (P>0.05). The highest b* value was measured in BSF 8% group. Egg yolk b* value showed similar results to a*, and b* increased in direct proportion to the increasing amount of BSF in the diet.

Table 3. The effect of dietary different levels of BSF on the egg quality, yolk L*, a* and b* values of breeder Japanese quails

	Control	BSF 4 %	BSF 8 %	P-Value
Eggshell breaking strength (N)	11.720 ± 0.514	12.133 ± 0.727	12.962 ± 0.543	0.357
Eggshell thickness (mm)	0.229 ± 0.008	0.221 ± 0.002	0.214 ± 0.001	0.135
Eggshell weight (g)	1.116 ± 0.084	1.103 ± 0.031	1.144 ± 0.017	0.856
Eggshell ratio (%)	9,453 ± 0,579	9,111 ± 0,274	9,472 ± 0,298	0,783
Egg shape index (%)	75.611 ± 0.328 ^{b}	77.337 ± 0.378 ^{a}	76.658 ± 0.571^{ab}	0.042
Albumen index (%)	6.3208 ± 0.163 ^{a}	5.6983 ± 0.102 ^{b}	6.0176 ± 0.130 ^{ab}	0.017
Eggyolk index (%)	47.052 ± 1.02	44.550 ± 1.59	48.430 ± 0.334	0.072
Haugh Unit	92.642 ± 0.649	90.546 ± 0.263	92.165 ± 0.799	0.069
L*	41.845 ± 0.83	42.905 ± 0.49	43.732 ± 0.78	0.211
a*	5.827 ± 0.08^{b}	6.1700 ± 0.27^{ab}	6.555 ± 0.13^{a}	0.039
b*	21.324 ± 0.79^{b}	23.079 ± 0.53^{ab}	24.641 ± 0.55^{a}	0.029

^{A,B}: The differences indicated by different letters on the same line are statistically significant, P<0,01

^{a,b}: The differences indicated by different letters on the same row are statistically significant, P<0.05

Hopley (2016) reported that the addition of BSF larvae and prepupal BSF to laying hen did not affect egg albumen height and albumen weight, yolk L*, significantly increased the a* value and increased the b* value numerically but not statistically significant. Addition of black soldier fly to laying hen diets has been reported to result in brighter egg yolk compared to the control group(Al-Qazzaz *et al.* 2016). It was shown that the addition of BSF larvae instead of fish meal to laying quail diets had no significant effect on egg weight, Haugh unit, yolk index and yolk color, but it significantly affected egg production and eggshell thickness(Purwanti and Nahariah 2020). Finke (2013) stated that black soldier fly larvae contain the carotenoids beta-carotene, lutein and zeaxanthin and that these carotenoids are generally responsible for the yolk pigmentation in feeds. This may be the possible reason for the increase in egg yolk a* and b* value.

The results of the effects of dried and ground black soldier fly larvae at 4% and 8% levels on the incubation parameters of broiler Japanese quail diets are given in Table 4.

Table 4. The effect of dietary different levels of BSF on incubation parameters of breeder Japanese quails

	Control	BSF 4 %	BSF 8 %	P-Value
Hatching weight (g)	7.93 ± 0.163	8.24 ± 0.116	8.35 ± 0.235	0.259
Hatchability (% of fertile eggs)	98.61 ± 1.39	97.10 ± 1.84	98.61 ± 1.39	0.733
Fertility (%)	98.61 ± 1.39	98.61 ± 1.39	97.22 ± 1.76	0.761
Hatchability(% of setting eggs)	97.22 ± 1.76	95.83 ± 2.85	95.83 ± 1.86	0.878

The treatment groups in terms of incubation parameters such as chick's weight, hatchability and fertility were found to be insignificant (P>0.05). It was reported that the addition of black soldier fly larvae to laying hen diets instead of 5% of soybean meal did not affect fertility and hatchability(Petkov *et al.* 2022). In this study, the addition of black soldier fly larvae to the diet did not have any negative effect on incubation parameters

4. Conclusions

In conclusion, the supplement of BSF meal to the diets of breeder Japanese quail did not have any detrimental impact on performance, egg quality and incubation parameters. There is a need for research on the use of black soldier fly, which has a high potential as an alternative feed source, in animal nutrition. As a result of the experiment, the use of dried and ground BSF meal 8% level in breeder Japanese quail diets can be recommended.

Author Contributions: The authors have an equal contribution. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Research Article

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General Characteristics of Konya Agricultural Machinery Manufacturing Industry

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HIGHLIGHTS

- The primary objective is to investigate general characteristics of Konya agricultural machinery manufacturing industry.
- Competitive power of the enterprises in international markets should be increased.

Abstract

A survey was conducted in this study to determine the general characteristics of the agricultural machinery manufacturing industry in the Konya region. To achieve this, 50 enterprises of various scales, considered representative of the sector, were selected and included in the study. When evaluating the enterprises based on size, it was found that 52% of them were small-scale enterprises. The average age of business owners is 53 years, with 35 years of industry experience, and 47% of them have primary school education. While a total of 1204 workers are employed in the enterprises participating in this study, there is an average of 24.5 workers per enterprise. Of the total personnel, 78.3% consisted of workers, followed by engineers with an average of 5.5%, accountants with 5.3%, marketers with 5% and technicians with 4%. Only 34% of enterprises cooperate with KOSGEB and 8% with TUBITAK in R&D and P&D activities. In addition, it was determined that 18% received consultancy from universities. Present findings shed light on the developments in the sector as well as understanding the situation of agricultural machinery manufacturing industry enterprises in the region.

Keywords: Agricultural machinery manufacturing industry, Agricultural machinery manufacture, Konya

1. Introduction

Agriculture is among the most important sectors in Türkiye and agricultural machinery manufacturing sector is a locomotive of this sector. According to 2022 data, agricultural production is carried out on an area of 23.8 million hectares in Turkey. About 69% of the total area is occupied by field crops, 31% by vegetables, ornamental plants, fruits and fallow lands (TUIK, 2022b).

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Agricultural mechanization plays a great role in reduction of labor needs in agricultural production. Agricultural mechanization is defined as the mechanization of plant and animal production activities using power sources and agricultural machinery.

Agricultural technologies and mechanization practices increase productivity in agricultural production and reduce total input costs. However, technology use in agriculture is affected by labor demand, climate parameters and land characteristics. Agricultural mechanization is an important tool for optimum use of input materials such as fertilizers, pesticides and seeds and provides significant contributions to yield and productivity (Özgüven ve ark., 2010).

With a land area of 1.78 million hectares, Konya constitutes about 7% of total agricultural lands of Türkiye (TUIK, 2022b). Agricultural machinery manufacturing sector is also highly important for Konya since the province is one of the important agricultural centers of the country.

Agricultural machinery manufacture in Konya is composed of manufacture of tractors, soil cultivation machines, planting machines, plant protection machines, fertilizing machines, harvest and threshing machines, irrigation machines, agricultural machinery sub-industry and spare parts, livestock mechanization machinery.

Majority of agricultural machinery companies operating in Konya province has quite a high export capacity and they export agricultural machinery to several countries. Agricultural machines produced by businesses operating in this sector are used both in Türkiye and around the world.

Agriculture is one of the largest export sectors in Türkiye and the agricultural machinery manufacturing sector of Konya constitutes an important part of this export. In short, Konya has an important place in the agricultural machinery manufacturing sector and this sector provides a significant contribution to agricultural sector and economy of the country.

2. Materials and Methods

Primary data obtained through questionnaire made with the agricultural machinery manufacturing enterprises of Konya constituted the primary material of this study. A questionnaire form was prepared to get relevant data from participating enterprises.

In 2022, 870 enterprises evaluated by the Central Bank of the Republic of Türkiye (CBRT) for the agricultural machinery manufacturing sector, 147 of them are joint stock companies and 721 of them are limited liability companies, 527 of which are micro, 265 are small, 63 are medium and 15 are large enterprises (TCMB, 2021).

Data of the Ministry of Agriculture and Forestry indicated that there were test reports of 797 enterprises (importer-manufacturer) in Türkiye between the years 2015-2020, 190 of them were operating in Konya province and Konya province was in the first place in terms of the number of enterprises that received test reports (Atasoy, 2021).

Questionnaires were applied through face-to-face meetings with managers of 50 different business of different scales, which are thought to represent the sector. Their working and production processes were observed by the researchers themselves and the necessary information and data were obtained. The data obtained through questionaries belong to the year 2022.

Within the scope of the questionnaires, questions were asked to gather information about the company, foreign trade, machinery and equipment inventory, number of employees and their competencies and problems encountered. Questionnaire forms were filled out based on the information provided.

With its rapidly developing and dynamic industry, Konya province has taken an important place in the industrial production of Türkiye. Parallel to development of the manufacturing industry; variety of industrial products has increased in the province and the product range that is subject to domestic and foreign trade has expanded.

In terms of the goods subject to trade in Konya province, agricultural and animal products continue to be important; agricultural machinery, automotive sub-industry, rubber-plastic products, shoes, furniture, agricultural products, iron-steel products, manufacturing machinery, textile-clothing, salt, aluminum, marble are industrial products that are the subject of trade (Özkul ve Güzel, 2020).

Konya province maintains its leading position in export of agricultural machinery, metalworking, metal casting, on-board equipment production, production and export of shotguns (Özkul ve Güzel, 2020).

In Konya province, there are 9 active and 2 still inactive organized industrial zones, 1 organized industrial zone at the establishment stage, 80 industrial sites and 2 industrial zones, 17 of which were built with the support of the Ministry of Industry and Technology. There are over 16.000 workplaces in industrial sites and approximately 114,000 people are employed (Anonymous, 2022a).

Providing suitable environments for investors at the investment and production stages, organized industrial zones, small industrial sites and other industrial sites in the form of collective workplaces are of great importance in the development of industry in Konya province.

Konya Agricultural Machinery Specialized Organized Industrial Zone held a site selection meeting for 313 ha area on 26/10/2017. Opinions of institutions have been completed, except for the Provincial Directorate of Agriculture and Forestry (it has been requested that the land be registered in the name of the Treasury by making a change in quality) and the establishment process is continuing (Anonymous, 2022a).

The largest agricultural machinery specialization Fair of Türkiye is also organized in Konya province. Konya Agriculture Fair has a total exhibition area of 90,000 m², of which 66,000 m² is closed in 7 different halls. In 2022, with the participation of 461 businesses and business representatives from 20 countries, it hosted 207.133 visitors from 90 countries and 81 provinces (Anonymous, 2023b).

The trend for foreign trade of Konya province for the years between 2013-2022 is presented in Figure 1.

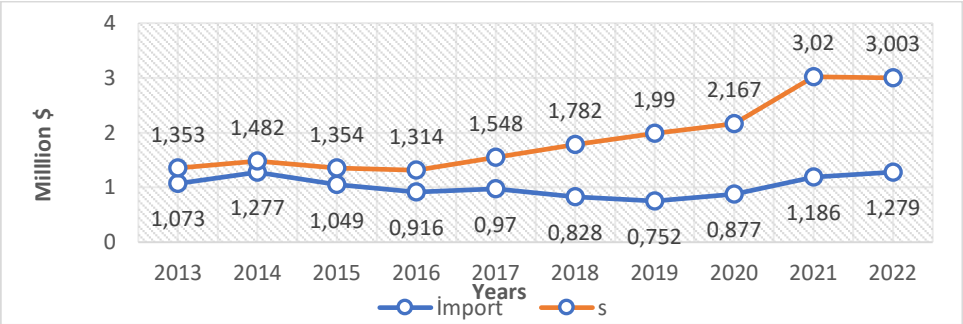

Figure 1. Foreign Trade Trend of Konya Province (2013-2022) (TUIK, 2022a)

According to foreign trade data of Türkiye for the year 2022; 3 billion dollars of 232.2 billion dollars export of the country were carrieda out by the businesses registered in Konya province. This value is the highest value reached by provincial exports.

Table 1. Foreign trade of Konya province based on top 10 countries (TUIK, 2022a)

Export		
Order	**Country**	**Ratio (%)**
1	Iraq	8.84
2	Germany	7.44
3	Russia	6.75
4	USA	5.84
5	Italy	3.5
6	Poland	3.19
7	Romania	2.71
8	Egypt	2.68
9	Israel	2.68
10	Algeria	2.61
	Total	46.2

Import		
Order	**Country**	**Ratio (%)**
1	China	23.6
2	Russia	11.6
3	Ukraine	6.71
4	Germany	4.42
5	South Korea	4.06
6	Italy	3.98
7	India	3.4
8	Saudi Arabia	2.78
9	Vietnam	2.44
10	Indonesia	2.33
	Total	65.3

Imports of Konya province showed an increasing trend until 2014 and then started to decrease. Provincial imports reached their highest value in 2014 with 1.3 billion dollars. According to the foreign trade data of 2022, 1,279 billion dollars of Türkiye's imports of 363 billion dollars were realized by businesses registered in Konya province(TUIK, 2022a).

Considering the foreign trade performance of the provinces in Türkiye in 2022; Konya ranks 12th with a 1.29% export share and 20th with a 0.38% import share. Konya province exports are mostly made to the European Union and Middle East countries and neighboring countries. Imports are made from South Korea, Russia and China. The foreign trade of Konya province on the basis of the first 10 countries is provided in Table 1. In 2022, the share of exports to the first ten countries in the foreign trade of Konya is 46.2%, while the share of imports is 65.3% (TUIK, 2022a).

The countries with the highest exports in 2022 are Iraq with 263.5 million dollars, Germany with 221 million dollars, the Russian Federation with 201.3 million dollars, the USA with 174 million dollars and Italy with 104 million dollars.

The countries with the highest imports are respectively ordered as China with 328.7 million dollars, Russian Federation with 162.3 million dollars, Ukraine with 93.5 million dollars, Germany with 61.6 million dollars and South Korea with 56.6 million dollars.

3. Results and Discussion

Agricultural machinery manufacturing enterprises participating into the survey in the Konya Region were evaluated based on the number of employees and classified as micro-scale, small-scale, medium-scale and large-scale enterprises.

Micro-enterprise: Businesses that employ less than ten people annually and whose annual net sales revenue or financial balance sheet does not exceed five million Turkish Liras. Small business: Businesses that employ less than 50 people annually and whose annual net sales revenue or financial balance sheet does not exceed fifty million Turkish Liras. Medium-scale enterprise: Businesses that employ less than 250 people per year and whose annual net sales revenue or financial balance sheet does not exceed two hundred and fifty million Turkish Liras. Large enterprise: Businesses that employ more than 250 people and whose annual net sales revenue or financial balance sheet exceeds two hundred and fifty million Turkish Liras (Anonymous, 2023a).

Agricultural machinery manufacturing enterprises participating into the survey in the Konya Region were evaluated based on the number of employees and it was determined that 32% were composed of micro-scale, 52% small-scale, 14% medium-scale and 2% large-scale enterprises.

Established in 1978, Turkish Agricultural Equipment and Machinery Manufacturers Association (TARMAKBİR) is an important non-governmental organization for the agricultural machinery manufacturing industry with its sector meetings, training projects and university-industry cooperations. It has 195 members in Türkiye and most of its members operate in Konya province (Anonymous, 2023c).

The membership status of the surveyed enterprises is presented in Figure 2. All of the businesses are registered with the Konya Chamber of Commerce. It was determined that 47% of the enterprises are also registered with the Konya Chamber of Industry. It was also determined that 12.5% of Micro-Scale enterprises, 54% of Small-Scale enterprises and all Medium-scale enterprises are registered with Konya Chamber of Industry. Only 30% of the enterprises are members of the Turkish Agricultural Equipment and Machinery Manufacturers Association.

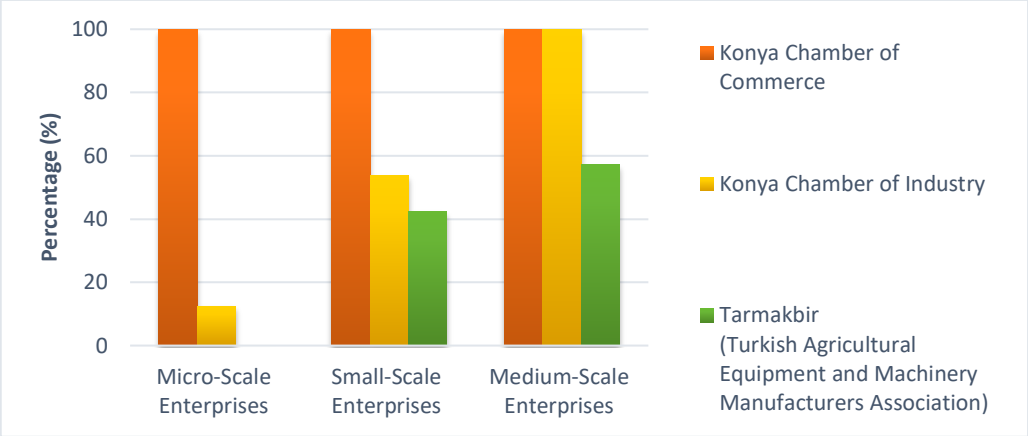

Figure 2. Membership status of surveyed enterprises to chambers and associations

In general, 90% of agricultural machinery manufacturing enterprises are operated with equity capital and 10% benefit from financial credit systems. This situation is shown in Table 2. It was determined that 20% of the enterprises benefiting from the financial credit system used 10% loans in their capital, 20% used 20% loans and 60% used 50% loans in their capital.

Table 2. Benefiting from Financial Credit Systems

	%	Number of Enterprises
Using credit in enterprise capital	10	5
Not-using credit in enterprise capital	90	45

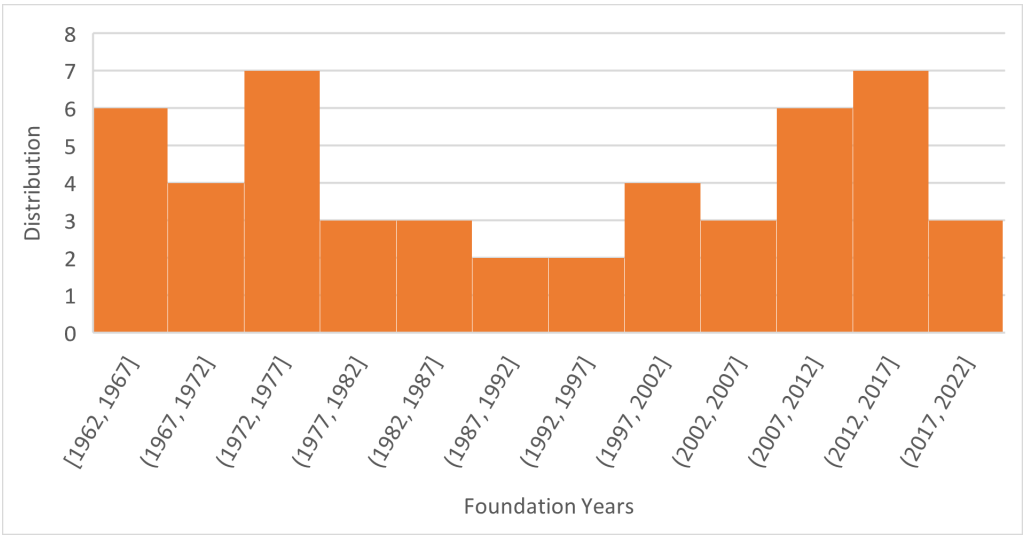

Figure 3. Frequency distribution of the enterprises based on foundation years

Establishment of agricultural machinery manufacturing enterprises of Konya province date back to 1692 and frequency distributions of the enterprises based on foundation years are presented in Figure 3.

Table 3. Corporate status of businesses

	%	Number of Enterprises
Non-corporate	46	23
Corporate	54	27

In terms of corporate status of the participating enterprises, it was determined that 46% of the enterprises are non-corporate and 54% are corporate enterprises. This situation is shown in Table 3. According to table 4,

it has been determined that 55% of the partnerships are between siblings, 22% are father-son/daughter partnerships, 18% are non-family partnerships and 5% are joint partnerships.

Table 4. Partnership status of the enterprises

	%	Number of Enterprises
Father – Son/Daughter	22	6
Siblings	55	15
Spouse	5	1
Non-family	18	5

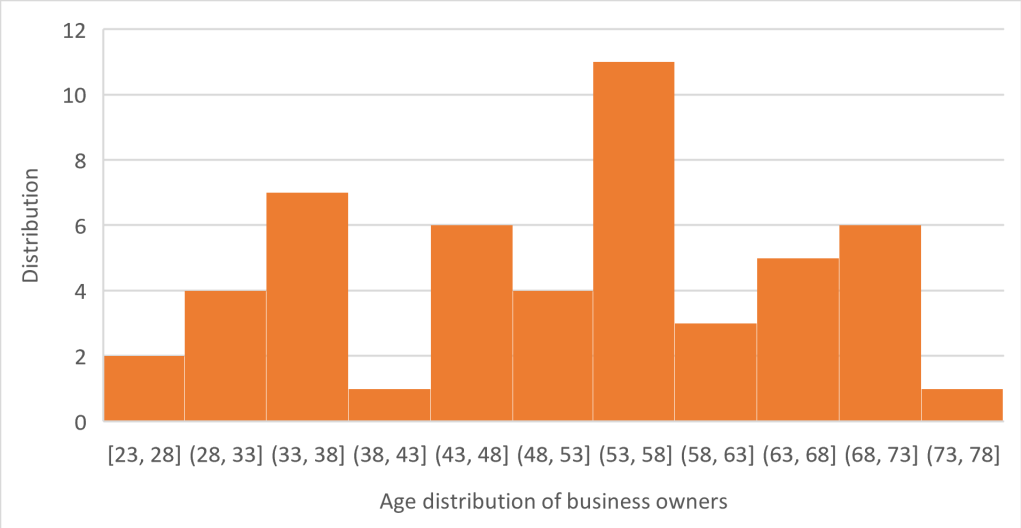

Figure 4. Frequency distribution of enterprises based on owner's age

Frequency distribution of agricultural machinery manufacturing enterprises based on owner's age is presented in Figure 4. It was determined that the average age of business owners was 53, the youngest business owner was 23 and the oldest was 78.

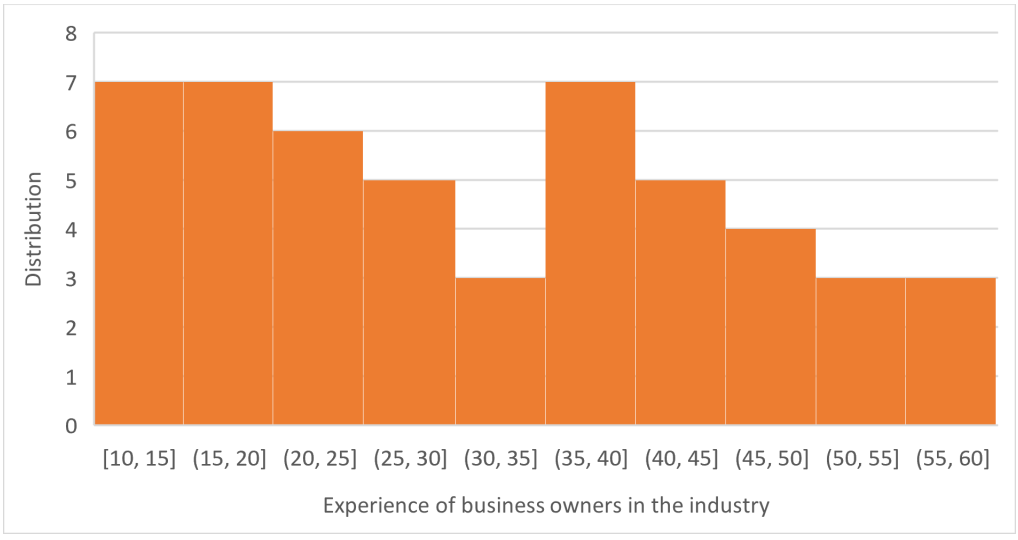

Figure 5. Frequency distributions of the sector experiences of the business owners

The frequency distributions of the sector experiences of the business owners are presented in Figure 5. It was determined that the average experience period was 35 years. The average sector experience was 33 years in micro-scale enterprises, 30 years in small-scale enterprises and 42 years in medium-scale enterprises.

When the agricultural machinery manufacturer business owners are examined in terms of education level, it was determined that 46% of the business owners were primary school graduates, 10% were secondary school

graduates, 24% were high school graduates, 12% had an undergraduate degree and 6% had graduate degree. It is given in Table 5.

Table 5. Educational level of business owners

	Primary School	Secondary School	High School	Undergraduate	Graduate
Micro-scale	7	4	4	1	
Small-scale	11	1	8	4	2
Medium-scale	6	-	-	1	1
Total	23	5	12	6	3
%	46	10	24	12	6

When the agricultural machinery manufacturing enterprises are evaluated in general, it was determined that 20.4% of the enterprises have professional managers, while 79.6% of them do not have professional managers. According to Table 6, while there are no professional managers in micro-scale enterprises, 19.3% of small-scale enterprises and 71.4% of medium-sized enterprises have professional managers.

Table 6. Professional administrator status of the enterprises

	Micro Scale	Small Scale	Medium Scale	Total %
Number of enterprises	-	5	5	10
%	-	19.3	71.4	20.4

It was determined that 40% of the enterprises are operating in rented facilities and 60% of them own their buildings. According to Table 7, it was determined that the rental rate was 56% in micro-scale enterprises, 30% in small-scale enterprises and 50% in medium-sized enterprises.

Table 7. Rental/ownership status of enterprise facilities

	Rental	Owner	Total
Micro Scale	9	7	16
Small Scale	8	18	26
Medium Scale	4	4	8
%	40	60	100

The average closed area is 4654 m^2 and the open area is 4740 m^2. The average closed area is 1216 m^2 in the micro-scale enterprises, 4275 m^2 in in the small-scale enterprises and 13430 m^2 in the medium-scale enterprises. The average open area is 1930 m^2 in the micro-scale enterprises, 4191 m^2 in the small-scale enterprises and 12000 m^2 in the medium-scale enterprises. It was determined that 12.5% of micro-scale enterprises do not have open space.

Workers constitute 78% of the total personnel working in the agricultural machinery manufacturing sector. As can be seen in Table 8, when all manufacturing enterprises are evaluated, it was seen that total number of workers is 1204. It was also determined that 101 workers were employed in micro-scale enterprises, 537 workers in small-scale enterprises and 566 workers in medium-scale enterprises. Number of workers per enterprise was determined as 6.3 workers in micro-scale enterprises, 20.6 workers in small-scale enterprises and 80.8 workers in medium-scale enterprises.

Table 8. Worker/Staff ratio of the enterprises

	Micro Scale	Small Scale	Medium Scale	Total
Total number of employees	137	704	695	1536
Total number of workers	101	537	566	1204
Worker/Staff Ratio (%)	73.7	76.2	81.4	78.3
Number of workers per facility	6.3	20.6	80.8	24.5

Number of workers is followed by engineers with 5.5%, accounting with 5.3%, marketers with 5% and technicians with 4%. Engineers (85) are composed of mechanical engineers with 56.5%, agricultural machinery engineers with 29.4%, mechatronics engineers with 5.9%, industrial engineers with 5.9% and electrical engineers with 2.4%.

Table 9. Distribution of engineers employed in enterprises

	Mechanical Engineer	Agricultural Machinery Engineer	Industrial Engineer	Mechatronics Engineer	Electrical Engineer
Micro Scale	7	-	-	-	-
Small Scale	22	11	-	-	-
Medium Scale	25	11	6	5	2

Estimated labor productivity for production varied between 40 - 70% on average. While the estimated labor productivity in micro-scale enterprises is 61.56% on average, it is 48.8% in small-scale enterprises and 57.14% in medium-scale enterprises.

It was determined that 43% of the malfunctions in production were due to workmanship, 43% of them due to materials along with workmanship and 14% of them only due to materials. Likewise, it was determined that 50% of the malfunctions in production of micro-scale enterprises and 57.7% of malfunctions in production of the small-scale enterprises were due to material and workmanship. On the other hand, in medium-scale enterprises, it was determined that the malfunctions were only due to workmanship with a high rate of 57.1%.

When businesses are evaluated in terms of their licensed software, it was seen that all businesses have at least one accounting software. As can be seen in Table 10, accounting software per enterprise was 1.06 in micro-scale enterprises, 1.15 in small-scale enterprises and 3.43 in medium-scale enterprises. It was also seen that number of design (CAD-CAM) software per enterprise is 0.63 in micro-scale enterprises, 1.5 in small-scale enterprises and 5.86 in medium-scale enterprises.

Table 10. Number of licensed software of the enterprises

	Micro Scale		Small Scale		Medium Scale		Total	
Type of Software	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio
Design Software	10	0.63	39	1.5	41	5.86	90	1.8
Accounting Software	17	1.06	30	1.15	24	3.43	71	1.4
Other Software	1	0.06	10	0.38	18	2.57	29	0.6

Number of workbenches of agricultural machinery manufacturers and workbenches per enterprise are provided in Table 11. There are 491 gas metal arc welding machines, 206 drill benches, 124 CNC lathes, 93 Eccentric Press benches, 86 Band saw benches, 80 Universal lathes in the enterprises. Number of the mentioned benches per enterprise is higher than the other benches. Per enterprise, there are 10.02 gas metal arc welding machines, 4.20 drill benches, 2.53 CNC lathes, 1.9 lathes, 1 Eccentric Press machine, 1.76 Band saw benches, 1.63 Universal lathes.

1

Table 11. Workbenches of the enterprises

		Micro Scale	Workbench Enterprise	Small Scale	Workbench Enterprise	Medium Scale	Workbench Enterprise	Total Benches	Total Bench Enterprise
Benches	**Type**	**Number**	**Ratio**	**Number**	**Ratio**	**Number**	**Ratio**	**Number**	**Ratio**
Welding	Gas-metal arc	83	5.19	258	9.92	150	21.43	491	10.02
	Electric arc	11	0.69	26	1.00	2	0.29	39	0.80
	Oxygen	12	0.75	17	0.65	3	0.43	32	0.65
	Spot	4	0.25	24	0.92	7	1.00	35	0.71
	Robotic	3	0.19	12	0.46	10	1.43	25	0.51
	Other	2	0.13	0	0.00	0	0.00	2	0.04
Drill		41	2.56	122	4.69	43	6.14	206	4.20
Universal Lathe		26	1.63	39	1.50	15	2.14	80	1.63
Mill		11	0.69	11	0.42	7	1.00	29	0.59
Saw	Circular	0	0.00	1	0.04	0	0.00	1	0.02
	Band	22	1.38	48	1.85	16	2.29	86	1.76
Press	Hydraulic	11	0.69	21	0.81	7	1.00	39	0.80
	Eccentric	19	1.19	49	1.88	25	3.57	93	1.90
	Press Brake	7	0.44	16	0.62	13	1.86	36	0.73
	CNC	2	0.13	10	0.38	0	0.00	12	0.24
CNC	Lathe	11	0.69	81	3.12	32	4.57	124	2.53
	Mill 3 axial	5	0.31	23	0.88	16	2.29	44	0.90
	5 Axes and more	0	0.00	0	0.00	2	0.29	2	0.04
Guillotine Shears		7	0.44	14	0.54	4	0.57	25	0.51
Borwerk Benches		2	0.13	0	0.00	2	0.29	4	0.08
Shaper		3	0.19	5	0.19	1	0.14	9	0.18
Pipe Bending	Mechanic	2	0.13	12	0.46	1	0.14	15	0.31
	CNC	0	0.00	0	0.00	0	0.00	0	0.00
Laser Cut		1	0.06	11	0.42	7	1.00	19	0.39
Plasma Cut		3	0.19	12	0.46	3	0.43	18	0.37

2

It was determined that 49% of businesses renew their products every year. It was also determined that 50% of micro-scale enterprises, 42.3% of small-scale enterprises and 71.4% of medium-scale enterprises renew their products every year.

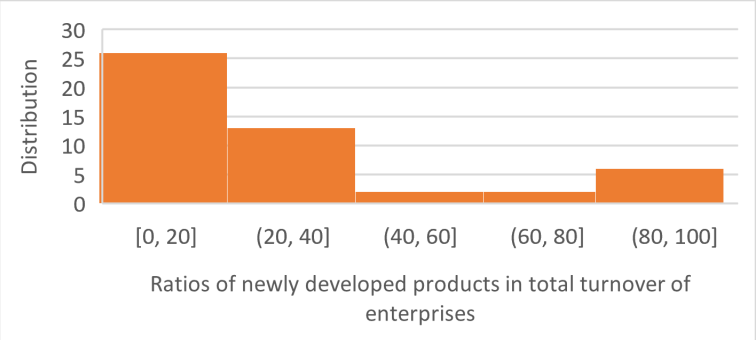

Figure 6. Ratios of newly developed products of enterprises in total turnover

Of the agricultural machinery manufacturing enterprises, 53% stated that the ratio of their newly developed products (last 3 years) in the total turnover ranged between 0 - 20%, 26.5% stated the ratio as between 20-40% and 20.5% stated as 40% or more.

Agricultural machinery manufacturing enterprises stated that the ratio of the budget allocated for R&D to the total budget is 6.02% on average. Such a ratio is 6.5% in micro-scale enterprises, 3.07% in small-scale enterprises and 8.5% in medium-scale enterprises.

It was determined that 24.5% of the agricultural machinery producing enterprises prefer imported materials, 26.5% prefer domestic materials and 49% prefer suitable materials regardless of any source of origin. It was determined that only 8.15% of the enterprises made a search from the websites while 73.5% preferred the material supply with references.

Of the participant enterprises, 57.1% stated that they could not make cost analysis of the products they produce. It was determined that 50.2% of micro enterprises, 65.3% of small enterprises and only 42.8% of medium-scale enterprises can perform cost analysis.

Table 12. Serial Production Works of the Enterprises

	Serial Production Works	
	Yes	**No**
Micro Scale	-	16
Small Scale	5	21
Medium Scale	5	2

According to table 12, while 79% of the enterprises stated that they did not have any works for serial productions, 21% stated that they had works for serial productions. While there is no work for serial production in all micro-scale enterprises, it was observed that 80% of small-scale enterprises and 71% of medium-scale enterprises have works for serial productions.

When the enterprises are examined in terms of their quality management systems, it was determined that only 55.1% of them have a quality management system and 55% of the enterprises that have a quality management system cannot fully implement the system.

When the enterprises producing agricultural machinery are evaluated in terms of the projects they carry out with institutions such as KOSGEB and TUBITAK and the number of consultancy received from universities, only 34% of these enterprises have cooperations with KOSGEB for R&D and P&D activities, 8% with TUBITAK. It was also determined that 18% of them received consultancy from universities.

Table 13. Industrial Property Documents Owned by Enterprises and KOSGEB, TUBITAK Collaboration Status

	Micro Scale	Small Scale	Medium Scale
Number of enterprises with cooperations with TUBITAK	-	2	2
Number of enterprises with cooperations with KOSGEB	5	7	5
Number of enterprises with cooperations with Universities	2	5	2
Number of enterprises with Patent Registrations	3	3	1
Number of enterprises with Utility Model Registrations	8	19	5
Number of enterprises with Industrial Design Registrations	3	2	4
Number of enterprises without any Industrial Property Documents	8	4	2

It was determined that 71% of medium-scale enterprises carry out R&D and P&D projects with institutions such as KOSGEB and TUBITAK, while 60% of these enterprises carry out KOSGEB and 40% both KOSGEB and TUBITAK projects, they also receive consultancy services from universities. When the medium-scale enterprises are evaluated considering their intellectual and industrial property documents, it was seen in Table 13 that 71% have utility model, 57% industrial design and 14% patent certificate.

It was also determined that 42.3% of small-scale enterprises carry out various projects with KOSGEB and TUBITAK, 72.7% of these enterprises cooperate with KOSGEB, 9% both KOSGEB and TUBITAK at the same time, 9% only with TUBITAK. It was determined that 54 of them received consultancy services from universities during these collaborations. When small-scale enterprises are evaluated considering their intellectual and industrial property documents, it was seen in Table 13 that 73% have utility models, 11.5% have patents and 7.7% have Industrial Design certificates.

It was observed that 31% of micro-scale enterprises carry out various projects with KOSGEB and 12% receive consultancy services from universities. When micro-scale enterprises are evaluated considering their intellectual and industrial property documents, it was seen in Table 13 that 50% have utility model, 18.75% have industrial design certificate and 18.75% have patent certificate.

Table 14. Distribution of Industrial Property Documents Owned by Enterprises and KOSGEB, TUBITAK Collaborations

	Micro Scale	Small Scale	Medium Scale
Number of TUBİTAK Projects		2	2
Number of KOSGEB Projects	4	8	5
Number of Consultancies from universities		6	2
Total number of collaborative projects	4	16	7
Number of Patent Registrations	3	7	10
Number of Utility Model Registrations	21	55	13
Number of Industrial Design Registrations	8	5	41
Total number of Intellectual and Industrial Property Documents	31	67	44

When the companies producing agricultural machinery are evaluated in terms of the projects they carry out with institutions such as KOSGEB and TUBITAK, number of consultancies received from universities and intellectual property certificates, it was determined that they carry out various projects TUBITAK (4 projects) and KOSGEB (17 projects) and 8 of them receive consultancies from universities. Table 14 shows that there are 31 intellectual property certificates in micro-scale enterprises, 67 in small-scale enterprises and 44 in medium-scale enterprises. It was determined that there are 3 patent certificates in micro-scale enterprises, 7 patents in small-scale enterprises and a total of 10 patent certificates in medium-scale enterprises. While Micro and Small-scale enterprises have the most Utility model certificates, medium-scale enterprises have the most Industrial design certificates.

Table 15. Quality positioning of the products of the enterprises in the International Markets

	Degree of Quality in International Markets		
	1st Quality (%)	2nd Quality (%)	3rd Quality (%)
Micro Scale	31.30	37.50	31.20
Small Scale	15.40	76.90	7.70
Medium Scale	28.60	57.10	14.30
Total Ratio (%)	22.40	61.30	16.30

Participant enterprises generally define their product quality as the 2nd quality with a rate of 61.3% in the international markets. Table 15 shows that while 76.9% of enterprises that define their products as 2nd quality are small-scale and 57.1% are medium-sized enterprises, only 37.5% of micro-scale enterprises define their products as 2nd quality.

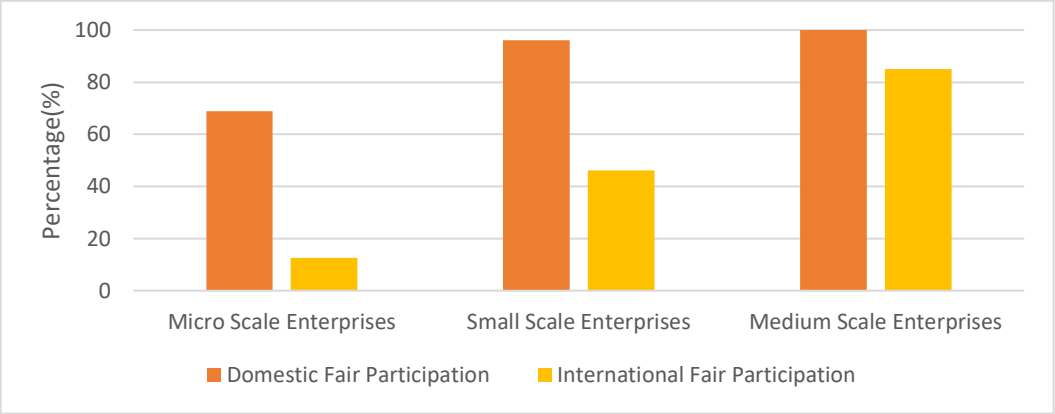

Figure 7. Participation status of enterprises in Domestic-Overseas Fairs

The participation status of agricultural machinery manufacturers in domestic fairs is shown in Figure 7. While 31.2% of micro-scale enterprises did not participate in domestic fairs, all medium-scale enterprises participated in domestic fairs. It was determined that 4% of small-scale enterprises do not participate in domestic fairs. It was also determined that 87.5% of micro-scale enterprises and 53.8% of small-scale enterprises do not participate in foreign fairs. It was observed that 85% of medium-scale enterprises participated in international fairs. It was found that especially the organizations held in Germany, Italy and Bulgaria are predominant in the participation of foreign fairs.

When the businesses are examined in terms of the management of their websites, it was determined that 75% of them receive service from another company for website management and 69.6% of these businesses control the website for more than 6 months.

In general, 98% of the enterprises producing agricultural machinery export directly or indirectly. About 93% of micro-scale enterprises and all of the small and medium-scale enterprises export. Exported countries include Russia, Turkic Republics, Middle East, North African countries and European countries such as Romania and Moldova.

Participant enterprises stated that for agricultural machinery manufacturing sector to reach a much better place in international markets, efforts should be made to reduce production costs and effective government supports should be carried out. Additionally, micro-scale enterprises stated that a corporate management system should be adopted. Small-scale enterprises and medium-scale enterprises stated that access problems to the ports should be eliminated, domestic quality assessment should be made between enterprises and studies on smart agriculture systems should be carried out.

4. Conclusions

When the general situation of the agricultural machinery manufacturers in Konya Region is examined, it was seen that about 84% of the enterprises consist of micro and small-scale enterprises, 90% of the enterprises work with their own capital and do not benefit from the banking credit systems. Such a case is largely resulted from the socio-cultural status of the business owners and religious belief. Therefore, businesses are expected to encounter various problems in financing.

About 46% of the enterprises are corporate businesses. Such a case brings about fragmentation and downsizing of the enterprises. Therefore, it becomes difficult to make forward-looking and growth-related decisions.

Average age of the business owners is 53 years and the average of their experience in the sector is 35 years. Such a case indicated that they started manufacturing at a young age and chose it as a profession. Although this situation is important in terms of professional experience, it caused the education level of business owners to decrease.

Business owners are managers at the same time, therefore, about half of the businesses do not have professional managers. Ratio of university-graduate business owners is 15%, therefore, they may have problems in terms of future planning.

Enterprises employ engineers with a ratio of 5.5% and technicians with a ratio of 4% in total personnel, therefore, employment support should be provided by organizations such as KOSGEB and İŞKUR, especially for small-scale enterprises.

It was seen that the adequacy level of the manufacturing technology owned by the agricultural machinery manufacturing enterprises is low. When the bench park of the enterprises is examined, it is necessary to increase the competitiveness of especially micro and small-scale enterprises, to renew the technologies and to modernize the enterprises.

Lack of R&D departments, quite a low number of projects and products developed with different institutions and resultant low numbers of intellectual property certificates revealed that they have difficulty in following the developments in the sector and cannot establish future scenarios.

It was seen that micro and small-scale enterprises are affected by the negative effects occurring in the country and have financial problems. However, they export at a rate of 80% and such a case caused them to be less affected by some negativities that will occur in the domestic market.

It was observed that especially micro and small-scale enterprises do not have sufficient corporations with organizations such as KOSGEB and TUBITAK for R&D projects. Therefore, micro and small-scale enterprises should be directed to organizations such as KOSGEB and TUBITAK for their R&D activities.

About 61.3% of businesses define their products as second quality in international markets. Especially small and medium-scale companies position their products as 2nd quality and such a case can be explained by high participation rate of these enterprises in foreign fairs and the problems experienced in production. On the other hand, micro-scale enterprises define their products as 1st quality since they don't participate foreign fairs much, thus are unable to compare their products with international competitor enterprises. It is necessary to increase the participation rate of micro and small enterprises into foreign fairs to follow international markets, competitors, innovations and practices.

Agricultural machinery manufacturers can promote their products and brands in different international fairs held in Turkey. Due to the capacities and costs of these fairs, the access of micro and small enterprises is not possible at a sufficient level.

While there are especially large and medium-scale enterprises in Konya Agriculture, Agricultural Mechanization and Field Technologies Fair, which is described as the largest agricultural machinery and technologies specialization fair of Türkiye, participation of micro and small-scale enterprises in this fair

cannot reach a sufficient level. In this sense, it is necessary to increase the capacity of the fairground and especially the micro and small enterprises should be supported for their participation into this fair.

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Research Article

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Determination of Draft Power, PTO Power and Fuel Consumption of Single Acting Disc Harrow Driven from PTO

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HIGHLIGHTS

- Measurement of fuel consumption of applications
- -Determination of the draft force and draft power of applications
- -Determination of the PTO torque and PTO power of applications
- -Determining the power requirement of applications
- -Determination of the ratio of disc circumference speed to tractor forward speed of applications

Abstract

In this study; A single-acting disc harrow was used, which takes its movement from the tractor PTO. PTO driven single-acting disc harrow was tested with disc diameters of 610 and 660 mm, disc direction angles of 16^0 - 23^0 and 30^0, and disc revolutions of 104.97 - 119.97 and 143.96 min^{-1}. Single-acting disc harrow moving from PTO was compared in terms of disc diameter, directional angle and disc revolutions. The lowest draft force requirement was obtained from $D_1N_1Y_1$, $D_2N_1Y_1$ and $D_1N_1Y_2$ applications, with values of 0.48 kW, 0.61 kW and 0.79 kW, respectively. The highest draft power requirement was obtained from $D_2N_3Y_3$, $D_2N_3Y_2$ and $D_1N_3Y_3$ applications with 7.80 kW, 6.97 kW and 5.25 kW values, respectively. The lowest PTO power take-off power requirement from the applications was obtained from the $D_1N_1Y_1$, $D_1N_2Y_1$ and $D_1N_3Y_1$ applications, with values of 22.80 kW, 22.88 kW and 24.82 kW, respectively. The highest PTO power take-off power requirements were obtained from the $D_2N_3Y_3$, $D_1N_3Y_3$ and $D_2N_3Y_2$ applications, with power requirements of 41.15 kW, 39.40 kW and 38.62 kW, respectively. In terms of fuel consumption, the lowest fuel consumption was determined in $D_1N_1Y_3$, $D_1N_1Y_2$ and $D_1N_1Y_1$ applications with 11.40 L ha^{-1} 11.63 L ha^{-1} and 11.95 L ha^{-1} values. The highest fuel consumption was obtained from 18.34 L ha^{-1}, 17.78 L ha^{-1} and 17.58 L ha^{-1}, respectively, and $D_2N_3Y_3$, $D_2N_3Y_2$ and $D_2N_3Y_1$ applications.

Keywords: Draft power, PTO power, Fuel consumption, Disc direction angle

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1. Introduction

The PTO-driven disc machine has less wheel slip due to the need for less draft force, which allows the use of lower- power tractors, which can directly help with less soil compaction and reduce tractor purchase costs Salokhe et al. 1994; Hann and Giessibl,1998).

Upadhyay and Raheman (2019) reported that the effect of increasing the working depth on the draft force is less pronounced in PTO driven disc harrows compared to free rotating disc harrows. They explained that the reason for this is that in cases where the working depth is high, the cutting soil strip section of the disc harrows moving from the PTO increases as the working depth increases and the forward thrust force increases due to the fact that the convex part of the disc contacts the incisal wall more with the increase in the working depth. In addition, in their study with PTO driven disc harrow and free moving disc harrow at different working depths and u/v ratios, they reported that the fuel consumption of PTO driven disc harrows is higher than free moving disc harrows. They stated that the reason for this may be the use of additional PTO power in the PTO driven disc harrow and its effect on more soil.

Nalavade et al. (2013) in their study with PTO and free-moving disc harrows, determined that at different working speeds, PTO-driven disc harrows achieve higher working depth, carry higher soil volume, and consequently consume higher draft power than free-running harrows. When both disc harrows were compared, they reported that the PTO - driven disc harrow gave lower draft force values per unit working depth.

Salokhe et al. (1994) reported that the battery direction angle, the number of passes, and the feed rate were determined by the specific determined that the torque requirement was significantly affected and explained that the specific torque requirement at the 28° battery direction angle was lower than the 33° group angle.

Hoki et al. (1988) observed a reduction in overall power requirements as well as a reduction in draft requirements during a preliminary test of an electric disc harrow. In addition, it was noted that the draft and power requirements were affected by the feed rate, PTO speed, tillage depth and soil condition.

Salokhe and Quang, (1995) reported that the slower the shear rate, the higher the soil's resistance to shearing, so higher torque is required.

Khadr (2000), stated in his research that fuel consumption, overall processing efficiency and specific energy increase with increasing agricultural processing speed. He also stated that the fuel consumption of the tractor depends on the draft force of the machine and the working depth.

Resarchers reported that in disc harrows, by enabling the discs to rotate by driving, the ratio of the circumferential speed of the discs to the forward speed and the appropriate adjustment of the disc angle, the draft force requirement and penetration resistance decreased significantly with the improvements in the physical properties of the soil (Hoki et al., 1988; İslam et al., 1994; Salokhe et al., 1994; Salokhe and Quang, 1995; Nalavade et al., 2010; Upadhyay and Raheman, 2018).

The researches made with the PTO driven disc tillage machines are based on the studies made using a single disc and in the soil channel. There is very limited information about the comparative field performance of tillage implements and machines with disc moving from the PTO. It is thought that it is important to determine the draft force requirement, draft power, torque requirement, PTO power and fuel consumption of the single-acting disc harrow driven by the PTO under field conditions, and to compare it with the single-acting disc harrow that takes its movement from the soil.

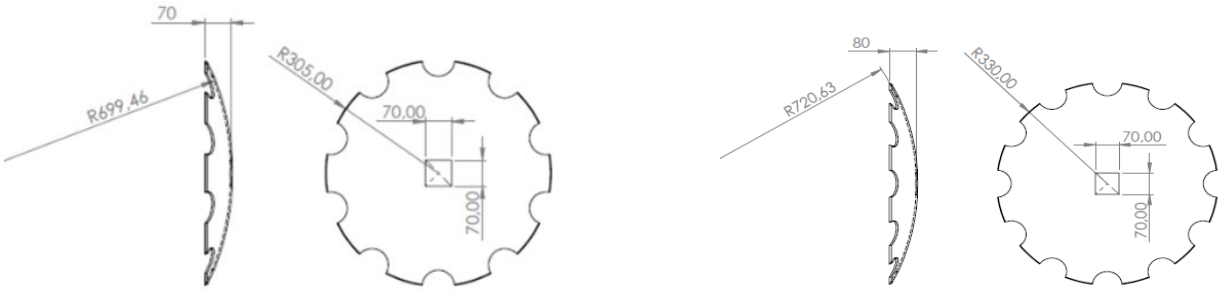
2. Materials and Methods

The specifications of the PTO driven single acting disc harrow used in the trials are given in Table 1 and Figure1. New Holland TD110 tractor was used in the trials. It is used as a disc harrow that takes its movement from the soil, by removing the sprocket mechanism connected to the movable single acting disc harrow disc shaft from the PTO shaft. The discs used in the trials are given in Figure 2.

Figure 1. PTO driven the single-acting disc harrow used in the trials

Table 1. Features of PTO driven single acting disc harrow

Features	D1	D2
Number of discs	8	8
Disc diameter (mm)	610	660
Direction angle (Y1-Y2-Y3)	16^0 -23° - 30^0	
Disc speed (N1-N2-N3) (1.min^{-1})	104.97 – 119.97- 143,96	
Distance between discs (mm)	260	260
Working depth (mm)	210	210
Machine weight (kg)	950	1000



(a) (b)

Figure 2. Features of the discs used in the trials. (a) D1:610mm (b) D2:660mm

The movement taken from the tractor PTO with the help of the articulated shaft is transmitted to the shaft to which the discs are connected by means of the chain-gear system (Figüre 3). Different disc speeds applied in the trials, in the sprocket system given in Figures 3.5 and 3.6, the gears with 10, 12, 14 and 16 gear were

changed and the disc revolutions were N1= 104.97 min^{-1} N2 = 119.47 min^{-1} and N3= 143.97 min^{-1} has been obtained.

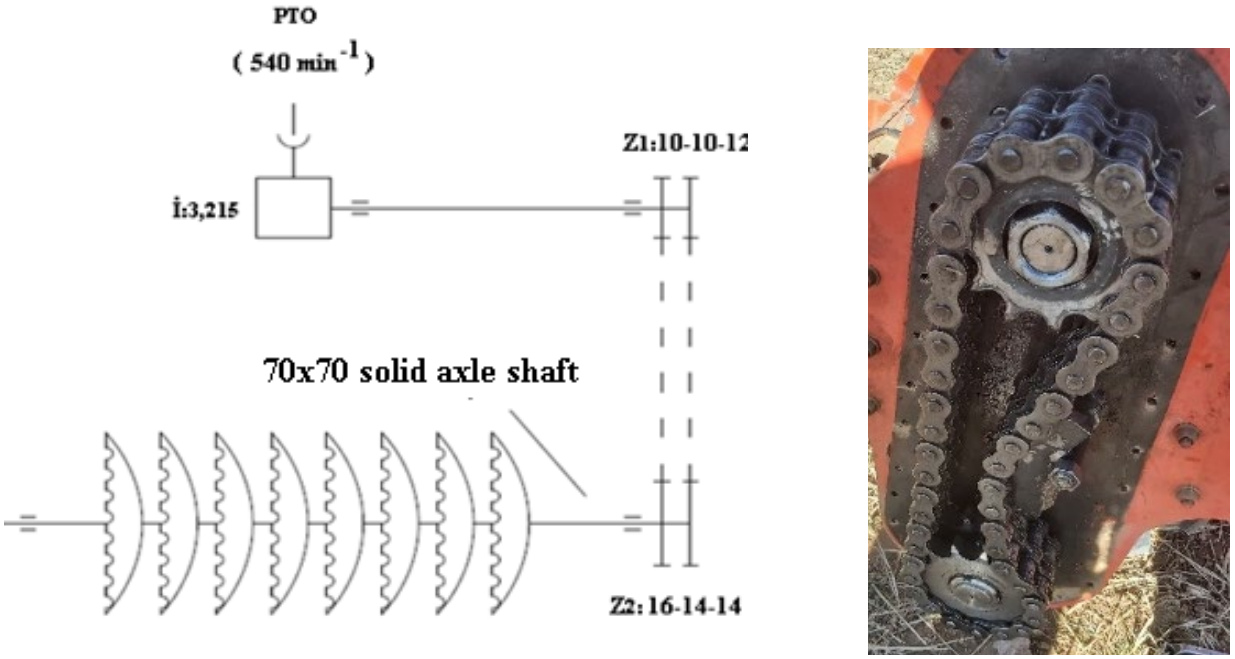


Figure 3. PTO driven single acting disc harrow motion transmission diagram chain-gear system

The soil properties of the area where the trials were carried out are given in Table 2.

Table 2. Soil properties of the field area

Parameters	Units	Values
texture class	loamy	
Hair	(%)	17.73
Plate	(%)	30.37
Annual	(%)	51.90
Volume Weight	(g cm^{-3})	1.35
Porosity	(%)	48.98
Organic matter	(%)	1.05
Moisture	(%)	16.8

Determination of draft force

The draft resistance values were measured in each application by connecting the single-acting disc harrow driven from the PTO to the three-point linkage system connection points of the tractor with three drafts (Figure 4). During the trials, the draft resistance values taken from three points were recorded in the data collector in kg. Datataker, which is used as a data collector, is set to record 2 data per second. The recorded results were then transferred to the Microsoft Excel program in the computer environment, and the results were evaluated and the draft power needs were calculated.

Determination of PTO power

Torque values of the single-acting disc harrow driven from the PTO were measured in Nm with a torquemeter connected to the PTO shaft of the tractor in each application (Figure 4). The torque and power consumption values obtained from the torquemeter during the trials were recorded in the data collector.

Datum brand digital display data collector was used as a data collector. The values recorded in the data collector were then transferred to the computer environment and evaluated.

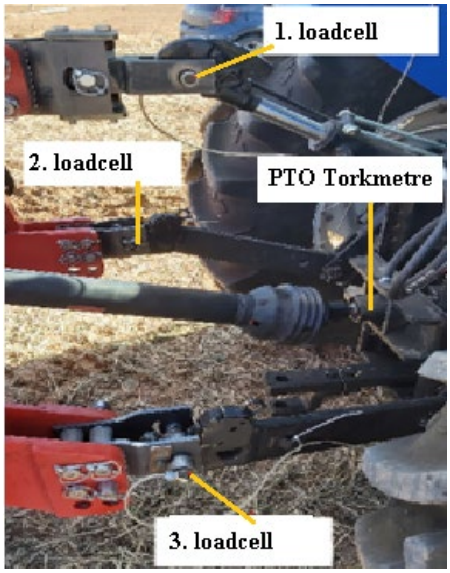


Figure 4. Connecting the draft pins and torque meter used in the trials to the tractor

Measuring Fuel Consumption

In order to clearly measure the instantaneous fuel consumption of the tractor during operation, one of the connections of the fuel meter is connected to the fuel line from the fuel tank to the engine and the other to the return line from the engine to the fuel tank (Figure 5). The fuel meter measures the amount of fuel going to the engine fuel line and from the return line to the fuel tank separately, and the net amount of fuel consumed by the tractor was measured instantaneously in L h^{-1}. The obtained hourly fuel consumption was calculated as L ha^{-1}.

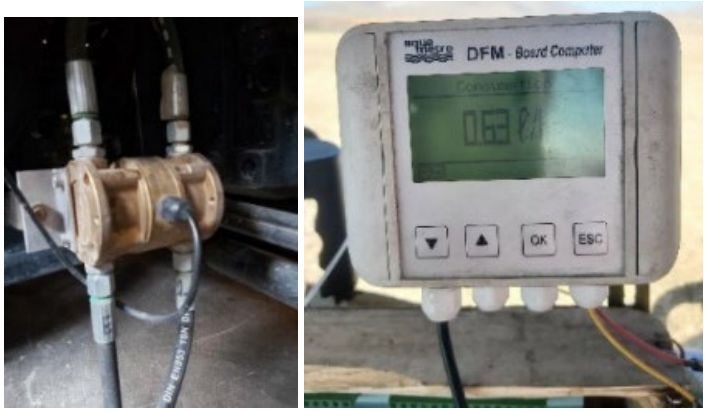


Figure 5. Fuel meter and data collector

3. Results and Discussion

The draft power values obtained for the D_1 diameter disc were obtained between 0.48 – 5.25 kW. The highest draft force value was obtained from the $D_1N_3Y_3$ application with 5.25 kW, followed by $D_1N_3Y_2$ with 4.10 kW and $D_1N_2Y_3$ with 2,32 kW, respectively. The lowest draft power values were obtained from $D_1N_1Y_1$,$D_1N_1Y_2$ and $D_1N_2Y_1$ applications with 0.48 kW, 0.79 kW and 0.93 kW, respectively Figure 6.

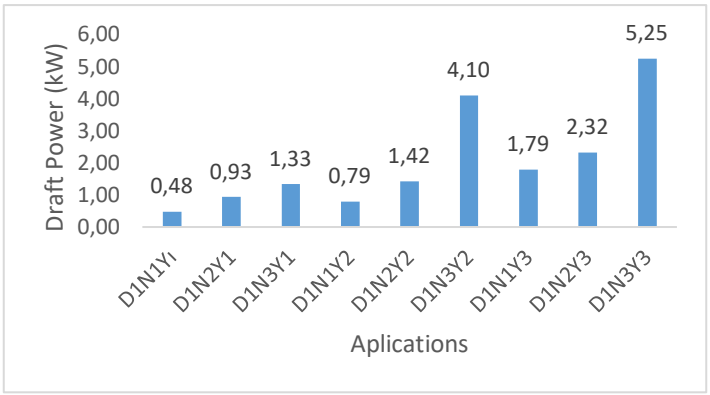


Figure 6. Draft force values of D_1 applications

The draft power values obtained for the D_2 diameter disc were obtained between 0.61 – 7.80 kW. The highest draft power value was obtained from the $D_2N_3Y_3$ application with 7.80 kW, followed by $D_2N_3Y_2$ with 6.97 kW and $D_2N_2Y_3$ with 3.68 kW, respectively. The lowest draft power values were obtained from $D_2N_1Y_1$, $D_2N_2Y_1$ and $D_2N_1Y_2$ applications with 0.61 kW, 1.27 kW and 1.57 kW, respectively Figure 7.

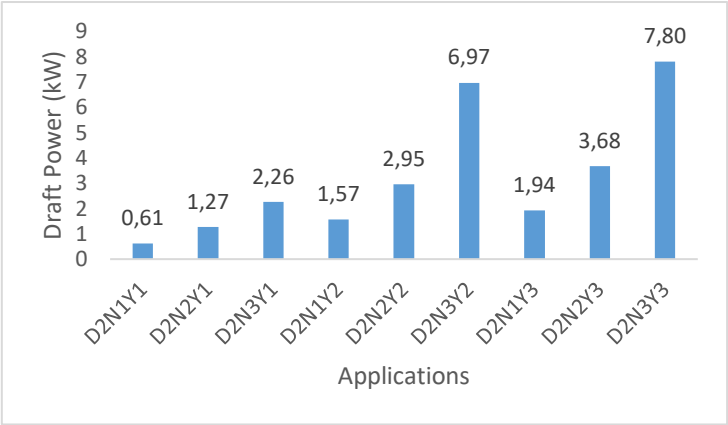


Figure 7. Draft force values of D_2 applications

PTO power values obtained for the D_1 diameter disc were obtained between 22.80 – 39.40 kW. The highest PTO power value was obtained from the $D_1N_3Y_3$ application with 39.40 kW, followed by $D_1N_2Y_3$ with 34.17 kW and D1N1Y3 with 31.54 kW, respectively. The lowest PTO power values were obtained from $D_1N_1Y_1$, $D_1N_2Y_1$ and D1N3Y1 applications with 22.80 kW, 22.88 kW and 24.82 kW, respectively Figure 8

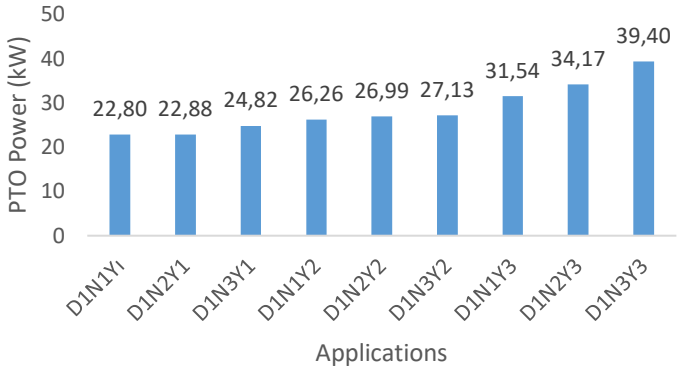


Figure 8. PTO power values for D_1 applications

PTO power values obtained for the D_2 diameter disc were between 28.33 – 41.15 kW. The highest PTO power value was obtained from the $D_2N_3Y_3$ applicationwith 41.15 kW, followed by $D_2N_3Y_2$ with 38.62 kW and $D_2N_2Y_3$ with 34.98 kW, respectively. The lowest PTO power values were obtained from $D_2N_1Y_1$, $D_2N_1Y_2$ and $D_2N_1Y_3$ applications with 28.33 kW, 28.51 kW and 29.49 kW, respectively Figure 9.

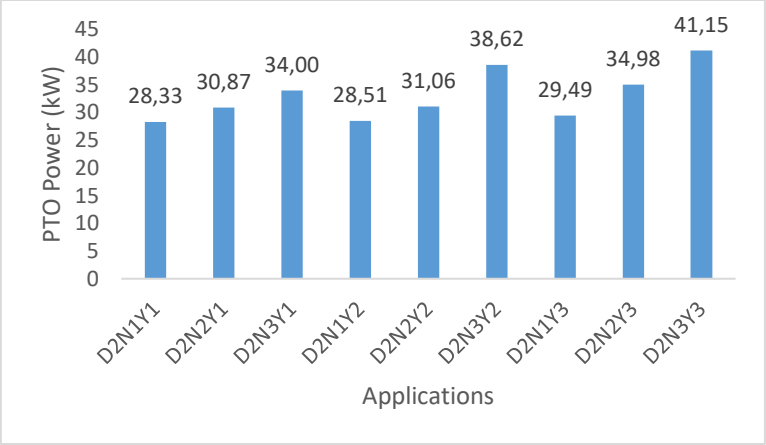


Figure 9. PTO power values for D_2 applications

The fuel consumption values obtained for the D_1 diameter disc were between 11.40 -15.46 L ha $^{-1}$. The highest fuel consumption value was obtained from $D_1N_3Y_3$ application with 15.46 L ha^{-1}, followed by $D_1N_3Y_2$ with 15.11 L ha $^{-1}$ and D1N3Y1 applications with 15.10 L ha $^{-1}$ respectively. The lowest fuel consumption values were obtained from the D1N1Y3, $D_1N_1Y_2$ L ha $^{-1}$ and $D_1N_1Y_1$ L ha $^{-1}$ applications, with 11.40, 11.63 and 11.95 L ha $^{-1}$ respectively Figure10.

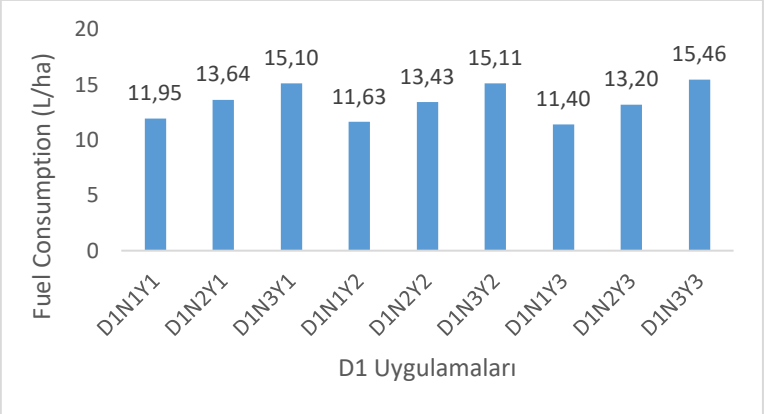


Figure 10. Fuel consumption values for D_1 applicationsThe fuel consumption values obtained for the D_2 diameter disc were between 13.99-18.34 L ha $^{-1}$. The highest fuel consumption value was obtained from the $D_2N_3Y_3$ application with 18.34 Lha $^{-1}$,followed by D_2N_3Y2 with 17.78 L ha $^{-1}$ and D_2N_3Y1 with 17.58 Lha^{-1} respectively. The lowest fuel consumption values were obtained from $D_2N_1Y_2$, $D_2N_1Y_1$ and $D_2N_2Y_2$ applications, with 13.99 L ha $^{-1}$, 14.01 L ha $^{-1}$ and 14.17 L ha $^{-1}$ respectively Figure 11.

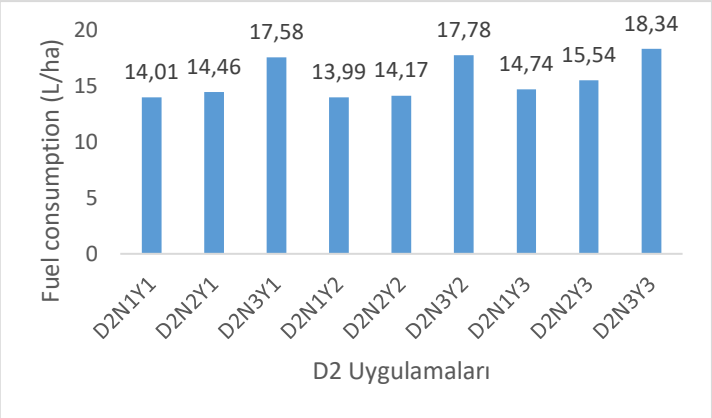


Figure 11. Fuel consumption values for D_2 Applications

4. Discussion

The tillage depth of the disc harrow increased as the disc direction angle and disc speed increase. While the working depth of the machine was determined as at least 95 mm at minimum direction angles, it was observed that it increased up to 205 mm at maximum direction angles.

The machine's gravitational force requirement and accordingly the gravitational force requirement increased at the same rate as the disc direction angles increased. As a result of the drive of the disc harrow discs from the PTO, it was observed that the discs create thrust in the opposite direction, especially at low direction angles where the direction angle is 160, and it was determined that the machine tried to push the tractor. The reason for this is that at low directional angles, as a result of low working depth, the discs encounter soil resistance and create a pushing force by holding onto the soil instead of cutting the soil. As the diameter and speed of the disc increased, the gravitational force increased and accordingly, the need for gravitational force increased. Although sufficient soil cultivation could not be done in Y1 direction angle applications, the efforts of the discs to hold on to the soil due to the notch caused the values to be taken in torque measurements in these applications.

The torque values measured from the PTO also increased with the increase of the machine's disc direction angles, working depth, disc revolutions and disc diameter. It can be said that the reason for this is that when the disc direction angle and disc diameter increase, the volume of the cut soil increases as a result of the increase in the working depth and the high disc speed. In all applications, the minimum PTO power is 22.80 kW and the maximum is 41.15 kW. Abernathy (1976) concluded from laboratory trials on an externally driven disk that the draft requirement can be reduced by 20%, but the total power requirement is 3-6 times greater than the total power requirement of freely rotating disks. Although the machine reduces the traction power by creating forward thrust, it has been observed that the thrust force due to the operation of the discs from the PTO provides an increase in the PTO power.creating forward thrust, it has been observed that the thrust force generated because the discs are actuated from the PTO provides an increase in PTO power.

As a result of the research, it was concluded that as the disc diameter, disc direction angle and disc speed of the disc harrow moving from the PTO increase, the fuel consumption also increases. Among the applications, the highest fuel consumption, direction angle, disc speed and disc diameter were obtained from the applications. Fuel consumption in all applications varied between 11.40 L ha-1 and 18.34 L ha-1. Upadhyay and Raheman (2019) stated that the reason for the high fuel consumption in PTO driven disc harrows may be the use of additional PTO power and its impact on the ground. Damanauskas et al. (2019), in their study with an individual mounted disc harrow, when they increase the forward speed from 1.4 ms-1 to 3.6 ms-1, the fuel consumption is 1 L ha-1, and when the disc angle is increased from 10° to 20°, it is 2 L ha.-1 reported that when

the working depth was increased from 5 cm to 8 cm (clay-loamy and loamy soil type), it increased by 0.25 L ha-1.

We can explain the low draft power, PTO power and fuel consumption results in Y1 applications as the low operating depth due to low directional angle and the inability of the discs to cut sufficient soil volume. The increase in the angle of direction ensures that the fragmentation is effective, as at the angles of 230 and 300 degrees, and the discs overlap the soil to leave a more even profile, thus making the tillage more efficient. Soil cultivation in accordance with agricultural technique is achieved by increasing the direction angle, but as a result, it causes higher draft power requirement, PTO power requirement and fuel consumption.

The single-acting disc harrow, acting from the PTO, provides better lateral displacement of the cut soil slice. The increase in the disc direction angle increases the volume of soil to be cultivated, which causes an increase in soil reaction forces.

According to the data obtained from the research results, it is necessary to adjust the desired direction angles in the structure of the disc harrow in order to tillage at different disc direction angles with the single-acting disc harrow driven by the PTO. High disk circumferential velocity and high u/v ratio are required as well as wide directional angles for optimum soil fragmentation. It should not be ignored that the working depth increases with the increase in the direction angles, and accordingly the required draft power and fuel consumption increase. Young (1976) made a pitch evaluation of a disc harrow driven by a power take-off and observed that increasing the disc peripheral speed increases the power requirement and reduces the pull requirement.

In addition to the appropriate working depth, the disc direction angle should be chosen between 230 -30° in order to make a soil cultivation in accordance with the agricultural technique. In general, the results of the research show that the direction angles of the disc harrow discs moving from the PTO should be adjusted with an adjustment scheme that the farmer can change as he wishes. Thus, it will allow working by adjusting the disc direction angles according to different soil conditions and agrotechnical requests.

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Research Article

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Magnetic Field Treatment in Barley: Improved Salt Tolerance in Early Stages of Development

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HIGHLIGHTS

- Increased salt stress tolerance by treatment of magnetic field
- Effect of magnetic field treatment exposure time on growth parameters
- Treatment of environmentally friendly and innovative method for stress tolerance in barley

Abstract

It is known that salinity is one of the most important abiotic stress factors reducing productivity in agriculture. High salt concentration has a negative effect on seed germination and seedling formation. Magnetic field treatment can be considered as an environmentally friendly, innovative and inexpensive method to increase tolerance to stress, as a productivity-enhancing factor in agriculture. In this study, germination rate, germination strength, shoot and root length, fresh and dry weights, chlorophyll a, b and total chlorophyll content and ion leakage values of three different barley varieties under the influence of 150 mT constant magnetic field at different time intervals were determined. In light of the results, it was determined that although it varies according to the barley varieties, it has a significant positive effect on the germination rate and strength depending on the duration of the applied magnetic field. This study revealed that the magnetic field seed treatment has different effects according to the treatment time and the severity of the salt stress.

Keywords: Barley, salt stress; magnetic field treatment, improved tolerance

1. Introduction

Barley cultivation is in second place after wheat among the cereals in Turkey. Although the purpose of growing barley is for feed and partial malting production, its use for food is increasing with its rich carbohydrate content (Tafresh et al., 2023). Malting barley production has a very small place in total barley production in Turkey. However, the export potential and prices of malted barley are higher than forage barley, which is an important indicator of increasing production (Anonymous, 2019). Turkey is the country with the highest amount of barley production in the world, the fact that the quality of malting barley produced is not in line with world standards causes difficulties in meeting the needs. For this reason, it is necessary to develop

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high quality barley varieties with high malt extract ratio and in accordance with standards in order to close the current gap (Kerpes et al., 2023). Obtaining varieties with superior characteristics can be achieved by breeding studies. The most important factors determining quality are the variety and environmental conditions. In order to produce malting barley in the required quantity and quality, it is necessary to determine the barley varieties suitable for the region where the cultivation will be carried out (Yu et al., 2023).

Abiotic stress, which is a factor that seriously affects yield and quality in plants, is one of the leading factors limiting agricultural production. Although stress factors are quite diverse, one of the most important stress factors is salt (Dawood et al., 2022). Plants can easily absorb the soluble salt in the soil. Salt compounds entering the body of the plant show negative effects when they exceed a certain density. With the increase in the salt concentration in the soil, it becomes difficult for the plant to take water from the soil, the structure of the soil deteriorates and the plant growth slows down or even stops (Yavuz et al., 2022). It is known that salinity is one of the most important abiotic stress factors reducing productivity in agricultural areas, and high salt concentration has a negative effect on seed germination and seedling formation (Cavdar and Yildiz 2021). Plants are more sensitive to salt in the early stages of their development than in other stages of development (Budaklı Carpıcı and Erdel, 2016). For this reason, it is appropriate to carry out studies in which salinity tolerance is determined at early developmental stages. The amount of salt in the environment has an important effect on the germination, healthy emergence and survival of the seed (Ozyazici and Acikbas 2021). Yavuz et al. (2023) reported that salt treatment is a fast and effective technique for the determination of salt-tolerant wheat cultivars during the germination and early seedling development period. The negative effect of salinity is observed in more than 20% of agricultural lands in the world (Hickey et al., 2019). In addition, the rehabilitation of saline areas is very expensive and it cannot be expected to be a permanent solution unless the factors causing salinity are eliminated. For this reason, the determination of salt-resistant varieties is important for plant cultivation in these areas. In terms of salt tolerance, there are differences between families, genera and species as well as among varieties belonging to the same species (Xu et al., 2023). The high salt levels in the soil and the quality of the irrigation water used are among the factors of concern for future agriculture. Therefore, it is important to develop effective strategies to increase yields through salt tolerance (Salim and Raza 2020).

Physical processes affect physiological and biochemical processes in seeds, thus contributing to vegetative growth, increasing crop yield and quality. Magnetic field (MF) pretreatment, which has been used in agriculture as an environmentally friendly technique, is one of the physical processes reported to increase the germination of seeds and yield of crops (Rathebe et al., 2023) and improve the performance of various crops (El-Gizawy et al., 2016). Many scientists have studied the positive and negative biological effects of MF on plants. MF treatment, in addition to contributing to vegetative growth by affecting the physiological and biochemical processes in various seed materials; is a physical process that also improves yield and quality as in potato and sorghum x sudangrass hybrids (El-Gizawy et al. 2016; Beyaz et al. 2020). MF exposure to various plant species may result in different biological effects at the cell, tissue, and organ levels (Bilalis et al. 2013). MF treatment has been carried out in important plant species such as wheat (Pietruszewski et al. 2001), soybean (Atak et al. 2003), maize (Vashisth and Joshi 2017), lentil (Aladjadjiyan 2010), sunflower (Vashisth and Nagarajan 2010; Matwijczuk et al. 2012), potato (Bahadir et al. 2020) and sorghum (Beyaz et al. 2020).

In the studies carried out so far, the magnetic field applied at low and high intensities increases the yield, increases the photosynthesis rate, decreases the antioxidant activity, reduces the effect of abiotic stresses (salinity, drought, heavy metal etc.), increases the germination rate and has positive effects on plant mass and root-stem development (Zuniga et al., 2016). However, magnetic field treatment exposure time is the most important factor for improved tolerance. With the agronomic and biotechnological studies to be done, the changes caused by the magnetic field in the plant can be determined, and environmentally friendly and inexpensive methods can be developed to increase the tolerance against environmental stresses.

In the current situation of the world, environmentally friendly and innovative agricultural approaches have become indispensable for ecological and sustainable life. There is a need for new solutions to alleviate the negative effects of abiotic stress factors such as drought, salinity and extreme temperature caused by the increasing global climate change in recent years. Although magnetic field treatments are not new, their effects

and application methods on plants have not been efficiently studied. In this study, it was aimed to increase salt stress tolerance in barley varieties by different exposure times of MF.In this study, an alternative method has been examined to reduce the damage of increasing soil salinity on the plant. In this context, the physiological and biochemical effects of constant magnetic field treatment on barley at different exposure times were investigated for improved salt stress tolerance.

2. Materials and Methods

Plant Material

Three forage barley varieties "Baronesse", "Bolayır" and the malted variety"Aydanhanım" were used in the study. Seeds of barley varieties were obtained from Konya Selcuk University, Department of Field Crops Department. The "Aydanhanım" variety is developed by the Central Research Institute of Field Crops. It is bending-resistant. Also, it has high tillering and a good response to water and nitrogen (Anonymous, 2021a). "Baronesse" is a two-row, high-yielding forage variety developed in Germany and widely produced in the USA, and is used as a gene source in breeding programs to increase the yield of barley varieties in the USA (Saygılı, 2019). "Bolayır" variety has been registered by Trakya Agricultural Research Institute. It has a high tillering capacity and yields potential. It is a variety with good malting quality (Anonymous, 2021b).

Magnetic Field Treatment

Barley varieties seeds of Aydanhanım, Baronesse and Bolayır cultivars were exposed to 150 mT (militesla) MF (magnetic field) force at different time periods (0-control, 24, 48 and 72 hours). Magnetic field treatment was used according to Tanaka et al. (2010) with some changes from the method stated. In the study, sintered magnets with Nd-Fe-B band N35 Atech, Beijing, China (https://www.atechmagnet.cn/) were used for magnetic field treatment. These magnets are in the form of squares with dimensions of 50 mm x 50 mm x 10 mm and an average surface magnetic strength of 1.2 T (Tesla). A distance of 2.8 cm was set between the seeds and the magnets to ensure that the seeds were exposed to 150 mT MF force. The accuracy and uniformity of the MF power were checked with a digital tesla meter (ref. 13610-93, PHYWE, Göttingen, Germany).

Growing Conditions

The research was carried out in the laboratories of Selcuk University and Erciyes University, Faculty of Agriculture, Department of Field Crops. For seed sterilization, barley seeds were washed with 5% commercial bleach in a magnetic stirrer for 15 minutes. Then it was rinsed 3 times with distilled water for 5 minutes. After the seeds were dried on blotting paper for 10 minutes, they became ready for salt stress treatments. Three different NaCl doses, T0: 0 (control), T1: 3 g/L NaCl and T2: 6 g/L NaCl, were determined for salt stress treatment. For the germination test, sowing was carried out in glass petri dishes containing sterile filter paper, with 15 seeds in each petri dish. After 5 ml of the prepared salt solutions were added to them, they were covered with a second filter paper and 3 more ml of salt solution was added. Sterile distilled water was used in control treatments. Germination tests were carried out in a growth chamber at 24±1°C under dark conditions and constant humidity (50%).

On the other hand, a pot experiment was established under greenhouse conditions to determine the chlorophyll a, b and ion leakage parameters. For this purpose, the experiments were carried out in three replications, one seed in each pot, in pots filled with 500 g sterile soil. In pot experiments, seeds exposed to 150 mT MF at 4 different times (0, 24h, 48h, 72h) intervals were used. The pots in the experiment were regularly watered with 50 ml of T0, T1 and T2 salt solution. Analyzes were performed using fresh tissues at day 21 post-germination.

Examined Features

The examined morphological features were obtained by measuring and counting on the 4th and 8th days in accordance with the ISTA rules. Seeds with rootlets exceeding 2 mm were considered germinated and counted (Fuller et al. 2012). "Germination rate" was determined by counting the seeds germinated on the 4th day, and "germination power", "root length", "shoot length", "wet weight" and "dry weight" were determined

by counting and measurements made on the 8th day. While determining the said characteristics, 10 seedlings randomly selected from each petri dish were used. Dry weight was determined by drying the wet shoots at 70°C for 24 hours (Atak et al., 2006; Saboora et al. 2006).

Analysis of chlorophyll a, chlorophyll b and total chlorophyll was performed according to Curtis and Shtty (1996). First, 50 mg of fresh shoot sample was kept in 3 ml of methanol at 23 oC' for 2 hours in a dark environment and homogenized. 0.3 ml of homogenized liquid was taken and optical density was measured in spectrophotometer at 650 and 665 nm, and the amounts of chlorophyll a, chlorophyll b and total chlorophyll were determined in µg chlorophyll/g fresh tissue and were calculated with the formulas given below.

Chlorophyll a = (16.5 x A665-8.3 x A650) x 3/ 0.05

Chlorophyll b = (33.8 x A650-12.5 x A665) x 3/ 0.05

Total chlorophyll = (25.8xA650-4.0 x A665) x 3/ 0.05

The ion leakage was done according to the method stated in Aydın (2012). After the fresh shoots (0.5 g) were washed with distilled water, they were kept in 10 ml distilled water at room temperature for 24 hours and the EC of the solution was measured (O.D1). Then, the ion leakage in the leaf tissues (O.D2) was calculated with the following equation after it was kept in the autoclave at 121 o C for 20 minutes, cooled and the EC was measured again.

% İon leake = (O.D1 / O.D2) x 100

Statistical analysis

The statistical analyses in this study were carried out on 3 repitations and 10 plants in each repitation. The data obtained for all the results that were measured and observed in the experiment were subjected to analysis of variance (ANOVA) in the JMP 13.2.0 program. The laboratory experiment was two factorial Split Plot Design. The salt stress treatment doses T0, T1, and T2 were the first factor, assigned to the main plots; and the different exposure times (0, 24h, 48h and 72h) of 150 mT MF treatment were the second factor allocated to the subplots (split plots). The differences between the means in the laboratory experiments were determined using the Duncan test at 0.05 level (Snedecor and Cochran, 1967).

3. Results and Discussion

Today, innovative and environmentally friendly approaches and strategies such as seed priming, organic and sustainable agricultural practices are of great importance (Oğuz et al., 2023). Similarly, MF techniques applied under suitable conditions are an innovative and environmentally friendly technique for improving seed germination, vegetative growth and different yield criteria in many species. In addition to contributing to vegetative growth, MF treatment affects physiological and biochemical processes in various seed materials; It is a physical process that also improves product yield and quality. (Beyaz et al., 2020; Bahadir, 2020). Many scientists have investigated the positive and negative biological effects of MFs on living organisms. MF exposure to various plant species can cause different biological effects at the cell, tissue and organ levels. (El-Gizawy et al., 2016). Biological effects of MF; It is directly related to the type, degree and exposure time of the MF treatment (Yildiz et al., 2017).

MF affects whole organism by modifying morphological, physiological and molecular properties (Pietruszewski and Martínez 2015). Exposure of seeds to higher MF strength increases seed germination and plant growth by increasing water assimilation and photosynthesis (Florez et al. 2007). Conversely, it has been reported that near-zero MF has a suppressive effect on biomass accumulation in generative period (Xu et al. 2013). This factor delays the floral transition, therefore to mature late and become vulnerable to negative effects of the environment. Seeds exposed to higher MF strength germinate not only faster but also more uniformly. Rapid and uniform growth is an indicator that plants will gain superiority against environmental conditions during vegetative development.

The results of Aydanhanım cultivar are given in Table 1. Germination rate of Aydanhanım cultivar decreased in parallel with increasing salt dose (Table 1). The germination rate, which was determined as 95.3% in T0 and MF control treatments, was measured as the highest value. According to MF exposure time in T0 stres treatment, the best germination rate was determined in control, 24 and 72 hours MF treatments. In T1 salt stress treatment, the best germination rate was determined at 93.0% in 72 h of treatment, while the best germination rate in T2 stress treatment was measured in 72 h of application with 93.0%. The difference between the germination rates at T0, T1 and T2 in 72 h magnetic field application is statistically insignificant (Table 1). The difference between salt stress treatments in germination power results was found to be statistically insignificant (Table 1). On the other hand, there is no statistical difference between the control, 24 and 72 h (95.3%, 98.4%, 96.9%, respectively) according to the effects of exposure to magnetic field on germination power; the germination power determined at 48 hours exposure time was the lowest (80.3%) (Table 1). The highest value in shoot length was determined at 13.6 cm in T0 and 72 h MF treatment (Table 1). The lowest shoot lengths in all MF application times were determined in T2 salt application. The difference between the root length values determined in T0 and MF control application (11.4 and 11.2 cm, respectively) and the root length (11.6 cm) determined in 72 hours MF application is insignificant. MF exposure time did not show a significant positive increase in the deterioration of root lengths compared to increasing salt stress treatments. The difference between the effects of magnetic field exposure times on wet weight values was found to be statistically insignificant. On the other hand, wet weight values decreased in increasing salt stress applications and the differences between them were statistically significant (Table 1). In dry weights, the difference between salt stress doses was found to be statistically insignificant, while the effects of magnetic field exposure times were significant. While the highest dry weight values were obtained in the 48 and 72 hours application, the difference between them and the control was insignificant. The mean values of chlorophyll decreased with the increase of salt stress. The highest chlorophyll value, which was measured as 273.2 µg/g in the T0 control application, decreased in T1 and T2 applications. The difference between T1 and T2 is statistically insignificant. The effects of magnetic field exposure times caused a significant increase compared to the.

Table 1. Effects of magnetic field treatment exposure times on the investigated parameters of Aydanhanım variety

MF exposure time	Germination rate				Germination power				Shoot length (cm)			
	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg
Control	95.3^{a}	84.3^{abc}	77.7^{bcde}	85.8	97.7	93.0	95.3	95.3^{a}	12.9^{abc}	12.6^{abcd}	11.2^{de}	12.2
24 h	93.0^{a}	80.0^{bcd}	70.7^{def}	81.2	100.0	100.0	95.3	98.4^{a}	9.5^{f}	7.8^{g}	5.8^{h}	7.7
48 h	73.0^{cdef}	64.0^{f}	66.3^{ef}	67.8	86.0	77.3	77.7	80.3^{b}	12.0^{bcd}	11.6^{cd}	11.3^{de}	11.7
72 h	86.3^{ab}	93.0^{a}	93.0^{a}	90.8	95.3	97.7	97.7	96.9^{a}	13.6^{a}	13.2^{ab}	10.0^{ef}	12.3
Avg	86.9	80.3	76.9		94.7	92.0	91.5		12.0	11.3	9.6	
MF exposure time	Root lenght (cm)				Wet weight (g)				Dry weight (g)			
	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg
Control	11.4^{a}	8.6^{b}	7.1^{d}	9.0	2.8	2.5	2.2	2.5	0.30	0.30	0.26	0.28^{ab}
24 h	11.2^{a}	9.0^{b}	8.8^{b}	9.7	2.8	2.4	2.0	2.4	0.26	0.30	0.20	0.25^{b}
48 h	8.4^{bc}	7.4^{cd}	7.1^{d}	7.6	2.8	2.7	2.4	2.6	0.30	0.30	0.33	0.31^{a}
72 h	11.6^{a}	8.3^{bc}	7.0^{d}	9.0	3.1	2.7	2.2	2.7	0.30	0.30	0.30	0.30^{a}
Avg	10.6	8.3	7.5		2.9^{a}	2.6^{b}	2.2^{c}		0.29	0.30	0.27	
MF exposure time	Chlorophyll a (µg/g)				Chlorophyll b (µg/g)				Total chlorophyll (µg/g)			
	T_0	T_1	T_2	Avg.	T_0	T_1	T_2	Avr.	T_0	T_1	T_2	Avg
Control	180,3	157,8	139,9	159.3^{c}	101.2^{de}	87,7^{e}	86,3^{e}	94.2	159,6^{f}	149,7^{f}	135,7^{g}	147.2
24 h	334,1	296,5	270,1	300.2^{a}	151,7^{bc}	148.0^{ab}	128,3^{cd}	141.6	269,0^{a}	244.1^{c}	222,4^{d}	246.3
48 h	270,7	225,5	218,6	238.2^{b}	132,9^{bcd}	123,2^{cde}	102,1^{de}	119.4	226,6^{d}	199,5^{e}	207,8^{e}	211.3
72 h	307,6	302,7	296,	302.4^{a}	146,3^{bc}	150,0^{bc}	195,6^{a}	163.9	253,5^{bc}	254,6^{bc}	263,8^{ab}	257.3
Avg	273.2^{a}	245.6^{b}	231.3^{b}		133,0	127,2	128,0		227.2	212.8	206.6	

*T_0 : 0 g/L NaCI (control) T_1 : 3 g/L NaCI T_2 : 6 g/L NaCI

*The differences between the values indicated with different letters in the properties examined are statistically significant ($p<0.01$)

Table 2. Effects of magnetic field treatment exposure times on the investigated parameters of Baronese variety

MF exposure	Germination rate (%)				Germination power (%)				Shoot length (cm)			
time	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg
Control	77.7^{bc}	82.0^{b}	64.0^{d}	74.5	95.3^{ab}	90.7^{abc}	86.3^{bc}	90.8	10.7^{bc}	10.8^{bc}	9.0^{d}	10.2
24 h	97.7^{a}	82.0^{b}	73.0^{c}	84.2	95.3^{ab}	84.0^{c}	90.7^{abc}	90.0	10.6^{bc}	7.8^{e}	6.3^{f}	8.2
48 h	80.0^{bc}	97.7^{a}	82.0^{b}	86.5	86.3^{bc}	97.7^{a}	84.0^{c}	89.3	11.3^{ab}	6.4^{f}	9.9^{cd}	9.2
72 h	95.3^{a}	90.7^{a}	97.7^{a}	94.5	95.3^{ab}	93.0^{abc}	100.0^{a}	96.1	12.1^{a}	11.3^{ab}	7.8^{e}	10.4
Avg	87.7	88.1	79.2		93.1	91.3	90.2		11.2	9.1	8.3	
MF exposure	Root lenght (cm)				Wet weight (g)				Dry weight (g)			
time	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg
Control	10.1^{b}	8.2^{cd}	7.1^{d}	8.9	2.6^{ab}	2.4^{bc}	2.0^{de}	2.3	0.23^{ab}	0.20^{bc}	0.23^{ab}	0.20
24 h	10.4^{b}	9.4^{bc}	7.3^{e}	9.0	2.7^{ab}	2.4^{bc}	1.8^{ef}	2.3	0.23^{ab}	0.13^{c}	0.23^{ab}	0.20
48 h	10.3^{b}	12.0^{a}	7.6^{de}	9.9	2.5^{bc}	2.6^{ab}	2.5^{bc}	2.5	0.20^{bc}	0.20^{bc}	0.23^{ab}	0.20
72 h	12.0^{a}	9.3^{bc}	7.8^{de}	9.7	2.8^{a}	2.2^{cd}	1.6^{f}	2.2	0.20^{bc}	0.30^{a}	0.20^{bc}	0.20
Avg	10.7	9.9	7.6		2,61	2,40	1,97		0.21	0.83	0.22	
MF exposure	Chlorophyll a (µg/g)				Chlorophyll b (µg/g)				Total chlorophyll (µg/g)			
time	T_0	T_1	T_2	Avg.	T_0	T_1	T_2	Avr.	T_0	T_1	T_2	Avg
Control	199.7	162.5	128.9	163.7^{c}	105.8	92.8	82.8	93.8^{b}	173.9	147.3	124.8	148.6^{b}
24 h	240.7	318.4	203.5	254.2^{a}	122.9	140.6	171.4	144.8^{a}	205.1	253.0	204.5	220.8^{a}
48 h	195.4	195.5	179.7	190.1^{bc}	106.7	106.4	100.1	104.4^{b}	172.9	172.6	136.5	160.7^{b}
72 h	246.2	242.3	232.6	240.3^{ab}	129.3	127.0	124.7	127.0^{a}	213.4	209.8	208.0	210.4^{a}
Avg	192.6^{b}	229.7^{a}	186.2^{c}		106.1^{c}	116.7^{b}	119.8^{a}		171.2^{b}	195.7^{a}	168.4^{c}	

*T_0: 0 g/L NaCI (control) T_1: 3 g/L NaCI T_2 : 6 g/L NaCI

*The differences between the values indicated with different letters in the properties examined are statistically significant ($p<0.01$)

Table 3. Effects of magnetic field treatment exposure times on the investigated parameters of Bolayır variety

MF exposure	Germination rate (%)				Germination power (%)				Shoot length (cm)			
time	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg
Control	75.3^{c}	86.3^{ab}	73.0^{c}	78.2	97.7^{ab}	97.7^{ab}	93.0^{ab}	96.1	11.9^{cd}	12.9^{ab}	12.0^{bc}	12.3
24 h	90.7^{a}	77.7^{bc}	50.7^{d}	73.0	93.0^{ab}	95.3^{ab}	91.0^{abc}	93.1	9.1^{f}	6.6^{g}	5.6^{h}	7.1
48 h	90.7^{a}	70.7^{c}	73.0^{c}	78.1	100.0^{a}	90.7^{bc}	82.0^{c}	90.9	12.6^{abc}	10.9^{de}	8.2^{f}	10.6
72 h	75.3^{c}	95.3^{a}	73.0^{c}	81.2	93.0^{ab}	93.0^{ab}	97.7^{ab}	94.6	13.5^{a}	12.5^{bc}	10.7^{e}	12.2
Avg	83.0	82.5	67.4		95.9	94.2	90.9		11.8	10.7	9.1	
MF exposure	Root lenght (cm)				Wet weight (g)				Dry weight (g)			
time	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg	T_0	T_1	T_2	Avg
Control	8.3^{f}	7.0^{gh}	7.7^{fg}	7.6c	2.9	2.7	2.6	2.7^{a}	0.20	0.20	0.23	0.21
24 h	12.4^{a}	10.7^{bc}	8.4^{ef}	10.5	2.7	2.6	2.0	2.4^{ab}	0.20	0.20	0.20	0.20
48 h	9.4^{de}	6.7^{h}	7.5^{fgh}	7.9	3.0	2.7	2.4	2.6^{ab}	0.23	0.23	0.20	0.21
72 h	9.8^{cd}	11.4^{ab}	6.8^{gh}	9.3	3.0	2.1	2.2	2.3^{b}	0.23	0.17	0.23	0.21
Avg	9.9	9.0	7.6		2.9^{a}	2.5^{b}	2.1^{c}		0.22	0.22	0.23	
MF exposure	Chlorophyll a (µg/g)				Chlorophyll b (µg/g)				Total chlorophyll (µg/g)			
time	T_0	T_1	T_2	Avg.	T_0	T_1	T_2	Avr.	T_0	T_1	T_2	Avg
Control	276.3^{d}	244.1^{e}	206.9^{g}	245.8	121.9^{ef}	117.2^{ef}	111.8^{f}	109.4	232.2	198,4	182.0	204,2^{b}
24 h	341.5^{b}	251.2^{e}	230.5^{f}	274.4	281.3^{a}	139.2^{d}	118.0^{ef}	180.6	328.6	226.9	227.1	260.9^{a}
48 h	179.7^{h}	168.4^{i}	162.3^{i}	170.1	98.3^{g}	97.6^{g}	96.5^{g}	97.5	159.1	153.9	151.2	154.7^{c}
72 h	364.4^{a}	308.4^{c}	202.5^{g}	291.8	201.3^{b}	130.3^{de}	170.4^{c}	167.3	315.7	243.0	201.5	253.4^{a}
Avg	293.0	243.0	200.6		178.2	122.0	115.8		258.9^{b}	350.7^{a}	190.4^{c}	

*T_0 : 0 g/L NaCI (control) T_1 : 3 g/L NaCI T_2 : 6 g/L NaCI

*The differences between the values indicated with different letters in the properties examined are statistically significant ($p<0.01$)

Table 4. Effects of magnetic field treatment exposure times on ion leakage (%)

MF exposure time	Aydanhanım			Baronesse			Bolayır		
	Ion leakage								
	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
Control	46,8^{f}	51,1^{d}	67,2^{a}	44.7^{cd}	55.1^{b}	65.4^{a}	47.1^{d}	52.0^{b}	63.8^{a}
24 h	31,7^{k}	38,6^{j}	50,0^{e}	56.4^{b}	43.3^{cd}	50.9^{bc}	35.8^{l}	38.0^{j}	40.0^{h}
48 h	38,5^{j}	45,0^{g}	55,9^{b}	32.0^{e}	41.4^{d}	45.2^{cd}	36.8^{k}	45.4^{f}	48.4^{c}
72 h	39,9^{j}	42,2^{h}	53,8^{c}	32.1^{e}	41.1^{d}	49.0^{bc}	38.2^{ı}	42.5^{g}	45.5^{e}

*T_0 : 0 g/L NaCI (control) T_1 : 3 g/L NaCI T_2 : 6 g/L NaCI

*The differences between the values indicated with different letters in the properties examined are statistically significant ($p<0.01$)

control application (159.3 μg/g). Chlorophyll a values determined in 24 and 72 hours applications are higher than 48 hours (300.2 μg/g, 302.4 μg/g and 238.2 μg/g, respectively). The highest value in chlorophyll b values was measured as 195.6 μg/g in T_2 salt and 72 hours MF application. On the other hand, the lowest chlorophyll b value was measured in the control MF application as 86.3 μg/g in the T_2 application. Accordingto these results, it was determined that 72 hours of MF application showed a positive significant increase on chlorophyll b value. The lowest Total chlorophyll values were determined in control MF applications at all salt stress doses. On the other hand, the highest total chlorophyll value was determined as 269.9 μg/g in 24 hours of exposure time. Although MF exposure times showed a significant positive effect in mitigating the negative effects of increased salt stress, the best positive effect was determined as 263.8 μg/g in 72 h of MF exposure time. This value gave good results even in applications without salt stress. It reveals the therapeutic effect of magnetic field application for 72 h exposure time. The highest value in ion leakage values was determined at the T_2 stress level of 67.2%. A low ion leakage value indicates tolerance to salt stress. In this context, the lowest ion leakage value was determined at a T_2 stress level of 53.8% in 72 hours of MF application. On the other hand, the lowest ion leakage determined at all stress levels was determined at 31.7% in the 24h application (Table 4).

Pietruszewski et al. (2001) determined the effects of a static magnetic field on germination capacity of wheat and reported that MF densities of 100 and 180 mT gave the best results. Another study was carried out on lentil seeds by Aladjadjiyan (2010). The 150 mT static magnetic field on lentil seed has concluded better germination. Carbonell et al. (2000), reported that 150 and 200 mT MF treatments on rice and barley seeds caused faster germination under field conditions. Florez et al. (2007) emphasized that corn seeds exposed to 125 or 250 mT continuously gave the best results in terms of germination and early growth of seedlings. In another study; treatment of maize seeds with 60-200 mT static magnetic field caused stem growth and yield increase in plants (Aladjadjiyan 2007). It has been stated that exposure of seeds to magnetic fields improves the integrity of the seed coat membrane and reduces cellular leakage and electrical conductivity (Vashisth and Nagarajan 2010). Similarly, in our study, MF treatment showed positive effects on important growing parameters.

Under salt stress, plants are severely damaged during their first development period, and their growth and development periods are significantly reduced (Okumus and Uzun 2022). Salt stress can kill the plant directly, as well as significantly reduce plant growth, seed germination, root length, plant height and grain yield (Yavuz et al., 2022), it is more effective in older leaves and causes chlorosis and necrosis by chlorophyll and cell membrane breakdown (Kalisz et al., 2023). The tolerance of plants to high salt doses is determined in the first developmental stages. In this period; germination percentage, germination rate and strength are important parameters that should be examined against salt stress (Zhu et al. 2018).

The results of Baronesse cultivar are given in Table 2. The germination rate of the Baronesse cultivar decreased with increasing salt stress doses (Table 2). The lowest germination rate was determined as 64.0% at T_2 stress treatment. This rate reached 97.7% at 72 h of MF exposure time. The results showed that 72 exposure time in MF application was successful in treating the negative effect caused by salt stress. Similarly, increasing salt stress doses had a negative effect on germination power results. MF applications have shown significant

positive effects in improving this negative effect. At the T_1 stress treatment, 48 h MF exposure time resulted in 97.7%, and at the T_2 stress level, 72 h MF exposure time resulted in 100% germination power. Shoot length values were best determined in 72 and 48 hours applications in the T_0 control application (12.1 and 11.3 cm, respectively). The highest value of root length in the T_0 control application was measured as 12.0 cm in 72 h exposure time. A similar, 12.0 cm root length was determined in 48 h exposure time at the T1 stress level and it was measured as the best root length in the all trial. While wet weight gain occurs with cell expansion (Ismail et al., 2023), dry weight increase occurs as a result of cell division and new material synthesis due to high photosynthetic activity (Khan et al., 2023). The highest value in the Baronesse cultivar, wet weight values was determined as 2.8 g in 72 h exposure time, but the difference between the control and 24 hours is important. The effects of MF applications in increasing salt stress levels did not show a statistically significant positive effect. In dry weight values, the highest value at the T_1 stress level was determined as 0.30 g in 72 hours of MF application. In dry weight values at T_2 and T_1 levels, MF applications did not give better results than the control. Chlorophyll a values were measured as 192.6, 229.7 and 186.2 μg/g at T_0, T_1 and T_2 stress levels, respectively. In MF applications, the best chlorophyll a values were determined on average at all stress levels in 24-hour and 72-hour applications (254.2 μg/g and 240.3 μg/g, respectively). The best results in terms of mean values on chlorophyll b values of magnetic field applications were measured as 144.8 μg/g in 24 hours and 127.0 μg/g in 72 hours. The difference between them was found to be statistically insignificant. The effect of salt stress on the total amount of chlorophyll was different. The total chlorophyll value of 171.2 μg/g determined in T_0 application was determined as 195.7 μg/g in T_1 and 168.4 μg/g in the T_2 stress treatment. This difference supports the idea that the plant tends to increase its photosynthetic capacity to provide stress tolerance. In addition, the decrease in the total amount of chlorophyll at the increased stress level can be interpreted as the plant is adversely affected by stress and loses its tolerance. Among the MF exposure times, the highest total chlorophyll amount was determined at all stress levels, on average, at 72 h and 24 h (210.4 μg/g and 220.8 μg/g, respectively). The highest ion leakage value was calculated as 65.4% at the T_2 stress treatment. The 45.2% ion leakage value determined in 48 h exposure time of MF determines that the treatment is successful. In addition, other MF practices had significant positive effects (Table 4).

The results of the Bolayır cultivar are given in Table 3. The highest germination rate in Bolayır cultivar was determined in T0 stress treatment at 24 h and 48 h exposure times (both 90.7%) (Table 3). There was no statistical difference between 72 h and control MF exposure time application in the T0 stress treatment. The best germination rate at the T1 stress level was 95.3% in 72 h exposure time of MF, but the difference between control MF exposure time (86.3%) was statistically insignificant. No significant positive effect of MF exposure time on germination rate was determined at the T2 stress level (Table 3). The difference between the effect of MF exposure time on germination power in T0 stress treatment is statistically insignificant. Similarly, the difference between control, 24h and 72h MF applications at T1 stress level was found to be insignificant. The same is true for the T2 stress level. The lowest germination power was determined in 48h MF application at T1 and T2 stress levels (90.7% and 82.0%, respectively). In general, the increased salt dose negatively affected the germination rate and strength parameters, and it shows parallelism with other studies (Benlioglu and Ozkan 2018, Okumus and Uzun 2022, Doruk Kahraman and Gokmen 2022). Studies have shown that magnetic field treatment reduces the damage caused by salt at high doses (Shabalkin et al., 2023). Considering other studies (Wang et al., 2016), increasing salt doses negatively affected shoot and root lengths. However, it has been determined that magnetic field treatment has a positive effect at high salt doses, and the results have shown parallelism in similar studies (Abdellaoui et al., 2004; Beyaz et al., 2020).

Shoot length values in Bolayır cultivar were determined as 13.5 cm in the T_0 application for 72 hours in the MF application (Table 3). With increasing salt stress, shoot length values decreased and could not be treated with MF applications. The best effect on root length was measured as 12.4 cm in 24 hours MF application. At T_1 salt stress level, the highest root length was determined in 72 hours of MF treatment. The best average value was determined in T_0 application in wet weight values (2.9 g). As the salt stress level increased, the wet weight value decreased (2.5 g and 2.1 g, respectively). There is no statistical difference between 24 h and 48 h MF exposure times and control. There was no statistical difference between the effects of salt stress and MF exposure times on dry weight values (Table 3). The best positive effect on chlorophyll a value at T_0 stress level was determined in 72 hours of application (364.4 μg/g). The best value at the T_1 stress level was again measured

as 308.4 μg/g in 72 hours of application. There was no positive effect of MF applications on T_2 stress level. The best result in chlorophyll b values was determined in the T_0 application during 24 hours of exposure to MF (281,3 μg/g). No significant positive effect of MF applications was found at other stress levels. Total chlorophyll values were measured as 258.9 μg/g in the T_0 control treatment, 350.7 μg/g in the T_1 stress treatment and 190.4 μg/g in the T_2 stress treatment. The increase in the total amount of chlorophyll in the T_1 stress level was interpreted as the low-level stress trying to increase the tolerance of the plant by creating a triggering effect. 24 and 72 hour applications on total chlorophyll amounts are the applications in which the best values are determined at all stress levels (Table 3). A decrease in the total amount of chlorophyll was determined in parallel with the increasing salt dose. Similar results were found in studies (Ru et al., 2023; Song et al., 2023). Chlorophyll content directly affects photosynthesis (Nalley et al., 2023) and is accepted as an indicator of photosynthetic capacity in tissues (Song et al., 2023). Since the high photosynthetic activity will increase the production of the substance, it also increases the yield (Ru et al., 2023). Chlorophyll coverage can be used as a measure of growth (Joshi et al., 2023). This shows that the magnetic field strength increases the rate of photosynthetic activity (Ercan et al., 2022; Hafeez et al., 2023). The highest ion leakage in Bolayır variety was determined at T_2 stress level and 63.8% in control MF application. The lowest ion leakage value at the T_2 stress level was measured as 40.0% in 24 hours of MF application. The lowest ion leakage values in the whole experiment were determined in the 24-hour MF application (Table 4).

All organisms are under the influence of the Earth's magnetic field, called the geographic magnetic field (GMF) (Maffei, 2014; Pietruszewski and Martínez, 2015). Although the density of GMF varies according to the regions; this value varies between 25 and 65 μT. (Occhipinti et al., 2014). Exposure of seeds to higher MF than GMF (from the current magnetic field of the environment) increases seed germination and plant growth by increasing water assimilation and photosynthesis (Cotrina Cabello et al., 2023). Seeds exposed to higher MF exposure than GMF not only germinate faster, but also more uniformly. Rapid and uniform development is an indication that plants will gain superiority against adverse conditions during vegetative development. Another important parameter in the MF effect is the MF intensity. Cecchetti et al. (2022); in their study to determine the effects of a static magnetic field on the germination capacity of wheat, it was reported that the density of 100 and 180 mT gave the best results. Besides, the study carried out by Aladjadjiyan (2010) on lentil seeds and 150 mT static MF showed better germination of lentil seeds. Florez et al. (2007) reported that 150 and 200 mT MF treatment of rice and barley seeds resulted in faster germination of seeds under field conditions. Florez et al. (2007) emphasized that maize seeds continuously exposed to 125 or 250 mT performed best in terms of germination and early growth of seedlings. The treatment of a constant magnetic field intensity of 150 mT in our study is important in determining the effect on the investigated parameters. Pietruszewski and Martínez (2015) stated that MF effect studies performed have reported that it varies in a wide range from μT to mT and T, and the results vary up to 109 times. However, with these wide differences in applied MF values, it is seen that their effects are similar to each other and result in positive results. It has been reported that the important factor here is the exposure time to the magnetic field. In the results of the current study; in addition to the yield increases obtained at certain exposure times, decreases were also observed. This result supports that the fixed MF effect does not always have a positive effect at different exposure times. In MF effect studies, in addition to magnetic field type and intensity, the most important parameter is exposure time. Vashisth and Joshi (2017); In the study, they investigated the effects of different MF doses on certain seedling parameters in maize plants. The increase in MF strength did not show parallelism with the increase in physiological development; similarly, they reported that there was no linear relationship between physiological development and exposure time. Therefore, it is of great importance to investigate the effect of continuous and constant MF at different time intervals on different physiological and molecular responses of the plant. In this way, it is predicted that results that will enable the elucidation and association of the MF effect mechanism will be obtained.

4. Conclusions

Environmentally friendly and innovative agricultural approaches have become essential for ecological and sustainable life in the current order of the world. Especially in the last years negative effects of fertilizers, pesticides, insecticides to environment have led to finding new solutions. Even though magnetic field treatments are not new, their effects and applications are still not common enough among people working in agriculture. Considering all the examined properties, the results revealed that the magnetic field treatment exposure time had a significant effect on seed germination, plant growth, seedling height and root length. In the light of the data obtained, results have been obtained that will provide important contributions to innovative biotechnological approaches to improve new variety development, yield and yield parameters needed in agricultural production. On the other hand, in the treatment of magnetic field to increase the tolerance against salt stress in barley, researchers are recommended to perform optimization studies for the exposure time and power of the magnetic field. It is suggested that magnetic field treatments be carried out on different plant species and varieties, especially considering the possible differences due to plant variety differences. Experimenting with this study with other plant species and cultivars in both laboratory and field conditions will increase the success rate.

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Effect of Organo Mineral Fertilization on Weed Infestation and Dynamic in Upland Rice Growth in The Southern Sudanian Zone of Burkina Faso

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HIGHLIGHTS

- Dynamism and specific diversity of weeds were determined;
- The impact of fertilisation on weed clustering was determined;
- The effect of weeds on upland rice yield was evaluated.

Abstract

Weeds are a major biotic constraint in rice production, causing crop yield losses. Fertilization system may be an effective control of weeds. This study aims to improve rice productivity through soil amendment and weed management in Burkina Faso. Method. The trial was set up in a Fisher block design with 4 replications and 10 fertilizer treatments. The effect of the fertilization was assessed on weeds at 45 days after planting through their abundance and dry biomass, and on rice through plant height and grain yield. weeds diversity at 65 days old has an average Shannon index of 2.9 bits. Three (03) weed clusters and their indicator species that are associated with the treatments studied were identified at 65 days after sowing. The Cyperus esculentus L. weed grouping was found to be associated with the organo-mineral fertilizer treatments. The application of poultry manure plus urea obtained simultaneously the lowest weed infestation rate (6.37 g/m²) and the highest grain yield (1.6 t/ha). Thus, to improve the productivity of upland rice, integrated weed management could be a combination of poultry manure treatments combined with mineral fertilization and specific control methods for the main species of the *Cyperus esculentus* L. group.

Keys words: Poultry manure, weeds, indicator species, diversity, up-land rice

1. Introduction

Rice cultivation feeds more than 50% of the world's population. In terms of global production, rice is the second most important cereal after corn, with 755.1 million tons produced in 2016 (FAO, 2018). In recent years, demand for rice has grown strongly at 6% per year in West and Central Africa (Rio and Brent, 2014). In Burkina Faso, rice is the fourth most produced cereal (Bazié et al. 2014). Moreover, rice is increasingly consumed with an average annual consumption of 35 kg per person in 2013 and about 50 kg/person/year in large urban centers (FAO, 2014).

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There are three types of rice cultivation, namely upland, lowland and irrigated rice, with a national production of 384,690 tons (INSD, 2017). Up-land rice, represents 23% of the rice growing area and contributes 5% to national rice production. This type of rice cultivation is only suitable for regions of Burkina where annual rainfall reaches or exceeds 800 mm (Ouédraogo et al. 2011). This type of rice cultivation. Several improved varieties from varietal selection programs are available for this type of rice cultivation, mainly, FKR 45N, FKR 59 and FKR 61. Crop rotation between rice and other cereals is low and irregular (Traoré, 2015).

Indeed, weeds are the second most important limiting factor in rice production after water (Deuse et al. 1980). They limit rice productivity through competition for nutrients (Anonymous, 1995). These two factors are a specific constraint in upland rice production. Crop rotation systems that could improve soil fertility upland rice production are almost non-existent in (Traoré et al. 2015; Sanou et al. 2019). Thus, it is necessary to propose integrated fertilizer management methods in this cropping system

The effect of environmental and agronomic factors on weed dynamics is a prerequisite for any cropping system (Barralis and Chadoeuf, 1980). It is in this context that this article aims to determine the effect of organo-mineral fertilization on weed infestation and dynamics in up-land rice production in western Burkina Faso.

2. Materials and Methods

The study was conducted at the Farako-Bâ research station, headquarters of the DRREA-Ouest, within the INERA Rice and Rice Culture Program. The station is located about 10 km south of Bobo-Dioulasso on National Road No. 07 (Bobo - Banfora axis), in the Sudanian zone of Burkina Faso. Its geographical coordinates are: 11°6' North latitude, 04°20' West longitude and 405 m altitude. The amount of rainfall varied from 59.1 to 262 mm between 4 and 14 days during the 2016, 2017 wet seasons (Figure 1 and 2).

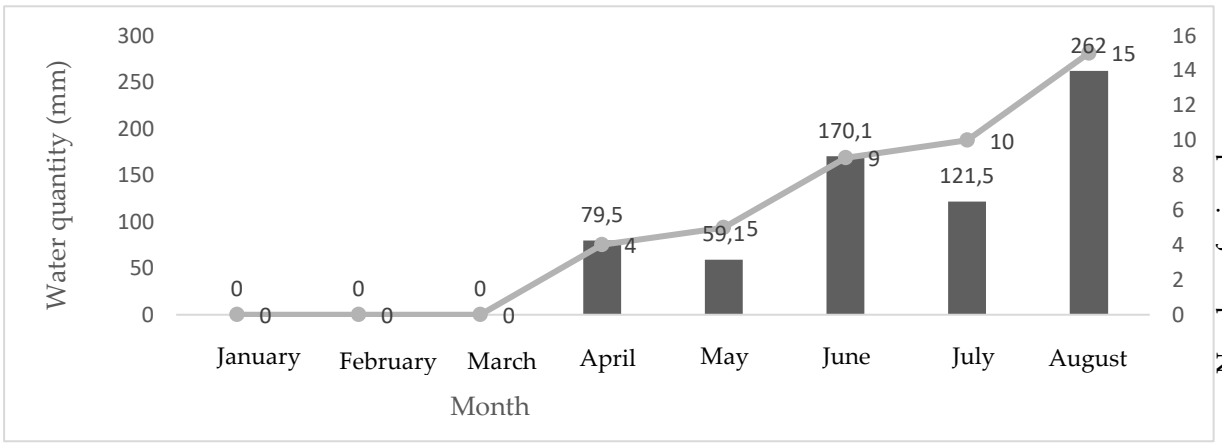

Figure 1: Rainfall of the Farako-Bâ station, 2016

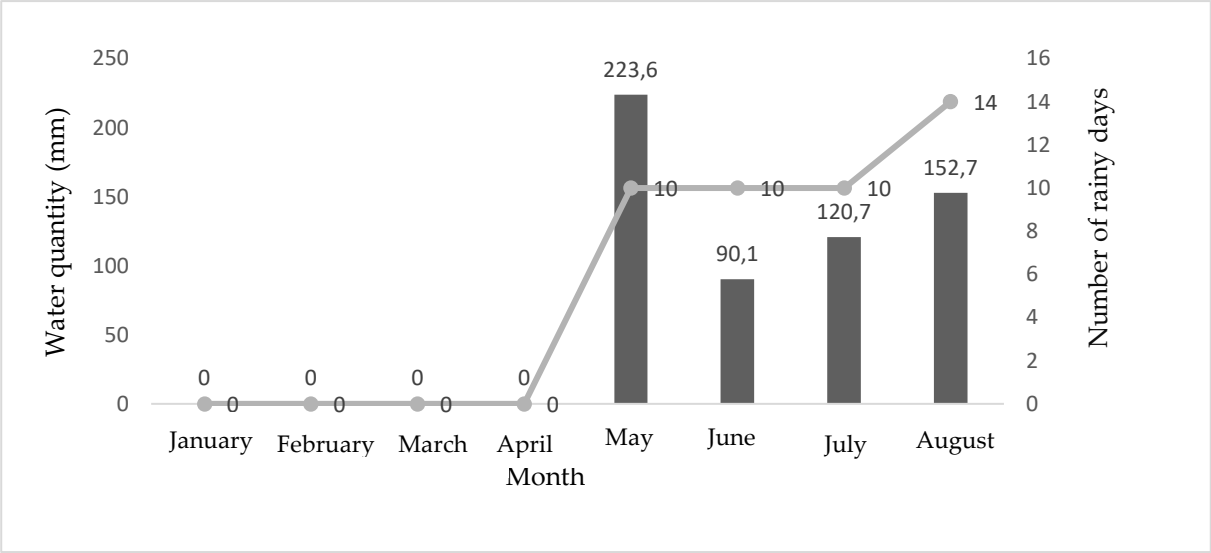

Figure 2: Rainfall at the Farako-Bâ station, 2017

The plant material used for the experiment is the rainfed rice variety FKR 59N with a sowing maturity cycle of 90 days from INERA. The fertilizers used were organic (poultry droppings, and compost and mineral (Burkina phosphate (BP, NPK). The organic matter was either rice straw harvested during the previous season or manure composed of animal dung produced at the Farako-Bâ.

The trial was conducted in a fully The Randomized Block Designing with 10 fertiliser treatments (Table 1) and 4 replications. The factor studied was the type of fertilisation at 10 levels (Table 1). The total trial area was 571.5 m2 and the individual plots were 9 m2 (3 m × 3 m) each. The unit plots were separated by 0.5 m rows and the blocks by 1 m rows. Ploughing was carried out by animal traction one week before sowing, followed by levelling and loosening of the soil to obtain a good seedbed. Various fertilisers such as poultry droppings as well as Burkina phosphate (BP) and compost were incorporated into the soil two (02) days before sowing. Sowing was carried out seven days after ploughing with a spacing of 20 cm between the rows and between bunches, followed by de-matting with two (02) plants per bunch at 14 days after sowing (DAS). The doses of fertilizers were applied in each elementary plot in accordance with the fertilization. NPK (14-23-14) was applied at sowing. Urea was applied in two fractions at 15 and 46 days after weeding. NPK (14-23-14) was applied at sowing (200 kg/ha). Urea was applied in two fractions at 15 (35 kg/ha) and 46 days after weeding (65 /ha).

Table 1: List of manures applied in the elementary plots

Treatments	Fertilization mode
F1	Absolute control (no manure)
F2	NPK (14 -23-14) + Urea
F3	Urea + BP (500 kg/ha)
F4	Poultry manure (7.5 t/ha)
F5	Poultry manure (7.5 t/ha) + Urea
F6	Poultry manure (7.5 t/ha) + BP (500 kg/ha)
F7	Poultry manure (7.5 t/ha) + BP (500 kg/ha) + Urea
F8	Rice straw compost (10 t/ha) + Urea extension
F9	Rice straw-based compost (10 t/ha) + BP (500 kg/ha)
F10	Rice straw-based compost (10 t/ha) + BP (500 kg/ha) + Urea

Data collection was done on weeds and rice crop:
- Weed biomass (g/m²): Weed biomass was determined after weeding at 45 days after harvesting in three 1 m² plots and drying for 24 hours at 60°C in the oven;
- Weed cover (%): was determined at 45 days after harvesting according to the Marnotte (1984) scale (Table 2);
- Weed abundance: was determined during the inventory at 65 DAS, using the Barralis (1976) scale (Table 2).
- Height (cm) of rice plants: This parameter was recorded on 12 plants along the diagonals within each elementary plot using a ruler;
- Number of rice tillers: This was counted on 12 plants along the diagonals in each
- diagonals of each elementary plot;
- Weight of 1000 grains: this was obtained by counting 1000 grains and weighing them with an electronic scale.
- Yield (t/ha): Harvests were made on the useful plots after removing the two border lines. Paddy yield was calculated and reported per hectare at 14% moisture content using the following formula: R (t/ha) = (14×10-2 ×R)/ (Th×Su) (R = Yield (t/ha) obtained from the useful plot; Th = Moisture content of harvested paddy; Su = Useful area).

Floristic surveys were used to characterize floristic richness/diversity in the plots:

(i) Shannon-Weaver index: The Shannon-Weaver (1949) index (H) is an indicator of diversity, taking into account not only species richness, but also the proportion of each species represented within the community. It varies between 1 and 5 (Lincy, 2003). The higher the Shannon index, the greater the diversity. This index can be used to determine the level of stand structure. Two stands may have the same diversity index but different taxonomic richness, and therefore different levels of structure.

The Shannon-Wiener index is calculated according to the following formula:

$\mathbf{Ish = -\Sigma \left(\frac{ni}{N}\right) \log_2 \left(\frac{ni}{N}\right)}$, where ni is the number of species i and N is the total number of species;

(ii) Simpson's index: Simpson's index (SI), which favors the most abundant species, expresses the probability that two (02) individuals chosen at random from an infinite population belong to the same species, and varies between 0 and 1. The higher the index, the lower the dominance within the group (Lincy, 2003).

Simpson's index is calculated according to the following formula:

$\mathbf{IS = \Sigma \left(\frac{ni}{N}\right)^2}$, where ni is the number of species i and N is the total number of species;

(iii) Piélou Equitability Index: The Piélou Equitability Index (Eq), measures the degree of diversity achieved by a stand in relation to its maximum value, and enables us to compare two groups that do not have the same number of species (Grall and Coïc, 2005).

It lies between 0 and 1. If Eq tends towards 0, then almost all individuals belong to a single species within the group, and when Eq takes the value 1, then all species have exactly the same coverage or importance within the group.

The Piélou Equitability Index was calculated using the following formula:

$\mathbf{Eq = \frac{ISh}{\log_2 N}}$, where N is the total number of species;

(iv) Weeds clustering and influence of treatments on clustering : Cluster analysis was used to determine weeds grouping. The influence of treatments on clustering was determined using the indirect Canonical Correspondance Analysis (CCA) method;

(v) Indicator species: The indicator species of the clusters were determined by Indicator Species Analysis (ISA), which uses a Monte Carlo test to give the following values: the indicator value of the species, the probability at the 5% threshold of the indicator species. The taxonomic names of the indicator species will be used to name the plant groupings (Dossou et al., 2012).

The Excel spreadsheet was used for data entry. Variance analysis was carried out using Genstat statistical analysis program, and comparisons of means were made using Duncan's test with a threshold of 5%.

PC-ORD version 5.0 ordination program was used to carry out the canonical correspondence analysis (CCA) and the phytosociological analysis of the weeds, making it possible to highlight the influence of treatments on weed distribution.

Table 2: Combined scale of Marnotte (1984) for scoring cover rate and Barralis(1976) and weed abundance

Class	P (%)	Recovery rate (%)	Abundance
1	**< 1**	Species present, but rare	< 1 individual/m2
2	**1-7**	Less than one individual per m2	1-2 individual /m²
3	**7-15**	At least one individual per m2	3-20 individual /m²
4	**15-30**	15-30% cover	21-50 individual /m²
5	**30-50**	30-50% cover	>50 individual m²
6	**50-70**	50-70% cover	
7	**70-85**	Strong cover	
8	**85-93**	Very little visible soil	
9	**93-100**	Full coverage	

3.Results and discussion

The weed flora recorded at 65 days before harvest on all the treatments is 20 species belonging to 20 genera and 08 families. The class of dicotyledons represents 70% and the dominant families are *Cyperaceae* (25%) and *Fabaceae* (25%) (figure 3). The canonical correspondence analysis (CCA) of the main matrix consisting of 20 species inventoried at 65 JAS, reveals that the two axes explain 26.9% of the existing relationship between species and treatments (Figure 4). The Monte Carlos test showed that the treatments had a significant effect on the distribution of 15% of the species on the first two axes (P<0.05%) with the formation of three weed plant groupings. The rate of weed cover at 45 days after planting varies according to the fertilization method (Table 3 and 4). The analysis of variance shows that there is a highly significant difference (P<0.001) between treatments in terms of plot coverage. The F1 treatment recorded the highest rate of coverage, i.e. 55%, compared to the F7 treatment, which recorded the lowest rate of coverage (27.5%). Weed dry biomass at 45 days after planting also varied among treatments (Table 4). Analysis of variance reveals that manures had a highly significant effect on weed dry biomass (P<0.001). Treatment F4 had the highest dry biomass (27.40g/m2). On the other hand the lowest was recorded with treatment F5 (6.37g/m2).

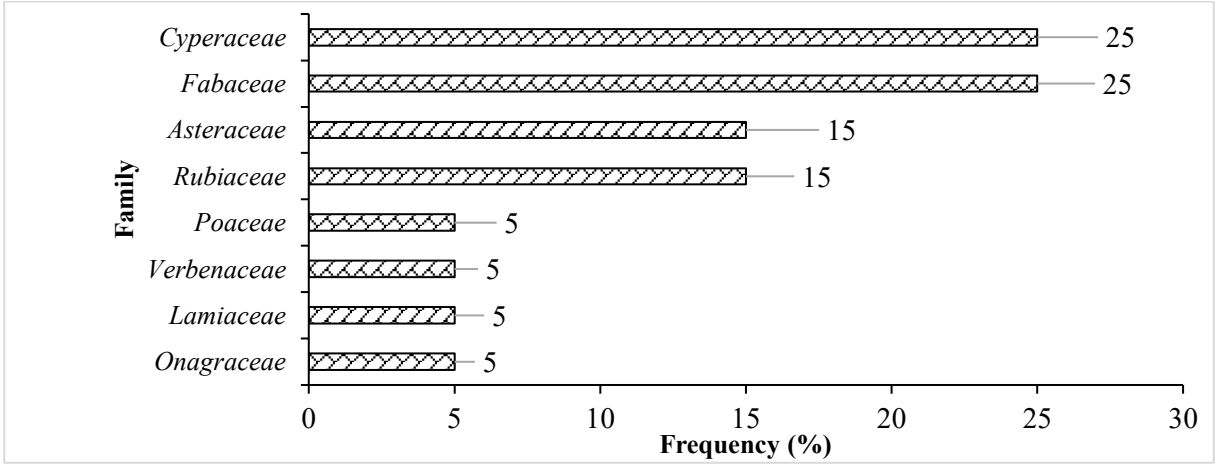

Figure 3: Frequency (%) of weed families inventoried at 65 DAS

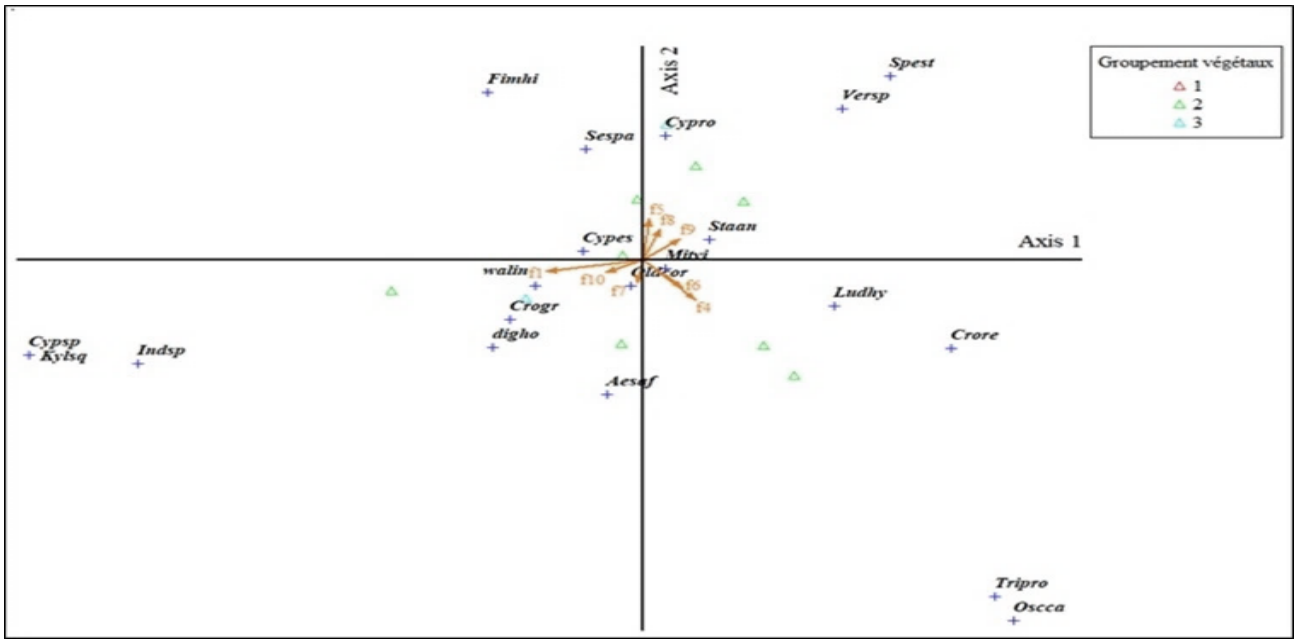

Figure 4: Distribution diagram of weed species inventoried at 65 DAS according to treatments.

Legend: Plant grouping: Grouping of weeds where the influence of the treatments is the most significant ; 1; 2, 3: Weed subgroups according to the influence of the most significant treatments

Table 3: Plant grouping characteristics of weed species inventoried at 65 DAS

Code	**Name of the grouping**	**RS**	**Indicator species**	**IVI**	**P-value (Monté Carlo)**	**Dominant family**	**Ish**	**IS**	**IE**
G1	*Sesbania pachycarpa* de Candolle	5	*Sesbania pachycarpa* de Candolle	60	0,0002	*Fabaceae*	2,9	0,93	0,96
G2	*Oldenlandia corymbosa* L.	13	*Oldenlandia corymbosa* L.	43,9	0,0344	*Cyperaceae*	2,9	0,93	0,95
						Fabaceae			
G3	*Cyperus esculentus* L.	2	*Cyperus esculentus* L.	54,2	0,0016	*Cyperaceae*	2,9	0,93	0,96
			Moyenne				**2,9**	**0,93**	**0,956**

SR: Species richness; IVI: Species indicator value; H: Shannon index; SI: Simpson index; IE: Piélou equitability index

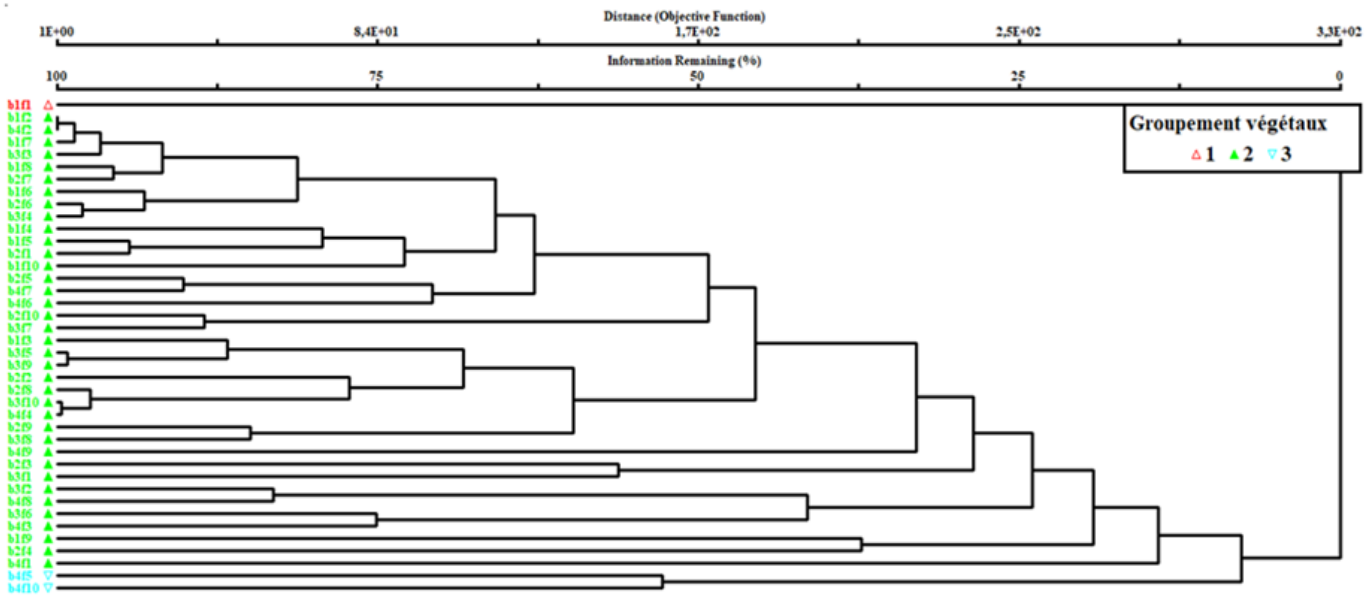

Figure 5: Classification dendrogram of weed species inventoried on the 65 DAS treatments at Farako-Bâ

Legend: Plant groupings. Weed groups where the influence of the treatments is most significant;1; 2, 3: Weed subgroups according to the influence of the most significant treatments.

Table 4: Coverage rate at 45 DAS of the plots and Dry biomass of weeds according to the fertilization mode, Farako-Bâ, wet season 2016 and 2017

Fertilization mode	Recovery rate (%) at 45 DAS	Dry biomass (g/m²)
F7 (Poultry manure + BP + Urea)	27,5^{a}	7,75^{a}
F8 (Compost + Urea)	35^{ab}	9,12^{a}
F3 (Urea + BP)	40^{abc}	10,02^{a}
F5 (Poultry manure + Urea)	40^{abc}	6,37^{a}
F10 (Compost + BP + Urea)	45^{bc}	13,45^{ab}
F2 (NPK + Urea)	45^{bc}	8,60^{a}
F4 (Poultry manure)	50^{bc}	27,40^{c}
F6 (Poultry manure + BP)	50^{bc}	19,05^{b}
F9 (Compost + BP)	50^{bc}	26,67^{c}
F1 (Control without manure)	55^{c}	11,67^{a}
P	0,009	<0,001
M	43,8	14,01
Cv (%)	9,5	16,6

3.1. Effect of treatments on yield parameters of rice

The number of tillers and the height of the rice plants varied according to the fertilization method at 45 days after planting (Table 5). For the number of tillers, there was a highly significant difference (P<0.001) between treatments. The F1 treatment (Control without fertilization) has the lowest average number of tillers (1.2 tillers). While treatment F5 (Poultry manure + Urea), recorded the highest average number of tillers (3.5 tillers). Treatments F10 (Compost + BP + Urea), F5 (Poultry manure + Urea) and F4 (Poultry manure) form a statistically homogeneous group.

The same tendency can be observed regarding the height of the plants. Indeed, the highest plant height (84.7 cm) was obtained with the F5 treatment (poultry manure) while the lowest (41.7 cm) was obtained with the F1 control treatment. Straw biomass and rice grain yield varied according to the fertilization method (Table

6). The results show a highly significant difference (P<0.001) between treatments for straw yield. Treatment F5 had the highest straw biomass (7.3 t/ha) while the T1 control had the lowest straw biomass (0.7 t/ha). Treatments F6, F4, F7 and F5 formed a statistically homogeneous group with treatment F5.

With respect to rice grain yield, the F1 control treatment had the lowest grain yield (0.3 t/ha). The highest grain yield was obtained with treatment F5 (1.6 t/ha), which forms a statistically homogeneous group with treatments F7 and F6.

Table 5 : Number of tillers and height (cm) of rice plants according to the fertilization method, Farako-Bâ, wet season 2016 and 2017

Fertilization mode	Number of tillers	Height (cm)
F1 (Control without fertilizer)	1,196^{a}	47,31^{ab}
F3 (Urea + BP)	1,870^{ab}	61,39^{c}
F9 (Compost + BP)	1,929^{b}	56,77^{abc}
F8 (Compost + Urea)	1,287^{ab}	44,82^{a}
F10 (Compost + BP + Urea)	3,331^{c}	81,47^{d}
F2 (NPK + Urea)	3,234^{c}	80,27^{d}
F5 (Poultry manure + Urea)	3,507^{c}	84,74^{d}
F4 (Poultry manure)	3,407^{c}	77,33^{d}
F7 (Poultry manure + BP + Urea)	1,817^{ab}	58,84^{bc}
F6 (Poultry manure + BP)	1,511^{ab}	51,97^{abc}
P	<0,001	<0,001
M	64,5	2,309
Cv (%)	9,0	15,8

Table 6: Straw biomass (kg/ha) and grain yield (t/ha) of rice according to mode, Farako-Bâ, wet season 2016 and 2017

Fertilization mode	Straw weight (t/ha)	Grain yield (t/ha)
F1 (Control without fertilizer)	0,7^{a}	0,3^{a}
F10 (Compost + BP + Urea)	1,1^{ab}	0,70^{ab}
F2 (NPK + Urea)	1,0^{b}	0,80^{ab}
F3 (Urea + BP)	0,8^{a}	0,40^{ab}
F4 (Poultry manure)	18,0^{c}	1,4^{c}
F5 (Poultry manure + Urea)	20,5^{c}	1,6^{c}
F6 (Poultry manure + BP)	1,73^{c}	1,5^{c}
F7 (Poultry manure + BP + Urea)	2,02^{c}	1,5^{c}
F8 (Compost + Urea)	11,0^{ab}	0,84^{b}
F9 (Compost + BP)	7,7^{a}	0,60^{ab}
P	<0,001	<0,001
M	1,36	0,98
Cv (%)	18,8	18,9

3.2. Discussion

At the end of the 65 DAS inventory, *Cyperaceae* (25%) and *Fabaceae* (25%) were identified as the most dominant families. In fact, a study of farmers' perceptions of the most harmful weed families revealed that the *Cyperaceae* is the most feared family in up-land rice production in Burkina Faso (Sanou, 2019). Regarding the distribution of classes where dicotyledons (70%) are the most frequent. Traoré and Maillet (1998) obtained a similar frequency for dicotyledons within cereal crops in Burkina Faso. Rahali et al. (2010) explain this dominance in part by the effect of tillage that is much less favorable to the development of monocotyledons. Three plant groupings were identified based on the effect of fertilisation at 65 JAS.

However, this influence is weak because fertilizer type and level explain only 26.9% of the grouping of weed species at 65 DAS. In lowland rice, the effect of fertilizer type and level on weed distribution is stronger, averaging 41% (Sanou et al. 2022). This could be explained by the fact that the lowland rice cropping system is a continuous rice cropping system. However, other factors not considered in this study contribute to the distribution of weeds in cultivated fields, namely crop growth and associated cropping practices (Freid et al. 2008).

In the present study, three (03) species were considered as characteristic species because of their strong indicator value within the plant groups: they are *Oldenlandia corymbosa* L.; *Sesbania pachycarpa* de Candolle and *Cyperus esculentus* L. Similar results were obtained by identifying plant groups and their characteristic species on the basis of their affinity to environmental conditions (Dossou et al. 2012) and specifically the mode of weeding and fertilization in rice cultivation (Sanou, 2022). In this study, the majority of species remain indifferent to fertilizers.

However, the *C. esculentus* group, despite a low species richness, seems to have an affinity for treatments F5, F10 respectively.

However, according to Colbach et al. (2013), the effect of fertilization on weed floristic composition is primarily related to the facilitation of nutrient availability that it allows, rather than the type of fertilizer. The floristic composition could be related to the fact that poultry dung-based fertilizers improve the amount and availability of soil nutrients. Indeed, poultry manure in combination with mineral fertilizer can contribute to the improvement of soil fertility and ensure the sustainability of cropping systems (Gomgnimbou et al. 2019).

The average diversity indices including the Shannon (2.9 bits), Simpson (0.93 bits) and Piélou (0.96 bits) indices recorded for these three (03) plants groupings are quite low. These indices differ from those obtained by Dossou et al. (2012) who reported indices of the order of 4.3 bits for the Shannon index and 0.65 for the Piélou Equitability index of the plant groups of the Agonvè swamp forest in South Benin. This difference could be explained by the disruptive effect of cultivation practices in the cultivated fields.

In terms of number of tillers, rice straw biomass, and height, all fertilizer treatments improved the vegetative development of rice plants compared to the no-fertilizer control. These results corroborate those of Agbede et al. (2008), who also obtained better vegetative development of sorghum using poultry dung. However, the addition of BP to poultry dung appears to have a more efficient effect on rice development. These results corroborate those of Mahasen et al. (2012) who obtained the similar results in cotton crop. Weed dry biomass (27.4 g/m2) is much higher under manure fertilization at 7.5 t/ha. However, this infestation does not affect rice yield compared to the control without fertilizer. This could be explained by the fact that organo-mineral fertilization improves soil water status, improves nutrient availability and nutrient content (Ouattara, 1994). These results corroborate those of Hien (2004), who estimates that increasing manure rates can maintain sorghum yields between 1.5 and 3.5 tons/ha. The mode and type of organo-mineral fertilization improved rice yields (Chanda et al. (2021); Sanou et al. (2022)).

3.3. Conclusions and perspectives

This study has contributed to the identification of the impact of soil fertility management on floristic diversity and weed infestation in rainfed rice. The fertilizers used had an effect on weed dynamics and infestation. Indeed, the control plot without fertilization had the highest cover and abundance rates.

Dry biomass was higher with the application of poultry manure at 7 t/ha. The fertilization treatments explain 26.9% of the vegetation grouping. Three (03) herbaceous communities were identified on the basis of their affinity with the treatments. With regard to the agro-morphological parameters of the rice, it appears that they varied according to the types of fertilizers applied. Indeed, these parameters were favored by organo-mineral fertilization, mainly urea, poultry droppings and Burkina Phosphate.

In the future, it will be interesting to:

- to study the effect of organo-mineral fertilizers on the dynamics of the seed stock of weeds;

- to determine the effect of the content of the main soil nutrients on the distribution of weeds.

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Research Article

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Determination of Essential Oil Yields and Composition Lavender and Lavandin Cultivars (*Lavandula sp.*) Cultivated in Tuzlukçu, Konya

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HIGHLIGHTS

- Lavender and lavandin cultivars were compared.
- As Konya and its surroundings are more ecologically arid, the impact of stress conditions on the constituents of lavender species was examined.
- The interpretation of the difference between the ratio of essential oils and the constituents of lavender.

Abstract

Lavender is an important medicinal plant, which used in many fields such as perfumery, cosmetics, medicine, aromatherapy and phytotherapy thanks to its high quality essential oils components. Hemus and Sevtapolis lavender cultivars of Lavandula angustifolia and Super A lavandin of Lavandula x intermedia were used in the study. The lavenders and lavandin were planted in 2022 in Tuzlukçu district of Konya province and harvested in 2023. Essential oils were obtained from the dried flowers of the plant. The essential oil components of the plant materials were measured by GC-MS. The essential oil yield of *Lavandula angustifolia* cv. Hemus was 2.65%, that of *Lavandula angustifolia* cv. Sevtapolis was 2.14% and that of *Lavandula x intermedia* cv. Super A was 7.95%. In the Hemus cultivar of lavender, the concentration of linalyl acetate was 19.76%, linalool was 39.09%, and camphor, an undesired component in lavender essential oil, amounted to 0.41%. Meanwhile, the Sevtapolis cultivars contained 10.64% linalyl acetate, 40.41% linalool and 0.47% camphor. In Lavandin (Super A) cultivar contained 30.39% linalyl acetate, 30.29% linalool, and 6.71% camphor.

Keywords: Lavender, lavandin, essential oil content, essential oil composition

1. Introduction

Lavender (*Lavandula* sp) is a perennial plant that produces essential oil and belongs to the Lamiaceae family. It has a semi-shrub form, grows between 20 and 60 cm in height and has lilac-blue flowers (Ceylan, 1996; Zeybek and Zeybek, 1994). The plant originates from the Mediterranean and grows naturally in the Balkans, as well as Southern Europe and North Africa, bordering the Mediterranean Sea. There are approximately 39 lavender species (*Lavandula* sp.). The lavender species with commercial value globally are Lavender (*Lavandula angustifolia* Mill. = *L. officinalis* L. = *L. vera* DC), Spike Lavender (*Lavandula spica* = *L. latifolia*

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Medik.), and Lavandin (*Lavandula intermedia* Emeric ex Loisel. = *L. hybrida* L.), known as hybrid and has high essential oil yield (Beetham and Entwistle, 1982).

The essential oil obtained from the flowers of the lavender is used in aromatherapy, perfumes, cosmetics and foods (Ceylan et al., 1996; An et al. 2001; Giray, 2018), calming nerves and reducing insomnia problems (Sönmez et al., 2018) thanks to its beautiful and relaxing scent. Besides, owing to its beautiful flowers, it is used as an ornamental plant in parks and gardens (Zeybek and Zeybek, 1994). In pharmacological studies indicate that lavender essential oil possesses antifungal, anti-inflammatory, antibacterial, antiviral (Kurt and Çankaya, 2021), and antioxidant (Hui et al, 2010) effects.

The flowers are the parts of lavender utilized to extract essential oil. The obtained essential oil from the plant's flowers has a colourless or slightly yellow. The essential oil content of lavender flowers is between 1-3%. The quality and market value of lavender oil depend on the composition of its essential oil, with high amount of linalool and linalyl acetate, and low amount of camphor being crucial for good quality lavender oil. In line with International Organization for Standardization (ISO) standards on lavender essential oil composition (ISO 3515:2002), lavender oil can only be used in the perfume industry if it contains a minimum of 25% linalool and linalyl acetate ratio and less than 0.5% camphor ratio (Anonymous, 2002). Lavender oil with a low camphor content finds its use in the food, cosmetic, and pharmaceutical industries, such as perfume, cream, and cologne. On the other hand, lavender oil with a high camphor content is utilized in the manufacturing of candles, soaps, and insecticides, among other products (Tisserand and Balacs, 1999).

It is widely acknowledged that the composition and yield of essential oil in lavender vary depending on their species and genotype of the plant (Tinmaz et al., 2012) as well as the ecological conditions of the region in which they are cultivated (Kara and Baydar, 2011), the methods used for their cultivation (Arabacı and Bayram, 2005), the parts of the plant used, harvest time, and post-harvest treatments (Arabacı and Ceylan, 1990; Balcı, 2019). The objective of this study was to assess the quality and yield of essential oil derived from lavender species cultivated in the Tuzlukçu district of Konya, in accordance with regional conditions, and to contribute the production of high-quality lavender oil to enhance the national economy.

2. Materials and Methods

2.1. Description of the research location

The study was carried out in the ecological surroundings of Tuzlukçu situated in Konya during 2022-2023. Tuzlukçu district falls in the continental climate zone characterized by warm and mild summers and cold and wet winters. The mean annual temperature equals 11.3 °C and the average annual precipitation stands at 484 mm. The location where the experiment was conducted is situated between 38°47' North latitude and 31°63' East longitude, with an average altitude of 1000 meters. The soil texture class of the test area was identified as clayey-loamy with a slightly alkaline pH of 7.6. Moreover, the soil was found to have a high calcium carbonate content of 29.6% and a moderate amount of soil organic matter at 2.14%. The content of phosphorus is low, whereas the content of potassium, iron, zinc, and copper are medium and sufficient. Additionally, the nitrogen content is also medium and adequate, although the content of manganese is high, and the content of iron is inadequate.

2.2. Plant materials

The lavenders and lavandin used in the study, were 'Hemus' and 'Sevtapolis' lavander cultivars of *Lavandula angustifolia* species and 'Super A' lavandin cultivar of *Lavandula x intermedia* species. Lavender and lavandin seedlings were sown in April 2022. However, in the first year, the lavender and lavandin bloomed less, it achieved its full development in the second year to obtain essential oil. When they reach the full flowering stage, lavender plants were manually harvested in July (4 July 2023) and subsequently transported to the laboratory. The freshly collected flower samples were weighed before being dried in the shade at room temperature for a two-week duration. Following the drying process, the weight of the resulting dry flowers was measured.

2.3. Essential Oils Distillation

The water-distillation process was employed to extract essential oils from 100 g of dry flower samples in 1L water, using a Clevenger apparatus, following the standard procedure outlined in the European Pharmacopoeia for determining oil content (v/w %) over a 3-hour period. GC-MS instrument was used to determine the essential oil components. The essential oils were stored at -20^{0}C until analyzed.

2.4. GC-MS Analysis

GC-MS analysis was performed on a Agilent 6890N Network GC system combined with Agilent 5975 C VL MSD Network Mass Selective Detector. The GC conditions were; column, DB Waxe tr; 60.0m x 0.25mm x 0.25μm; oven temperature programme: The column held initially at 60 ^{0}C for 10 min after injection, then increased to 22 ^{0}C with 4 ^{0}C min-1 heating ramp for 10 min and increased to 240 ^{0}C with 10 ^{0}C min-1 heating ramp without hold; inject or temperature 250 0C; carrier gas; He; inlet pressure, 9.60 psi; linear gas velocity, 7 cm sec-1; initial flow 0.3 ml min-1; split ratio,65.0:1; injected volume 1.0 μl. Computer matching against commercial libraries (Wiley GC–MS Library, Adams Library, MassFinder 3 Library) as well as MS literature data was used for the identification of essential oil components (European Pharmacopoeia 7.0).

3. Results

The percentage of essential oils extracted from the flowers of *Lavandula angustifolia* species 'Hemus' and 'Sevtapolis' and *Lavandula intermedia* species 'Super A', harvested at full bloom, were measured. Essential oil components and their amount obtained with GC-MS in lavenders and lavandin are shown in Table 1. The total amount of each cultivars were 100 %. Essential oil compound was assessed at levels above 0.4%.

The essential oil yields of lavender species of 'Hemus' and 'Sevtapolis' were determined as 2.65% and 2.14%, respectively. The essential oil yield of lavandin variety of the 'Super A' from the *Lavandula x intermedia* species was 7.95%. Although it is established that the yield of *Lavandula angustifolia* essential oil yield is between 1-3% (Demirezer et al. 2011). The studies conducted by Arabacı and Bayram (2005) reported 1.5 - 2.3% and Atalay (2008) reported 2.1 - 2.6%. Baydar (2009) documented that the essential oil content of the lavandin variety Super A varied between 5 - 6%, whereas Renaud et al. (2001) noted that it ranged between 7.1 - 9.9% in dry stemless flowers. The essential oil ratios of lavender and lavandin varieties obtained in this study, showed findings consistent with the literature.

Linalool, linalyl acetate were determined as the main compounds in the essential oil of lavender and lavandin cultivars. In the research, while the highest linalool content was determined in Sevtapolis (43.3 %), this order is followed by the Hemus (39.09%) and then Super A (30.29%) cultivars. The highest linalyl acetate content in the experiment was determined from Super A (30.39 %), and then Hemus (19.76%), and the lowest linalyl acetate content was obtained from Sevtapolis (10.64%). In both lavender cultivars and lavandin examined in the study, the linalool content exceeded the linalyl acetate content. The highest 4-terpineol content was determined from Sevtapolis and Hemus cultivars (6.89 and 6.53 %, respectively), it was found to be less than 0.4 % from Super A. Camphor, an undesirable component in lavender and lavandin, was obtained from Sevtapolis, Hemus and Super A. The camphor content of Sevtapolis and Hemus cultivars were measured 0.47% and 0.41%, respectively and the camphor content of Super A cultivar was measured 6.71%.

According to the quality standards outlined by the International Organization for Standardization (ISO 3515:2002) for lavender essential oil composition, the percentages of linalool, linalyl acetate, cymene, 4-terpineol, and camphor should fall within the ranges 25.0-38.0%, 25.0-45.0%, 4.0-10.0%, 2.0-6.0%, and 0-0.5%, respectively (Anonymous, 2002). And according to the European Pharmacopoeia (2011), the camphor content is restricted to a maximum of 1.2%. The linalyl acetate content of the Sevtapolis cultivar was lower than the normal values, and the camphor content of the Super A cultivar was significantly higher than the desired amount. Further, the camphor content of the cultivars, except for Super A, comply with the ISO 3515:2002 lavender oil standards and the European Pharmacopoeia (2011). In addition, the amount of 4-terpinol was measured in a very small amount in The Super A. In a study carried out in L. angustifolia Mill. essential oil, linalool was measured as 25.1 - 59.9 % and linalyl acetate was measured as 25.8 - 54.8 % (Arabacı and Bayram,

2005). Kara and Baydar (2011) stated that the ratio of linalool ranged from 34.3% to 54.6%, while the ratio of linalyl acetate ranged from 2.0% to 29.0%. Additionally, the ratio of camphor ranged from 1.6% to 6.0%, and the ratio of borneol ranged from 6.7% to 0.8%.

Secondary metabolites in medicinal and aromatic plants are affected by various factors. These include genotype (Marotti et al., 1989), cultivation techniques, ecological conditions (Atalay, 2008), harvest time, distillation method and drying method (Arabacı and Ceylan, 1990; Kara and Baydar, 2013). It can be said that the differences in the amount of essential oil composition are due to the application of water distillation in essential oil extraction, analysis environment and conditions, ecological factors, soil structure, excessive rainfall in Tuzlukçu district in the summer period of 2023, differences between cultivars, harvest and post-harvest treatments.

Table 1. Chemical composition of essential oil of lavender and lavandin species (%)

Compound	*L. angustifolia*		*L. intermedia*
	Sevtapolis	Hemus	Super A
Amylethyl ketone	0,47	0,94	0,67
Myrcene	1,81	1,08	0,72
ρ - Cymene	0,58	-	-
Limonene	0,61	0,47	-
cis-Ocimene	4,11	4,23	-
β-Ocimene	1,85	2,15	0,99
Linalool oxide	1,56	0,50	0,41
Linalool	**40,41**	**39,09**	**30,29**
Camphor	**0,47**	**0,41**	**6,71**
Lavandulol	0,69	0,75	-
Endo-borneol	1,07	1,13	4,36
4-Terpineol	6,89	6,53	-
Cryptone	-	0,51	0,66
α-Terpineol	5,68	4,83	2,94
Nerol	0,91	0,76	0,75
Linalyl acetate	**10,64**	**19,76**	**30,39**
Geraniol	2,11	-	-
Lavandulyl acetate	5,14	4,91	2,64
Neryl acetate	1,38	1,21	0,69
Geranyl acetate	2,56	2,16	1,15
β-caryophyllene	0,64	1,21	0,85
Farnesene	0,41	0,81	0,48
Germacrene D	-	-	0,47
Caryophyllene oxide	1,34	0,48	0,24
1,8 cineole	1,24	1,07	7,31
Acetic acid	-	0,66	-
α-Bisabolol	-	-	0,89

4. Conclusions

In the research, the ecological conditions of Tuzlukçu district are suitable for cultivating lavender and lavandin cultivars. Especially Hemus cultivars are identified as quality cultivars for lavender oil standard based on their high contents of linalool, 4-terpineol and low camphor levels, in accordance with ISO 3515:2002 lavender oil standards.

Based on the results of the research, Super A exhibited a higher essential oil yield than the other cultivars. The cultivars Sevtapolis and Hemus were identified as better quality due to their low camphor content. In conclusion, Super A is recommended for floriculture and landscape decoration purposes, while Sevtapolis and Hemus are recommended for essential oil compounds and essential oil quality. Lavender and Lavandin cultivars (*Lavandula* sp.) demonstrate agricultural suitability and sustainability within the ecological conditions of the Tuzlukçu district. There is a demand for the advancement of lavender and lavandin

agriculture in the Tuzlukçu district of Konya province, due to its significant production potential. Furthermore, it is important to explore and examine various lavender and lavandin cultivars in this region to meet the efficiency and quality standards set by global markets.

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Research Article

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Effects of Egg Albumin Film Containing Coriander Extract on Some Quality Properties of Chicken Drumsticks during Refrigerated Storage

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HIGHLIGHTS

- Coating treatment had no negative effect on the *L** values of samples during storage.
- Coating had no negative effect on the pH values of samples (except for EWF2, EWF6).
- Coating with and without CLE decreased the TBA values of samples during storage.

Abstract

In this study, the effects of the egg white film coatings containing different levels (0%, 2%, 4%, and 6%) of coriander leaf extracts (CLE) and 0.1% BHT on some quality parameters of chicken drumsticks were investigated during refrigerated storage for 7 days. Chicken drumsticks were formed as follows: the uncoated chicken drumsticks (Control), chicken drumsticks coated with the egg white film solutions (EWF), chicken drumsticks coated with the EWF solutions with 2% CLE (EWF2), chicken drumsticks coated with the EWF solutions with 4% CLE (EWF4), chicken drumsticks coated with the EWF solutions with 6% CLE (EWF6), and chicken drumsticks coated with the EWF solutions added 0.1% BHT (EWFBHT). TBA, colour, and pH values were measured on the 1st, 3rd, 5th, and 7th days of the chicken drumsticks. Excluding EWF2 and EWF6, no significant changes were found regarding the pH values of uncoated and coated chicken drumsticks during refrigeration for 7 days ($P > 0.05$). TBA values of samples showed an increase during the refrigerated storage. The highest TBA values were calculated on the 7th day ($P < 0.05$). *L** values of chicken drumsticks were not affected by coating treatment ($P > 0.05$). With regard to Control and EWF4, the highest *a** values were found on the first day, and then the value decreased ($P < 0.05$). In terms of *b** values, except for the EWF6 group, the *b** values of samples increased during the refrigerated storage. The highest *b** values were measured on the 7th day ($P < 0.05$). The results state that EWF solution with and without CLE could be a potential natural antioxidant coating to enhance some quality attributes of chicken drumsticks during refrigerated storage.

Keywords: Colour; *Coriandrum sativum* L.; Egg white film; Natural antioxidant; Oxidative stability.

1. Introduction

Consumers prefer chicken meat to other types of meat throughout the world, considering their high population, price advantage and desirable nutritional composition for human life (Ilansuriyan et al. 2015; Pereira and Vicente 2013). Chicken drumstick as raw meat contains nutritional components such as protein, vitamins and minerals, and fatty acids which are inclined to oxidative degradation (Domínguez et al. 2019).

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Now that chicken is recognized as perishable, the poultry industry and researchers have set sights on adopting new approaches to enhance the quality attributes of poultry meat, especially chicken meat (Khorshidi et al. 2021).

Taking into account the fact that conventional packaging from synthetic materials is non-biodegradable and non-renewable, curious scientists and innovative food industries are closely interested in edible coatings aimed to enhance meat quality as well as products from meat by decreasing moisture loss, and lipid oxidation giving rise to the rancidity and colour degradation of meat as well as poultry meat (Bharti et al. 2020; Campos et al. 2011).

Concerning easily accessible protein resources, egg white is of significance and versatility (Mihalca et al. 2021). Ovalbumin, ovotransferrin from egg proteins and lysozyme from egg white display antioxidant attributes, in which ovalbumin is the main component of egg white (Benedé and Molina 2020). Chang et al. (2018) reported that the antioxidant, antimicrobial and anticancer activities of peptides from egg call attention to contribute to human health.

Protein-based films are not only exceptional barriers to oxygen but they are also able to carry antimicrobials, and antioxidants to prolong the shelf life of meat, poultry as well as seafood (Janes and Dai 2012; Ustunol 2009). The coriander (*Coriandrum sativum* L.) is grown mainly in the Middle Eastern and Mediterranean regions. It is regarded as a healing plant, whose leaves show antioxidant activity (Nadeem et al. 2013). According to Boby et al. (2021), 1% long coriander leaf extract was suggested as a natural antioxidant for chicken meatballs.

Although there are several studies on egg albumen coatings in the reduction of lipid oxidation in cooked and uncooked poultry (Armitage et al., 2002), egg white protein-based coatings on frozen atlantic salmon (Rodríguez-Turienzo et al., 2016), egg white protein powder on chicken patties (EL-Sayed et al., 2018), there is a lack of study in which egg albumin film containing CLE as natural antioxidants on the quality parameters of refrigerated chicken drumsticks. Therefore, the aim of the present study is to analyse the effects of egg albumin film containing coriander extract as natural antioxidants on lipid oxidation, and the physicochemical, pH and colour attributes of chicken drumsticks during refrigerated storage for 7 days.

2. Materials and Methods

2.1. Materials

The coriander (*Coriandrum sativum* L.) used in the study was purchased from the local greengrocers in Konya, Türkiye. Chicken drumsticks were obtained from a local market in Konya. Egg white protein powder was obtained from a company (Alfasol Kimbiotek Chemical Agents Inc., Istanbul, Türkiye). There is no need to obtain an ethics committee number. 1.0 N NaOH solution, glycerol and all other chemicals needed for solution preparation are of analytical purity and were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of coriander extracts

Coriander leaf extract was obtained using the method described by Konak (2018) with a slight modification. After washing the coriander leaves for 5 seconds under drinkable water, they were dried under laboratory conditions (18-20 °C) and in the shade for a week. The dried coriander leaves were ground into powder, and then water was added to the coriander leaves. 2.5 g of ground coriander leaves were blended with 50 mL of distilled warm water. The mixture was stirred for 30 minutes with a magnetic stirrer (750 rpm) and filtered with Whatman No. 1 filter paper.

2.3. Preparation of egg white film solution

Edible EWF solution was prepared by modifying the study of Armitage et al. (2002). 80 mL of distilled water was added to 7 g of egg white protein in 1 L glass beaker and stirred for 1 hour at room temperature with a magnetic stirrer (150 rpm), and then 2 mL of glycerol was added. Then, the pH of the solution was

adjusted to 9.0 using 1.0 N NaOH. The solution was kept in a water bath (Nuve BM 402, Türkiye) at 60 °C for 40 min.

2.4. Application of coatings on chicken drumsticks

Different levels (0%, 2%, 4%, and 6%) of CLEs and BHT (0.1%) were added to the EWF solutions. Six different groups were prepared, including the uncoated control group. Then, the drumstick samples were immersed in the solutions containing different levels of coriander extracts and BHT for one minute and the coating procedure was repeated in duplicate.

2.5. Experimental design

Six groups of chicken drumsticks were formed: the uncoated chicken drumsticks (Control), chicken drumsticks coated with the egg white film solutions (EWF), chicken drumsticks coated with the EWF solutions with 2% CLE (EWF2), chicken drumsticks coated with the EWF solutions with 4% CLE (EWF4), chicken drumsticks coated with the EWF solutions with 6% CLE (EWF6), and chicken drumsticks coated with the EWF solutions added 0.1% BHT (EWFBHT). TBA, colour and pH analyses were performed on the 1st, 3rd, 5th and 7th days of the chicken drumsticks samples stored at 4 °C.

2.6. Proximate composition

Moisture (hot air oven), fat (ether extraction) and protein (Kjeldahl) contents of the Chicken drumsticks were determined according to standard methods of the AOAC (AOAC, 2000). Moisture (%) was determined after drying a 3 g sample at 105 °C to maintain a constant weight. By appealing to the Kjeldahl method, protein (g protein/100 g sample) was analysed. Factor 6.25 was used for the conversion of nitrogen to crude protein. By using a Soxhlet fat extractor, fat content (g fat/100 g sample) was determined.

2.7. pH measurement

The pH values of the drumstick samples in each group were determined by using a pH meter (Testo 205 T-Handle pH Meter, Germany) (Lambooij et al. 1999).

2.8. Determination of TBARS number

10 g drumstick samples were blended with 97.5 mL distilled water using an Ultra-Turrax homogenizer (WiseTis HG–15D, Daihan Scientific Co., Seoul, Korea). The mixture was transferred to a 250 mL balloon. After adding 2.5 mL HCl, 3-5 glass beads and 3 drops of antifoam, the balloon was heated. After collecting 50 mL of distillate, 5 mL of distillate was taken with the help of variable adjustable volume pipettes and transferred to screw cap glass test tubes. Then 5 mL of TBA reagent was added into the tube and was kept in a boiling water bath for 35 min. The absorbance was read according to the method described by Tarladgis et al. (1960) spectrophotometrically (Optizen POP, UV/VIS Spectrophotometer, Mecasys Co., Korea) at 530 nm against a reagent blank. The TBA numbers of drumstick samples were recorded as milligrams of malonaldehyde per kilogram of samples.

2.9. Colour measurements

Colour measurements of the chicken drumstick sample were carried out with a colorimeter (Konica, Minolta CR 400, Osaka, Japan). L^* (lightness), a^* (redness), and b^* (yellowness) parameters were read according to Hunt et al. (1991). The results were averaged as means of the values measured from five different parts for each chicken drumstick sample.

2.10. Statistical analysis

A completely randomised factorial design was employed to compare the six treatments (Control, EWF, EWF2, EWF4, EWF6 and EWFBHT). Analysis results were subjected to analysis of variance (ANOVA) using the generalized linear mixed model. MINITAB for Windows Release 16.0 was used to evaluate the results. Tukey Multiple Comparison Test was applied to determine if the differences between group means were significant at a 95% confidence level ($p < 0.05$)(Snedecor and Cochran 1989). The results were expressed as

the mean ± standard error. Each parameter experienced two replications with triplicate samples for a total of 36 samples each day.

3. Results and Discussion

3.1. Proximate composition of chicken drumsticks

The proximate composition of chicken drumsticks was calculated as: 72.75% moisture, 18.36% total protein, and 5.08% crude fat. Our results were similar to the findings of the previous studies (Ananey-Obiri et al. 2020; Demirhan and Candoĝan 2017; Khorshidi et al. 2021).

3.2. pH values

In terms of pH values, uncoated and coated chicken drumsticks refrigerated for 7 days are shown in Table 1. Excluding EWF2 and EWF6, no significant changes were found in the pH values of uncoated and coated chicken drumsticks during refrigeration for 7 days ($P > 0.05$). Similarly, Rodríguez-Turienzo et al. (2016) found that egg white protein-based coating did not affect the pH values of Atlantic salmon (*Salmo salar*) after 4 months of frozen storage ($P > 0.05$). Results from our study are similar to the findings of Harliani et al. (2020), in which no significant effect was made on the pH of beef nuggets with egg white ($P > 0.05$). However, for the group of EWF2 and EWF6, while the lowest pH values were determined on the first day, the highest pH values were read on day 7 ($P < 0.05$). A possible explanation for the differences in pH values is likely to the differences in the initial microflora of samples (Katiyo et al. 2020). In the present study, changes regarding the pH values of chicken drumsticks were insignificant except for the 3rd day. On the 3rd day, the highest pH value was read in the EWF6 ($P < 0.05$).

3.3. TBA values

TBA values of uncoated and coated chicken drumsticks during refrigerated storage for 7 days are presented in Table 2. TBA values of samples showed an increase during the refrigerated storage. On the 7th day of the storage, the highest TBA values were calculated ($P < 0.05$). Similarly, a significant increase in TBA values was obtained by Mansour et al. (2023) in chicken drumsticks coated at 50 °C with alginate edible coating.

Generally, TBA numbers of the EWF group that was coated and the groups that were added coriander extract at different levels with the coating (EWF2, EWF4, EWF6) were found to be lower than that of the Control as well as EWFBHT groups. Similarly, Mansour et al. (2023) informed that alginate edible coating and lauric arginate (LAE) exhibited lower TBARS values than uncoated chicken drumstick samples. A similar trend in TBA numbers was observed by Aboul-Anean, El-Sayed, and Bakhy (2018) who applied edible film with egg white protein powder on chicken patties during 30 days of cold storage. Similarly, Yerlikaya and Şen Arslan (2021) determined that the highest TBA numbers were found on control groups with no extract while the lowest TBA numbers found on the samples with 10% EEP (ethanolic propolis extract) on chicken sausages. The calculated lower TBA numbers could arise from the differences between the preparation and application of edible coating methods.

3.4. Colour properties

Colour parameters of uncoated and coated chicken drumsticks during refrigerated storage for 7 days are given in Table 3. Coating treatment did not affect L^* values of uncoated and coated chicken drumsticks during refrigerated storage for 7 days ($P > 0.05$). Concerning L^* values, the differences in colour parameters of whey protein isolates (WPI) and egg white powder protein (EP) groups were measured to be insignificant by Dursun and Erkan (2014) who prepared edible coating from concentrate WPI and EP to prolong the shelf life of hot-smoked rainbow trout for a period of 6 weeks refrigerated storage.

With regard to Control and EWF4, the highest a^* values were found on the first day, and then the value decreased ($P < 0.05$). A similar trend in a^* value was charted by Venkatachalam and Lekjing (2020) who used chitosan-based edible film and added clove essential oil and nisin to extend the pork patties' shelf-life during

15 days of refrigerated storage. This reduction was referred to as the aggregation of metmyoglobin (Papuc et al. 2017) as well as the appearance of oxidative reactions regarding meat and meat products (Munekata et al. 2020).

In terms of *b** values, except for the EWF6 group, the *b** values of samples increased during the refrigerated storage. The highest *b** values were measured on the 7th day ($P < 0.05$). On the contrary, Olcay and Sarıçoban (2022) listed the lowest *b** values of control and egg white edible films added 5% hops (*Humulus lupulus* L.) extract on hamburgers during refrigerated storage for 7 days. A possible reason for the adverse trend would be the differences in the variety of extracts and colour of edible films, additional levels, and samples.

4. Conclusion

The present study proved to be beneficial to enhance the quality parameters of chicken drumsticks during refrigerated storage for 7 days. The egg albumin film coating with and without CLE decreased the TBA numbers of chicken drumsticks in comparison to control and EWFBHT groups during refrigerated storage for 7 days, which is much lower than the spoilage limit. Coating treatment had no negative effect on the *L** values of chicken drumsticks as well as pH values of samples (except for EWF2 and EWF6) during refrigerated storage. Egg white film coating containing CLE, used as a natural antioxidant in the study, may contribute to scientific studies to reduce or limit the use of synthetic antioxidants. In this way, it can be useful for the emergence of new edible film materials with different tastes and flavours. EWF coating would play an important role in the coating of protein-rich innovative products.

Table 1. pH values of uncoated and coated chicken drumsticks during refrigerated storage for 7 days

Treatment	pH (Sample numbers for each day are 36)			
	Day 1	Day 3	Day 5	Day 7
Control	6.04 ± 0.10^{Aa}	6.04 ± 0.04^{Ab}	6.33 ± 0.08^{Aa}	6.56 ± 0.20^{Aa}
EWF	6.16 ± 0.01^{Aa}	6.09 ± 0.001^{Ab}	6.35 ± 0.10^{Aa}	6.29 ± 0.03^{Aa}
EWF2	6.18 ± 0.01^{Ba}	6.14 ± 0.04^{Bab}	6.41 ± 0.04^{Aa}	6.46 ± 0.05^{Aa}
EWF4	6.29 ± 0.11^{Aa}	6.16 ± 0.04^{Aab}	6.42 ± 0.10^{Aa}	6.44 ± 0.03^{Aa}
EWF6	6.18 ± 0.05^{Ba}	6.41 ± 0.10^{ABa}	6.22 ± 0.03^{Ba}	6.60 ± 0.05^{Aa}
EWFBHT	6.13 ± 0.27^{Aa}	6.06 ± 0.01^{Ab}	6.41 ± 0.08^{Aa}	6.50 ± 0.31^{Aa}

Mean±std. error.

Different capital letters (A–B) in the same row and lower-case letters (a-b) in the same column indicate significant ($P < 0.05$) differences.

Control: uncoated chicken drumsticks, EWF: chicken drumsticks coated with the egg white film solutions, EWF2: chicken drumsticks coated with the egg white film solutions with 2% coriander leaf extract, EWS4CLE: chicken drumsticks coated with the egg white film solutions with 4% coriander leaf extract, EWF6: chicken drumsticks coated with the egg white film solutions with 6% coriander leaf extract, EWFBHT: chicken drumsticks coated with the egg white film solutions added 0.1% BHT.

Table 2. TBA values of uncoated and coated chicken drumsticks during refrigerated storage for 7 days

Treatment	TBA (mg MA/kg sample) (Sample numbers for each day are 36)			
	Day 1	Day 3	Day 5	Day 7
Control	0.07 ± 0.02^{Ba}	0.04 ± 0.005^{Ba}	0.22 ± 0.02^{Ba}	0.72 ± 0.06^{Aa}
EWF	0.02 ± 0.004^{Ba}	0.06 ± 0.01^{Ba}	0.12 ± 0.01^{Ba}	0.61 ± 0.09^{Aa}
EWF2	0.04 ± 0.01^{Ba}	0.08 ± 0.002^{Ba}	0.12 ± 0.01^{Ba}	0.60 ± 0.10^{Aa}
EWF4	0.04 ± 0.01^{Ba}	0.05 ± 0.001^{Ba}	0.12 ± 0.02^{Ba}	0.65 ± 0.03^{Aa}
EWF6	0.06 ± 0.02^{Ba}	0.04 ± 0.01^{Ba}	0.16 ± 0.04^{Ba}	0.52 ± 0.02^{Aa}
EWFBHT	0.08 ± 0.02^{Ba}	0.10 ± 0.03^{Ba}	0.21 ± 0.03^{Ba}	0.56 ± 0.06^{Aa}

Mean±std. error.

Different capital letters (A−B) in the same row and lower-case letters (a-b) in the same column indicate significant ($P < 0.05$) differences.

Control: uncoated chicken drumsticks, EWF: chicken drumsticks coated with the egg white film solutions, EWF2: chicken drumsticks coated with the egg white film solutions with 2% coriander leaf extract, EWS4CLE: chicken drumsticks coated with the egg white film solutions with 4% coriander leaf extract, EWF6: chicken drumsticks coated with the egg white film solutions with 6% coriander leaf extract, EWFBHT: chicken drumsticks coated with the egg white film solutions added 0.1% BHT.

Table 3. *L**, *a** and *b** values of uncoated and coated chicken drumsticks during refrigerated storage for 7 days

Analyses	Storage Periods	Treatment (Sample numbers for each day are 36)					
		Control	EWF	EWF2	EWF4	EWF6	EWFBHT
*L**	Day 1	65.67 ± 1.31^{Aa}	63.65±1.95^{Aa}	64.87±1.47^{Aa}	63.41±0.78^{Aa}	63.85±0.40^{Aa}	68.89±0.15^{Aa}
	Day 3	64.36±1.69^{Aa}	62.29±1.77^{Aa}	63.77±0.77^{Aa}	63.26±1.56^{Aa}	64.89±1.13^{Aa}	63.51±1.03^{Aa}
	Day 5	64.19±2.39^{Aa}	63.89±2.65^{Aa}	63.20±1.12^{Aa}	65.98±3.03^{Aa}	64.93±1.01^{Aa}	65.37±1.17^{Aa}
	Day 7	67.32±0.31^{Aa}	66.33±1.63^{Aa}	65.61±0.28^{Aa}	66.40±0.07^{Aa}	65.52±0.18^{Aa}	68.52±1.45^{Aa}
*a**	Day 1	4.60±0.54^{Aa}	3.02±2.17^{Aa}	1.40±0.60^{Aa}	2.37±0.09^{Aa}	2.35±0.02^{Aa}	1.72±0.41^{Aa}
	Day 3	0.82±0.09^{Bb}	1.09±0.07^{Aab}	1.57±0.06^{Aab}	2.17±0.14^{ABa}	1.36±0.47^{Aab}	1.90±0.28^{Aab}
	Day 5	1.92±0.55^{Ba}	0.74±0.04^{Aa}	1.13±0.74^{Aa}	1.37±0.30^{ABa}	0.79±0.42^{Aa}	1.29±0.27^{Aa}
	Day 7	0.66±0.06^{Ba}	1.11±0.29^{Aa}	0.85±0.01^{Aa}	0.99±0.24^{Ba}	1.06±0.06^{Aa}	1.55±0.68^{Aa}
*b**	Day 1	3.04±0.45^{Ba}	2.47±0.93^{Ba}	3.09±0.25^{Ba}	2.68±0.36^{Ba}	2.90±0.86^{Aa}	3.73±0.24^{ABa}
	Day 3	3.43±0.71^{Ba}	1.83±0.98^{Ba}	0.92±0.25^{Ba}	1.89±0.64^{Ba}	1.53±0.35^{Aa}	2.71±0.34^{Ba}
	Day 5	1.82±0.87^{Ba}	3.24±0.73^{Ba}	8.32±2.60^{ABa}	3.00±0.32^{Ba}	3.78±0.65^{Aa}	2.63±1.58^{Ba}
	Day 7	8.92±1.12^{Aab}	10.34±1.02^{Aab}	12.38±0.22^{Aa}	12.20±1.18^{Aab}	5.86±1.42^{Ab}	10.10±1.49^{Aab}

Mean±std. error.

Different capital letters (A−B) in the same column and lower-case letters (a-b) in the same row indicate significant ($P < 0.05$) differences.

Control: uncoated chicken drumsticks, EWF: chicken drumsticks coated with the egg white film solutions, EWF2: chicken drumsticks coated with the egg white film solutions with 2% coriander leaf extract, EWS4CLE: chicken drumsticks coated with the egg white film solutions with 4% coriander leaf extract, EWF6: chicken drumsticks coated with the egg white film solutions with 6% coriander leaf extract, EWFBHT: chicken drumsticks coated with the egg white film solutions added 0.1% BHT.

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Research Article

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Determination of Effects the Different Holes Position in the Vacuum Discs of Pneumatic Precision Vegetable Planters on Emergence and Uniform Plant Distribution in Black Carrot Production

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HIGHLIGHTS

- Pneumatic precision planter using for vegetable seeds should be focused on subject to improve planting quality.
- Searching solution of the problems encountered in the precise planting of vegetable seeds.
- Significant both researchers and producers in terms of field emergence rate and seed placement.

Abstract

The objective of this study was to determine the seed releasing characteristics of seed metering discs used in the precision seeding of black carrot seeds. High precision vegetable seeder was tested in the field conditions using black carrot seeds in Kuzukuyu village of Ereğli-Konya in 2018. Three different seed metering discs that have holes in three different circle positions were used in the experiments. These applications referred to seeding techniques, ST_1, ST_2 and ST_3. The seed metering discs with three trajectories, each diameter was 210, 185, and 155 mm respectively. Each trajectory on vacuum plate using in ST_1 had equal number of holes (96 holes), while P_1 and P_2 hole positions had same number of holes in ST_2 ve ST_3, however, 25% and 50% decrease in the number of holes in the P_3 (bottom hole position), respectively. The downforce on the press wheel was kept constant throughout all trials. In these three different seeding techniques, the planting performance of the machine under field conditions was evaluated and the coefficient of variation values expressing the seed distribution uniformity, mean of the emergence rates were determined. According to the results, the mean of emergence rates and coefficient variation values were determined as %59.86 ve %76.84, %68.91 ve %75.31, %66.26 ve %72.48for ST_1, ST_2 ve ST_3, respectively. Given hole positions (planting rows), emergence rates and CV values were obtained %59.71 ve %81.94 for P_1, %62.69 ve %74.49 for P_2 and %72.60 ve %68.20 for P_3.

Keywords: Precision vegetable seeder; vacuum plate; black carrot seed; emergence rate; coefficient variation of plant distribution uniformity

1. Introduction

Currently the most widely used machine for precision seeding of small seeds is vacuum type. The capture of seeding by vacuum plate in seed metering unit of vacuum seeder should be performed precisely without doubling or missing. Habitat quality of the seed; seed bed preparation before sowing depends on variables

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such as seeder features. seed quality and ground speed. It is clear that the cost of seed. which is one of the most important inputs in vegetable production. can only be reduced by using a minimum number of seed. This is only possible by using advanced single seed sowing machines that provide the seed to be fed into the soil according to the agrotechnical demands. In the cultivation process carried out in this way. providing an equal amount of living space to the seed is one of the important advantages. The seed releasing characteristics of a seed meter units affect the seeding quality and metering unit design. In the literature there are a few published studies on vegetable seeders. One of these was conducted by Bracy et al. (1999). using the cabbage seed in Gaspardo brand vacuum type pneumatic precision seeder with 10 holes at 2.4 km h^{-1} forward speed and using a planter plate with a hole diameter of 1.0 mm. at six different seed spacing (38, 77, 114, 153, 229 and 284 mm) conducted laboratory tests. In the results of these experiments the miss indexes for 38, 77, 114-, 153-, 229- and 284-mm seed spacing were 60%, 35%, 38%, 26%, 32% and 18% respectively. As they obtained multiple indexes 19%, 24%, 12%, 7%, 10% and 10% and quality of feed indexes determined 21%, 41%, 49%, 68%, 58% and 73%. The other study conducted by Karayel and Özmerzi (2001) on the sowing of melon and cucumber seeds with a vacuum-based single seed planter under laboratory conditions at four different forward speeds (0.5, 1.0, 1.5 and 2.0 m s^{-1}) and three different seed spacing. They determined the forward speed and the seed spacing on the row statistically affect the sowing quality and that the best sowing quality for both seeds was achieved at 1.0 and 1.5 m s^{-1} forward speeds and 64 cm sowing distance. Additionally, Parish and Bracy (2003) tested the standard machine situation on the sticky belt test stand with seed orientation applications by using a guiding plate between the Gaspardo brand vacuum type precision vegetable planter and leaving the seed from the planter disc to the furrow and adding a tubular apparatus they call the seed tube. The acceptable seed spacing rates for cabbage. onion and mustard seeds at 76, 76 and 51 mm in-row planting distances, respectively, were 59.8%, 58.7% and 76.3% for cabbage, onion and mustard seeds, respectively and 48.2% for seed path application. They obtained 56.3% and 66.0% and 40.6%, 48.3% and 45.1% in seed tube application and reported that seed guiding plate and seed tube applications did not have a positive effect on the distribution uniformity. All of these studies were conducted in the sticky belt test stand. There is no study on these planters capable of sowing vegetable seeds in field conditions. The aim of this study is to determine by comparing the seed distribution uniformity criteria in the changes in the number of holes at different linear velocity of different hole positions on vacuum plate in field experiments. The objective of this study was the comparison of the seed distribution uniformity criteria in order to seed releasing from the disc with changes in the number of holes as a result of the change in linear velocities at different hole positions.

2. Materials and Methods

2.1. Precision planter and system components

Four-row pneumatic vegetable seeder with a chain-sprocket (gearbox) transmission system operating based on the vacuum principle was used within the scope of this study. Technical properties of the high precision vegetable planter (Şakalak Inc.) used in the experiments were given in Table 1. This planter equipped with four row unit was provided by project of TUBITAK (Project number was 115O111).

Figure 1. View of pneumatic precision vegetable seeder

During the planting operations, Hattat brand A78 model tractor was used and 210/95 R28 and 210/95 R44 sized front and rear rubber wheels with narrow track width were used in accordance with the tire tread spacing.

Table 1. Technical properties of the precision planter

Technical properties		
Number of row unit	4	
Width	1650 mm	
Height	1450 mm	
Length	2500 mm	
The min. row unit spacing	260 mm	
The width of runner opener	150 mm (75+75)	
The volume of seed hopper	4 x 2 L	
Mass	650 kg	
PTO speed	540 min^{-1}	
The properties of closing wheels	**Front and back closing wheels**	**Narrow closing wheels**
Material	Tyre	Tyre
Diameter	250 mm	180 mm
Width of closing wheels	200 mm	30 mm
The mass of closing wheels	72.9 N	10.6 N

2.2. Vacuum-type precision metering unit

In the experiments. a vacuum-type precision metering unit with triplet vacuum plate was used and with a ground-driven wheel with 0.65 m diameter that transfers the motion to vacuum plate with a combination of gears was used.

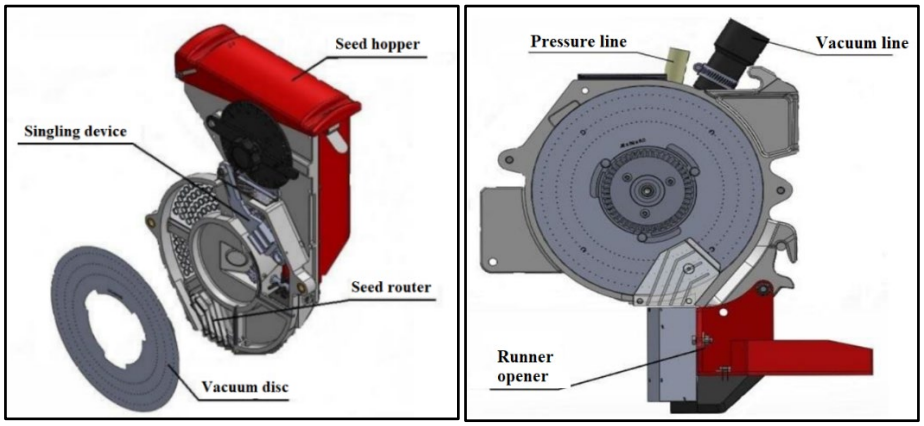


Figure 2. Seed metering unit

2.3. Vacuum plate used in the experiments

Seeds are released from the vacuum disc, and then pass through the seed router (Fig. 3) and finally, they are delivered in to the three different seed slides of runner opener (Fig. 4). The holes were drilled in three different trajectory such as P_1, P_2 and P_3 (which are on the orbit of 0.210, 0.185 and 0.155 mm, respectively) diameters. In first seeding technique (ST_1), the vacuum plate for P_1, P_2 and P_3 had 96 holes, while both ST_2 and ST_3 had the same number of holes (96) on P_1 and P_2 positions. However, it was found on P_3 position that 72 holes for ST_2 and 48 holes for ST_3. The negative pressure applied in vacuum line was 30 mbar and the positive pressure (pushing seeds to the furrow) in pressure line was 10 mbar.

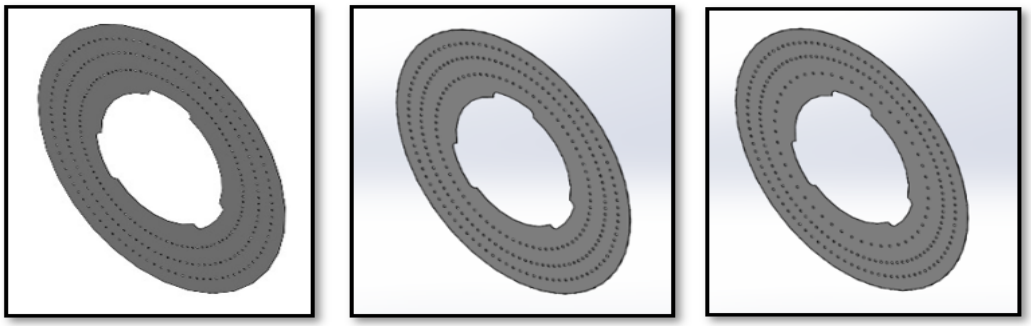


Figure 3. Disc plates used in field trials

2.4. Runner opener used in trials

The seed slide followed by the seeds release from different hole positions through the seed router (Fig. 2) on the runner opener was shown in Fig. 4. Runner opener has three different seed slides (P_1, P_2 and P_3) and angle between slides was 36°.

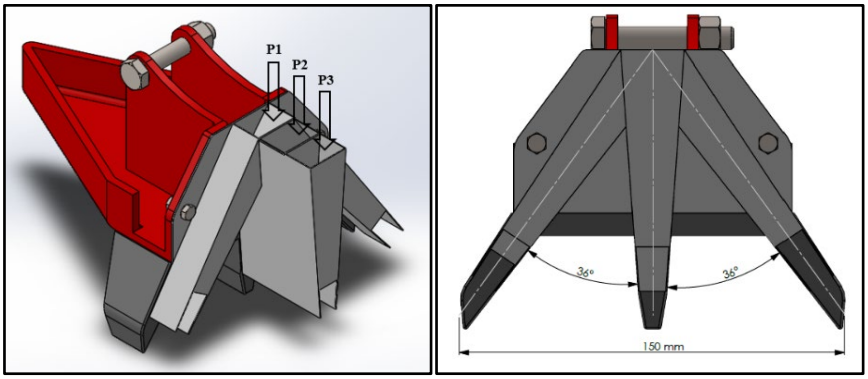


Figure 4. Runner opener and seed trajectories (P_1, P_2 and P_3)

2.5. Field description and layout

Field trials were conducted on 6 da sized loamy-sand soil at Ereğli-Konya in Turkey in 2018. Field coordinates were 37°41'51.683" N and 33°57'21.575" E (Fig. 5, no. 1). In the experiments, high precision vegetable planter was operated at the suggestion of producer optimum ground speed 0.48 m s^{-1} using black carrot seeds.

Target spacing in planting on ridge rows were adjusted for Z_1 (30.1 mm), Z_2 (47.3 mm) and Z_3 (66.5 mm). Decrease number of holes in the smallest orbit of vacuum plate, target spacing value of mid rows (P_3) in ST_2 and ST_3 increased in rate of 25% and %50 respectively.

Figure 5. Location of experiment field

The soil structure of the field, clay content was determined to be 12%. with a silt content of 1.60% and a sand content 86.40%. The soil texture was classified as Sandy-clay-loamy. In addition to these, properties of soil, pH, EC and mineral values at 30 cm soil depth where the root development of the black carrot takes place were shown in Table 2.

Table 2. Soil properties at root development area

Parameters	Units	Value
pH	-	8.45
EC	($\mu S\ cm^{-1}$)	315
Organic substance	%	0.78
Lime ($CaCO_3$)	%	40.32
Clay	%	12.00
Silt	%	1.60
Sand	%	86.40
NH_4-N	mg kg^{-1}	20.66
NO_3-N	mg kg^{-1}	31.32
P	mg kg^{-1}	23.43
K	mg kg^{-1}	362
Ca	mg kg^{-1}	3947
Mg	mg kg^{-1}	403
Na	mg kg^{-1}	378
Fe	mg kg^{-1}	1.42
Zn	mg kg^{-1}	1.67
Mn	mg kg^{-1}	8.01
Cu	mg kg-1	1.21

Ereğli local population black carrot seeds were used in the trials. These seeds have only been sift through a sieve and not calibrated. The thousand grain mass of uncoated black carrot seeds was determined as 1.65 g and the laboratory germination percentage was determined as 90%.

The average daily, minimum and maximum temperature values were determined as 20.6ºC, 13.6ºC and 28.5 ºC, respectively, after planting date of black carrot (16.05.2018) to the date of final emergence (16.06.2018). The average, minimum and maximum soil temperature values at 5 cm soil depth were occurred as 21 ºC, 13.8 ºC and 28.6 ºC, and there was a total of 34.8 mm of precipitation during the emergence period (TSMS, 2018).

The agricultural operations were applied to the experimental plots and the amount of water given during emergence were given in Table 3. DAP fertilizer at 40 kg da^{-1} fertilizer norm was distributed on the field surface with a centrifugal fertilizer in each plots and the seed bed was prepared with rotary cultivators with vertical axes. Planting ridges were created with a pneumatic precision vegetable planter before planting (Figure 6).

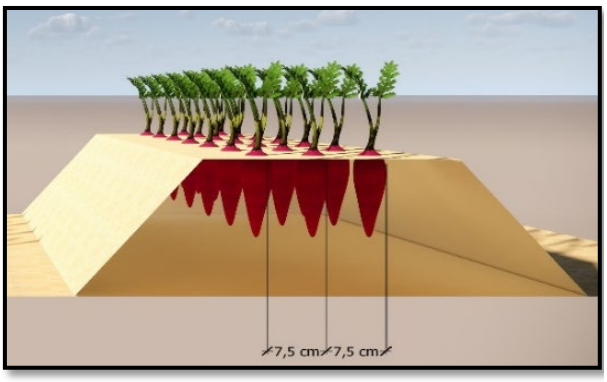
Table 3. Agricultural operations and irrigation applied on experimental field

Date	Agricultural operation
20.02.2018	Tillage with chisel
23.04.2018	Tillage with mouldboard plough
03.05.2018	Seed bed preparation with rotary cultivators with vertical axes
13.05.2018	Centrifugal fertilizer spreading machine (DAP at 40 kg da^{-1} fertilizer norm)
14.05.2018	Rotary cultivators with vertical axes (after rain)
15.05.2018	Preparation of planting ridges
16.05.2018	Planting using pneumatic precision vegetable planter
19.05.2018	1st irrigation using sprinkler (80 mm)
21.05.2018	2nd irrigation using sprinkler (32 mm)
25.05.2018	3rd irrigation using sprinkler (32 mm)
05.06.2018	4th irrigation using sprinkler (48 mm)
10.06.2018	5th irrigation using sprinkler (48 mm)

Split plot in randomized complete block design with three replicates. Dimensions of plots were 75 m in length and 2.8 m (210 m^2) in width. Measurements of plant spacing, were collected from 5 m sample strips

determined on the row. Analysis of variance was performed to field emergence rates and coefficient of variation values which express the uniformity of plant distribution using the MINITAB 16 program.

LSD analysis was performed using the MSTAT-C package program on features with a 1% and at least 5% significance level variance between applications (Düzgüneş ve ark., 1983). Obtained field emergence values of black carrots were converted into angular transformation and analysis of variance was applied (Önal, 1983).



The distance between the rows is 7.5 cm seeding techniques.

Distance between ridges: 72 cm

Width of ridges: 30 cm

Height of ridges: 25 cm

Figure 6. Measurements of ridges

2.6. Field data collection

In measurements with a penetrometer, a penetrometer tip with a base area of 1 cm^2 and a peak angle of 30° was used, and the measurement range was 0-250 N cm^{-2}. These values were evaluated in Excel. Otherwise, uniformity of plant placement terms such as variance coefficient of seeding uniformity was calculated to assess the seeding performance of the centralized seed-metering device based on the standard ISO-7256/2 (ISO, 1984). The variance coefficient of seeding uniformity was calculated as follows (i=1. 2. 3. …. n) (Lei et al., 2021). In the spacing measurements of plants, the distances between sequential plants in the row were measured, starting from the first plant. Measurements were made 30th day of after emergence was completed, and for each measurement, they were carried out with the help of tape measure on 10 m long, in five rows, on randomly selected rows from the plots. Average plant spacing and coefficient of variation values were calculated using the equations were given below.

$$Z_{avg} = \frac{1}{n} \sum_{i=1}^{n} X_i$$

$$SD = \left\{ \sum_{i=1}^{n} [(X_i - \overline{X})^2]/(n-1) \right\}^{\frac{1}{2}}$$

$$CV = \frac{SD}{Z_{avg}} \times 100$$

$$FER\ (\%) = \frac{N_x}{N_0} \times 100$$

Z_{avg} : Average of the plant spacing (mm)
SD : Standard deviation
CV : Coefficient variation (%)
FER : Field emergence rate (%)
N_x : Number of plants emerged per meter
N_o : Number of seeds planted per meter

3. Results and Discussion

3.1. Variation of penetration resistance

After seed bed and ridge preparation, the penetration resistance value was measured on the gap ridges as 2.5 Mpa on average at 25 cm tillage depth. After the ridges were formed by disc ridger, the average of the highest penetration resistance values of the press wheels at a planting depth of approximately 1 cm varied between 0.21 and 0.25 MPa (Fig. 7).

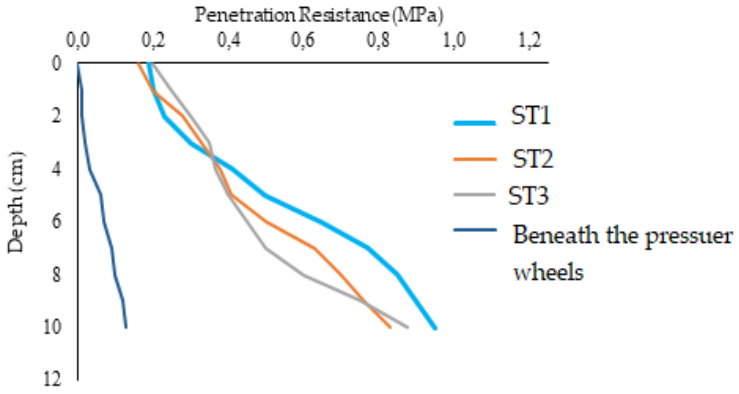


Figure 7. Variation of penetration resistance on the ridges

3.2. Field emergence rate

As a result of the trials, the variation of emergence values was given in Table 4. According to the Table 4, the emergence values obtained in ST_1, ST_2 and ST_3 showed variation between 46.79% and 70.23%, 52.24% and 77.71%, finally 48.04% and 77.62%, respectively. In general, higher germination rates were obtained in the middle row (P_3) for all three seeding techniques. Considering the theoretical seed spacing, 116% and 125%, 107% and 114%, 116% and 125% respectively adjusted Z_2 and Z_3 theoretical spacing compared to Z_1 theoretical spacing in all seeding techniques 114%, more emergence rates were obtained.

Table 4. Emergence values in different seeding techniques (%)

ST_1				ST_2				ST_3			
Target spacing	**Hole positions**	**FER (%)**		**Target spacing**	**Hole positions**	**FER (%)**		**Target spacing**	**Hole positions**	**FER (%)**	
Z_1	P_1	50.12		Z_1	P_1	52.84		Z_1	P_1	48.04	
	P_2	46.79			P_2	65.59			P_2	54.96	
	P_3	61.17			P_3	74.86			P_3	73.75	
	Avg.	***52.69***			***Avg.***	***64.43***			***Avg.***	***58.92***	
Z_2	P_1	61.09		Z_2	P_1	63.46		Z_2	P_1	62.43	
	P_2	57.53	59.86$_c$		P_2	66.26	68.91$_b$		P_2	63.33	66.26$_a$
	P_3	64.56			P_3	77.71			P_3	76.67	
	Avg.	***61.06***			***Avg.***	***69.14***			***Avg.***	***67.48***	
Z_3	P_1	61.11		Z_3	P_1	72.11		Z_3	P_1	66.22	
	P_2	65.90			P_2	70.54			P_2	73.33	
	P_3	70.23			P_3	76.84			P_3	77.62	
	Avg.	***65.74***			***Avg.***	***73.16***			***Avg.***	***72.39***	
Mean of seeding techniques										***LSD=2.479***	

According to the results of variance analysis applied to emergence rate, it was established a statistically significant difference between seeding technique (F=19.22), plant spacing (F=29.38), hole position in the disc (F=40.52) and plant spacing x hole position interaction (F=2.78). A relationship has been identified. When emergence averages were evaluated in terms of seeding techniques, the highest field emergence value was

obtained as 68.91% in ST_2 and the lowest was 59.86% in ST_1. In other words, there was no statistical difference in field emergence averages between ST_2 (68.91%) and ST_3 (66.26%) ($p<0.01$). Obtaining high germination values in ST_2 and ST_3 may be caused by the 25% and 50% decrease in the number of holes in the P_3 hole position in these seeding techniques.

The emergence values were determined as 58.68%, 65.89% and 70.43% (LSD = 2.479), respectively. There was a significance among the emergence rates obtained at all three theoretical spacing ($p<0.01$). The highest field emergence was obtained at the theoretical seed spacing of Z_3 (70.43%) and there was no statistical difference between it and Z_2 (65.89%). The reason is that decrease in the transmission rate of the pneumatic precision vegetable seeder, and therefore the rolling and drifting of the seed in the furrow is reduced due to the decrease in the disk peripheral velocity. Given the hole positions (in other words plant rows), the average of emergence obtained in P_1 (upper position), P_2 (mid-position) and P_3 (bottom position) were obtained as 59.71%, 62.69% and 72.60%, respectively (LSD = 2.479) and a statistical difference was determined between them. There was no statistical difference between the P_1 and P_2 hole positions, and the emergence value (72.60%) were stated in P_3 (bottom position-mid row) was found to be statistically significant compared to the other two positions. When the row distance x hole position interaction was examined, the highest emergence rates (LSD=1.856) were determined as 74.89% in the Z_3K_3 parameter ($p<0.05$). In triplet sowing, the field emergence rate was found to be higher because the P_3 (mid-row) hole position was on the lower axis of the seed plate, left less distance on the seed guide, and the seeds on the lower axis dropping to the furrow vertically in the seed trajectory.

3.3. Uniformity of plant distribution (Z_{avg} and CV)

The variance in the average plant spacing and coefficient of variation values, which express uniformity of seed distribution of three-row seeding techniques in the field conditions, were specified collectively in Table 2. According to the results achieved from field conditions, average in-row theoretical plant spacing between 6.13 and 9.81 cm were obtained in different hole positions in the ST_1 seeding technique. It was determined that the mean of plant spacing values increased by 2.10, 1.49 and 1.39 times, respectively. Considering the hole positions, increases of 2.21, 1.61 and 1.44 times in the Z_1, Z_2 and Z_3 planting distances were determined in the P_1; 2.07, 1.47 and 1.49 times in the P_2, and 2.02, 1.39 and 1.26 in the P_3, respectively. As the ST_1, the coefficient of variation values obtained in different rows and theoretical plant spacing, which express the uniformity of seed placement on the row, showed an alteration between 65.49% and 86.77%. When it is taken into consideration all three hole positions, it was determined that the coefficient of variation values of the P_3 was lower than the others and were 73.17% at Z_1, 65.49% at Z_2 and 70.43% at Z_3 plant spacing. With the increase in the theoretical plant spacing, the average coefficient of variation value of plant distribution was found to be higher at Z_3 (80.68%). Considering the averages of the hole positions, coefficient of variation value of the hole positions, at the theoretical plant spacing Z_1, Z_2 and Z_3 was determined as 82.10%, 78.73% and 69.70%, respectively.

In ST_2, Z_{avg} were determined between 4.59 cm and 10.50 cm in different hole and target spacing. It was determined that the Z_{avg} values of each plant row increased by 1.50, 1.32 and 1.19 times in Z_1, Z_2 and Z_3 planting distances, respectively. Considering the hole positions, it was determined that there were increases of 1.75, 1.52 and 1.21 in the P_1, for Z_1, Z_2 and Z_3 target spacings, 1.51, 1.26 and 1.17 in the P_2, and 1.37, 1.21 and 1.19 in the P_3, respectively. In general, the average of the CV values of the each rows with ST_2 showed a change between 66.44% and 88.36%. There was a decrease in the coefficient of variation values determined due to the increase in the target seed spacing. These values for each theoretical seed spacing were obtained as 77.12%, 76.95% and 70.31% on average. When three different plant rows were examined separately, the CV values obtained in the left row (P_1) were determined to be the highest with 88.36%, 85.53% and 74.01% depending on target seed spacing, and the middle rows (P_3) were determined to be the lowest with 73.66%, 70.62% and 66.44% at the target seed spacing. In ST_2, the average of CV values each seed spacing were determined as 82.63%, 73.07% and 70.24% in the P_1, P_2 and P_3, respectively. In the ST_3 seeding technique, Z_{avg} were obtained between 5.27 cm and 17.87 cm, depending on the target spacing, and Z_{avg} values were determined as 1.58, 1.37 and 1.28 times higher for Z_1, Z_2 and Z_3, respectively, compared to the theoretical spacing. Given the hole

positions, 1.91, 1.56 and 1.42 increase rates were determined in the P_1, 1.74, 1.51 and 1.34 in the P_2, and 1.34, 1.21 and 1.28 in the P_3, for each theoretical spacing, respectively.

Table 5. Average spacing and coefficient of variation values of uniformity of plant distribution in three-row seeding techniques

Seeding Techniques	Target spacing	Hole positions	Transmisyon oranı (i)	V_p (m s^{-1})	K	Z_t (cm)	Z_{avg} (cm)	CV (%)
		P_1		0.110	96	3.00	6.64	82.50
	Z_1	P_2	0.760	0.096	96	3.03	6.27	76.73
		P_3		0.080	96	3.04	6.13	73.17
		Avg.				**3.02**	**6.34**	**77.47**
		P_1		0.070	96	4.71	7.59	77.04
ST_1	Z_2	P_2	0.484	0.061	96	4.73	6.97	74.69
		P_3		0.052	96	4.74	6.58	65.49
		Avg.				**4.73**	**7.05**	**72.41**
		P_1		0.050	96	6.59	9.47	86.77
	Z_3	P_2	0.348	0.044	96	6.60	9.81	84.78
		P_3		0.036	96	6.75	8.53	70.43
		Avg.				**6.65**	**9.27**	**80.68**
		P_1		0.110	96	3.00	5.24	88.36
	Z_1	P_2	0.760	0.096	96	3.03	4.59	75.44
		P_3		0.080	72	4.06	5.55	73.66
		Avg.				**3.36**	**5.05**	**77.12**
		P_1		0.070	96	4.71	7.15	85.53
ST_2	Z_2	P_2	0.484	0.061	96	4.73	5.97	73.29
		P_3		0.052	72	6.31	7.64	70.62
		Avg.				**5.25**	**6.93**	**76.95**
		P_1		0.050	96	6.59	7.99	74.01
	Z_3	P_2	0.348	0.044	96	6.60	7.71	70.48
		P_3		0.036	72	8.79	10.50	66.44
		Avg.				**7.33**	**8.73**	**70.31**
		P_1		0.110	96	3.00	5.73	83.87
	Z_1	P_2	0.760	0.096	96	3.03	5.27	74.12
		P_3		0.080	48	6.26	8.37	70.02
		Avg.				**4.09**	**6.45**	**74.90**
		P_1		0.070	96	4.71	7.33	82.55
ST_3	Z_2	P_2	0.484	0.061	96	4.73	7.12	70.13
		P_3		0.052	48	9.75	11.76	62.81
		Avg.				**6.39**	**8.74**	**71.83**
		P_1		0.050	96	6.59	9.33	76.84
	Z_3	P_2	0.348	0.044	96	6.60	8.84	70.78
		P_3		0.036	48	13.92	17.87	61.17
		Avg.				**9.04**	**11.53**	**69.59**

When the coefficient of variation values in transplantation with ST_3 are examined, it is seen that a change occurred between 61.17% and 83.87%. It is seen that the CV obtained in three plant rows have the highest values in the P_1, and the lowest values in the P_3. The coefficients of variation obtained in the P_1 were determined as 83.87% for Z_1, 82.55% for Z_2 and 76.84% for Z_3. In addition to these, it was observed that the lowest CV values were determined in the P_3, at rates of 70.02%, 62.81% and 61.17% for Z_1, Z_2 and Z_3 target spacing, respectively. In ST_3, the average CV values for the hole positions in the upper, middle and lower axis at the target seed spacing of Z_1, Z_2 and Z_3 were found as 81.09%, 71.68% and 64.67%. The average coefficient of variation of the seeding techniques was determined as 76.84%, 75.34% and 72.48% in ST_1, ST_2 and ST_3, respectively. Variance analysis was performed on the CV values obtained to compare the plant distribution uniformity of the triple seeding techniques of black carrot. As a result of the variance analysis, only the hole

position parameter was found to be statistically significant (F=17.29). There is no significance on other parameters and their interactions. As a result of the variance analysis, the average of the coefficient of variation value acquired at the P_1 hole position was determined as 81.94% and was found to be higher than the other positions, and no statistical difference was determined between the P_2 (74.49%) and P_3 (68.20%) positions. The fact that there are 72 holes in the P_3 position in ST_2 and also 48 holes in the P_3 position in ST_3 was effective in the low CV values. Önal (2011) emphasizes that the number of holes in the vacuum plate should be chosen as 64 for lint-free cotton seeds and 27 for corn seeds, and that the use of a 72-hole vacuum plate for nappy free cotton seeds should not be preferred due to the formation of a continuous vacuum zone in the hole orbit. Therefore, depending on the hole positions in the three rows, it is stated that the CV values decreases due to the decrease in the effect of the vacuum trajectory in the K_3 (the number of holes is 25% in ST_2 and 50% in ST_3).

4. Conclusions

Among the three-row planting techniques, ST1 was applied by producer in the region. In the ST2 seeding technique, field emergence rates (68.91%) were higher than ST1 (59.86%), and in terms of coefficient of variation values, which express the uniformity of plant distribution, lower values were found compared to ST1. Considering the hole positions (planting rows), the average field emergence rates obtained in P1 (upper), P2 (mid) and P3 (lower) hole positions were obtained as 59.71%, 62.69% and 72.60%, respectively, and there was no statistical difference between them. In triplet seeding techniques, the field emergence rates were found to be higher because the P3 (mid-row) hole position was on the lower trajectory of the vacuum plate, traveling less distance on the seed router, and the seeds on the lower axis falling vertically from the seed slide to the furrow. The average of the coefficient of variation values obtained at the P1 hole position was determined as 81.94% and was found to be higher than the other two positions, and no statistical differences were determined between the P2 (74.49%) and P3 (68.20%) locations. Depending on the hole positions in the three rows, able to be stated that the coefficient of variation values decreases due to the decrease in the effect of the vacuum trajectory in the lower row (P3) (the number of holes is 25% in ST2 and 50% in ST3). For this reason, currently, it has gained importance to use pneumatic precision vegetable planters with three separate units (planting each narrow row independently). It should be encouraged domestic production of these planters. Additionally, using calibrated and coated black carrot seeds will increase planting quality.

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Bibliometric Analyzes of Some Major Effect Genes Associated with Meat Yield Traits in Livestock

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HIGHLIGHTS

- Bibliometric analysis is commonly used to guide decisions regarding research funding and the creation of research policies and offers scientists helpful information about research trends, patterns, and impact.
- This study presents a detailed bibliometric analysis of 4085 documents belonging to 464 different sources between 1981 and 2023 worldwide.
- The scientific community has recently been particularly interested in investigating some major effect genes associated with meat yield traits in livestock.

Abstract

Bibliometric analysis is commonly used to guide decisions regarding research funding and the creation of research policies and provides scientists with helpful information about research trends, patterns, and impact. Thus, researchers can track collaborators in this subject and find prospective scientific alliances. Additionally, researchers can develop new research themes by constantly monitoring the most recent trend study topics in this area. Therefore, we performed a comprehensive bibliometric analysis of 4085 documents scanned in the Web of Science (WoS) database on some major effect genes associated with meat yield traits in livestock between 1981 and 2023. The analysis shows that interest in this topic has recently grown. The fact that numerous scholars participated in the investigations, which major research groups conducted, demonstrates the growth of this field's collaborative working culture. The publication of studies in this field in high-impact journals such as Meat Science, Journal of Animal Science, and Animal Genetics reveals the scientific impact of this field. Keywords used in studies in this field are generally related to investigating the genetic factors affecting livestock's growth, muscle development, and meat quality characteristics. In country-based studies, China and the United States have the most studies in this field. The citation records of articles reveal the significant impact of this field in literature. The scientific community has recently been particularly interested in investigating some major effect genes associated with meat yield traits in livestock.

Keywords: Bibliometrics; Livestock; Myostatin; Calpastatin; Meat Yield; Meat Traits

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1. Introduction

The livestock industry is a global industry that has been meeting the animal protein needs of human beings for many years (Meissner et al., 2013; Sohaib and Jamil, 2017). However, increasing meat yield per animal is becoming essential to meet the animal protein demand (Herrero and Thornton, 2013; Rauw et al., 2020). Meat refers to an animal's consumable parts used for food (Boler and Woerner, 2017). Meat is one of the valuable food sources for humans. It contains proteins, vitamins, and minerals necessary for the growth and development of the human body (Baltic and Boskovic, 2015; Pereira and Vicente, 2013). The main component of meat is proteins, which are responsible for tissue growth and repair (Singh and Bharti, 2021). In this regard, meat contains essential amino acids for the human body (Amirkhanov et al., 2017; Samicho et al., 2013). Meat also includes significant amounts of B vitamins and minerals such as copper, manganese, phosphorus, and iron (Strazdiņa et al., 2013).

Today, meat yield and quality are the key factors consumers consider before purchasing meat products (Resurreccion, 2004; Testa et al., 2021). Meat yield is the live weight ratio to the processed carcass weight (Motoyama et al., 2016). Therefore, meat yield strongly correlates with livestock system efficiency (Capper, 2013; Pethick et al., 2021). Meat yield is a complex trait, and various genetic and environmental factors affect this trait (Filipčík et al., 2020; Grosso et al., 2010). In this respect, genetic factors significantly affect livestock meat yield (Burrow et al., 2001; Case et al., 2010). However, meat yield is a quantitative trait that emerges with the common effects of polygenes (Hagen et al., 2005; Wang et al., 2022). Therefore, identifying the functional genes related to meat yield can increase yield per animal and contribute to the sustainability of the livestock industry (Gibbs et al., 2009; Gura, 2007).

To date, many significant genetic markers related to meat yield have been identified by Genome-wide association studies (GWASs) (Raza et al., 2020; Song et al., 2016). Myostatin is one of the most significant genetic markers associated with livestock muscle development (Aiello et al., 2018; Bellinge et al., 2005). Myostatin negatively regulates muscle growth (Amthor et al., 2002; Thomas et al., 2000). Moreover, mutations in the myostatin gene increase muscle mass and meat yield (Aiello et al., 2018; Bellinge et al., 2005). However, IGF1 (insulin-like growth factor 1), MYOD1 (myogenic differentiation 1), LEP (leptin), CAST (calpastatin), CAPN1 (calpain 1), FABP4 (Fatty acid binding protein 4), GHR (growth hormone receptor), SCD (stearoyl-CoA desaturase), and PPAR-γ (Peroxisome proliferator-activated receptor gamma) are other important genetic markers related to meat yield traits in livestock (Boucher et al., 2006; Mwangi et al., 2022; Özşensoy and Kara, 2019; Ramiah et al., 2016; Raziye, 2019; Sato et al., 2012; Telegina et al., 2018; Yan et al., 2018; Zhang et al., 2012).

Recently, bibliometric analysis has become very popular in scientific research (Merigó and Yang, 2017). Today, bibliometric analysis is a powerful tool for evaluating the valuable insights from scientific publications (Coimbra et al., 2019; Muhuri et al., 2018; Sweileh, 2020). Although it has many varieties, it generally provides detailed information such as publication year, citation, and collaboration numbers, as well as the scientific journals in which studies were published, journal impact factors, and other related information (Mishra et al., 2018; Thanuskodi, 2010). In this study, we have performed a comprehensive bibliometric analysis to highlight studies about some significant effects of genes associated with meat yield traits in livestock.

2. Materials and Methods

This study obtained 4,085 documents related to the use of major genes in meat yield and quality, indexed in the Web of Science (WoS) database between 1981 and 2023 as of April 1, 2023 (Table 1). To access the target studies related to the subject in the Web of Science database, both keywords and journals were carefully selected as criteria. Subsequently, the data related to the subject were downloaded from the Web of Science

(WoS) database system in plain text format and then organized using the "convert2pdf" package in the R software (R Core Team, 2016).

Table 1. Document structure of primary data

Document Types	**n**
Article	3668
Book Chapter	32
Data Paper	7
Early Access	25
Proceedings Paper	101
Publication with Expression of Concern	1
Correction	3
Addition (Correction)	1
Editorial Material	24
Book Chapter (Editorial Material)	1
Meeting Abstract	6
Note	11
Proceedings Paper	47
Review	156
Book chapter review)	1
Early access (review)	1

Bibliometrics is a quantitative analysis method used to explore the social network of scientific research (Onder and Tırınk, 2022). By leveraging quantitative data, this method facilitates the identification of historical trends in scientific studies and potential research subject areas in the future. It is examined through various aspects, including word frequency, co-citation, co-authorship, shared keywords, and the number of institutions or countries involved in the research (Çelik Ş., 2020). In this context, bibliometric analysis was conducted using the shiny application of the 'bibliometrix' package in R software (Aria and Cuccurullo, 2017).

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3. Results and Discussion

The primary data shows that articles are the most common document type among the 4,085 documents. It can be observed that the second most common document types after articles are Reviews and Proceedings Papers, numbering 156 and 101, respectively. Moreover, the remaining document types make up a small percentage of the total documents. According to the comprehensive bibliometric data analysis of the actual data, the analyses reveal a consistent increase in published articles related to major effect genes associated with meat yield traits in livestock between 1981 and 2023. The increase in the number of articles can be traced back to 1980, with a peak occurring in 2000. There was another noticeable surge in the number of articles between 2000 and 2010, followed by a peak again in 2010. It is also evident that the increase in the number of articles has continued from 2010 to 2023. The annual distribution of published articles is presented in Figure 1.

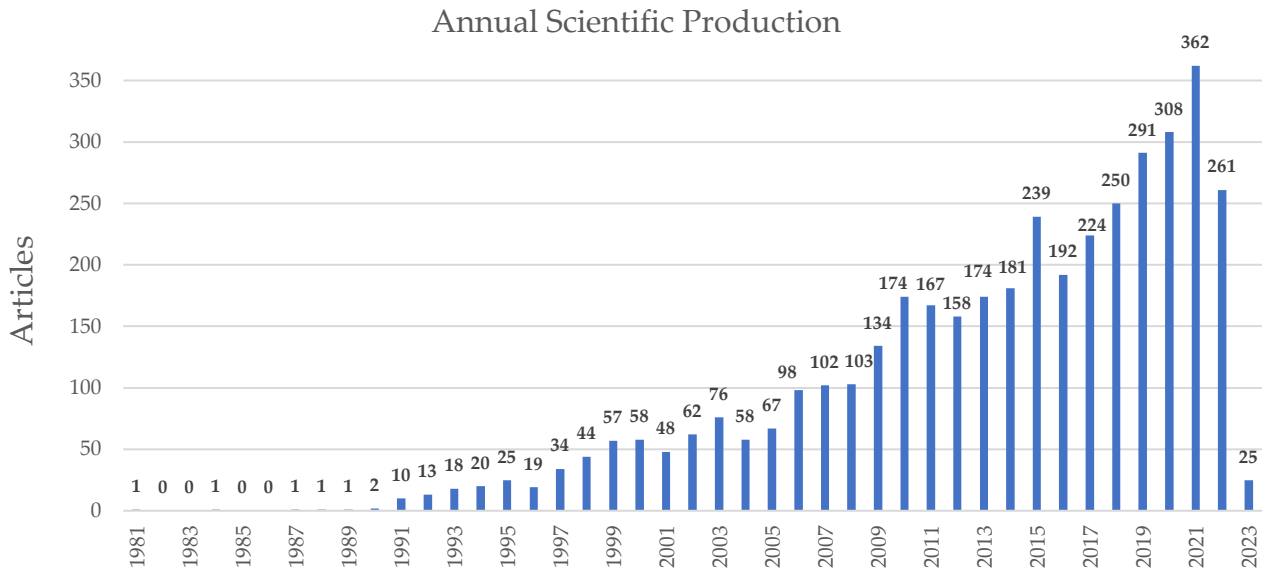

Figure 1. Published articles by year

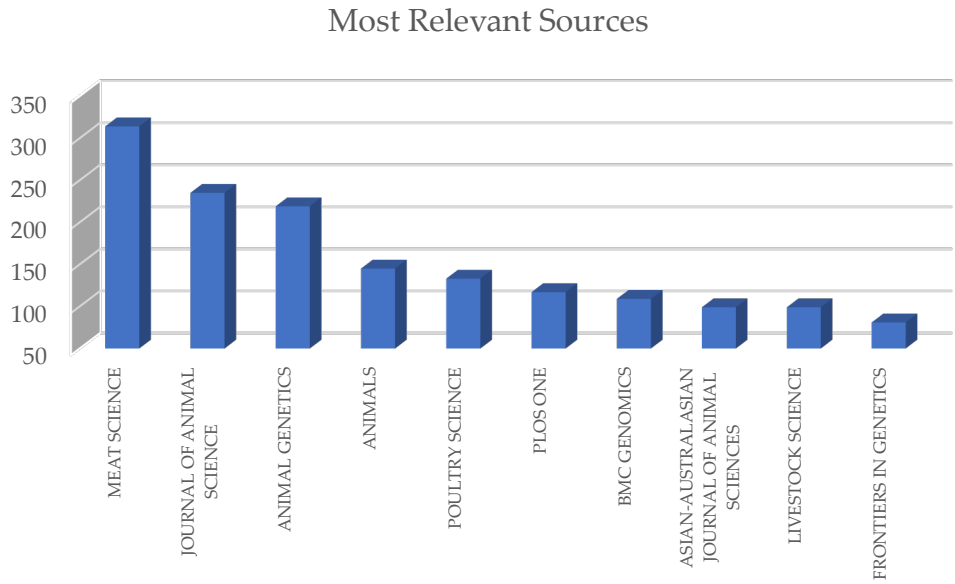

Figure 2. The journal sources of published articles

Figure 2 shows the journal sources of published articles in this field. First, it can be observed that the journal with the most articles published is 'Meat Science,' with 314 articles. Following that, it was determined that the most published journals were the 'Journal of Animal Science,' with 235 articles, and 'Animal Genetics,' with 219 articles. Notably, the publications in this field are included in the most impactful journals in the field of Animal Science.

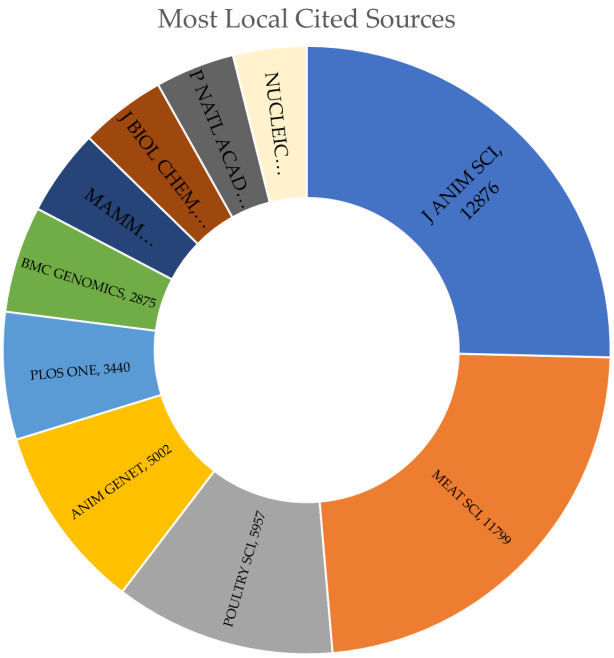

Figure 3. The most locally cited journals

The most locally cited journals are given in Figure 3. The Journal of Animal Science and Meat Science occupy the top two positions among the cited journals. Considering all cited journals, essential clues are obtained regarding the scientific impact of the publications in this field.

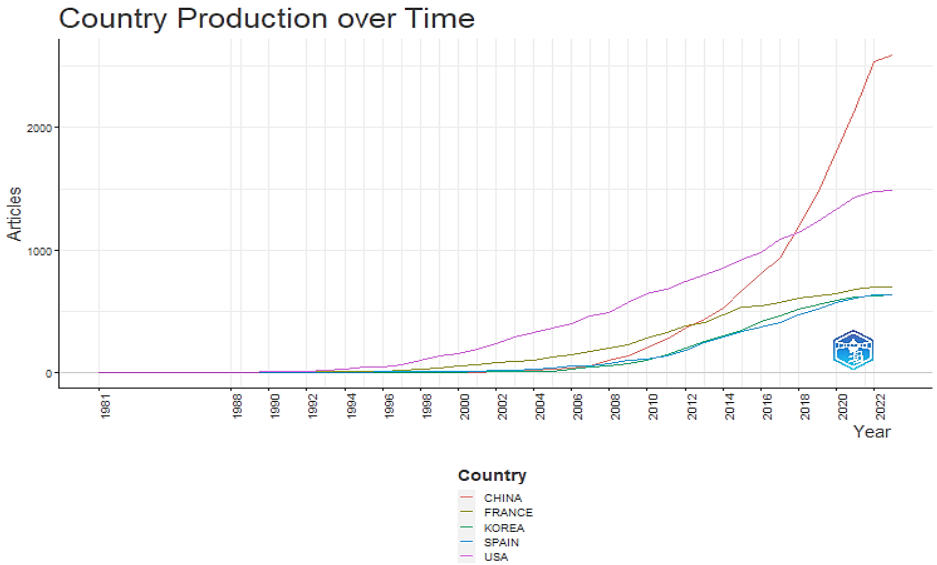

Figure 4. Country production by year

Figure 4 provides information about country-based production between 1981 and 2022. Considering country-based production, the highest production is in China and the United States; France, Spain, and Korea follow it. It is also seen that the studies conducted in China have increased rapidly, especially in recent years. The fact that country-based production continues to increase over the years reveals that studies in this field are a trend.

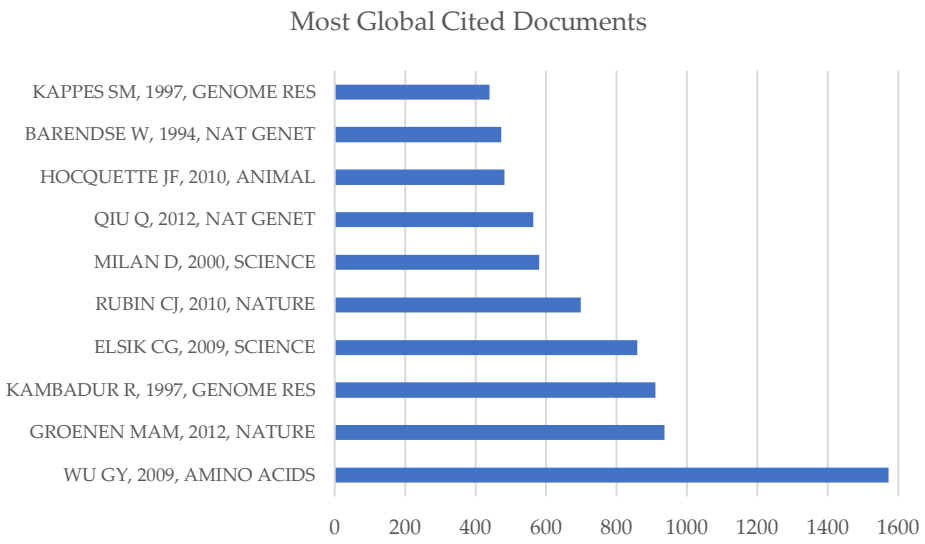

Figure 5. Globally most cited publications

The most cited studies worldwide are given in Figure 5. When the ten most cited studies in this field are evaluated, it is seen that 7516 citations have been made to these studies. Among these studies, the first four most cited studies are Wu GY, 2009, The Journal of Amino Acids (1573), Groaenen Mam, 2012, the Journal of Nature (936), Kambadur R, 1997, The Journal of Genome Research (936) and Elsık Cg, 2009, The Journal of SCIENCE (859).

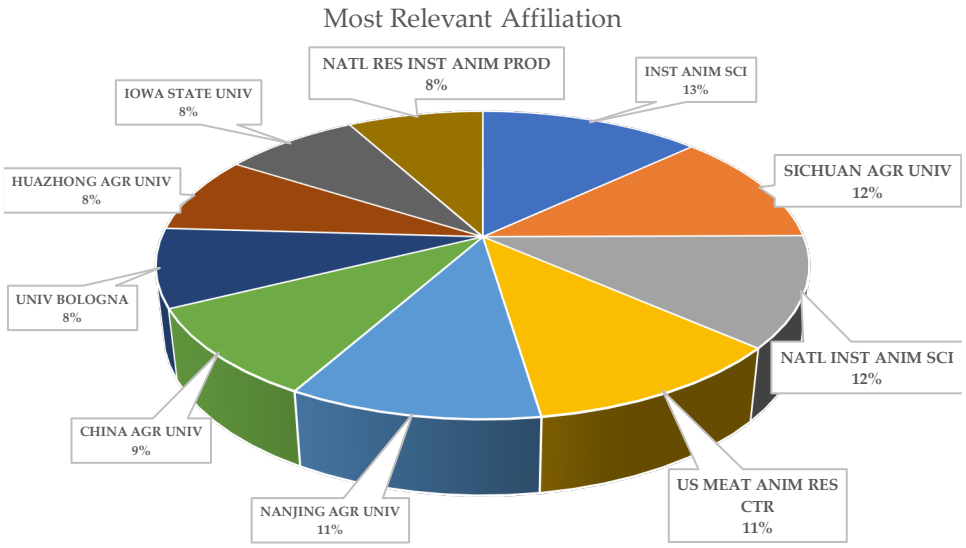

Figure 6. Most relevant affiliations

The ten educational institutions that have published the most scientific articles are depicted in Figure 6. The Institute of Animal Science ranks first among educational institutions, with 165 articles produced. Following this institution, Sichuan Agricultural University (149), National Institute of Animal Science (146), US Meat Animal Research Center (141), and Nanjing Agricultural University (133) are among the educational institutions with the highest number of articles. When evaluating educational institutions, it becomes evident

that institutions in China and the USA occupy the top positions as the most prolific article producers.

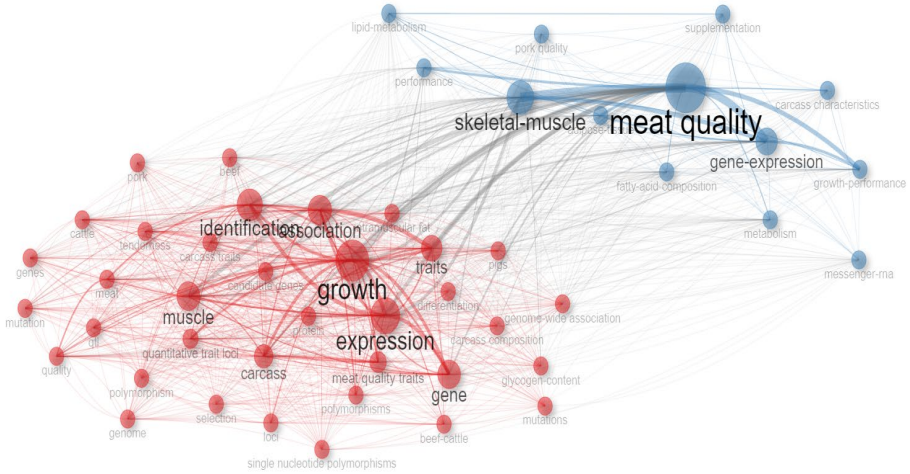

Figure 7. The closeness of trend topic keywords

The closeness of the trend topic keywords used in the studies is illustrated in Figure 7. When evaluating the keywords employed in this study field, it is apparent that they fall into two distinct groups. In the first group, keywords such as "expression," "growth," "muscle," "identification," "association," and "gene" are closely related to one another in terms of their connections. The central keyword in this group is "growth," which exhibits a typical relationship with all the other keywords. In the second group, keywords like "meat quality," "skeletal muscle," and "gene expression" have "meat quality" at their center, and these keywords are relatively less connected to the keywords in the first group. When considering both groups, it can be concluded that they are related to articles investigating the genetic factors influencing the growth, muscle development, and meat quality characteristics of livestock.

4. Conclusions

Bibliometric analysis provides valuable information to scientists about research trends, patterns, and impact, and it is frequently used to inform decisions regarding research funding and the development of research policies (Akhavan et al., 2016; Zahra et al., 2021). Therefore, this study presents a detailed bibliometric analysis of 4,085 documents from 464 different sources, including journals and articles, published worldwide between 1981 and 2023. Consequently, this study serves as a vital research resource encompassing all aspects of work conducted in the field of animal science up to this point. In this regard, researchers can identify potential scientific partnerships and stay updated on colleagues working in this field. Furthermore, researchers will also be able to identify new research topics by closely monitoring the latest trends in this field.

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Data Availability Statement: The data were accessed from the Web of Science (WoS) database on 01.04.2023 using the keywords "gene" and "meat quality traits". The data of this study in the field of animal husbandry will be provided by the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest

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Research Article

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Planning The Highest Income-Generating Labor Use in The Agriculture Sector According to Production Activities

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HIGHLIGHTS

- Lack of labor can cause economic losses.
- Insufficient labor reduces production efficiency.
- Labor planning is important for agricultural production.

Abstract

In all production processes, making detailed analyses and thinking about using the resources at an optimal level is defined as planning. For this reason, it is crucial to analyze and plan the external effects during the production process and the constraints that occur due to the use of inputs, especially in the agricultural sector, compared to other sectors. The main purpose of enterprises is to reach maximum profit with optimal resource use or to achieve optimal production by using minimum input. Therefore, planning should be done to ensure the optimization of limited resources and to realize the best distribution among various options to achieve a specific purpose. The factor of production that prevents capital and natural resources from being passive and contributes to using resources with their qualifications and abilities is human. For this reason, the planning of human resources in agricultural production will directly influence the use of other factors. Within the scope of this study, production patterns in Konya's agroecological regions were designated, and optimal use of the labor was planned to achieve maximum income. It has been determined that economic losses are due to underemployment in the agroecological third, fourth, and fifth regions. Policy recommendations have been developed to solve the problems identified in the study.

Keywords: Agriculture; Agricultural Employment; Linear Planning; Labor Force; Agroecology.

1. Introduction

The macro- and micro-scale interaction between economies is bidirectional. While the policies implemented at the macro scale affect the production and marketing processes of the enterprises, they are important in maintaining production and increasing competitive power at the international level. On the micro-scale, enterprises need to plan production, use their resources efficiently and effectively, and create new employment areas with the production income. The success of both economic structures at the scale level will contribute to price stability in the long run.

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Price changes at a predictable level predicate the enhancement of consumer welfare and, thus, success in the development process. The most important element representing a country's competitiveness and welfare level on a global scale is "access to adequate secure food." In order to achieve this global success, it is necessary to use resources more effectively and to develop production models that can provide sufficient food for the growing population (Godfray et al. 2010; Smith et al. 2018; Güler and Saner 2021).

As part of economic activities, every business organization works to produce and market goods and services and obtain the highest profit with the lowest input costs. In order to achieve this goal, the factors of production must be operated continuously and effectively. To use the resources effectively, the buildings, machinery, equipment, inputs, labor, etc., must be analyzed accurately. Production planning judgments include decisions such as determining the labor force level, determining the priorities in the production phase, and making the division of labor (Graves 1999; Demirdöğen and Güzel 2009). Concisely, production planning refers to the decision-making processes regarding the resources of the enterprise and the use of resources for producing products of desired quality and quantity in the future (Magee 1967; Bilici 2010). Labor planning is the organization of the amount and quality of labor needed in business activities in line with future expectations, depending on time. Businesses have to perform a systematic study and manage resources properly in terms of labor planning. It is essential to work systematically, particularly in plant production processes carried out in limited times due to natural resources and climatic changes. For this reason, planning and properly managing the labor in agricultural enterprises ensure efficient and effective work.

"Linear programming method" is preferred to plan the labor per the production pattern in agricultural enterprises. The program has been used frequently in planning studies for the agricultural sector. Heady and Chandler (1958) adapted the planning methods to the agricultural sector. They examined both the solution to the problems in the enterprises with the method of maximization and minimization and the methods to obtain optimum results in the face of changing prices. Meijaard (1972) aimed to determine the most profitable production method for agricultural enterprises using the budgeting method. Aksöz (1971) contributed to optimal planning for agricultural enterprises in Nebraska. In the study, the contributions of the working capital at different levels to the business processes were determined, and the optimal operating capacities were calculated according to the scales. Tekeli and Ergün (1983) carried out a study covering the provinces of Ankara, Konya, Şanlıurfa, Tokat, Eskişehir, Mersin, Samsun, and İzmir. Data were collected on input usage, input-product and price relationship, optimum product component, and the adequacy of inputs for optimum production. Özçelik (1985) used the linear program method by comparing the existing technology and advanced technology within the scope of the study carried out in Eskişehir, optimum enterprises were specified, and their success levels were determined. Akay (1996) identified the problems related to the full and effective use of business resources in the study conducted in Tokat. Tatlıdil (1992) determined the optimum size of farms in the Beyşehir and Ereğli districts of Konya, considering the labor force, revenue assets, and tractor capacity of agricultural enterprises in irrigated and dry lands. Aksoyak (2004) worked on the economic analysis and planning of agricultural enterprises in Konya, Sarayönü district, and calculated the optimum product component and the size of the enterprise with sufficient income. Within the scope of the study, the enterprise size that will provide sufficient income for the family, which is an agricultural enterprise, was determined as 134.38 da. Çevik (2006) completed a labor plan that will provide minimum cost for a company operating as a family business in Tokat province. Within the model's scope prepared for business activities, a shift system and the number of workers were determined to minimize personnel costs by preventing insufficient or idle personnel. Khan et al. (2005) analyzed crop cultivation areas, production quantities, and income by establishing a linear programming model. With the planning made in agricultural enterprises, the cultivation areas decreased by 1.76%, while the optimum income increased by 3.28%. Şahin and Miran (2008) determined that within the scope of the planned labor study, labor costs can be minimized in conjunction with optimum planning, labor can be used more effectively, and open and disguised unemployment can be directed to production through support policies. Uysal (2008) aimed to determine agricultural enterprises' economic structures and annual activity results in the Dikbıyık town of Samsun. According to the planned production pattern, it has been determined that the production of forage crops will increase depending on the increase in livestock activities. By extension, the gross profit will increase by 218.00%.

2. Materials and Methods

Agroecological regions refer to sub-fields with similar characteristics, such as the environmental characteristics of the land, potential yield, and land suitability (Soylu 2011).

Table 1. Agro-Ecological Regions of Konya Province

Regions	Province in the Region	Field (hectare)	Rate (%)	Yearly Precipitation (mm)
Region 1	Çumra, Karatay, Meram, Selçuklu	704.649	16,9	<400
Region 2	Akören, Ahırlı, Bozkır, Güneysınır, Hadim, Taşkent, Yalıhüyük	525.234	12,6	>400
Region 3	Akşehir, Ereğli, Halkapınar, Ilgın, Tuzlukçu	597.982	14,3	>400
Region 4	Beyşehir, Derbent, Derebucak, Doğanhisar, Hüyük, Seydişehir	589.385	14,2	<400
Region 5	Altınekin, Cihanbeyli, Çeltik, Emirgazi, Kadınhanı, Karapınar, Kulu, Sarayönü, Yunak	1.752.150	42,0	<400
Total	31 District	4.169.400	100	-

Source: Çelik et al. 2015.

Konya is among the provinces of Türkiye well-known for its high agricultural production potential. The province comes to the fore in terms of its product diversity and its agricultural employment diversity. It is known that more than 120,000 agricultural workers are employed only in Konya's livestock sector. This study was carried out to determine the perspective on the permanently and seasonally employed labor in various jobs. A stratified random sampling method was used to determine the number of enterprises to be surveyed, and it was studied with 5% error and 90% confidence limits. Yamane's (1967) formula was used in determining the strata distribution and the number of questionnaires. In line with the results obtained, 375 surveys were conducted in 2022. Questionnaires were obtained from face-to-face data. Accordingly, the distribution of the number of enterprises to be surveyed according to districts and strata was determined by proportioning the number of enterprises in each district and the "k" value of the strata (number of establishments/k).

The linear programming method was used in labor planning in agricultural enterprises. The linear programming method is a mathematical method used to determine the optimum business plan by evaluating various constraints for agricultural enterprises (Uysal 2008). The linear programming model has three basic elements. These are the determined decision variables, the goal to be optimized, and the business constraints. Within the scope of this study, gross profit in agricultural enterprises was determined as the decision variable. The purpose of planning is to use the labor at an optimal level.

As linear programming deals with allocating limited resources among alternatives, it is impossible to refer to negative activities or resource use. The last part of linear programming models consists of the boundary that ensures that the decision variables are not negative (Büyükkeklik 2007). For any problem to be the subject of linear programming, it must meet certain conditions (Cinemre 2011). Within the scope of work;

- The decision-maker must have a goal that he or she wants to achieve.
- At least one must have alternative strategies to achieve the goal.
- Resources should be limited.
- The purpose should be explained mathematically, and the resources' limits should be shown in equality and inequalities. All of these equations or inequalities must be linear.

Provided that these conditions are met, the problem can be expressed mathematically. As in all other numerical decision-making methods, establishing linear programming models begins with defining the problem. Then, the defined problem is observed in the system, and the parameters affecting the problem are determined. Using these parameters establishes a linear programming model consisting of the problem's objective function and constraints set. By solving this model, the values of the decision variables that satisfy all boundary conditions and make the value of the objective function optimum are obtained (Ekmekçi 2015).

In agricultural enterprises, the need for labor is intensified according to the type of work and seasonal characteristics. In order to determine the labor need, a production season was divided into periods and

analyzed by region. There is continuity in animal production processes due to living materials, and activities continue for 365 days. For this reason, plant production was taken as a basis while classifying working periods and hours.

Table 2. Working Periods Used for Businesses

Period	Date	Work Type
Term 1	February 1 - April 15	Spring tillage and planting
Term 2	April 16 - June 30	Care for anchor plants
Term 3	July 1 - September 15	Harvesting and care
Term 4	September 16 - November 30	Harvesting and sowing winter cereals

Source: Bayramoğlu et al., 2006.

3. Results

The values of production activities determined as a result of planning include fractional results. Values are fractional due to the divisibility condition of the linear programming method used to determine the optimal business organization. Fractional expressions are converted into integers and included in the planning process, considering the capacity levels and limitations in the implementation process (Bayramoğlu et al. 2006).

It is observed that there are changes between the current situation and the post-planning situation in terms of the products examined in Table 3. For the enterprises in the first region to succeed using their limited resources, they must halt the irrigated farming of barley, oats, sugar beet, potatoes, dried beans, and chickpeas. Moreover, cattle and sheep and goat farming activities should be halted to increase the gross profit in the region. As a result of the focus on profit maximization and optimal use of resources, the production of dry wheat and confectionary sunflowers also needs to be reduced. Due to the reduction of dry wheat areas by 5.40 da and confectionary sunflower by 2.68 decares per enterprise, the resources acquired will be used in other areas planned for production, thus increasing the gross profit. The products expected to increase production areas after planning are irrigated wheat, grain maize, dry barley, silage maize, clow, Hungarian vetch, sunflower for oil, zucchini for snacks, and onions. With this production pattern, the business enterprises in the first region, which continue their agricultural activities, will be able to increase their gross profits from 443,775.21 TL to 764,269.30 TL.

In line with the results obtained with the linear program method, marginal revenue for each product included in the production plan and marginal loss for products not included in production were calculated. Marginal losses represent the decreases in earnings due to the inclusion of these products in the production activities of the enterprise. For certain products included in the planning, the value of marginal revenues is calculated as zero and therefore is not indicated in the table. The marginal income for the existing land assets of the enterprises is calculated as 230.86 TL. This value represents that if the enterprises continue their activities without changing their production areas, a contribution of 230.86 TL will be made to the gross profit of the enterprise. When all effective products in marginal income are examined, it is determined that the product that makes the most substantial contribution to profit is an onion with 1,962.84 TL. Dry onions, which are included in the production pattern of enterprises, make an important contribution to ensuring economic sustainability. The intensive labor requirement of onion production during harvest periods is important in terms of the employment of the rural labor and the creation of alternative income sources. When an evaluation is made, including the enterprises located in the first region for the working hours of the labor, it is possible to say that the resources are used sufficiently for the first period. However, the same is not the case for other employment periods. Wages are important in increasing the labor's motivation, creating social and economic welfare conditions, and maintaining the workflow. Determining an optimal wage for workers and agricultural operators (employers) is necessary to sustain employment and production. In order to increase the gross profits of agricultural business managers in the first region, it was determined that the wages for the second period should not exceed 35.78 TL per hour. Labor fees for the third term are determined as 56.16 TL/hour and for the fourth term as 98.48 hours. Wages also increase when packaging/sacking operations intensify during the harvest period.

Once the business structures in the first region are examined in terms of marginal losses, it has been determined that irrigated barley, oats, potatoes, dried beans, and chickpeas should not be produced. The production of irrigated barley in one decare for the enterprises will cause a loss of 373,76 TL in the total gross profit. This value is 695.95 TL for oats, 843.74 TL for potatoes, 798.76 TL for dried beans, and 667.31 TL for chickpeas. Potato is the product that causes the greatest loss due to production activities in the region. The main reason for this situation is that using pesticides and fertilizers increases input costs. When the enterprises in the first region for livestock activities are examined, it has been determined that the cattle stock is 8.95 heads, and sheep and goats are 5.54 heads per enterprise. Under the current circumstances, the protection of animal existence is important for businesses. Therefore, it has been determined that reducing or not including livestock activities within the business planning will increase the gross profit of the business enterprises. The gross profit, which was calculated as 443,775.21 TL under the current conditions, will increase to 764,269.30 TL as a result of the continuation of the activities.

Table 3. First Region Production Plan

Products	Unit	Available Status	Planning Result
Wheat (irrigable land)	Decare	30,76	35,01
Barley (irrigable land)	Decare	17,11	0,00
Oats	Decare	9,84	0,00
Grain Maize	Decare	33,51	34,44
Dry Wheat	Decare	29,69	24,29
Dry Barley	Decare	28,91	30,37
Silage Maize	Decare	5,40	17,23
Clover	Decare	7,56	17,23
Hungarian Vetch	Decare	1,36	6,07
Oil Sunflower	Decare	6,43	24,15
Sunflower for Snack	Decare	21,03	18,35
Sugar Beet	Decare	34,49	0,00
Potato	Decare	0,68	0,00
Dry Beans	Decare	2,78	0,00
Zucchini for Snack	Decare	0,65	8,61
Dried Onion	Decare	0,65	17,23
Chickpea	Decare	2,14	0,00
Fallow	Decare	4,55	4,55
Bovine Animal	Head	8,95	0,00
Small Bovine Animal	Head	5,54	0,00
Total Gross Profit	TL	443.775,21	764.269,30

Table 4 demonstrates the enterprises' production activities and planning results in the second region. According to the planning results, the use of marginal lands in agricultural production will increase enterprise profitability. As a result of the planning, it was determined that dry wheat and zucchini for snacks cultivation areas, which are included in the production pattern of the enterprises, should be reduced. Before the planning, the business planning did not include irrigated wheat, irrigated barley, peach, walnut, cherry, pear, apple, strawberry, and lavender. Due to the limited resources in the production processes of agricultural enterprises, the distribution of existing resources should be carried out at an optimal level to reach the highest profit. Therefore, only products that will contribute to the optimal use of resources should be included in business planning. It has been recommended to increase the fields of grain maize, dry barley, oats, grapes, silage maize, Hungarian vetch, dried beans, and zucchini for snacks among crops for businesses in the second region. Increasing livestock activities in the region is one of the activities planned for the planning process. The cattle stock in agricultural enterprises is 7.29 heads, and the stock of sheep and goats is 30.87. As a result of the implementation of the planning made by considering the enterprises' economic and technical conditions, the gross profit from 232,955.41 TL is expected to increase to 473,963,20 TL. Gross profit growth is expected to be 49.15% in businesses in the region. Suppose these enterprises produce with the planned production pattern and maintain their existing business sizes for their irrigated lands. In that case, gross profit will increase by 2,490.90 TL, and dry land assets will increase by 307.15 TL. The product that will contribute the most to the

enterprises' gross profit within the planned production pattern is pumpkin for snacks with 2,139,80 TL. In order to use the resources effectively and increase the business's profitability, according to the planned production pattern, there should be 1.94 decares of confectionary squash. While diversifying the production pattern and creating alternative sources of income for businesses will contribute to the distribution of economic risks, it will also improve business management. Among the products included in the production planning, the product that is expected to have the largest land asset is oat with 31.02 da. The marginal income that oats will provide for each decare within the planned business model is 505.61 TL. In the second region, the marginal value of the labor force for the fourth period has been determined as 172.10 TL/hour, and it is expected that the wage value of the labor to be hired will not exceed 172.10 hours. Otherwise, there will be decreases in gross profit. According to the planning results, the use of marginal lands in agricultural production will increase enterprise profitability.

Table 4. Second Region Production Plan

Products	Unit	Available Status	Planning Result
Wheat (irrigable land)	Decare	3,70	0,00
Barley (irrigable land)	Decare	2,94	0,00
Grain Maize	Decare	4,00	7,76
Dry Wheat	Decare	26,71	24,81
Dry Barley	Decare	24,30	31,02
Oats	Decare	0,60	6,20
Grape	Decare	2,91	3,88
Peach	Decare	0,30	0,00
Walnut	Decare	0,49	0,00
Cherry	Decare	11,46	0,00
Pear	Decare	0,28	0,00
Apple	Decare	1,64	0,00
Strawberry	Decare	0,30	0,00
Silage Maize	Decare	2,30	3,88
Hungarian Vetch	Decare	0,24	3,88
Dry Beans	Decare	1,54	1,94
Zucchini for Snack	Decare	1,89	1,94
Chickpea	Decare	8,29	3,88
Sunflower for Snack	Decare	1,28	11,64
Lavender	Decare	0,66	0,00
Fallow	Decare	11,11	11,11
Bovine Animal	Head	5,84	13,13
Small Bovine Animal	Head	4,32	35,19
Total Gross Profit	TL	232.955,41	473.963,20

Table 5 presents the enterprises' current production activities and planned production patterns in the third region. It has been determined that reducing the number of silage maize and cattle in planning within the planned business activities will contribute to the resource use of the enterprises. Since silage maize is generally used for feeding in cattle breeding, a parallel decrease in both factors is an admissible result. Irrigated wheat, irrigated barley, dry wheat, cherry, apple, sour cherry, plum, Hungarian vetch, sugar beet, and poppy are the products that are not included in the planned new production activities of the enterprises. The products whose production is planned to be increased are grain maize, dry barley, rye, tomatoes, alfalfa, sunflower for oil and confectionary sunflower, confectionary pumpkin, and small cattle. It is seen that the gross profit can rise to 645,146,20 TL with the new production pattern as a result of changing the operating plan while maintaining the technical and economic conditions in the enterprises. The business profitability in the third region will increase by 53.32% due to the new business plan. When the production potential and labor needs in the third region are evaluated, it has been determined that there is a labor shortage in terms of periods. In this context, the labor force needed in the enterprises has been scripted and included in the planning. Working times in one decare area for all products produced were calculated within the scope of the study. In the current situation, a study includes 748.23 hours in the first period, 55.42 hours in the second period, 544.80 hours in the third

period, and 419.77 hours in the fourth period. Based on these working hours, the cultivated land in the enterprises is calculated as 105.64 decares. In the region, which has a total operating area of 155.65 decares, the current working hours cause the land to remain idle at 50.01 decares. Agricultural lands may remain inactive due to legal, environmental, technical, social, and economic reasons. As a result of assumptions such as the seasonal labor force in the region is not sufficient, the demand for employment in agriculture decreases, the income of agricultural workers is not sufficient by the workers, the labor costs are perceived as high by the agricultural operators, the labor force is decreasing, and the labor capacity in the enterprises is insufficient for production. This situation is reflected in the working hours and results in the idleness of the lands. Business planning and employment planning should be done to transform idle lands into cultivated lands due to economic and social reasons. If only family labor remains in the production processes in the region, the presence of idle land will increase.

Table 5. Third Region Production Plan

Products	Unit	Available Status	Planning Result
Wheat (irrigable land)	Decare	24,34	0,00
Barley (irrigable land)	Decare	11,68	0,00
Grain Maize	Decare	6,78	20,40
Dry Wheat	Decare	24,89	0,00
Dry Barley	Decare	25,25	25,06
Rye	Decare	0,48	25,06
Cherry	Decare	4,94	0,00
Apple	Decare	0,24	0,00
Cherry	Decare	1,95	0,00
Plum	Decare	0,85	0,00
Tomato	Decare	0,97	8,06
Silage Maize	Decare	11,45	10,20
Hungarian Vetch	Decare	0,68	0,00
Clover	Decare	3,69	10,20
Oil Sunflower	Decare	5,15	30,60
Sunflower for Snack	Decare	11,10	17,45
Sugar Beet	Decare	9,69	0,00
Potato	Decare	0,45	0,00
Poppy	Decare	6,39	0,00
Zucchini for Snack	Decare	0,68	5,10
Fallow	Decare	3,52	3,52
Bovine Animal	Head	13,69	5,14
Small Bovine Animal	Head	11,51	35,29
Total Gross Profit	TL	301.130,89	645.146,20

The production planning results for the fourth region are analyzed in Table 6. As a result of the planning, it was foreseen that the presence of irrigated wheat, dry wheat, zucchini, sheep, and goats produced in the region should be reduced. Irrigated barley, dry barley, safflower, sugar beet, potatoes, dried beans, and chickpeas grown in the region should be excluded from the production plan. To increase the total gross profit of the enterprise, the plant production pattern of the enterprise should include grain maize, oats, grapes, strawberries, melons, silage maize, clover, Hungarian vetch, poppy, lentils, and lavender. Increasing the stock of cattle in enterprises will also contribute to profit maximization. The products included in the planning will contribute to the region's more effective use of scarce production factors. Fallow areas are included in the planning, provided that their widths remain constant. The reason why the fallow areas remain stable is to create a strategy for the preservation of soil fertility in dry agricultural lands. If the businesses continue to produce using their existing land assets, the gain will be 267.55 TL for each unit of land width. In return for the continuation of production on irrigated agricultural lands, a contribution of 1,207,64 TL will be made to the gross operating profit for each decare of land. Silage maize provides the most profitable production activity for businesses in the fourth region with 2,111,35 TL. Other products included in the business planning are grain maize, clover, Hungarian vetch, poppy, strawberries, and dried wheat. Similar to the fact that grapes are

included in the production pattern in the second region, the presence of strawberries in the fourth region is important for research. Because Hüyük, one of the districts in the fourth region, stands out with its strawberry production and adds value to agricultural production activities by creating brand value in the region. The marginal income to be obtained for each decade of strawberries in the enterprises operating in the fourth region is 1,495.53 TL. The product included in the production planning but with the lowest marginal income is dry wheat, with 103.89 TL. Production activities in dry lands are important to prevent unirrigated agricultural lands from remaining idle.

When the current situation and the result of the planning are compared, it is planned to increase the number of cattle to 18.68 heads and to decrease the number of sheep and goats in the enterprises to reach 9.08 heads. The main reason for the decrease in the presence of sheep and goats is to ensure the effective use of scarce production factors in cattle breeding activities. Depending on the increase in cattle stock for the planned business activities, a marginal income increase of 102.22 TL will be achieved due to each unit change in the enterprises. The gross profit of cattle breeding activities is greater than the gross profit of small cattle breeding activities. As a result of the implementation of the planning results by the businesses in the region, it is expected that the gross profit, which was 98,389.86 TL, will increase more than three times and reach 312,815.90 TL.

As a result of the production activities in the fourth region, the working hours required by the enterprises for each unit were calculated. Similar problems were detected in the fourth region as in the third region. The existing labor prevents the full-capacity use of agricultural lands in enterprises. In the current situation, the labor need of the enterprises in the first period is 793.68 hours; in the second period, it is 583.13 hours; in the third period, it is 594.21 hours and in the fourth period, it is 563.99 hours. With the current working hours, businesses can only cultivate 87.17 decares of land, considering their total land assets. The total farm widths in the region are 91.44 decares, and in the current situation, 4.27 decares of land remains idle for each farm. The working hours needed according to the periods were calculated to include the uncultivated lands in the region in the business planning. Calculated values were used to increase the efficiency of the planning process. According to the calculation results, the required working hours are the same for the first and second semesters. According to the planned production activities, the labor need of the enterprises is 844.81 hours for the third period and 1.063.99 hours for the fourth period. There are marginal losses for sugar beet, potato, and dried beans due to labor shortages in production activities in the third and fourth periods. As a result of meeting the seasonal labor needs in the region, the marginal losses of the enterprises will be transformed into marginal revenues, and the enterprises' gross profits will increase.

The products that cause the highest marginal losses per decare in agricultural enterprises are sugar beet with 1.049.58 TL and dried beans with 1.082.82 TL. Meeting the seasonal labor needs during periods when the need for labor in agricultural enterprises is intensified will increase the areas of activity that increase workers' income and the gross profitability for the agricultural enterprises. However, due to the seasonality of production in agricultural enterprises, the periods when labor demand is concentrated in each geographical region will be different. In particular, the mobile seasonal labor is present in the areas where the workload is concentrated for a short time (3-7 days) in line with the work routes they have determined in May and June, and they fulfil the requirements of the job during this period. Therefore, the business operator creates a demand for every production activity that requires labor during the production period and experiences losses in terms of product yield and income, particularly during periods when it cannot meet the labor demand that is concentrated during the harvest period. To minimize these losses, the resident labor in the region should take an active role in agricultural production activities.

Table 6. Fourth Region Production Plan

Products	Unit	Available Status	Planning Result
Wheat (irrigable land)	Decare	6,54	3,13
Barley (irrigable land)	Decare	2,61	0,00
Grain Maize	Decare	0,48	4,74
Dry Wheat	Decare	35,55	29,30
Dry Barley	Decare	21,93	0,00
Oats	Decare	0,28	30,40
Safflower	Decare	0,05	0,00
Grape	Decare	0,05	1,20
Strawberry	Decare	0,19	0,42
Melon	Decare	0,05	0,36
Silage Maize	Decare	0,93	2,37
Clover	Decare	0,49	2,37
Hungarian Vetch	Decare	0,36	2,37
Sugar beet	Decare	5,78	0,00
Potato	Decare	2,73	0,00
Zucchini for Snack	Decare	3,46	1,82
Dry Beans	Decare	0,08	0,00
Poppy	Decare	0,31	2,37
Lentil	Decare	0,84	1,20
Chickpea	Decare	1,88	0,00
Lavender	Decare	0,02	2,37
Fallow	Decare	7,02	7,02
Bovine Animal	Head	7,66	18,68
Small Bovine Animal	Head	11,14	9,08
Total Gross Profit	TL	98.389,86	312.815,90

The production planning of the fifth region is examined in Table 7. According to the obtained planning results, it was determined that irrigated barley and dry wheat production areas should be reduced to use the resources more effectively. The production of irrigated wheat, oats, canary grass seed, dry barley, oil sunflower, sugar beet, canola, dry beans, lentils, and sheep and goats is not included in the planning result. Production of grain maize, rye, silage maize, clover, Hungarian vetch, sunflower for snacks, pumpkin for snacks, onions, chickpeas, cumin, poppy, and grass and cattle breeding activities are planned. In order to reach the highest gross profit of the enterprises, it is necessary to produce confectionary sunflowers on 50.95 decares for the planned production activities. Among the planned business activities, the product with the highest land width is the confectionary sunflower. This is followed by grain maize with 33.97 decares and rye with 20.13 decares. For the region, the ovine stock should be removed from the operation plan, the bovine stock should be increased, and the 5.39 headstock should be increased to 30. As a result of the implementation of the planning, the gross profit of the enterprises will increase from 383,479.81 TL to 934,785.10 TL, increasing approximately three times.

The average land width of the enterprises in the region is 231.90 decares, and as a result of continuing the production activities with the same land size, a contribution of 755.14 TL will be provided to the marginal income of the enterprises for each decade. The marginal income for the irrigated agricultural lands in the region is 1,083.30, and the fact that the enterprises have one more decare of irrigated lands will contribute to the increase in gross profit. Plant products included in the operation plan are grain maize, silage maize, clover, Hungarian vetch, confectionary sunflower, onion, grass, rye, chickpea, and cumin. The product with the highest marginal revenue in business planning is onion, with 3,277,83 TL. Dry onions are followed by grass with 2,321.42 TL and grain maize with 2,273.84 TL.

In the agricultural enterprises located in the fifth region, the marginal return of labor is equal to zero in the first and fourth periods. In these periods, an inactive labor force is accumulated in enterprises. In the second and third periods, the marginal value of the labor force increases due to the increase in working hours. The

labor requirement for the current business activity is 1,622.92 hours in the first period, 1,129.71 hours in the second period, and 1183.20 hours in the third and fourth periods. With the current labor working hours, production can only be made for an area of 144.58 decares. The land width of the enterprises in the region is 231.9 decares on average, and 87.32 decares of land remain idle in the current situation. When the working hours needed in the region are increased to 1,583.20 hours in the third region, all of the idle lands can be included in production. In this period, the cost for one labor unit employed as outsourcing will be 7.49 TL per hour.

Table 7. Fifth Region Production Plan

Products	Unit	Available Status	Planning Result
Wheat (irrigable land)	Decare	35,77	0,00
Barley (irrigable land)	Decare	23,77	8,49
Oats	Decare	0,28	0,00
Bird Feed	Decare	1,05	0,00
Grain Maize	Decare	20,19	33,97
Dry Wheat	Decare	21,75	15,10
Dry Barley	Decare	23,75	0,00
Rye	Decare	0,60	20,13
Silage Maize	Decare	7,87	16,98
Clover	Decare	5,60	16,98
Hungarian Vetch	Decare	0,12	16,98
Oil Sunflower	Decare	10,60	0,00
Sunflower for Snack	Decare	16,59	50,95
Sugar Beet	Decare	27,69	0,00
Canola	Decare	4,70	0,00
Zucchini for Snack	Decare	0,51	5,33
Dry Beans	Decare	6,07	0,00
Dried Onion	Decare	0,80	8,49
Chickpea	Decare	2,55	5,03
Lentil	Decare	0,60	0,00
Cumin	Decare	2,11	10,06
Poppy	Decare	1,13	3,15
Grass	Decare	2,11	8,49
Fallow	Decare	11,75	11,75
Bovine Animal	Head	5,39	30,00
Small Bovine Animal	Head	37,84	0,00
Total Gross Profit	TL	383.479,81	934.785,10

4. Discussion

When an evaluation is made in terms of labor presence, it has been determined that there are losses in production areas and processes due to missing labor in the third, fourth, and fifth regions. Agricultural operators cannot make effective production decisions because they cannot access the labor to employ, especially during the harvest periods when labor intensity increases. For this reason, production areas remain idle. In order to evaluate the use of resources and production capacity, the need for labor in agricultural enterprises should be determined by taking into account the working hours and production patterns. For this reason, there is a need for production planning to be done throughout Türkiye. Furthermore, an organizational model that will contribute to the supply of seasonal labor in times of increased labor force needs to be established in the agricultural sector and to play a facilitating role in terms of employment during the implementation phase. In the organizational process, it should be encouraged to determine the type of labor force according to production activities, to provide specialization for the job, to determine the labor needs of the operators, and develop the communication network between the worker and the employer. While organizing activities represent a system that facilitates access to personal rights for workers, it will also make

a significant contribution to the reduction of unregistered employment in the agricultural sector. The organized employment structure in agriculture will also create value on a macroeconomic scale by reducing problems such as disguised unemployment and structural unemployment.

Meeting the seasonal labor needs during periods when the need for labor in agricultural enterprises increases provides income-increasing activity areas for workers and increases the gross profitability for agricultural operators. Due to the seasonality of production activities in agricultural enterprises, the periods when labor demand is concentrated in each geographical region are different. In particular, the mobile seasonal labor is in certain periods, mainly in the months of May and June, in the areas where the work is concentrated in line with the work routes they have determined for a short time (3-7 days) and they leave the region by fulfilling the requirements of the job during this period. Therefore, the operator demands labor for every production activity that requires labor during the production period, and losses occur in the product and income, especially during the periods when the labor force cannot be met, which is concentrated during the harvest period. In order to minimize these losses, the resident labor in the region should take an active role in agricultural production activities. For this, a special working system should be established to encourage the participation of local labor in production. Within this system, a monitoring and follow-up system should be established, job notifications should be made, and a special insurance system should be applied to the people registered in the system.

There are problems arising from both working methods and time in agricultural enterprises. For this reason, the distribution and division of labor according to the production activity in the enterprises become difficult. Especially for the seasonal mobile labor, the short working hours cause the problem of specialization. In the evaluations made on sustainability, it was determined that personal factors affect labor productivity. Therefore, to achieve success in the production processes, it is necessary to implement training activities for specialization and to create a notification system that will contribute to the employment of the labor in the enterprises in the region. This system, which can be integrated into the organizational model, can be expanded through agricultural chambers operating in the region.

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Research Article

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Germination Parameters of Different Types of Black Carrot Seeds of Ereğli Local Population

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HIGHLIGHTS

- Black carrot is an important agricultural product for nutrition.
- Protecting gene resources in seed production should be a priority.
- It is important to cover the seeds for field emergence and yield.

Abstract

Consumer interest in healthy foodstuffs and vegetables has continuously been increasing. In this sense, quality has become a prominent issue in the production, consumption and marketing of vegetables. Seeds and planting processes significantly affect the quality of vegetables. Black carrot production is an important source of income for local farmers of Ereğli and Karapınar Districts of Konya province. Bare seeds of Ereğli local population are commonly used in black carrot production. Farmers produce their own seeds. These seeds undergo only one sieving and are not calibrated. In this study, uncalibrated bare, calibrated and coated seeds were planted and germination parameters were investigated. Experiments were conducted in Kuzukuyu village of Ereğli District, at three different planting distances (2.5, 5.0 and 7.5 cm) in randomized blocks design (3x3) with 3 replications. Planting was done in three rows on a ridge (with 7.5 cm row spacing) at a forward speed of 0.64 m s^{-1}. Mean germination time was calculated as 19.90 days for uncalibrated bare seeds, 20.14 days for calibrated bare seeds and 20.79 days for coated seeds; germination rate index values were respectively calculated as 0.431, 0.761 and 0.656 [seeds (m days)$^{-1}$]; field emergence rates were respectively calculated as 37.13%, 60.97% and 55.19%; number of plants per unit area were respectively determined as 42.35, 69.14 and 59.61 plants m^{-2}. It was concluded based on present findings that calibrated bare and coated seeds should be used in planting process of black carrot seeds of Ereğli local population. Farmers should be trained on black carrot seed production.

Keywords: Black carrot, number of plants, mean germination time, germination rate index, germination ratio

1. Introduction

Carrots have been cultivated all around the world for centuries. It is largely cultivated also in Turkey. Black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) originates from Turkey, Middle and Far East and has been cultivated for at least 3 000 years (Montilla et al., 2011). In Turkey, black carrots are produced

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especially in Ereğli and Karapınar Districts of Konya province and Kırıkhan District of Hatay province. Generally local populations are used as seed materials.

Worldwide, carrot (including black carrot) is produced on 1.125 million hectares and annual production is around 40.95 million tons with an average yield of about 36.38 t ha^{-1} (FAO, 2021).

In Konya Region, in 2021, a total of 353 700 tons of carrots were produced from 4 990 ha land area and the average yield was 70.9 t ha^{-1} (TUIK, 2021). It is estimated that in 2019, concentrate companies made approximately 120-130 thousand tons of black carrot purchase contracts in the region. In other words, about 1/3 of the carrot production of Konya Region is constituted by black carrot production. It can be emphasized that this production quantity is used as fermented beverage and concentrate. Therefore, there is an increasing interest in black carrot production and cultivation areas are becoming widespread in Ereğli and Karapınar Districts. Black carrot has an economic importance for these districts.

There is no registered black carrot variety and carrot seeds of Ereğli local population are used in production. Agricultural enterprises of the region also produce black carrot seeds of Ereğli local population. These seeds are passed through a single sieve, cleaned and classified (calibrated).

In Turkey, number of studies on seed production and planting of black carrots is limited. Kiracı (2013) created a gene pool for different types of purple carrots grown in Konya province and surroundings and used in industry, made the selection of the lines in the gene pool and identified the carrot lines that are preferred for industrial consumption and have high anthocyanin, sugar and β-carotene contents. Lökoğlu (2019) conducted a study to overcome seed production problems of black carrots and investigated the effects of root size and storage conditions on seed yield and quality in the first phase and the effects of planting spacings on seed yield and quality traits in the second phase of the study. Örnek et al. (2018) conducted a study with bare black carrot seeds of Ereğli population with a thousand-seed weight of 1.64 g and a laboratory germination rate of 91% under field conditions and planted seeds at 46.50 mm plant spacing and 0.84 m s^{-1} forward speed. The average germination time was reported as 18.33 days, germination rate index as 0.665 [(seeds (m day)$^{-1}$] and field emergence rate as 49.17%. Bülbül (2017) used a vertical spindle rototiller to prepare seedbeds for black carrot seeds and planted seeds with the use of different pressure wheels, plant spacing (2.5, 5 and 7.5 cm) and a forward speed of 0.75 m s^{-1}). Mean germination time was reported as 19.68 days, germination rate index as 0.479 [seeds (m days)$^{-1}$] and field emergence rate as 43.11%. Önal and Hacıseferoğulları (2022), in their research the bare and covered Kırıkhan population determined the average germination time, germination rate index and field emergence values as 9.40 and 9.18 days, 1.688 and 1.547 [seeds (m days)$^{-1}$], and 54.15% and 52.39%, respectively, in sowing using black carrot seeds.

The angle that a seed makes with the horizontal while falling onto the line is expressed as the impact angle. In planting uncoated (bare) and coated seeds with vacuum-type perforated planters, seed displacements on the line relatively decrease at impact angles above 40° and get minimum values at impact angle range of 75°-85° (Önal, 2011). The coating-induced increase is seed weight increases impact angles and these seeds fall onto the line close to the vertical direction and less spatter on the line. In this case, a more uniform seed spacing is achieved (Barut, 2006). Sowing depth uniformity of coated seeds is better than uncoated seeds, and seed germination is also affected by properties and thickness of the coating material.

There is no research on calibrated bare and coated black carrot seeds of Ereğli population in Konya region. In this study, bare seeds were cleaned and sorted before sowing and sorted seeds were also coated. These seeds in three different structures were sown at three nominal sowing distances by using a vacuum-type pneumatic precision vegetable planter that can plant in narrow row spacing in field conditions and field emergence characteristics were evaluated.

2. Materials and Methods

A vacuum-type pneumatic precision vegetable planter with four planter units was used for planting black carrot seeds (Figure 1). Planting was performed at narrow row spacing. In this research, a stainless-

steel press wheel at the front, a rubber press wheel at the rear and medium triple narrow intermediate rubber wheels were used for sowing black carrot seeds (Figure 2).

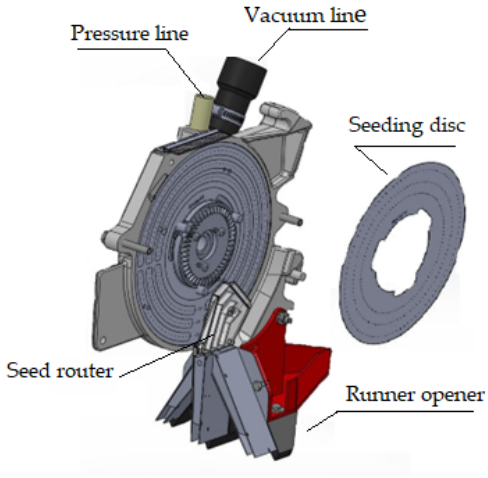

Figure 1. Schematic view of a planter unit and a planter disc

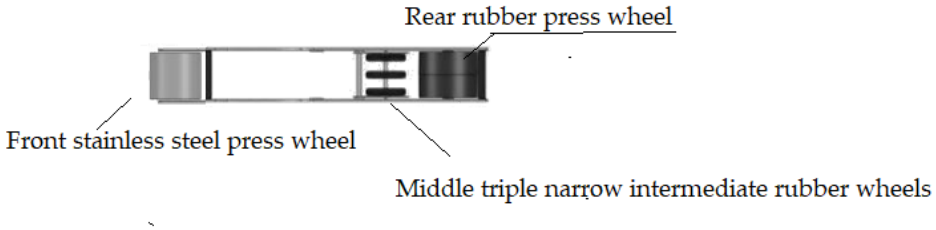

Figure 2. Schematic view of press wheel

Planter disc has a diameter of 235 mm and a thickness of 0.25 mm. In sowing, the diameter of the planting disc used is 235 mm and the thickness is 0.25 mm. There are 96 holes on planter disc arranged in three rows. From the top-hole axis, the hole axes have diameters of 210, 185 and 155 mm, respectively. Although the linear velocity values of three rows on planter disc are different, planting is done at the same planting spacing. Planting was carried out at an average vacuum pressure of 35 mbar and an air pressure of 15 mbar.

Black carrot seeds produced under farmer conditions in 2020 were used as the seed material of the study (Figure 3). Uncalibrated bare seeds (T_1) used in black carrot production contain foreign matter and weak grains. The average 1000-grain weight of these bare seeds (T_1) was 1.71 g and the germination rate was 76%. As a second process, these seeds were calibrated, passed through an oblong sieve of 1.75 mm, 1.50 and 1.25 mm, subjected to gravity and classified based on their specific gravity (T_2). The average 1000-grain weight of these classified seeds was 2.04 g and the germination rate was 86%. The classified bare seeds were coated with a special recipe integrated with a polymer structure by processing (subjecting to special processes) different materials (T_3). The germination rate of resultant coated seeds was determined as 84% and the sphericity value was determined as 0.62.

In present experiments, 3-row planting discs with a hole diameter of 0.5 mm were used for planting uncalibrated bare seed, 0.7 mm for calibrated bare seeds and 1.2 mm for coated seeds. Experiments were conducted in Kuzukuyu neighborhood of Ereğli District. All operations were carried out with the use of Hattat A78 brand tractor. Experimental soils were loamy-sand in texture with 4% clay, 10% silt and 86%

sand. Seed bed had a pH of 8.96, was strong alkaline with a lime content of 39.2% and organic matter content of 1.31%. Bulk density of 0-15 cm soil profile was 1.38 g cm^{-3} and average moisture content was 9.99%.

Normal bare seeds (T_1) Calibrated bare seeds (T_2) Coated seeds (T_3)

Figure 3. Seeds used in present experiments

From planting to end of germination of black carrot seeds for one month duration, daily average, minimum and maximum temperatures were respectively measured as 19.6 ºC, 11.3 ºC and 27.6 ºC; average, minimum and maximum soil temperatures at a depth of 5 cm were respectively measured as 25.6 ºC, 21.0 ºC and 30.7 ºC and there was no precipitation during the germination period (TSMS, 2021).

The agronomic practices and amount of irrigation water applied throughout emergence period are given in Table 1. In previous year, wheat was planted in the research area. Experimental plots were first plowed with a moldboard plow, 30 kg da^{-1} NPK 12-30-12 compound fertilizer was applied to soil surface with a centrifugal fertilizer spreader and seed bed was prepared with a vertical spindle rototiller.

Table 1. Agronomic practices and irrigations

Date	Agronomic practices
26.04.2021	Plowing with mold board plow
28.04.2021	Fertilizer application with centrifugal fertilizer spreader (30 kg da^{-1} NPK 12-30-12 composed)
28.04.2021	Seed bed preparation with vertical rototiller
29.04.2021	Preparation of planting ridges
29.04.2021	Planting
	Irrigations
30.04.2021	1st Sprinkler irrigation (72 mm)
03.05.2021	2nd Sprinkler irrigation (54 mm)
08.05.2021	3rd Sprinkler irrigation (36 mm)
12.05.2021	4th Sprinkler irrigation (36 mm)
18.05.2021	5th Sprinkler irrigation (36 mm)
22.05.2021	6th Sprinkler irrigation (54 mm)
27.05.2021	7th Sprinkler irrigation (54 mm)

Planting ridges were created with a pneumatic precision vegetable planter before planting (Figure 4). Sprinkler irrigation was applied 7 times from planting to last day of emergence. Totally, 342 mm of irrigation water was given.

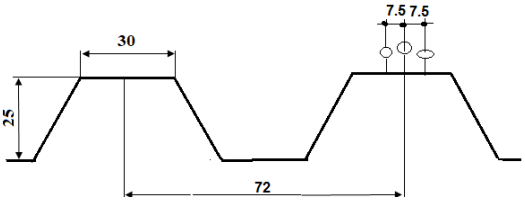

Figure 4. Schematic view and dimensions of planting ridges (cm)

Planting was done on three narrow rows with 7.5 cm spacing between the rows. The nominal planing distances on each row were chosen as 2.5, 5.0 and 7.5 cm and forward speed was chosen as 0.64 m s^{-1}.

Experiments were conducted with Ereğli local population black carrot seeds subjected to 3 different processes (T_1, T_2 and T_3) and 3 different planting distances (Z_1, Z_2 and Z_3) in randomized blocks design (3x3) with 3 replications. Experimental plots were 125 m long and 2.8 m wide (350 m^2). Soil penetration resistance was measured from the plots formed after seed bed was prepared and empty planting ridges formed, as well as from the tracks of pressure wheel formed on the ridge after planting process. Five measurements were made from five randomly selected plots with a penetrometer with a base cone area of 2 cm^2 and an apex angle of 30º.

To determine germination parameters of the seeds, 1 m long sections of randomly selected 3 planting ridges of each plot were selected and carrot sprouts emerging to soil surface were counted throughout the germination period and mean germination time (MGT), germination rate index (GRI) and field emergence rate (FER) values were calculated with the following equations (Erbach, 1982; Işık et al., 1986).

$$MGT=\frac{N_1D_1+N_2D_2+\ldots N_nD_n}{N_1+N_2+\ldots+N_N} \quad \text{(day)}$$

$$GRI=\frac{\text{Number of germinated seeds per meter}}{MGT} \quad [\text{seeds (m day)}^{-1}]$$

$$FER=\frac{\text{Number of germinated seeds per meter}}{\text{Number of planted seeds per meter}} \times 100 \quad (\%)$$

N: Number of germinated seeds in each count

D: Number of days passed after planting

To determine the number of plants per unit area, the plants in randomly selected 1.4 m sections of each row (1 m^2) was counted in 5 replications. About 32 days after planting, main root lengths and plant heights of randomly selected carrot plants were measured in 10 replications (Eker, 1988).

Data normality was checked with the use of Shapiro-Wilk test. Experimental data were subjected to analysis of variance with the use of MINITAB 16 software. Significant means were compared with the use of LSD analysis of MSTAT-C software.

3. Results and Discussion

3.1. Penetration resistance

Penetration resistance values measured from the seed beds are presented in Figure 5. It was observed that penetration resistance measured at 25 cm soil tillage depth passed over 2 MPa value.

Penetration resistance values measured from empty ridges and pressure wheel tracks are presented in Figure 6. Penetration resistance at 1 cm planting depth was about 0.15 MPa.

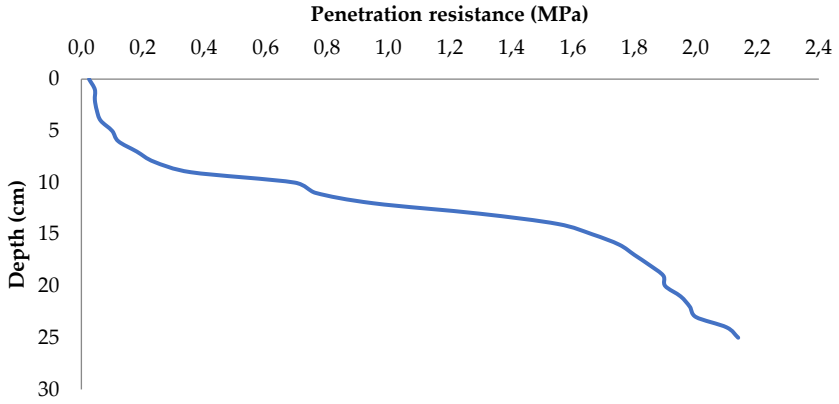

Figure 5. Penetration resistance curve of seed bed

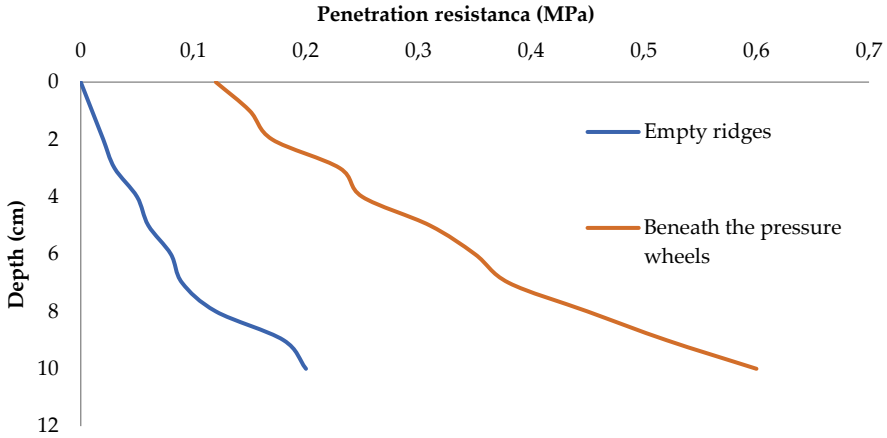

Figure 6. Penetration resistance of empty ridges and pressure wheel tracks

3.2. Germination and growth parameters

Change in germination parameters (mean germination time - MGT, germination rate index - GRI, field emergence rate - FER) with experimental treatments are provided in Table 2.

Table 2. Germination parameters of black carrot seeds

Seeds	Planting distance	MGT (day)	GRI [seed (m day)$^{-1}$]	FER (%)	Mean MGT	Mena GRI	Mean FIR
T_1	Z_1	20.16	0.544	27.46	19.90	0.431_b	37.13_b
	Z_2	19.75	0.382	35.98			
	Z_3	19.80	0.367	47.95			
T_2	Z_1	20.46	1.157	57.98	20.14	0.763_a	60.97_a
	Z_2	20.11	0.649	61.64			
	Z_3	19.85	0.483	63.27			
T_3	Z_1	22.03	0.961	52.01	20.79	0.658_a	55.19_a
	Z_2	19.34	0.592	54.50			
	Z_3	21.03	0.422	59.07			
						LSD=0.251(p<0.01)	LSD=8.183 (p<0.01)

Mean germination time was calculated as 19.90 days for T_1, 20.14 days for T_2 and 20.79 days for T_3. While coated black carrot seeds (T_3) had higher values, uncalibrated bare seeds (T_1) had lower values. Since T_1 seed

was not calibrated, germination was completed early due to weak and undersized seeds and germination was completed later in T_3 seed due to coating. However, there was no statistical difference between the mean germination times of the seeds. However, differences in mean germination time values of black carrot seeds were not found to be significant. A duff layer is usually formed in seed bed; thus 7 sprinkler irrigations were performed throughout the germination period to keep soil surface moist and prevent formation of a duff layer. Such a case resulted in insignificant differences in mean germination times. Bülbül (2017) reported mean germination time values of uncalibrated bare black carrot seeds of Ereğli local population as 18.82 days in 2015 and 19.38 days in 2016.

The highest germination rate index (0.763) was obtained from T_2 seeds and the lowest (0.431) from T_1 seeds. Germination rate index of T_3 seeds was calculated as 0.658 [seeds (m days)$^{-1}$]. Differences in germination rate index values of the seeds were found to be significant (F=7.58). Germination rate index values varied also with nominal planting distances and values were calculated as 0.887 for Z_1, 0.541 for Z_2 and 0.424 [seeds (m day)$^{-1}$] for Z_3. Resultant differences were found to be significant (F=15.27). Bülbül and Hacıseferoğulları (2016) used different types of pressure wheels and reported germination rate index values as between 0.194 - 0.971 [seeds (m days)$^{-1}$]. Örnek et al. (2018) planted uncalibrated bare black carrot seeds at 22.36, 46.50- and 68.70-mm nominal distances and reported germination rate index values as 1.007, 0.616 and 0.467 [seeds (m days)$^{-1}$], respectively. Present germination rate index values were similar with those earlier findings.

Field emergence rates of uncalibrated bare seeds were calculated as 37.13% for T_1, 60.97% for T_2 and 55.19% for T_3. Differences in field emergence rates were found to be significant (F=7.55). For nominal planting distances, the lowest field emergence rate (45.81%) was obtained from Z_1, followed by Z_2 (50.70%) and the highest value (56.76%) was obtained from Z_3. However, resultant differences were not found to be significant. Bülbül and Hacıseferoğulları (2016) conducted a study with a pneumatic precision vegetable planter with a front and rear pressure wheels and a triple narrow tire in the middle with adjustable pressure and reported field emergence rates as between 33.33 - 48.07%. Örnek et al. (2018) used a pneumatic precision vegetable seed drill at 0.84 m s^{-1} forward speed and 22.36, 46.50- and 68.70 mm nominal planting distances and reported field emergence rates as 55.24%, 49.17% and 54.42%, respectively.

Change in plant height and root depths of black carrot seeds with experimental treatments are provided in Table 3.

Table 3. Plant height and root depths of different black carrot seeds

Seeds	Planting distance	Plant height (mm)	Root depth (mm)	Mean plant height (mm)	Mean root depth (mm)
	Z_1	55.35	142.34		
T_1	Z_2	52.78	158.34	56.08$_b$	156.17$_b$
	Z_3	60.12	167.82		
	Z_1	65.41	183.08		
T_2	Z_2	64.55	166.86	66.23$_a$	179.18$_a$
	Z_3	68.72	187.59		
	Z_1	63.64	190.06		
T_3	Z_2	63.60	178.48	63.69$_{ab}$	182.10$_a$
	Z_3	63.84	180.75		
				LSD=7.822 ($p<0.01$)	LSD=19.620 ($p<0.05$)

Average plant height was determined as 56.08 mm for T_1, 66.23 mm for T_2 and 63.69 mm for T_3. The lowest plant height was obtained from T_1 seeds because of high rate of undersized seeds since they were not calibrated. Differences in plant heights of the seeds were found to be significant. Plant heights also varied with nominal planting distances. Average plant height was determined as 61.47 mm for Z_1, 60.31 mm for Z_2 and 64.23 mm for Z_3, but resultant differences were not found to be significant. Bülbül (2017) used a V-channel triple casting pressure wheel with sheet metal front wheel, rubber rear wheel and middle wheel, pressure of which can be adjusted with a spring, and nominal planting distances of 2.5 cm, 5.0 and 7.5 cm. Plant heights were reported as between 40.23 – 42.27 mm. Those values were greater than the present ones. Kayışoğlu (1993) applied a pressure from the bottom of pressure wheel in sunflower planting and obtained a plant height of 7.41 cm.

Root length was determined as 182.10 mm for T_3, 179.18 mm for T_2 seed and 156.17 mm for T_1 seeds. The differences in root lengths of T_2 and T_3 seeds were not found to be significant, but differences from T_1 seeds were found to be significant. Root length was measured as 171.83 mm for Z_1, 168.89 mm for Z_2 and 178.72 mm for Z_3. Kayışoğlu (1993) emphasized that there should be a strong ground on which the plant receives support while applying a pressure from pressure wheel and sheet metal pressure wheel in front of vacuum-type pneumatic vegetable planter provided a sufficiently compacted hard zone under the seed bed and three-row narrow rubber wheel in the middle-performed compaction at planting depth, thus obtained higher root lengths. Plant root length values were found to be high due to compaction at the level of the plant. Root length values of T_1 seeds (uncalibrated bare seeds) were found to be lower than the other two seeds. Bülbül (2017) planted black carrots with different types of pressure wheels and reported root length as 72.54 mm for seed beds prepared with vertical spindle rototiller. Present root lengths were greater than this value. Kayışoğlu (1993) reported root length of sunflower as 7.70 cm when a pressure applied from farrow bed and 6.84 cm when a pressure was applied from the surface.

3.3. Number of plants per unit area

Number of plants per unit area values obtained from triple ridge planting of different seeds and at different planting distances is provided in Table 4.

Table 4. Number of plants per unit area

Seeds	Planting distance	Number of plants per unit area (plant m^{-2})	Mean number of plants per unit area (plant m^{-2})
	Z_1	55.21_{bc}	
T_1	Z_2	41.77_{cde}	42.35_{c}
	Z_3	30.08_{e}	
	Z_1	101.53_{a}	
T_2	Z_2	58.88_{b}	69.14_{a}
	Z_3	47.11_{bcd}	
	Z_1	97.85_{a}	
T_3	Z_2	45.67_{cde}	59.61_{b}
	Z_3	35.32_{de}	
		LSD=16.97 ($p<0.01$)	LSD=9.508 ($p<0.01$)

The highest number of plants per unit area (69.14 plant m^{-2}) was obtained from T_2 seeds, followed by T_3 seeds with 59.61 plant m^{-2} and the lowest value (42.35 plant m^{-2}) was obtained from T_1 seeds. Differences in number of plants per unit area were found to be significant (F=33.17). Number of plants per unit area (1 m^2) decreased with increasing planting distances. Number of plants per unit area was determined as 84.86 plant m^{-2} for Z_1, 48.74 plant m^{-2} for Z_2 and 37.50 plant m^{-2} for Z_3. Resultant differences in number of plants per unit area values were found to be significant (F=112.14). In terms of interactions, the greatest number of plants per unit area was achieved in T_2Z_1 and T_3Z_1 combinations and there were significant differences (F=8.09). For Karapınar region of Konya province, number of plants per unit area should be between 60-90. However, number of plants obtained with T_1 seeds was found to be far from these values. With these seeds, even at Z_1 nominal planting distance, 60-90 plant m^{-2} could not be reached. Bülbül (2017) used 3-row planter disc with 96 holes and reported number of plants at 2.38, 4.65 and 6.78 cm nominal plant distances respectively as 46.82, 31.60 and 28.93 plant m^{-2} in 2015, as 52.55, 47.45 and 40.87 plant m^{-2} in 2016. Present values obtained from T_2 and T_3 seeds were greater than those earlier values.

4. Conclusions

Black carrot seeds of Ereğli local population should be cleaned and sorted before planting. Local producers do not produce seeds under primitive conditions and seed characteristics differ from producer to producer. In particular, they do not perform the cleaning process effectively. In this case, the hairs in unclassified seeds (due to insufficient cleaning) can cling onto disc holes and cause clumps in seed storage due to static electricity. Thus, it prevents the mixer from doing its job. Mixer should be checked at certain intervals during planting. Such a case has a negative effect on both field emergence and uniform distribution

on a row. Further research should be conducted to improve coating properties of calibrated bare seeds. Research should be done on different coating materials and ratios. Studies should be conducted on hole diameters for different seed characteristics. Further research is also recommended on singling unit of domestically-made machine since singling of three hole rows is done with a single piece manufactured as singling unit. In this case, especially the holes in upper row cannot be singled out effectively. Local farmers should be trained on black carrot seed production. Calibrated bare and coated black carrot seeds should be used in black carrot farming.

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