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Research Article

Fungal Contamination and Toxigenicity of *Aspergillus flavus* on Postharvest Cacao Beans in Northern Sumatera, Indonesia

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Fungal population,
Toxigenic

Abstract: The study was carried out to enumerate fungal contamination, and toxigenicity of *Aspergillus flavus* strains on cacao beans during drying and storage. As many as 3500 g cacao beans during drying and storage were purchased from smallholder farmers on the plantation areas at Karo Regency, Northern Sumatera. The percentage of the beans contaminated by fungi was conducted using direct plating. Fungal populations on soil and beans were determined using dilution followed by pour plated in dichloran 18% glycerol agar (DG18) and *Aspergillus flavus* and *parasiticus* agar (AFPA). The mycological evaluation was carried out based on morphological characteristics. Results showed eighteen genera of soil fungi were isolated at the cacao plantation; genera of *Aspergillus* sp., *A. niger, A. flavus,* and *Penicillium citrinum* were the most important contaminants. Six species of the fungi were associated with contamination on cocoa beans during drying *i.e. Aspergillus* sp*., Candida tropicalis, Saccharomyces* sp*., A. niger, Penicillium* spp*.,* and *Fusarium* spp. Whereas three fungal species were associated during storage *i.e. A. niger*, *A. flavus,* and *P. citrinum*. The percentage of cacao contaminated during drying and storage was dominated (>40%) by *Aspergillus* sp. *Fusarium* sp. *A. niger, A. flavus,* and *P. citrinum,* respectively. Among 21 strains of *A. flavus*, 3 strains (15%) were isolated from soil, and 18 strains (85%) were isolated from beans during storage. Among toxigenic *A. flavus*, both strain scaf6 isolated from soil and strain cbaf5 isolated from beans during storage were the highest aflatoxin producers (30.0 ppb). Preventing soil contamination during harvesting, drying, and storage of cacao beans was a prerequisite to minimise fungal contamination.

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1. Introduction

Cacao (*Theobroma cacao* L.) is one of the most important commodities in North Sumatera, Indonesia. According to Directorate General of Estate Crops (2021) area of cacao plantations in North Sumatera was estimated at 53397 hectares and most of the plantations were dominated by small scale farmers where harvest and warehouse storage condition out of control and often damage quality of the beans (Martins et al., 2010). High relative humidity promote improper stored dried beans absorb water

vapour from the environment. This process leads to an increase in beans moisture that results in accelerated fungal growth. Among microorganisms, fungi are the most contaminated on dried-stored commodities, cause physical damage, are contaminated by mycotoxins (Martins et al., 2010; Copetti et al., 2014; Dharmaputra et al., 2015). Fungal contamination occurred during preharvest, harvesting, and postharvest handling. Traditional drying is common in tropical countries, particularly among subsistence farmers where postharvest cacao beans were poor processing and lack of proper facilities. During sun-drying the beans were exposed to the air wind, dust and may be contaminated by fungi. Fagbohun et al. (2011) reported that mouldy cacao beans during storage were contaminated by *Aspergillus niger*, *A. flavus*, *Botryodiplodia theobromae*, *Fusarium* spp., *Mucor* spp., *Neurospora* spp., *Penicillium* spp. and *Phytophthora palmivora*. Genus *Aspergillus* section *Flavi* particularly *A. flavus* is a natural mycobiota in cacao beans and has the potential to produce aflatoxins (Sánchez-Hervás et al., 2008). The fungal propagules germinate when the bean moisture content is suitable for their growth. Soil is the main source of fungal inoculums causing disease or contamination on agricultural products (Ehrlich, 2014; Dharmaputra et al., 2018). Other environmental factors that affect fungal infection on crop products include fungal strains and substrates (Daou et al., 2021). Therefore, an integrated approach that starts in the field prior to harvest and throughout the whole chain is required, so good agricultural practices such as preharvest and postharvest handling minimised fungal contamination in every step to deliver safe cacao commodities to consumers. The purpose of the current study was to enumerate the fungal contamination, and toxigenicity of *Aspergillus flavus* strains on cacao beans during drying and storage.

2. Material and Methods

2.1. Soil sample and cacao beans

Soil samples were obtained from the area of one hectare of a smallholder cacao plantation located in Karo Regency, North Sumatera, Indonesia. Sampling was conducted randomly by dividing each areas into 100 sampling plots. Each plot (1×1 m^2) was divided into 10 points, and 20 g of soil sample was obtained for each point. All of the soil composite samples obtained were mixed thoroughly and placed into a sterile bag, then stored in the cold for further use. At the same time as soil sampling, 3500 g of cacao beans during drying (following harvesting) and storage (±1 month after sun-drying) were purchased from five smallholder farmers at the site of the plantation.

2.2. Beans moisture content

The drying oven method was conducted to determine cacao beans' moisture according to Standard Nasional Indonesia (2008).

2.3. Determination of the percentage of beans contaminated by fungi

The percentage of cacao beans contaminated by fungi was conducted by direct plating technique in dichloran 18% glycerol agar (DG18, NEOGEN®, Lansing, MI, USA) medium. The beans were superficially disinfected to remove the surface contaminants using sodium hypochlorite (NaOCl) for 1 minute. Beanswere then aseptically placed on DG18 medium (pH 5.6) in a petri dish (9 cm diameter) with 5 cacao beans per petri. All plates were incubated for 7 days at 29ºC. Ten replications were made for each sample.

2.4. Fungal population on soil and beans

Fungal population contaminated on beans and soil was determined using dilution technique followed by pour plated in DG18 medium. As much as 25 g of soil sample in erlen meyer 1000 ml was diluted in sterilised distilled water until the volume was up to 250 ml. Then, the soil suspension was homogenised using shaker Kottermann 4020, Hanigsen, W. Germany 250 rpm for 2 minutes, and a serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) was made. As much as 1 ml of each of the suspension in a petri dish (9 cm diameter) was pour plated DG18 medium. Three replicate plates were made for each dilution.

The fungal population on beans was determined as follows: every 3500 g of cacao beans was ground for 30 seconds (25 000 rpm) using Mill Powder RT-04 no Serie 980923, Taiwan. The ground

beans were then divided into sub-samples for the determination of moisture content and fungal population. As much as 25 g of the ground bean in a flask of 1000 ml was diluted in distilled water until the volume was up to 250 ml. The suspension was then homogenised using shaker Kottermann 4020, Hanigsen, W. Germany 250 rpm for 2 minutes, and a serial dilution was made. As much as 1 ml of each of the suspension in a petri dish (9 cm diameter) was then pour plated with DG18 medium. Three replicate plates were made for each dilution. All plates were incubated for 7 days at 29ºC. The colony of each fungal species was counted, isolated, and cultured for 7 days at 28ºC in potato dextrose agar (PDA), czapek yeast extract agar (CYA), or czapek yeast extract agar with 20% sucrose (CY20S). Fungal identification was made based on morphological characteristics according to Samson et al. (2002) and Pitt and Hocking (2009).

2.5. Isolation of *Aspergillus flavus*

To isolate *A. flavus* strains, the homogenised suspensions of soil and cacao beans were inoculated in a*spergillus flavus* and *parasiticus* agar (AFPA) medium in petri dishes (9 cm diameter). All plates were incubated for 7 days at 29°C. The presence of an orange color on the reverse side of the medium was indicated as *A. flavus* (Pitt and Hocking, 2009).

2.6. Determination toxigenic and non-toxigenic *A. flavus* strains

All *A. flavus* strains were further isolated on potato dextrose agar (PDA, Oxoid Ltd, Basingstoke, Hants, UK) in petri dishes (diameter 9 cm) and incubated for 7 days at 29ºC. The toxigenicity of each *A. flavus* strain was determined using 10% coconut agar medium (CAM) according to Lin and Dianese (1976). The medium was sterilised for 20 minutes at 120 ºC. Each plate was then inoculated by *A. flavus* strain. All plates were incubated for 5 days at 29ºC. The presence of yellow pigment on the reverse side of the medium indicates aflatoxin producer strains (Lin and Dianese, 1976; Davis et al., 1987).

2.7. Aflatoxins determination

Assessment of aflatoxin B_1 (AFB_1) production was done using the thin layer chromatography (TLC) method. A colony of *A. flavus* (from a CAM plate incubated at 29ºC for 5 days) was mixed with 50 ml of ethanol in a waring blender; the suspension was extracted for 30 minutes and filtered using filter paper (Whatman #1). The filtrate was then transferred to a 250 ml separating funnel and extracted twice with 50 mL of n-hexane, and cleaned with 50 ml of chloroform. The extract was then dehydrated in a vial and filtered using anhydrate sodium sulfate (Na_2SO_4). Using a microsyringe, 10 µl of the residue was spotted onto a TLC plate (MERCK # 1.05554, Silica gel 60, F254) and ran for 20 minutes. The developing solvent was chloroform: acetone (9: 1). Commercially available aflatoxins were used as standards (Sigma-Aldrich Chemical Company, USA).

3. Results and Discussion

3.1. Soil fungi and cacao beans

A total of 18 fungal species were isolated from the soil. However, only six of the species contaminate cacao beans during drying, and three fungal species contaminate during storage (Table 1). *Trichoderma* spp. followed by *Aspergillus chevalieri, A. flavus, Aspergillus* sp*., Drechslera tritici-repentis, A. Sydowii*, *Mucor circinelloides,* and *P. Corylophilum* were among the soil fungi commonly isolated. During drying (sun-drying) (bean moisture content 39.16%), the fungi most frequently isolated were *Aspergillus* sp., *Candida tropicalis, Saccharomyces* sp., *A. niger*, *Penicillium* spp*.,* and *Fusarium* spp. Whereas during storage, beans' moisture content declined to 7.02%, and the fungal contaminations were dominated by *A. niger, A. flavus*, and *P. citrinum,* respectively. As shown in Table 1, more fungal species were observed on soil than on cacao beans. The population of *Aspergillus* sp. (7×10^7 cfu g^{-1}) was the most contaminant cacao beans during sun-drying followed by *Candida tropicalis* (4.3×10^7 cfu g^{-1}). Whereas, during storage, *Aspergillus niger* was the most found (6×10^3 cfu g^{-1}), followed by *A. flavus* (6×10^2 cfu g^{-1}) and *P. citrinum* (1.4×10^3cfu g^{-1}). It seems that *A. niger*, *A. flavus,* and *P. citrinum*

are the most important contaminant on cacao beans produced by smallholder cacao beans at Karo Regency.

Fungal contamination on the cacao during drying and storage seemed to originate from the soil of the plantation or might cross-contamination occurred due to the practice of sun-drying by traditional farmers using tarpaulin or cemented pavement in an open area close to the ground. A previous study by Fagbohun et al. (2011) reported that *A. flavus*, *A. niger,* and *Rhizopus* spp. were the most contamination occurred on dried cacao beans stored on the bare floor. Dharmaputra et al. (2018) reported more fungal infection and aflatoxin contamination on storage nutmeg kernel collected from falling on the ground than that of nutmeg by picking from the tree. A similar finding was reported by Copetti et al. (2011), who reported the highest numbers of fungi contaminating cacao beans during drying and storage in comparison to during processing cacao into chocolate.

Table 1. Fungal population (cfu g^{-1}) isolated from soil at cacao plantations and cacao beans during drying and storage

Fungal species	Fungal population (cfu g^{-1}) on cacao beans		
	Soil	During drying	During storage
***Acremonium* sp.**	2×10^2	0	0
***Aspergillus* sp.**	5×10^2	7×10^7	0
A. flavus	5×10^2	0	6×10^2
A. niger	0	3×10^5	6×10^3
A. sydowii	4.8×10^2	0	0
A. tamarii	1.4×10^2	0	0
A. chevalieri	1×10^3	0	0
Botrytis cinerea	1×10^2	0	0
Candida tropicalis	0	4.3×10^7	0
Fusarium oxysporum	1×10^2	0	0
***Fusarium* spp.**	1×10^2	1×10^2	0
Drechslera tritici-repentis	5×10^2	0	0
Mucor circinelloides	3×10^2	0	0
***Penicillium* spp.**	0	3×10^5	0
P. citrinum	0	0	1.4×10^3
P. corylophilum	3×10^2	0	0
***Saccharomyces* sp.**	0	1.8×10^7	0
***Trichoderma* spp.**	1.7×10^3	0	0

cfu g^{-1} = colony forming unit per gram.

As a terrestrial habitat, conidia of fungi are abundant in plantation areas, and they are dormant, disperse or grow in organic matter and contaminate agricultural products during harvesting (Krijgsheld et al., 2012). Risk of fungal infection in small scale farms might be due to improper harvest and storage methods by the farmers. Open sun-drying by spreading the beans using tarpaulin or plastic sheet and close to the ground was a high risk of cross contamination. Storage with high relative humidity and temperature might favor fungal growth. As previously studied by Sardar et al. (2019) that farm households who had low education were likely less awareness to raise knowledge of agro-ecosystems in related to their agricultural products. None of the yeasts was observed on the soil and storage beans. The highest population of yeasts (*C. tropicalis*) (4.3×10^7 cfu g^{-1}) and *Saccharomyces* sp.(1.8×10^7 cfu g^{-1} was isolated on beans only during drying. Environmental contamination by yeasts is caused by cacao pods during pod breaking, insects, fermentation boxes, equipment used, and the highest number of yeasts occurred spontaneously at the beginning of fermentation (Copetti et al., 2011; De-Vuyst & Weckx, 2016; Mota-Guiterrez et al., 2018). The other soil fungi, *A. sydowii*, *A. tamarii*, *Trichoderma* spp., and *A. Chevalieri,* decreased in their viability during storage.

3.2. The percentage of cacao beans contaminated by fungi

Moisture content of cacao beans determines the percentage of the beans contaminated by fungi. As many as 5 fungal species were contaminated with cacao bean during drying, and four species were found during storage. *Aspergillus* sp. (72%) had the highest percentage on cacao during drying followed by *Fusarium* sp. (48%), *Penicillium* spp. (24%), *A. niger* (20%) and *Saccharomyces* sp. (8%). High moisture content (39.16 %) of cacao during drying promotes the growth of field fungi, particularly *Fusarium* sp. and yeast (*Saccharomyces* sp.).

Table 2. Moisture content and the percentage of cacao beans contaminated by fungi during drying and storage

Cacao beans	Moisture content (%)	Fungal species	Percent beans contaminated by fungi
During drying	39.16	*Aspergillus* sp.	72
		Penicillium spp.	24
		Fusarium sp.	48
		Aspergillus niger	20
		Saccharomyces sp.	8
During storage	7.02	*Aspergillus niger*	100
		A. flavus	72
		P. citrinum	40

High percentage of *Aspergillus* sp. (72%) on cacao during drying was consistent with the findings of Copetti et al. (2011), who reported that high diversity of fungi, especially *Aspergillus* sp. contaminated cacao beans at the farm during drying and storage. *Fusarium* sp. was the second contaminant (48%) and found only on cacao during drying while the beans' moisture content was still high. The presence of *Fusarium* sp. as a contaminant at the beginning of storage was reported on peanut (Santos et al., 2016), nutmeg (Nurtjahja et al., 2017), and maize (Carbas et al., 2021). A previous study by Yuan et al. (2020) reported that *Fusarium* is ubiquitous in terrestrial ecosystems. The occurrence of *Fusarium* on the soil at cacao plantations might cause the mold to be contaminated during harvesting and then grow on cacao beans during drying. Previously studied by Ploetz (2006) and Rosmana et al. (2013) reported that *Fusarium* caused disease in cacao. The absence of *Fusarium* sp. on the bean during storage indicated that bean moisture content (7.02%) inhibited the fungal growth. In line with this study, Nurtjahja et al., (2017) reported that *F. semitecum* and *F. verticillioides* were found only at the beginning of storage nutmeg with water activity (a_w) 0.80 and 0.97. Similar to *Fusarium*, the presence of *Saccharomyces* sp. on cacao during drying showed that the rest of the yeast might still be present after the natural fermentation process by farmers, as previously reported by De-Vuyst & Weckx (2016).

The highest percentage of cacao beans during storage was contaminated by *Aspergillus niger* (100%) followed by *A. flavus* (72%) and *P. citrinum* (40%). As shown in Table 1, the population of *A. niger* has become an important fungal contaminant on dried and stored cacao. A previous study by Fagbohun et al. (2011) showed that *A. niger* was most found on mouldy cacao beans during storage.

A total of 21 strains of *A. flavus* were isolated, which consisted of 3 strains isolated from soil at the cacao plantation, and eighteen strains isolated from cacao beans during the storage (Table 3). None *A. flavus* was found on cacao during drying. Among toxigenic (aflatoxin producers) *A. flavus*, strain scaf6, isolated from soil at cacao plantation was the highest aflatoxin producer (30 ppb). Whereas 18 toxigenic strains were isolated at cacao during storage, strains cbaf5 and cbaf11 produce aflatoxin 30.0 and 12.9 ppb, respectively.

Even though the population of *A. flavus* strains on the soil at cacao plantation was only 5×10^2 cfu g^{-1} (Table 1), certain strains of the toxigenic *A. flavus* isolated from soil (scaf6) might potential as a source of aflatoxin contamination on cacao during storage. Soil is the main source of fungal contamination, spoilage, and cause disease in agricultural products (Ehrlich, 2014; Dharmaputra et al., 2018; Winter & Pereg, 2019; Serumaga et al., 2020). The population of the toxigenic *A. flavus* on cacao beans might increase during improper preharvest, harvesting, and postharvest such as drying and storage. Even though fungal contamination on agricultural products can not be avoided (Lane et al., 2018), preventing beans from contact with soil and proper drying and storage of cacao are important to minimise deterioration and aflatoxin contamination.

Table 3. Toxigenic and non-toxigenic *A. flavus* strains on soil and cacao beans during drying and storage

	Aspergillus flavus strains	Toxigenicity on CAM	Aflatoxin B_1 (ppb) detected by TLC
Soil at cacao plantation	scaf3	-	0
	scaf6	+	30.0
	scaf12	-	0
Cacao beans during drying	0	0	0
Cacao beans during storage	cbaf1	+	< 3.01
	cbaf2	-	0
	cbaf3	-	0
	cbaf4	+	< 3.01
	cbaf5	+	30.0
	cbaf6	+	< 3.01
	cbaf7	+	< 3.01
	cbaf8	+	< 3.01
	cbaf9	+	< 3.01
	cbaf10	+	< 3.01
	cbaf11	+	12.9
	cbaf12	-	0
	cbaf13	-	0
	cbaf14	+	< 3.01
	cbaf15	-	0
	cbaf16	+	< 3.01
	cbaf17	-	0
	cbaf18	+	< 3.01

CAM = coconut agar medium; TLC = thin layer chromatography.

4. Conclusion

High population of fungi at cacao plantation was the potential to contaminate cacao beans. Some of the fungi were associated with contamination of postharvest cacao beans during drying and storage. The fungal genera such as *Aspergillus* sp, *A. niger,* and *P. citrinum* were the most important contaminants, and their population was increased in cacao beans during storage. The study results showed that proper drying and storage by smallholder farmers were required to minimize fungal infection and potential aflatoxin contamination.

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Research Article

Effect of Substrates, Planting Period, Explants Nodal Level and Arbuscular Mycorrhizal Fungi on Sweetpotato Vine Cutting Production in Soil and Soilless Systems

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Soil farming,
Vine cutting

Abstract: In West Africa, little attention has been given to the rapid propagation of sweet potato with much dependence on the use of tuber part as seed. This study thus investigated the propagation and establishment of a different number of nodal segments of sweet potato vines in soil and soilless system under the influence of Arbuscular Mycorrhizal Fungi (AMF). Cut vines (double and single nodal explants) from growing sweet potato plants were planted in the following substrates; Topsoil, Cocopeat, and Cocopeat + 5 g AMF (4 kg each fertigated with 250 g poultry manure). The experiment was conducted twice (March to July and August to December 2021). The experiment was a 2 (nodal explants) x 3 (substrates) x 2 (periods) factorial arranged in a completely randomized design with three replicates. The agronomic and yield parameters were collected and analysed using Analysis of Variance (ANOVA), and means were separated using Duncan Multiple Range Test (DMRT) at 5% level of significance. At 4 Weeks after Planting (WAP), the number of new leaves produced by the nodal explants was insignificant. At 8 WAP, the number of nodes produced differed significantly among substrates and ranged from 17.67±0.42 (cocopeat) to 20.17±0.42 (cocopeat+5g AMF). Number of tubers produced differed significantly between planting periods and ranged from 3.28±0.41 (March to July) to 5.06±0.41 (August to December). For efficient vine rooting, planting of single node vines of sweetpotato in cocopeat substrate fertigated with poultry manure and AMF between the August to December period of the year is thus recommended.

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1. Introduction

Sweet potato (*Ipomea batatas*) is the second most cultivated tuber crop after cassava in the world predominantly due to its high dry matter and nutrient content (Nedunchezhiyan et al., 2012). The tuber part is consumed after boiling or fried as a source of carbohydrates, vitamins, and proteins, or it can be sold to other end users, making it a source of cash for the producer (Magagula et al., 2010). However, in West African countries, Sweet potato has received little attention in terms of production by the local

farmers relative to other tuber crops like yam and cassava. This is probably due to the fact that the crop is largely considered as poor man's food and as a dessert to the rich rather than a staple for all and source of income to the farmers and gross domestic product to the nations (Essilfie et al., 2016). The above factors have contributed largely to the low production of sweet potato in West Africa, coupled with the production technique that is solely dependent on the use of tuber part, which is also the edible part.

Vine cutting technology is the propagation of crops through the nodal parts. This process increases the propagation ratio of clonally propagated crops against the sole use of tuber parts, as demonstrated in yam (Maroya et al., 2014) and sweet potato (Essilfie et al., 2016). However, the position of the shoot, number of nodes in the cut section, nutrient content of the rooting substrate, and the substrate type play an important role in the successful establishment of the technology (Nair, 2006). The younger shoots are normally considered a better option for the vine cutting establishment as the older shoots tend to lose totipotency easily over time (Balogun and Gueye, 2014). The higher the initial number of nodes in the establishing cutting, the faster rate of its establishment, but this reduces the propagation ratio obtainable from a growing plant. However, it is yet to be established the yield variability obtainable from the use of different explant numbers.

The use of soil as an establishing substrate has the advantage as a cheap source, but it is usually constrained by disease transmission, and heterogeneity of the soil nutrient content has resulted in crops low productivity relative to substrate based hydroponics (Maboko et al., 2009). Substrates like cocopeat, usually deployed in a hydroponics system, is another good rooting source, but it is nutrient inert and expensive to source. Hence the use of the cocopeat substrate means it has to be fortified with an additional nutrient source which could be synthetic or organic (Ossai et al., 2020). While the organic requires the addition of microbes to aid the decomposition or left for a longer time to completely mineralize before its eventual use (Alaboz et al., 2022). Different beneficial bacteria like the AMF have been frequently deployed to aid mineralization (Bunn et al., 2019), but their mode of action in unlocking the nutrient contained in organic manure has not been so clear as researchers have considered their role as a symbiotic and catalytic relationship with other enterobacteria in the environment. But to which extent their role will be beneficial in the quick establishment of the sweet potato vine cutting over just the use of the manure alone is yet to be established.

This study thus investigated the propagation and establishment of a different number of nodal segments of sweet potato vines in soil and soilless system under the influence of AMF in unlocking the organic fortified nutrient status of the substrates.

2. Material and Methods

2.1. The experimental site and environmental location

The experiment was conducted in a greenhouse condition at Idi-Ose, opposite International Institute of Tropical Agriculture, IITA, Ibadan, located at Latitude 7.5014° N and longitude 3.9099° E.

2.2. Source of planting materials

Single and double node vines were cut from two months old orange sweet potato varieties growing at the Soilless FarmLab, Abeokuta, Ogun State, Nigeria. Buffered cocopeat blocks were purchased from the Afri-Agri Company, Lagos, Nigeria. Topsoil was collected from the Teaching and Research Farm, Faculty of Agriculture, University of Ibadan. The AMF was purchased from the Botany Department, University of Ibadan, Nigeria, while poultry manure was sourced from Ajayi Farm Limited.

2.3. Experimental procedure

The topsoil was sterilized using an autoclave at a temperature of 121^{o}C for 15 minutes and kept in a bowl overnight to cool. The cocopeat blocks were dissolved in water (30 litres per block). Both the sterilized soil and dissolved cocopeat were weighed into a hydroponics trough and arranged in the following treatment composition;

Treatment One (T1): 250 g Poultry manure + 4 kg Topsoil,
Treatment Two (T2): 250 g Poultry manure + 4 kg Cocopeat,
Treatment Three (T3): 250 g Poultry manure + 4 kg Cocopeat + 5 g AMF

The substrates were left for 2 weeks, and single node and double node cuttings, respectively, of the orange fleshed sweet potato variety were planted in the treatment arrangements. The experiment was conducted in two (2) periods: 1. March to July 2021 as cycle one and August to December 2021 as cycle two.

2.4. Experimental design and data collection

The experiment was a 2 (nodal cuttings) x 3 (substrates) x 2 (periods) factorial arranged in a completely randomized design with three replicates. The number of new leaves produced at 2 and 4 weeks old, plant height, number of nodes and internodes at 2, 4, 6, and 8 weeks old, days to flowering, number of tubers, and tuber weight were collected.

2.5. Data analysis

Data collected were analysed using Analysis of Variance (ANOVA) (SAS 9.3 version), and means were separated using Duncan Multiple Range Test (DMRT) at 5% level of significance.

3. Results

3.1. Effect of substrate type on the growth and yield of vine cutting established sweet potato

Results obtained showed a gradual increase in the number of new leaves produced by the vine cutting established sweet potato from two (2) Weeks After Planting (WAP) to 4WAP, where the vines established in the cocopeat substrate mixed with 5 g AMF (4.42±0.26) was significantly higher than the rest substrates (Table 1). The number of nodes produced also increased from 2WAP to 8WAP, where the number of nodes produced by the vines established in cocopeat substrate mixed with 5 g AMF (20.17±0.42) was significantly higher than the rest substrates. Table 2 shows a gradual increase in the height of the established vines in the substrates from 2WAP to 8WAP. At 8WAP, the height of the plants in the cocopeat substrate mixed with 5 g AMF (26.00±0.42) was significantly higher than the cocopeat substrate alone (23.17±0.42). However, the internodes at 2WAP were highest in soil (0.99±0.06) which was significantly higher than the vines grown in the mixture of cocopeat and 5 g AMF (0.78±0.06). The sweet potato plants grown in the mixture of cocopeat and 5 g AMF took the shortest number of days (71.25±0.69) to produce flowers, and this was significantly lower than the 74.00±0.69 it took for the plants grown in soil (Table 3). The substrate effect was insignificant in the number of tubers produced and the tuber weight, respectively.

3.2. Effect of planting period on the growth and yield of vine cutting established sweet potato

At 4WAP, the number of new leaves produced by the established vines within the period of March to July (3.83±0.22) was significantly higher than the ones established between August and December (3.06±0.22). Also, the number of nodes produced at 4WAP (5.33±0.22) and 6WAP (13.44±0.23) in the March to July planting period was significantly higher than in August to December planting period. The effect of the planting period on the plant height and internode length was insignificant. However, the number of days taken by the plants grown in the August to December planting period to produce flowers (70.56±0.56) was significantly shorter than the ones planted in the March to July planting season (75.00±0.56), and the number of tubers produced in the August to December planting period (5.06±0.41) was significantly higher than the March to July planting period (3.28±0.41).

3.3. Effect of planting explant's nodal level on the growth and yield of vine cutting established sweet potato

The number of new leaves produced by the established vines also increased from 2WAP to 4WAP where the new leaves produced by the double nodes explants had 4.11±0.22 which was significantly higher than the single node planting explants (2.78±0.22). Also, the number of nodes produced after establishment increased gradually from 2WAP to 8WAP. However, at 6WAP the number of nodes produced by the double node explant (14.11±0.22) was significantly higher than the single node explant (11.89±0.22). At 8WAP, the height of the plants produced by the double node planting

explant (25.78±0.34) was significantly taller than the single node planting explant (23.11±0.34). Also, at 2WAP the internode length of the plants raised through the double node explant (0.94±0.05) was significantly longer than the single node planting explant (0.79±0.05). However, the number of nodes in the planting explant was insignificant in the days to flowering, number of tubers, and tuber weight, respectively.

3.4 Interactions between substrate types, period of establishment and explants nodal level on the growth and yield parameters of vine cutting established sweet potato

At 8 WAP, the interaction between substrate and explant nodal level on the number of nodes produced and plant height was significant. On the number of days to flowering, the interaction between substrate and explant nodal level, and between substrate and period of planting were significant, while the interaction between substrate and period of planting was significant on the number of tubers produced by the sweet potato plant.

Table 1. Effects of substrates, period of establishment, and explants node on the production of leaves and nodes by vine cutting established sweet potato

SUBSTRATES	Number of new leaves		Number of nodes			
	WEEK2	WEEK4	WEEK2	WEEK4	WEEK6	WEEK8
Cocopeat	1.00b	2.67b	2.00b	4.17b	12.17b	17.67b
Cocopeat+AMF	1.58a	4.42a	2.58a	5.92a	13.92a	20.17a
Soil	1.08b	3.25b	2.17ab	4.75b	12.92a	18.67b
SE	0.17	0.26	0.15	0.26	0.28	0.42
PERIOD						
MAR - JUL	1.39a	3.83a	2.39a	5.33a	13.44a	19.22a
AUG - DEC	1.06a	3.06b	2.11a	4.56b	12.56b	18.44a
SE	0.14	0.22	0.12	0.22	0.23	0.35
NODES						
DN	1.44a	4.11a	2.50a	6.11a	14.11a	20.72a
SN	1.00b	2.78b	2.00b	3.78b	11.89b	16.94a
SE	0.14	0.22	0.12	0.22	0.22	0.35
INTERACTIONS						
SUB*Period	NS	NS	NS	NS	NS	NS
SUB*Nodes	NS	NS	NS	NS	NS	**
SUB*Period*Nodes	NS	NS	NS	NS	NS	NS

Means with the same letter down the column are not significantly different from each other at 5% significance level. SE: Standard error, SUB: Substrate, DN: Double nodes, SN: Single nodes, NS: Not significant, * and **: Significant at 5% and 1% levels of significance. AMF: Arbuscular Mycorrhizal Fungi.

Table 2: Effects of substrates, period of establishment and explants node on plant height and internode length of vine cutting established sweet potato

SUBSTRATES	PH2	PH4	PH6	PH8	INTER2	INTER4	INTER6	INTER8
Cocopeat	2.16b	4.67b	14.73b	23.17b	0.84ab	1.28a	1.27a	1.68a
Cocopeat+AMF	2.85a	5.92a	16.37a	26.00a	0.78b	1.21a	1.25a	1.77a
Soil	2.56ab	5.17ab	15.48ab	24.17b	0.99a	1.40a	1.31a	1.56a
SE	0.2	0.28	0.42	0.42	0.06	0.07	0.07	0.09
PERIOD								
MAR - JUL	2.72a	5.72a	15.87a	24.94a	0.86a	1.29a	1.32a	1.72a
AUG - DEC	2.32a	4.78a	15.18a	23.94a	0.88a	1.31a	1.23a	1.61a
SE	0.16	0.23	0.35	0.34	0.05	0.06	0.06	0.08
NODES								
DN	2.99a	5.89a	16.23a	25.78a	0.94a	1.37a	1.29a	1.72a
SN	2.05b	4.61b	14.82b	23.11b	0.79b	1.22a	1.26a	1.61a
SE	0.16	0.20	0.35	0.34	0.05	0.06	0.06	0.08
INTERACTIONS								
SUB*Period	NS	NS	NS	NS	NS	NS	NS	NS
SUB*Nodes	NS	NS	NS	*	NS	NS	NS	NS
SUB*Period*Nodes	NS	NS	NS	NS	NS	NS	NS	NS

Means with the same letter down the column are not significantly different from each other at 5% significance level. SE: Standard error, SUB: Substrate, DN: Double nodes, SN: Single nodes, NS: Not significant, * and **: Significant at 5% and 1% level of significance, PH2, PH4, PH6, and PH8 represents plant height at 2, 4, 6 and 8 weeks after planting, respectively. INTER2, INTER4, INTER6, and INTER8 represent Internodes at 2, 4, 6, and 8 weeks after planting, respectively. AMF: Arbuscular Mycorrhizal Fungi**.** PH and INTER were measured in cm.

Table 3: Effects of substrates, period of establishment and explants node on days to flowering and yield of vine cutting established sweet potato

SUBSTRATES	D2F	NOT	TW (g)
Cocopeat	73.08ab	4.25a	2.78a
Cocopeat+AMF	71.25b	4.83a	3.78a
Soil	74.00a	3.42a	3.50a
SE	0.69	0.5	0.25
PERIOD			
MAR - JUL	75.00a	3.28b	3.09a
AUG - DEC	70.56b	5.06a	3.34a
SE	0.56	0.41	0.2
NODES			
DN	72.5a	4.39a	3.27a
SN	73.06a	3.94a	3.17a
SE	0.56	0.41	0.2
INTERACTIONS			
SUB*Period	**	**	NS
SUB*Nodes	**	NS	NS
SUB*Period*Nodes	NS	NS	NS

Means with the same letter down the column are not significantly different from each other at 5% significance level. SE: Standard error, SUB: Substrate, DN: Double nodes, SN: Single nodes, NS: Not significant, * and **: Significant at 5% and 1% levels of significance. D2F: Days to flowering, NOT: Number of tubers and TW: Tuber weight. AMF: Arbuscular Mycorrhizal Fungi.

4. Discussion

4.1. Effect of substrate type on the growth and yield of vine cutting established sweet potato

The emergence of new leaves by the rooted vine seedlings of sweet potato showed that the vines were successfully established in the three substrate types (Maroya et al., 2014). However, the variation in the number of new leaves is as a result of differences in the establishment rate in the substrates, as the substrate in which a higher number of new leaves was observed was where the vines established faster. In this case, it was the addition of 5 g AMF to the cocopeat, which contained 250 g poultry manure, that was the better substrate. This could be that within the two weeks period the substrates were kept before planting, the AMF along with the environmental biota acted on the poultry manure to aid mineralization, thereby making nutrients more available for the early formed roots to assimilate adequate nutrients for the continued crop growth (Kumar et al., 2019). Due to the adequate nutrient available for the rooted vines in the AMF fortified cocopeat, the growth and development of the rooted vines in the substrate were faster than the ones grown in the soil or cocopeat substrate alone despite the insignificant differences in their internode lengths. Due to the fastened physiological development, the plants in the AMF fortified cocopeat substrate developed flowers within a shorter day interval as the plants rooted in the soil developed flowers late. This could also be attributed to the delay in the plants access to the nutrients locked in the poultry manure (Akpeji et al., 2021).

4.2. Effect of planting period on the growth and yield of vine cutting established sweet potato

Vines established in the first half of the year developed faster than the ones set up in the 2nd half. This could be because before the emergence of new shoots and root development, the harsh weather condition has started to disappear gradually, allowing the plants to thrive in a considerably cool atmosphere (Kipkori, 2016). Whereas, the ones established in the second half enjoyed cool weather at the establishment stage but the atmosphere gradually became hotter towards the end of the year. However, in terms of the reproductive and yield parameters, the plants established in the second half of the year produced flowers faster, and the number of tubers produced was superior to the ones established in the 1st half of the year. This could be because the considerable cool environmental condition they enjoyed earlier pave the way for their early physiological development, and before the emergence of harsh weather conditions, the root of the plants had already bulked (Egbe, 2012).

4.3 Effect of planting explant's nodal level on the growth and yield of vine cutting established sweet potato

The rate of establishment and agronomic performance of the double node planting explants was faster than the single node planting explants. This is as a result of the additional node advantage in the double node allowing for double point of contact with the substrate allowing for more points of root formation and shoot emergence. This finding agrees with that of Dumbuya et al. (2017), who stated that the longer the cut explant, the faster the rate of establishment and yield. However, in this study, the higher number of nodal point in the planting explant only favored the fast establishment and agronomic performance but the yield relative to the single node cuttings were the same, which is contrary to the findings of Essilfie et al. (2016) who stated that the explants with a higher number of nodes produced longer tubers compared to the ones with smaller nodal points.

4.4 Interactions between substrate types, period of establishment and explants nodal level on the growth and yield parameters of vine cutting established sweet potato

The significant effect of the interaction between substrate and explant nodal level on the agronomic and yield parameters show that the type of substrate and availability of nutrient is an essential factor to be considered in establishing sweet potato vine cutting in order to improve the propagation ratio.

Conclusion

Sweetpotato is an important tuber crop and staple worldwide. However, the low propagation ratio relative to cereals like maize has constrained the full realization of the economic importance. Rooted vine cutting technology has been deployed in improving the propagation ratio of different clonally propagated crops, as successfully demonstrated in this study. However, although the number of nodal points in the planting explants played a significant role in the agronomic parameters, the yield compared with the explants with single nodal points was statistically the same, with the latter having a higher propagation ratio considered more beneficial. But the substrate type is an important factor to consider in the establishment as the use of a hydroponics system with cocopeat substrate fertigated with poultry manure adequate for the production in the presence of AMF that act on the manure with the help of other biotas to aid the release of the minerals locked in the manure.

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Research Article

Understanding Farmer Perception and Impact of Seasonal Climate Event on Rice Farming in Indonesia: Implication for Adaptation Policy in Local Level

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Abstract: The study aimed to understand how farmers perceive seasonal climate events (SCE) and assess the impact on farming and how farmers' attitudes and efforts to adapt in dealing with SCE. This study was conducted in Kuantan Singingi Regency, Riau Province, Indonesia, in August - December 2020. Total of 297 farmers was selected purposively to be interviewed. Descriptive statistics analysis was used to analyze socio-demographics, farm characteristics, the impact of SCE on rice production, and farmers' responses in adapting to SCE as an effect of climate change. Friedman's test was used to analyzed the importance of climate over other non-climate-related stressors. Ordinal regression analysis was performed for the determination of possible association of farmers' socio-demographics and farm characteristics to the perceived extent of SCE impact. Flood was the climatic factor that most often caused rice production failure. Farming experience, education, gender, farm size, cultivation period, rice varieties, land management, fertilization, rice field type, and farming purpose have a significant effect on farmers' perceptions of SCE impact. Adjusting the planting season, the use of chemical fertilizers, and controlling pests and diseases were the most dominant responses by farmers in dealing with SCE. Implications: The availability of weather information must be done massively, induction of flood-tolerant varieties needs to be carried out, female farmers with higher education and long experience in farming can be used as cadres as extension officers to farmer groups, planting twice a year was an option to increase rice production, the number of farmers get assistance from extension officers could be increased.

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1. Introduction

The earth's surface temperature has increased by 0.85°C over the last 100 years and is predicted to continue to increase by 1.5°C until the 21st century (IPCC, 2014). This condition causes climate change which has a negative impact on the agricultural sector, such as a decrease in cereal crops production and becoming more susceptible to disease and weeds (Asseng et al., 2011). This condition will certainly burden the agricultural sector in meeting global food needs, which are expected to double by 2050 compared to current needs (FAO, 2015). The agricultural sector, such as rice farming, is one of

the sources of food and economy for people in developing countries in tropical climates. This condition causes many of these farmers to live in areas that are at high risk from the impact of climate change events, such as a change in seasonal climate events, which causes a decrease in rice yield. The condition of farmers in developing countries is also limited in facilities and knowledge, so that they have limited ability to respond to the impacts of climate change (Fierros-González and López-Feldman, 2021). Responding to these conditions, it is necessary to adapt efforts for rice farmers in the face of changes in seasonal climate events.

These adaptation efforts can be further realized in the line of policies. In making these policies, it is necessary to pay attention to farmers' perceptions of climate change as an effort to ensure that adaptation efforts can be implemented at the farmer level (Karki et al., 2020; Jha and Gupta, 2021). Many studies on rice farmers' perceptions of climate change have been carried out in developing countries in tropical climates (Mishra et al., 2018; Akhtar et al., 2019; Islam et al., 2020; Khan et al., 2021; Ojo et al., 2021). However, from the previous study that has been done, no one has examined the perception of rice farmers about climate change, such as seasonal climate events, to recommend as a basis for making policies on adaptation efforts for rice farmers at the local level. Mahfoud and Adjizian-Gerard (2021) state that there are several reasons why it is important to develop adaptation efforts in the agricultural sector based on local contexts, 1) climate change impacts are mostly manifested at the local level of a region, which affects activities and the economy, 2) vulnerability and capabilities adaptation systems in a given area are determined by local conditions which may be completely different from the national and regional levels, 3) adaptation activities are often better observed, measured and more effective at the local level. In addition, the perception of local farmers when combined with modern knowledge will strengthen farmers in facing climate change (Apraku et al., 2021). IPCC (2001), Sardar et al. (2019) and Singh, (2020) suggest that climate change management in the agricultural sector can run effectively if it is integrated at the local to national level.

Indonesia, like other developing countries in Southeast Asia, uses rice as a food crop and also as a source of income, where the agricultural sector accounts for 13% of the national gross domestic product (GDP). Of the 25 million farming households, 17 million are rice farmers with average land ownership of 0.6 ha (BPS, 2019). Indonesia, which is located in a tropical climate, is one area that is vulnerable to the negative impacts of climate change especially change in dry and wet season events. Yuliawan and Handoko (2016) report that in tropical countries such as Indonesia, every 1°C increase in temperature will reduce rice production by 11% on irrigated rice fields and 14% decrease in production on rainfed rice farms. Meanwhile, the Ministry of Agriculture (2018) reports that in Indonesia, there are approximately 8 million hectares of rice farming land, and only about 58.13% of the total land has irrigation infrastructure.

Responding to the threat of climate change, the Indonesian government created a National Action Plan (NAP) to mitigate risks and adapt to climate change as an effort to maintain rice production to fulfill national food needs. Policy recommendations for adaptation for rice farmers in Indonesia have been reported by Rondhi et al. (2019), but the policy recommendations given are for the national level, which still needs to be studied further to determine whether they can be adopted at the local level. In the NAP it was also stated that local governments need to plan and solve problems related to climate change problems at the local level (Ministry of Development and National Planning, 2019). Understanding farmers' perceptions at the local level can support the sustainability of a policy in the agricultural sector (Granco et al., 2022). In addition, a risk assessment of climate change at the local level will also facilitate the preparation of adaptation plans at the local level (Kirby, 2021; Sainz de Murieta et al., 2021). Based on the explanation above, this study aims to understand 1) how farmers perceive on seasonal climate events and assess the impact on farming, and 2) how farmers' attitudes and efforts to adapt in dealing with seasonal climate events. Then the perception and knowledge of farmers about seasonal climate events can be used as recommendations in making adaptation policies at the local level.

2. Material and Methods

2.1. Conceptual framework

The conceptual framework (Figure 1) was compiled from various literature sources to examine rice production systems in local contexts, namely small farmers at the individual or group level who are

affected by climate change in the microenvironment of rice farmers (Mahfoud and Adjizian-Gerard, 2021). Climatic conditions that disrupt the agricultural environment will cause disruption of plant reproduction, physiological functions, and nutrient availability in the soil, which will then have an impact on production costs and production quantities (Li et al., 2015; Wang et al., 2018; Muehe et al., 2019; Mukamuhirwa et al., 2019). The framework refers to the idea that climate change impacts will be better determined from farmer perceptions of the events experienced related to the impact of the climate change on the rice farming system. In conveying the perceptions and events encountered, it is closely related to the conditions of socio-demographic and farm characteristics, cognitive factors, and experience processes (Xie et al., 2019). These farmer perceptions can be used as support for making policies related to climate change, behavioral intentions, and attitudes to address climate change (Sullivan and White, 2019).

Definition of perceptions referred to Wolf and Moser (2011) that perceptions were the views and interpretations of individuals on climate change issues based on beliefs, experiences, and understandings. In particular, we explore the experience of farmers to identify the impacts of climate change on rice production systems. Karki et al. (2020) suggest that the experience of farmers is important to be included in efforts to adapt to climate change; usually, farmers have different explanations and respond appropriately in dealing with climate change. Many researchers have shown that people attribute climate change to their personal experiences of an increased occurrence of extreme weather events and perceive this risk to be related to climate change (Carlton et al., 2016). Therefore, this study focuses on understanding possible adaptation responses to climate change through farmers' experiences of what they perceive about climate change and its associated impacts.

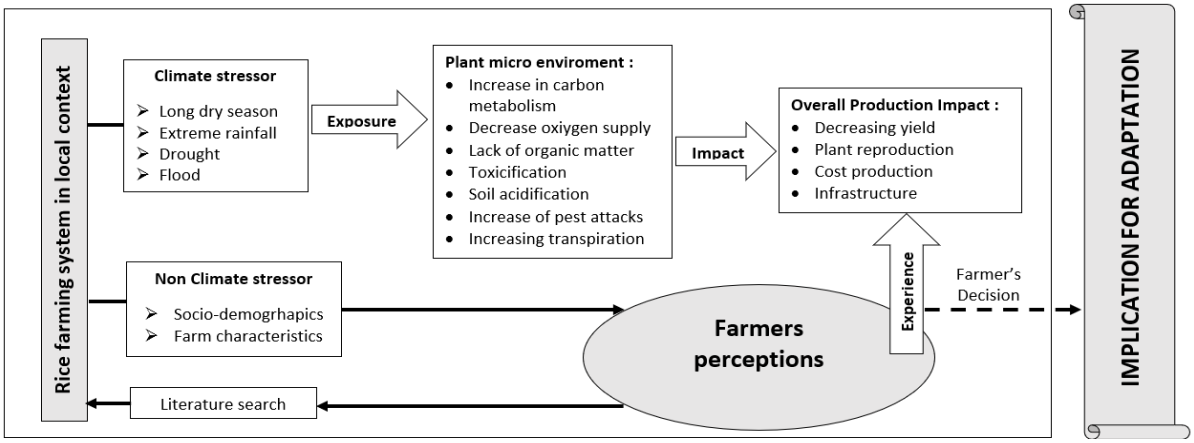

Figure 1. Conceptual framework linking farmers' perceptions on climate change risk, perceived impacts and adaptation behavior.

2.2. Study area and data collection procedure

This study was conducted during August - December 2020 at 22 villages spread over ten sub-districts along the Batang Kuantan river, which was located in Kuantan Singingi Regency, Riau Province, Region of Sumatra island, Indonesia. The ten sub-districts were Kuantan Mudik, Gunung Toar, Kuantan Tengah, Sentajo Raya, Benai, Pangean, Kuantan Hilir, Kuantan Hilir Seberang, Inuman, and Cerenti (Figure. 2). The reason for choosing this location as the study area was because, first, this area was an agricultural area that was dominated by rice farming because the soil conditions are quite fertile. The second reason, although this area has fertile soil, since 2011-2018 it has always experienced flooding during the peak of the wet season due to the overflowing of the Batang Kuantan river (BPS Riau, 2019), however during the dry season, the rice farms also experienced a drought. The third reason was that study on rice farmers' perceptions of climate change in Indonesia was mostly carried out on the Java island, which was dominant in rice farming (Rondhi et al., 2019), but in marginal areas such as on the Sumatra island, it was still rarely done, even though rice farming in the tropics was equally important in supplying food needs.

Collecting the data was used a structured questionnaire which was administered through face-to-face interviews with Farmers. Total 1245 farmers were registered in the village farmer group. From

the list of farmer groups, a total of 297 farmers were selected purposively to be interviewed. Interviews were conducted on the farms when the farmers rested after working. The interviews were carried out by surveyors who had been trained beforehand. The survey was conducted in Indonesian and then translated into English.

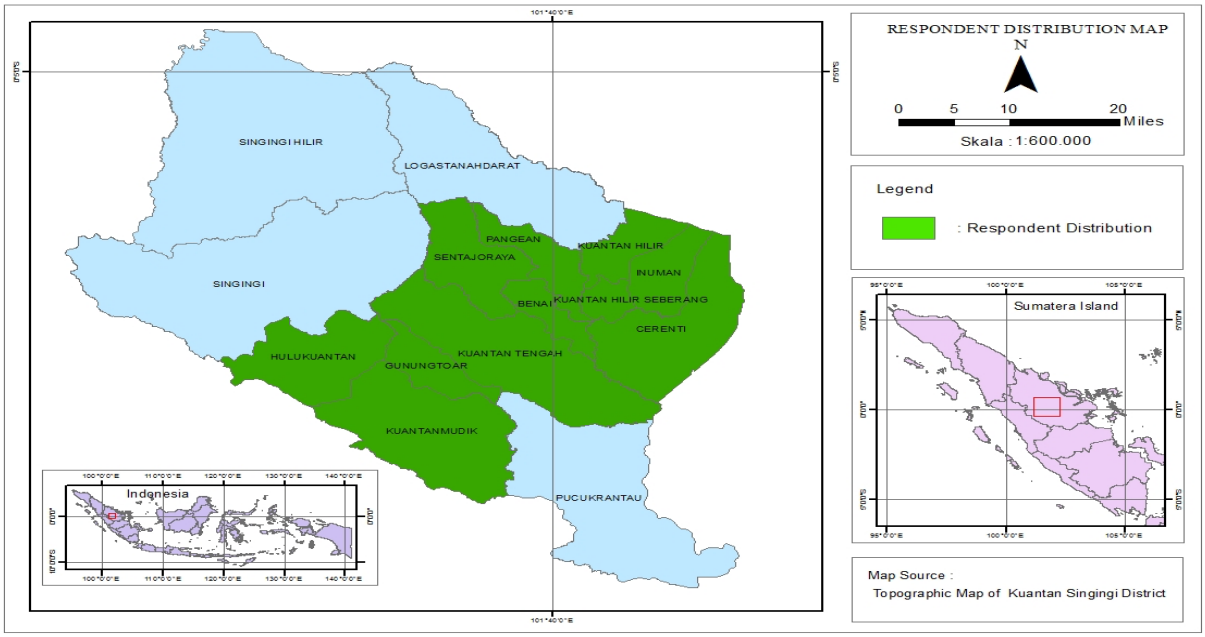

Figure 2. Map of the study area showing location and respondents distributions.

2.3. Questionnaire

The proposed questionnaire consists of 3 parts with closed questions: 1) socio-demographic characteristics and rice farming system, 2) perceptions about climate change in general, 3). Perceptions of the impact of floods and droughts due to climate change on rice production. Open questions for farmers' responses in mitigating and adapting to climate change. Perception data was collected by asking respondents whether they know about climate change and how the climate conditions are; they were asked about the perceived changes in seasonal climate events patterns in the last 5 years (2016-2020) with categorical answers ("no change", "changed"). The effects of seasonal climate events were compared with other stressors rated on a 5-point scale from 1 (no impact at all) to 5 (extremely strong impact).

2.4. Data analysis

Descriptive statistics analysis was used to analyze socio-demographics, farm characteristics, the impact of climate change on rice growth production, and farmers' responses in mitigating and adapting to climate change. To establish the importance of climate change over other non-climate related stressors to farmers' livelihoods, ratings were analyzed using Friedman's test (K-related-samples test) followed by Wilcoxon signed ranks test for pair-wise post hoc comparisons when appropriate. Ordinal regression analysis was performed to determine a possible association of farmers' socio-demographic conditions and farm characteristics to the perceived extent of climate change impact. The outcome variable captured responses on the perceived severity of seasonal climate events on overall production, an ordered variable coded as 0 (no impact), 1 (moderate impact), and 2 (strong impact). The dummy for socio-demographic variables for were: age (1 = 18-40 year ; 0 = other), farming experience (1 = <20 year; 2 = other), number of house-hold member (1 = <4; 2 = other), gender (1 = male; 0 = female); education (1 = junior high school; 0 = other), and land tenure (1 = private; 0 = other). The dummy for socio-demographic variables for were: Farm size (1 = less than 0.5 ha; 0 = other), Rice production (1 = less than 0.5 ton; 0 = others), cultivation period (1 = one a year; 0 = other), rice varieties (1 = hybrid; 0 = other), land management method (1 = traditional; 0 = other), fertilization (1 = chemical; 0 = other),

farm type (1 = irrigation; 0 = other), farming purpose (1 = for sale; 0 = other), getting assistance from extension officer (1 = getting assistance; 0 = other)

3. Results

3.1. Socio-demographic and farm characteristics

A total of 297 data from farmers as respondents in this study were used to analyze socio-demographic conditions and farms' characteristics. The socio-demographic and the farms' characteristics in the study area are shown in Table 1.

Table 1. Summary of socio-demographic conditions (n = 297)

Variable	Mean/SD
Socio-demographic conditions	
Age (year)	49.7 ± 11.1
Max	70
Min	18
Farming experience (year)	18.4 ± 10.4
Gender	
Male	91 (30.6%)
Female	206 (69.4%)
Education	
Elementary school	129 (43.4%)
Junior High school	87 (29.3%)
Senior High school	81 (27.3%)
Land tenure	
Private	254 (85.5%)
Otherwise (rent, shared)	43 (14.5%)
Farm Charactersitics	
Farm size (ha)	0.5 ± 0.3
Rice production (ton/ha)	1.1 ± 0.2
Cultivation period	
One a year	211 (71.0%)
Twice a year	86 (29.0%)
Rice varieties	
Hybrid	66 (22.6%)
Indigenous	231 (77.4%)
Land management method	
Traditional method	101 (34.0%)
Machine mechanization	196 (66.0%)
Fertilization	
Chemical	122 (41.1%)
Organic	175 (59.9%)
Farm type	
Irrigated	42 (14.1%)
Rain-fed	255 (85.9%)
Farming purpose	
For sale	28 (9.4%)
Self-consumption	269 (90.6%)
Assistance from the extension officer	
Get assistance	116 (39.1%)
Not getting assistance	181 (60.9%)

Based on the data collected, it was known that the average age of farmers was 49.7 years, and the average experience of farming was 18.4 years. Respondents of female farmers were higher than male farmers. Farmers' education was dominated by farmers with elementary education followed by junior education and senior education. The land used for rice farming with private ownership was higher than land leased or profit-sharing.

With an average land area owned by farmers of 0.5 hectares, the farmers' were able to produce rice production of 1.1 tons per year. The cultivating period carried out by farmers was dominated by planting 1 time compared to 2 times in a year. Farmers were more interested in using indigenous varieties than in using hybrid varieties. The land management method was dominated by machine mechanization

compared to using traditional methods by using tools such as plows with cattle or hoes. Organic fertilizers from livestock manure were used more by farmers than chemical fertilizers. The types of rice fields owned by farmers were almost dominated by rainfed, while the irrigated rice fields were only slightly. The rice yields obtained were generally used for own consumption compared to the purpose of selling. In general, more farmers did not receive assistance from extension officers than those who received assistance.

3.2. Farmers' perceived of seasonal climate events and impact on rice production

Several factors, which were climate, pests, diseases, and environmental damage as stressors in rice production, were asked to the farmers. From these factors, farmers consider that climate is the main factor as stressor that can cause failure in rice production, followed by factors of environmental damage, pests, and diseases. This was indicated by a significant difference in the median ranking ($p < 0.001$) among factor caused stressors. Furthermore, when we asked what climatic factors most influence rice production, 83% of the 297 respondents reported that flooding was the climatic factor that most often caused rice production failure (Figure 3.). More farmers stated that the wet season had not changed in the last 5 years, as well as in the dry season (Figure 4).

Table 2. Friedman's tests result showing rankings of the level of stressors on rice production

Stressors	Mean	SD	Mean rank[a]	Friedman test
Climate	3.4	0.45	3.3	$p < 0.001$
Pest	2.5	0.43	2.3	
Diseases	2.0	0.16	1.9	
Enviroment damages	2.6	0.45	2.4	

[a] The higher the mean rank of, the greater the level of concern as an a stressor.

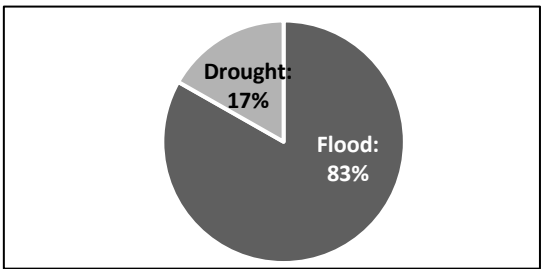

Figure 3. Climatic factors that cause rice production failure.

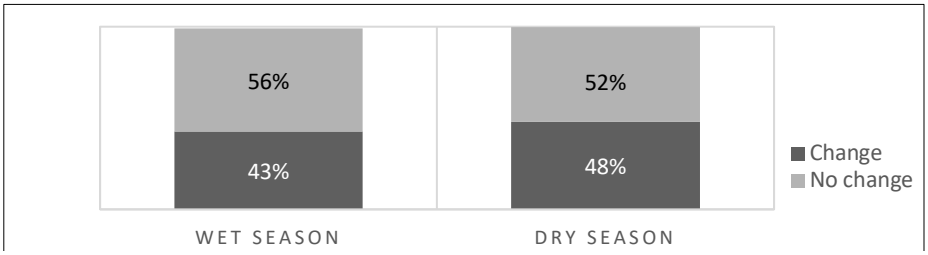

Figure 4. Farmers' perceptions of climatic conditions in the last five years.

Farmers were asked to share their perceptions related to flooding (Table 4) and drought (Table 5) impact on rice crops. Based on the farmer's perception, the most frequent flood events were for 3 days and caused the rice to wither and the possibility of rice to was low survival rate. Farmers reported that if the flood lasted for more than 3 days, the rice would rot and then perish. More farmers experienced less than 4 weeks of drought and a less severe impact on rice. Some farmers reported that they had experienced a drought of more than 4 weeks which had a negative impact on paddies, such as stunting, not bear, and yellowing leaves. Almost of farmers reported that the vegetative phase was a phase that is prone to failure due to flooding and drought compared to the seed phase and the generative phase (Figure 5).

Table 3. Farmer perceived of flood in rice production

Flood time (days)	Number of respondents	%	Impact
1	13	4.4	No impact
2	39	13.1	Low stress, high survival rate
3	124	41.8	Withers, low survival rate
4	49	16.5	Perish
5	27	9.1	Perish
6	6	2.0	Perish
7	17	5.7	Perish
>7	22	7.4	Perish

Table 4. Farmer perceived of drought in rice production

Drought time (weeks)	Number of respondents	%	Impact
1-2	135	45.5	No impact
3-4	125	42.1	Low stress, high survival rate
>4	37	12.4	Stunting, fail to grain

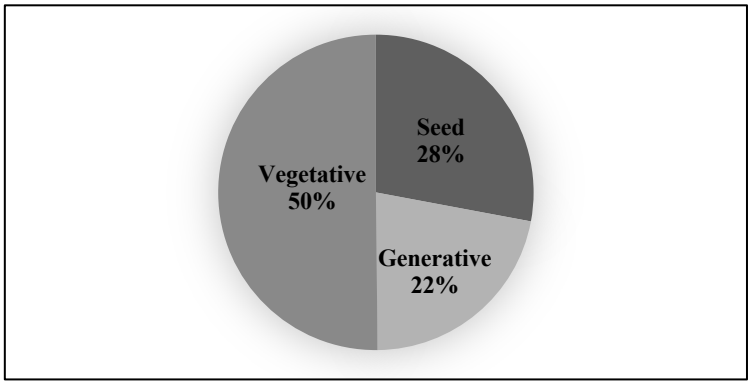

Figure 5. the most vulnerable rice growth stages due to flooding and drought based on farmers perceived.

3.3. Determinants factor of farmers' perceived seasonal climate events impacts

Of a total of 297 farmers, around 47.1% stated that the seasonal climate event had no impact on rice production, 32.3% of farmers stated it had a low impact, and 20.6% stated a strong impact. Logistic regression analysis of socio-demographic conditions on perceptions of seasonal climate events shows that farming experience (negative), education (negative), and gender (positive) have a significant effect on farmers' perceptions of climate change impact. Meanwhile, other factors such as age and land tenure did not affect farmers' perceptions of climate change impact (Table. 5). Farm characteristics in relation to perceptions of climate change show that farm size (negative), cultivation period (negative), rice varieties (positive), land management (positive), fertilization (positive), rice field type (positive) and farming purpose (negative) have a significant effect in determining farmers' perceptions in seasonal climate event. Meanwhile, rice production and assistance from extension officers did not affect farmers' perceptions of climate change (Table. 6).

Table 5. The relationship between socio-demographic and farmers' overall perceptions of seasonal climate event

Outcome variable	Estimate	SE	Wald	P Value
[Perceived of climate change = moderate impact]	-0.89	0.41	4.82	0.03**
[Perceived of climate change = strong impact]	-0.63	0.41	2.40	0.12
Location				
Gender (male)	0.42	0.24	3.10	0.07*
Age	0.08	0.40	0.04	0.83
Farming experience	-0.72	0.26	7.41	0.00***
Education				
Elementary school	-0.74	0.36	4.26	0.03**
Junior high school	-0.61	0.35	2.99	0.05**
Land tenure	-0.19	0.34	0.33	0.56
Overall model evaluation	$x^2 =$ 12.53	$p\ value$ 0.08*	R^2 Nagelkerke 4.7%	

***, **, and * denote significant at 1%, 5%, and 10% levels, respectively.

Table 6. The relationship between farm characteristics and farmers' overall perceptions of seasonal climate events

Outcome variable	Estimate	SE	Wald	P Value
[Perceived of climate change = moderate impact]	-2.99	0.89	11.20	0.00**
[Perceived of climate change = strong impact]	-1.22	0.88	1.94	0.16
Location				
Rice production				
Less than 0.5 ton	-0.10	0.75	0.01	0.89
More than 0.5 ton	-0.29	0.49	0.36	0.54
Farm size				
Less than 0.5 ha	-2.85	0.74	14.86	0.00***
More than 0.5 ha	-2.67	0.71	14.22	0.00***
Cultivation period	-0.72	0.26	7.73	0.00***
Rice varieties	0.92	2.87	10.43	0.00***
Land management method	0.75	0.26	7.79	0.00***
Fertilization	0.64	0.25	6.20	0.01**
Farm type	0.64	3.72	3.03	0.08*
Farming purpose	-1.43	0.49	8.54	0.00***
Assistance from extension officer	-0.23	0.27	0.75	0.38
Overall model evaluation	$x^2 =$ 68.51	$p\ value$ 0.00***	R^2 Nagelkerke 23.5%	

***, **, and * denote significant at 1%, 5%, and 10% levels, respectively.

3.4. Rice farmers' adaptive responses

Farmers were given open-ended questions about adaptative responses to minimize production failures due to climate change (Figure 5). Paying attention to the planting season, such as not planting in the rainy season, was the most common effort made by farmers to avoid flood events. Some other farmers increase the use of chemical fertilizers to fertilize crops and the use of pesticides to control pests and diseases. Some farmers repair irrigation canals to regulate the irrigation process. However, there were also many farmers who give up after a failure compared to farmers who replant if their rice fails to grow due to flooding or drought and irrigate with machines if there is a water source around the fields. A few farmers used local varieties that were more resistant to flood and drought conditions.

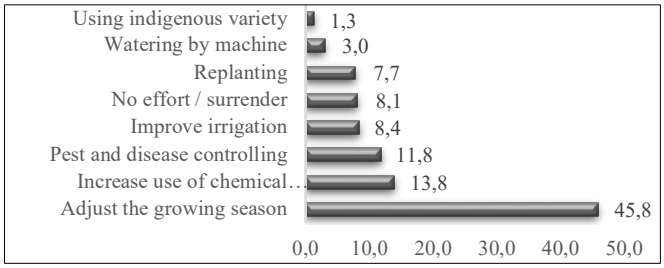

Figure 6. Rice farmers' adaptive responses.

4. Discussion

4.1. Socio-demographic and farm characteristics

In general, socio-demographic conditions and agricultural characteristics in the study were similar to some conditions in Indonesia (national level), as reported by Rondhi et al. (2019). The similar conditions were the average age of farmers being 49 years, the dominant type of rice fields being rainfed, the planting period being 1-2 times a year, land ownership being generally privately owned, and fewer farmers were receiving assistance from extension officers. However, there were a few points that differed at the national level in this field of study. Although the number of female farmers was low compared to the national level, the number of female farmers was higher in the current study area. In this study area, men usually acted as oil palm or rubber farmers, while women were rice farmers. Purpose of rice farming was used for family consumption, and oil palm and rubber farming as a source of economic income. The study area also has a higher proportion of farmers using organic fertilizers than farmers in Indonesia (at the national level), who predominantly use chemical fertilizers.

The average land area in the study of 0.5 ha is also almost similar to the data reported by BPS (2019). The average land area of rice farmers in Indonesia is 0.6 ha. Rice farmers in this study area were more interested in using local rice varieties than hybrid varieties. The reason being that farmers perceive local varieties were more resistant to flooding and drought than hybrid varieties ones. Rice production in the study area was low and only 1.1 tons/ha because, according to BPS (2021), in Indonesia, one hectare can produce rice of around 5.2 tons. In this study area, the farmers used the dominant local varieties and organic fertilization, and the irrigation system was not optimal because it used rainfed rice fields, which were thought to be some of the causes of the low rice production. The more dominant farmers' use of organic fertilizers was actually something positive for the sustainability of rice farming and climate change mitigation. Arunrat et al. (2021) reported that rice farming in Thailand using organic fertilizers contributed less negative impacts on climate change than rice farming using chemical fertilizers. The use of organic fertilizers does not always have a negative impact on rice production, Salam et al. (2021) reported in Bangladesh, rice farming which was dominantly using organic fertilizers intensively, results in higher rice production, with notes that the government provides sufficient access to organic fertilizers.

4.2. Farmers' perceived of seasonal climate events and impact on rice production

In general, farmers already know that climate has a big impact on rice farming. Farmers think that pests and diseases can be controlled with pesticides and the use of chemical fertilizers, but climate problems such as floods and droughts are difficult to overcome. However, although the climate was the main concern for farmers regarding the causes of failure of rice production, farmers consider that in the last five years, the rainy and dry seasons have not changed. To confirm the farmer's statement, we have collected rainfall data for the last five years from the Meteorology and Geophysics Agency station at the study site, and the results were known in the last five years the rainfall pattern was different every year (Figure 7).

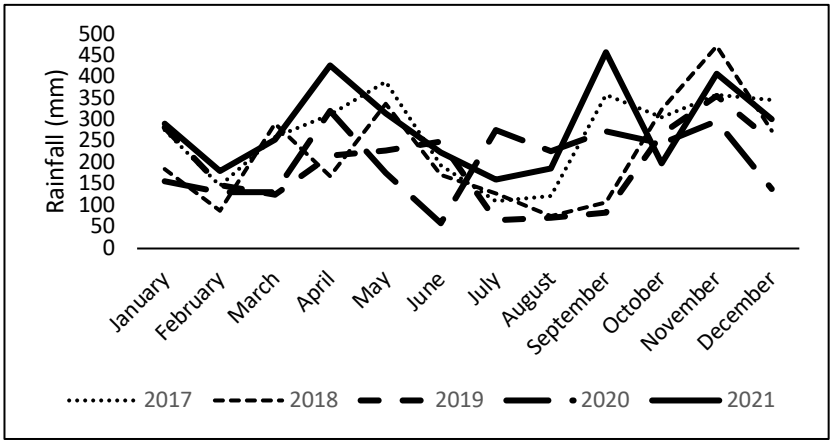

Figure 7. The rainfall trend in 2017-2021.

The climatic factor that was considered the biggest cause of rice production failure was flooding. This is also consistent with what was reported by Rondhi et al. (2019) that rice farmers in Indonesia consider climate factors such as floods to have the greatest impact on rice production failure compared to drought and other factors. According to farmers, if the flood recedes in two days, it is highly likely that the rice will continue to grow and bear, but if the flood does not subside within three days and the entire plant is submerged, then it is highly likely that the rice will fail to grow and perish. This condition is different from that reported by Manzanilla et al. (2011) that the average rice crop in Southeast Asia, such as in the Philippines, Laos, Indonesia, and Southern Vietnam, if flooding with a height of > 100 cm and more than 2 weeks can cause failure production by 40-77%. Floods cause many complex abiotic stresses on rice plants. Losses that may be caused to rice plants can vary depending on flood characteristics such as temperature, turbidity, water depth, oxygen and carbon dioxide concentration, and light intensity (Panda and Barik, 2021). The period of vegetative growth was a growth period that was prone to failure due to flooding and drought. This is also in accordance with what was conveyed by Hendrawan and Komori, (2021) that the vegetative to reproductive period is a growth phase that is prone to failure due to flooding.

4.3. Determinants factor of farmers' perceived seasonal climate events and farmers' adaptive responses

Farmers' perceptions of the impact of climate change, such as seasonal climate events on rice farming, were varying, and this was something natural because individual responses can vary (Grunblatt and Alessa, 2017). Based on socio-demographic conditions, factors that influence perceptions of the impact of climate change were gender, farming experience, and education. Female farmers believe that seasonal climate events can have a negative impact on rice farming. The longer the experience of farming and the higher the education of the farmer, the more likely he was to believe in the negative impacts of seasonal climate events on rice farming. The influence of gender on perceptions of climate change can be different in each region. Therefore, it was necessary to look at specific contexts and locations (Jamal et al., 2021). In several previous studies, such as in Africa reported by Ojo et al. (2021) and Poortinga et al. (2019) in Europe and specifically Rondhi et al. (2019) in Indonesia, it was stated that female farmers believed in climate change and were more adaptive in dealing with the negative impacts of climate change. Farming experience and education have a positive relationship to the perception of climate change. Education was able to increase the ability to receive information about the climate, which has an impact on cognitive abilities. The more experienced and educated farmers are, the better their ability to adapt to climate change (Ojo and Baiyegunhi, 2021; Appiah and Guodaar, 2021).

Based on farm characteristics, the larger the land owned by the farmers, the more belief they will be on the impacts caused by seasonal climate events. This was in contrast to the study reported by Amare and Simane. (2017) in Ethiopia and Koirala et al. (2022) in Nepal, reported that farmers with larger land perceived that climate change had less impact on agriculture than those with smaller land. However, another study conducted by Thoai et al. (2018) in Vietnam and Ahmed et al. (2021) in

Bangladesh reported that farmers who have large lands believe in the negative impacts caused by climate change. These farmers were also more adaptive in dealing with climate change than farmers who have small lands. Based on the results of previous studies and the results of this study, the influence of land area needs to be studied specifically according to the location and context. In the study area, the rainy season usually lasts longer than the dry season, and at least floods occur every year. Therefore, farmers who plant rice more than once a year have experienced flooding, and flooding due to seasonal climate events has a negative impact on rice production.

Farmers who cultivate the land using traditional mechanization believe in seasonal climate events. This was similar to that reported by Jha and Gupta, (2021), who reported that farmers who cultivate land in the traditional way believe that climate change is occurring and its impact on land cultivation becomes more difficult. Farmers who use chemical fertilizers believe in climate change, and this was also in accordance with a previous study result reported by Martey and Kuwornu, (2021) in Ghana that farmers who use chemical fertilizers believe that climate changes have an impact on soil fertility, the intents of use of chemical fertilizers was an effort to fertilize the soil. Farmers with irrigated farming types have a stronger belief in the negative impact of seasonal climate events than farmers with rain-fed farming types. The results in this study differ from those reported by Hein et al. (2019) in Myanmar reported that rice farmers with irrigated types of agriculture have a lower perception of the impact of climate change than farmers with rain-fed types. This could be because at the location of this study, farmers with rain-fed farming systems have not experienced extreme drought conditions so agricultural crops are not too dependent on irrigation. Farmers with rain-fed agriculture believe in climate change if they experience extreme drought conditions and low rainfall (Aliyar et al., 2022).

Farmers who grow rice for family consumption believe that seasonal climate events has an impact on rice production. The results of this study were also in accordance with the study of Mekonnen et al. (2021), who reported that farmers believe drought and extreme rains as a result of climate change affect the harvest of agricultural crops, which have an impact on food security at the family level. Assistance from extension officers does not affect farmers' perceptions of the impact of climate change. This is because there were still few farmers who received assistance from extension officers. Farmers who do not receive assistance from extension officers have limited knowledge about climate change (Jost et al., 2016). In addition, this can also indicate the weak role of extension officers in providing information about climate change to farmers. It was necessary to strengthen extension officers to provide information on the impacts of climate change on agriculture as well as adaptation efforts that can be adopted at the local level (Afsar and Idrees, 2019). Previous studies conducted by Zakaria et al. (2020) in Ghana and Anik et al., (2021) in Bangladesh show that farmers who have access to extension workers have good adaptability in dealing with climate change.

Adjusting the planting season followed by the use of chemical fertilizers and controlling pests and diseases was the most adaptive response by farmers to prevent yield failure due to seasonal climate events. The results of this study were the same as reported by Zama et al. (2021) in Cameroon. Farmers prefer the strategy of adjusting to the planting season because it is considered the most effective for reducing yield failure due to climate (Wang et al., 2022). The adaptive response carried out by farmers needs to get further support, such as assistance from extension officers. In addition, adaptation strategies by planting rice varieties that were more tolerant to flood and drought conditions can also be considered. In this study, the choice of variety was the least response chosen by farmers. Anik et al. (2021) reported that in Bangladesh by strengthening information services on climate, measurable use of fertilizers, use of flood or drought-tolerant varieties, and regular assistance from agricultural extension officers can help rice farmers in dealing with climate change.

Conclusion

Farmers believe that climate was the biggest stressor on rice production compared to environmental damage, pests, and diseases. Floods were climatic events that most often caused the failure of rice production. However, farmers were more dominant in stating that neither the dry season nor the rainy season has changed. The vegetative growth phase was a phase that was prone to failure due to flooding and drought.

Farmers who believe seasonal climate events had a moderate to strong impact on rice production are more likely than those who think seasonal climate events had no impact. The perception of farmers

in assessing seasonal climate events from socio-demographic conditions was determined by gender, farming experience, and education. Meanwhile, farm characteristics were determined by land area, cultivation period, rice variety, land management method, fertilization, type of rice field, and the purpose of farming. Adjusting to the planting season, the use of chemical fertilizers, and controlling pests and diseases were the most dominant responses by farmers in dealing with seasonal climate events.

The implications that can be given to the government at the local level were. 1) The availability of information about the weather must be done massively with the current technology so that farmers easily get information about the weather (rainy season and dry season); 2) Induction of rice varieties that are more flood-tolerant needs to be carried out to farmers so that they can adapt when a flood event occurs; 3) Female farmers with higher education and long experience in farming can be used as cadres as extension officer to farmer groups through intensive training on climate change adaptation and mitigation efforts; 4) Planting twice a year was one option to increase rice production, but farmers need to be accompanied by extension officers who are capable of paying attention to the planting season, the use of fertilizers and the selection of rice varieties.

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Research Article

Determination of the Physicochemical Properties and Fatty Acid Composition of Some Cheese Types with Geographical Indication in Thrace Region

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Malkara aged cheese

Abstract: The purpose of the present investigation was to assess the differences in the quality characteristics of the famous cheese types produced in Thrace region and labeled with a geographical indication or not. The different physicochemical properties, mineral content, and fatty acid composition of Ezine Cheese and Edirne Feta Cheese, and Malkara Aged Kaşar Cheese with geographical indication were determined. The obtained data were compared with some of the physicochemical specifications stated in the geographical indication registration documents and also with the cheese types of the same category but without the geographical indication. For this purpose, 90 cheese samples from 23 different local producers in Edirne, Tekirdağ, Çanakkale, and Kırklareli were supplied in sealed packages. Dry matter (%), ash (%), salt (%), protein (%), titratable acidity (% lactic acid), acid count (mg KOH/g fat), color (L, a, b) values were determined. Additionally, fatty acid compositions and some mineral contents of the cheese were analyzed by using gas-chromatography and inductively coupled plasma-optic emission spectrometry (ICP-OES). This study aims to contribute to registration documents of geographical indication, which cover the characteristic specifications, including fatty acid composition, protein amount, acid count (free fat acidity), and color (L*, a*, b*) values. It is advised to revise the registration documents of the mentioned cheese types by including aroma-active components to the aroma characterization and texture specifications to the characteristic features through a broader study.

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1. Introduction

Due to its geographical location, Turkey is a country where not only agricultural and animal product range is wide but also a rich cuisine culture is developed as a result of multi-cultural communities coming together. Cultural richness has a significant effect on traditional food products throughout the country (Kantaroglu and Demirbas, 2018). In order to protect this rich local heritage, registration procedures and application of geographical indications are currently handled by the Turkish Patent and Trademark Office. The purpose of the geographical indication and traditional specialty is to

protect both the product and its production method. Consumers may prefer products associated with a regional identity compared to any other product relying on their trust in the related region. Therefore, geographical indication or traditional specialty both have a major contribution in protecting the regional identifications that have become a symbol of a certain level of quality. , The geographical indication concept is being used to name products that owe their quality and fame to a certain geographical region (Saygin Alparslan and Demirbas, 2019). Types of geographical indications are "protected designation of origin" (PDO) and "geographical indication" (GI). In our country, local food products protected under geographical indication display the existence of a production potential which is very important for the national economy (Kantaroglu and Demirbas, 2018).

There are 688 registered (geographically indicated) products in Turkey. Additionally, there are 716 more products that are still under process. As far as the geographical indication is concerned, 62.3% of them have GI, and 37.2% of PDO. Furthermore, a traditional specialty is used for 0.5% of the products. In this respect, there were 427 products with GI, 255 with PDO, and 6 with a traditional specialty by the end of 2020. When the proportional distribution of geographical indication over product groups is considered, cheese occupies 3.1% with a quantity of 21. On the other hand, dairy products other than cheese and butter have a rate of 0.9% with a quantity of 6 (Turk patent, 2021). Definition of cheese with GI are listed in the definitions section of the Turkish Food Codex Cheese declaration (Anonymous, 2015). Accordingly, it is defined as the cheese which is registered after their production region, method, and characteristic specifications are certified by relevant national or international institutions.

Although there is a huge capacity of cheese production by industrial and classical methods, registered Ezine cheese (PDO), Edirne Feta Cheese (GI), and Malkara Aged Kaşar Cheese (PDO) are also produced in Thrace region, which carries significant potential in terms of dairy products. Determination of some distinguishing physicochemical specifications of the mentioned products with a further display of nutrition specifications like mineral content and fatty acid composition, determination of whether they have been produced according to the technical specifications covered in the registration, and comparison of specifications with the unregistered cheese types of the same group, all show the importance of the study. Some physicochemical properties of the mentioned cheese types with GI were studied through this study, thus displaying their characteristic value and contributing to the geographical indication protection by means of scientific data.

2. Material and Methods

In this study, samples were supplied in sealed packages from 23 different producers. Among these, Ezine Cheese, Edirne Feta Cheese, and Malkara Aged Kaşar Cheese samples with GI carrying diverse maturity time and milk type as declared on their label, were supplied from the authorized producers in their own region, whereas aged kaşar samples without GI and cheese stated as Ezine Cheese and Edirne Feta Cheese on their label were supplied from producers in Edirne, Tekirdağ, Çanakkale, and Kırklareli. The contact information of the companies authorized with GI registration was provided by Edirne Chamber of Commerce and Industry, Çanakkale Commodity Exchange, Ezine Cheese, and Dairy Farmer Protection, Development and Promotion Association, Malkara Chamber of Commerce and Industry, and related institutions of Ministry of Agriculture and Forestry. Special attention was paid during sampling to make sure that labels had logos and certification numbers on them. The related cheese samples were then divided into 6 groups as such: 6 samples with geographical indication named as ÇC1-6 (PDO), 6 without geographical indication but carrying Ezine cheese statement on the label named Ç1-6; 5 samples with geographical indication named as EC1-5 (GI), 7 without geographical indication named E1-7 but carrying Edirne feta cheese statement on the label; 2 samples of Malkara Aged Kaşar Cheese samples with geographical indication shoved as MC1-2 (PDO), 4 aged kaşar cheese without geographical indication showed as M1-4. Sampling was performed on a triplicate basis. 1 kg samples of each cheese type were provided during the study, using 90 samples in total. Cheese samples have been supplied during the April 2020 – October 2020 time interval. Cheese samples were provided in their original packages.

Physicochemical analysis and fatty acid composition determination methods applied to the samples are referred to below. Dry matter content (AOAC, 1990), ash content (AOAC, 1990), titratable acidity (lactic acid %) (AOAC, 1990), pH (AOAC, 1990), color analysis (Bhale et al., 2003), salt content

(Bradley et al., 1992), fat content with Gerber method (Anonymous, 2008), acid count (Renner, 1993), protein content with Kjeldahl method (AOAC, 1990) were performed. Mineral content analysis (Bakircioglu et al., 2011) and fat extraction method (Folch et al., 1956) were done according to described methods.

First, fat extraction was performed in order to do the fatty acid composition analysis for the cheese samples. Later, the obtained fat samples were transformed into methyl ester forms and processed for composition analysis with GC-FID. Fatty acids were determined by comparing the retention time of 37 FAME standard solution (Nu-Check-Prep, Inc., Elysian, MN, USA; Supelco, Inc., Bellefonte, PA, USA). The esterification of extracted fat samples for chromatographic analysis was done by acid-base methylation method. 2 mL sodium methoxy was added to 100 µL of the sample. The sample was then mixed with vortex for 2 minutes, followed by a 50°C hot water bath for 10 minutes. Later, 1 mL 14% boron trifluoride was added to the sample, which was once again mixed with vortex for 2 minutes and followed by 50°C hot water bath for 10 minutes. The sample was then vortexed again after adding 5 mL distilled water and vortexed once more after adding 5 mL hexane. A phase separation in the tube was observed. The obtained upper phase was transferred to an amber-colored vial filtered by a 0,45 µm syringe and kept at 20°C until the analysis (Özer et al., 2016).

All the obtained data are displayed as average ± standard deviation. The data was assessed by using one-way analyses of variance method (One-way ANOVA) through JMP statistical software (version 5.0.1.a SAS Institute. Inc. Cary, NC, USA). In cases where a statistically significant difference was observed between the sample means, multiple comparison was done by using Tukey test. Significance level (α value) was determined as 0.05 in all statistical assessments. The significance level between the mean data was given as a letter system in the tables. In order to obtain accurate results, every analysis and sampling was performed on a triplicate basis.

3. Results and Discussion

The results of proximate analysis for cheese samples were shown in Table 1. Dry matter content (%) of Malkara Aged Kaşar Cheese, Ezine Cheese, and Edirne Feta Cheese products with geographical indication complied with the values given in the related registration documents and were higher than the other cheese types without the geographical indication in the same type of cheese. Ash content (%) of Malkara Aged Kaşar Cheese, Ezine Cheese, and Edirne Feta Cheese products were higher than the other cheese types without the geographical indication in the same product group. However, this difference was statistically significant ($p<0.05$) only for Edirne Feta Cheese with geographical indication. There were no defined criteria for ash content % in registration documents and related notifications.

The average salt content % of the dry material of all the studied cheese types were within the value range given in the registration documents of the cheese with geographical indication except Malkara Aged Kaşar Cheese with PDO. Furthermore, it was observed that all cheese types, except Malkara Aged Kaşar Cheese with PDO, displayed variability within their product group in terms of salt % and salt in dry matter content % ($p<0.05$) (Turk patent, 2007,2017,2020).

Table 1. Physicochemical analysis of all cheese samples

Sample	Code	% Dry matter	% Ash	% Salt	% Salt in dry matter	% Fatty	% Fatty in dry matter	% Protein	Titratable acidity (lactic acid %)	pH	Acid number
Malkara aged Kaşar Cheese with PDO	MC1	65.99±0.05 b	3.84±0.13 a	3.84±0.13 a	5.91±0.08 a	29.8±0.35 b	48.2± 0.28 b	31.82±0.22 b	0.8±0.05 b	4.91±0.00 b	2.38±0.02 b
	MC2	71.85±0.49 a	3.91±0.10 a	3.91±0.10 a	6.15±0.07 a	33.8±0.49 a	54.07± 0.24 a	33.20±0.02 a	0.93±0.03 a	5.77±0.00 a	2.83±0.02 a
Malkara aged Kaşar Cheese	M1	61.18±0.11 d	2.79±0.16 c	2.79±0.16 c	4.21±0.15 d	25.6±0.70 a,b	42.30± 0.28 c	31.46±0.00 a	0.72±0.07 c	5.10±0.01 c	2.45±0.06 c
	M2	62.90±0.07 b	3.46±0.32 a,b	3.46±0.32 a,b	6.10±0.14 b	26.2±0.56 a,b	47.50± 0.42 b	32.11±0.36 a	0.7±0.06 c	5.49±0.01 a	2.39±0.06 c
	M3	61.48±0.04 c	3.10±0.04 b,c	3.10±0.04 b,c	5.12±0.02 c	24.8±0.42 b	40.45± 0.05 d	32.29±0.56 a	0.92±0.02 b	5.39±0.01 b	2.75±0.06 b
	M4	65.63±0.04 a	3.68±0.16 a	3.68±0.16 a	6.73±0.04 a	28.1±0.77 a	52.05± 0.42 a	31.92±0.24 a	1.15±0.07a	4.86±0.00 d	3.46±0.02 a
Ezine Cheese with PDO	ÇC1	56.95±0.07 c	5.62±0.24 a	4.69±0.03 a	6.69±0.01 a	28.4±0.21 c	51.2±0.07 c,d	22.87±0.06 b,c	1.29± 0.07 a	4.4±0.00 f	2.92±0.03 a,b
	ÇC2	63.81±0.08 a	5.36±0.02 a,b	4.45±0.05 a,b	6.30±0.07 b	33.7±0.28 a,b	54.75±0.16 b,c	24.03±0.17 a,b	1.17±0.07 a	4.62±0.00 d	2.2±0.01 b,c
	ÇC3	59.15±0.21 b	4.84±0.02 b,c	4.27±0.2 b,c	6.11±0.02 b	28.5±0.56 c	48.3±0.12 d,e	25.18±0.34 a	0.97±0.03 b	4.82±0.01 b	2.9±0.04 b
	ÇC4	55.37±0.09 d	4.69±0.26 c,d	3.77±0.05 d	5.32±0.02 d	28.1±0.28 c	44.2±0.05 e	22.29±0.61 c	0.99±0.07 b	4.54±0.00 e	2.28±0.05 b,c
	ÇC5	59.21±0.15 b	4.32±0.04 c,d	3.91±0.16 c,d	5.78±0.14 c	31.8±0.98 b	58.52±1.05 a,b	22.69±0.14 c	0.94±0.05 b	4.98±0.00 a	1.92±0.09 c
	ÇC6	59.15±021 b	4.23±0.02 d	3.92±0.19 c,d	5.79±0.02 c	34.1±0.14 a	62.77±1.41a	20.21±0.21 d	0.95±0.03 b	4.72±0.05 c	3.66±0.13 a
Ezine Cheese	Ç1	50.72±0.16 c	5.26±0.05 a	4.94±0.08 a	7.10±0.01 a	23.5±0.63 c	36.8±0.08 c	21.78±0.04 b	1.18±0.03 a	4.86±0.01 b	3.03±0.11 a,b
	Ç2	50.01±0.72 c	4.21±0.02 c	3.15±0.12 c	5.23±0.04 d	24.8±0.42 b,c	39.72±0.25 b,c	20.19±0.04 c	0.92±0.05 b,c	4.58±0.02 d	2.68±0.09 a,b
	Ç3	53.14±0.04 b	4.77±0.02 b	4.01±0.11 b	6.22±0.38 b	26.6±0.63 a,b	44.28±0.04 a,b	21.54±0.26 b	1.07±0.05 b	4.94±0.00 a	2.37±0.09 b
	Ç4	50.56±0.08 c	3.77±0.02 d	2.95±0.10 c	4.94±0.22 e	26.4±0.49 a,b	45.47±0.02 a,b	19.87±0.21 c	0.77±0.09 c,d	4.7±0.00 c	2.23±0.03 b
	Ç5	55.60±0.14 a	3.67±0.02 d	3.04±0.35 c	5.15±0.18 d	27.6±0.21 a	43.4±0.11 a,b	23.66±0.09 a	0.65±0.02 d	4.63±0.05 c,d	2.23±0.04 b
	Ç6	53.08±0.11 b	4.65±0.04 b	3.85±0.06 b	5.66±0.07 d	25.9±0.56 a,b	47.02±0.04 a	21.69±0.45 b	1±0.03 b	4.18±0.01 e	3.48±0.55 a
Edirne Feta Cheese with GI	EC1	60.23±0.04 c	5.23±0.04 a	4.78±0.24 a	6.95±0.04 a	31.4±1.27 a,b	52.25±0.47 a	23.21±0.16 a,b	1.23±0.05 a	4.46±0.01 c	3.65±0.03 a,b
	EC2	56.04±0.05 d	3.72±0.08 c	3.29±0.16 b	5.15±0.14 b	29.25±0.49 b	44.9±0.77 a	22.73±0.37 b	0.62±0.01 a	4.5±0.01 c	1.78±0.049 d
	EC3	60.91±0.15 b	4.51±0.09 b	4.12±0.18 a	7.05±0.77 a	32.4±0.21 a	57.2±3.28 a	23.66±0.55 a,b	1.1±0.05 a	4.63±0.00 b	3.8±0.07 a
	EC4	60.81±0.08 b	4.05±0.21 b,c	3.23±0.11 b	5.66±0.28 a,b	32±0.77 a,b	56.35±2.13 a	24.09±0.07 a	0.87±0.07 b	4.76±0.00 a	2.51±0.02 c
	EC5	63.21±0.15 a	5.18±0.11 a	4.40±0.50 a	6.26±0.05 a,b	33.7±0.14 a	54.2±1.30 a	24.24±0.12 a	1.25±0.05 c	4.63±0.05b	3.53±0.03 b
Edirne Feta Cheese	E1	51.09±0.11 d	3.76±0.02 b	3.08±0.21 c	5.42±0.02 d,e	24.7±0.21 b,c	43.24±1.12 b	22.41±0.14 b	0.68±0.06 a	4.54±0.01 e	2.14±0.04 c
	E2	51.91±0.14 c	3.94±0.05 b	3.46±0.21 b,c	5.83±0.04 b,c	25.9±0.21 b	48.1±0.4 a	21.73±0.14 b	0.95±0.05 a	4.51±0.01 e	3.32±0.11 a
	E3	51.02±0.02 d	3.76±0.01 b	3.19±0.13 b,c	5.55±0.05 c,d	23.9±0.35 c	43.11±0.85 b	22.81±0.07 b	0.73±0.07 a	4.74±0.00 c	1.69±0.10 d
	E4	52.90±0.07 b	3.85±0.00 b	3.35±0.22 b,c	5.13±0.04 e	25.9±0.56 b	41.29±0.26 b	22.49±0.10 b	0.93±0.07 a	5.3±0.02 a	2.87±0.03 b
	E5	50.01±0.01 e	3.74±0.07 b	3.29±0.28 b,c	5.87±0.09 b	24.8±0.42 b,c	42.2±0.62 b	20.57±0.04 c	0.66±0.01 b	4.51±0.01 e	1.93±0.04 c,d
	E6	56.92±0.02 a	4.46±0.15 a	3.70±0.14 a,b	6.10±0.12 b	27.6±0.07 a	45.99±1.52 a	24.52±0.16 a	0.98±0.05 b	4.67±0.00 d	3.56±0.07 a
	E7	57.12±0.02 a	4.45±0.06 a	4.01±0.27 a	7.18±0.10 a	27.45±0.21 a	47.58±0.77 a	25.02±0.69 a	1.05±0.05 b	5.07±0.02 b	3.28±0.04 a

a,b,c,d,e,f (↓) Within each type of cheese group, the values indicated by different letters in each column are statistically different from each other at the $p<0.05$ level.

Average fat content % in dry matter of Malkara Aged Kaşar Cheese, Ezine Cheese and Edirne Feta Cheese products with the geographical indication complied with the values given in the related registration documents and were higher than the other cheese types without the geographical indication in the same product group as seen in Table 1. However, this difference is statistically significant ($p<0.05$) only for Ezine Cheese and Edirne Feta Cheese with geographical indication. There were no defined criteria for protein content % in registration documents and related notifications (Turk patent, 2007; 2017; 2020).

Average protein content % in dry matter of Malkara Aged Kaşar Cheese, Ezine Cheese, and Edirne Feta Cheese products with the geographical indication were higher than the other cheese types without the geographical indication in the same product group. However, this difference was statistically significant ($p<0.05$) only for Ezine Cheese with the geographical indication (Table 1).

Titratable acidity rate (% lactic acid) of Malkara Aged Kaşar Cheese was the same as the other aged kaşar (matured) cheese. However, Ezine Cheese and Edirne Feta Cheese products with the geographical indication showed a statically significant difference ($p<0.05$) when compared with the other cheese types without the geographical indication in the same product group. On the other hand, in terms of pH values, only Edirne Feta Cheese with geographical indication indicated a statically significant difference ($p<0.05$) when compared with the other cheese types without the geographical indication in the same product group. As far as pH values of registered products were concerned, it can be stated that a sufficient amount of acidity development has been achieved for securing food safety. In this case, lowering the acidity values might be considered beneficial, bearing in mind that the acidity values stated in the registration may lead to a sourer perception of Edirne Cheese hence creating an unfavorable condition for the product in general. Likewise, the incompliance of all values with the registration criteria supported the idea that consumers may lead the producers in this direction.

Acid count (mg KOH/g fat) average values of Malkara Aged Kaşar Cheese and Ezine Cheese with geographical indication were lower compared to other cheese types without the geographical indication in the same product group, but it was higher for Edirne Feta Cheese (Table 1). Nevertheless, the mentioned differences were not statically significant ($p>0.05$). Additionally, acidity (mg KOH/g fat) has shown variabilities within the all-cheese groups. There are no defined criteria for acid count/free fatty acidity value in the registration documents of the mentioned cheeses.

The fatty acid compositions of all type of cheese mentioned in this study were shown in Table 2 (Malkara aged cheddar cheese), Table 3 (Ezine cheese), and Table 4 (Edirne Feta cheese samples). A significant amount of lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids exist in the saturated fatty acids group. Oleic acid (C18:1) rate within the unsaturated fatty acids group was quite high compared to other unsaturated fatty acids, among which palmitoleic (C16:1), linoleic (C18:2), and linolenic (C18:3) acids occupied a significant place as unsaturated fatty acids. When cheeses were compared on the basis of having geographical indication or not, significant differences ($p<0.05$) between lauric acid rates among Malkara Aged Kaşar Cheese and other kaşar cheese, 5 important fatty acid rates (caprylic, capric, myristic, stearic and oleic acid) among Ezine cheeses and 5 important fatty acid rates (butyric, caprylic, capric, lauric and linolenic acids) among Edirne cheeses were determined.

Regarding Malkara aged kaşar cheese, there was not a significant difference between the fatty acid profile of samples with PDO and without PDO except for lauric acid and palmitoleic acid. The lauric acid content of the Malkara, aged kaşar cheese with PDO was higher than the cheese sample without PDO. However, the palmitoleic acid was lower in the cheese with PDO than the cheese sample without PDO. For Ezine cheese samples, there were statistically significant differences in saturated and unsaturated fatty acids. The saturated fatty acids of Ezine cheese with PDO was 70.03 %, while it was 68.49 % for Ezine cheese without PDO. Anyhow, the polyunsaturated fatty acid profile of both cheese samples with and without PDO was not significantly different. When Edirne feta cheese samples were examined, there was no significant difference in the saturated and unsaturated fatty acids, but the polyunsaturated fatty acids in cheese without GI was higher than in cheese with GI.

Table 2. Comparison of mean fatty acid compositions (%) of Malkara aged kaşar cheese with PDO and other aged cheddar cheese (ripened) cheese samples without PDO

FATTY ACIDS (%)	with PDO	without PDO
	AVERAGE (%)	AVERAGE (%)
Butyric acid (C4:0)	2.17±0.01 a	2.21±0.04 a
Caproic acid (C6:0)	1.90±0.25 a	1.84±0.16 a
Caprylic acid (C8:0)	1.68±0.11 a	1.56±0.24 a
Capric acid (C10:0)	4.80±0.31 a	4.35±0.86 a
Lauric acid (C12:0)	3.58±0.08 a	3.35±0.13 b
Myristic acid (C14:0)	11.26±0.45 a	11.31±0.12 a
Palmitic acid (C16:0)	30.70±0.77 a	31.27±0.81 a
Palmitoleic acid (C16:1)	1.18±0.03 a	1.30±0.26 b
Stearic acid (C18:0)	12.82±0.47 a	13.22±0.43 a
Oleic acid (C18:1)	23.12±0.10 a	22.87±0.43 a
Linoleic acid (C18:2)	2.38±0.05 a	2,35±0.05 a
Linolenic acid (C18:3)	0.56±0.04 a	0.49±0.13a
Total saturated fatty acids Σ SFA	71.46±0.24 a	71.56±0.16 a
Total unsaturated fatty acids Σ UFA	28.55± 0.24 a	28.44±0.31 a
Monounsaturated fatty acids Σ MUFA	25.34±0.11 a	25.31±0.20 a
Polyunsaturated fatty acids ΣPUFA	3.2±0.33 a	3.13±0.09 a

a,b (→) The values shown with different letters in each row are statistically different from each other at the $p<0.05$ level.

Table 3. Comparison of mean fatty acid compositions (%) of Ezine cheese samples with PDO and without PDO

FATTY ACIDS (%)	with PDO	Without PDO
	AVERAGE (%)	AVERAGE (%)
Butyric acid (C4:0)	2.32±0.37 a	2.35±0.18 a
Caproic acid (C6:0)	1.94±0,22 a	1.88±0.12 a
Caprylic acid (C8:0)	1.62±0.38 a	1.27±0.08 b
Capric acid (C10:0)	4.58±1.70 a	2.99±0.22 b
Lauric acid (C12:0)	3.49±0.24 a	3.37±0.15 a
Myristic acid (C14:0)	11.23±0.55 b	11.69±0.28 a
Palmitic acid (C16:0)	30.89±1.95 a	30.70±1.80 a
Palmitoleic acid (C16:1)	1.26±0.25 a	1.36±0.23 a
Stearic acid (C18:0)	11.57±0.63 b	12.19±1.21 a
Oleic acid (C18:1)	24.32±1.60 b	25.66±1.09 a
Linoleic acid (C18:2)	2.40±0.40 a	2.50±0.14 a
Linolenic acid (C18:3)	0.39±0.22 a	0.31±0.05 a
Total saturated fatty acids Σ SFA	70.03±1.86 a	68.49±1.10 b
Total unsaturated fatty acids Σ UFA	29.97±1.86 b	31.53±1.10 a
Monounsaturated fatty acids Σ MUFA	26.79±1.85 b	28.39±0.89 a
Polyunsaturated fatty acids ΣPUFA	3.18±0.36 a	3.15±0.22 a

a,b (→) The values shown with different letters in each row are statistically different from each other at the $p<0.05$ level.

Mansson (2008) stated that the mean values of lauric, myristic, and palmitic fatty acids in cow milk are 3.30%, 10.9%, and 30.60%, respectively, in sheep milk 5.37%, 10.18%, and 22.04%, goat milk. It was determined as 7.64%, 11.94%, and 26.40% in milk. Blasi et al. (2008) 3.9% lauric acid, 13.1% myristic acid, and 31.6% palmitic acid in cow milk, 3.8% lauric acid, 8.8% myristic acid, and 23.1% palmitic acid in goat milk, they determined lauric acid 3.0%, myristic 7.0% acid and palmitic acid 19.8% in sheep milk. Caprylic fatty acid (C8:0) content in cow's milk is 1.69% (Ahmad et al., 2013), 1.4% (Mansson, 2008); 1.92% in sheep milk (Ahmad et al., 2013); In goat milk, it can vary in values of 3.66% (0.463%-9.722%) (Saroha et al., 2014). When compared with the literature studies, it was determined that the caprylic fatty acid ratios found were in line with the results of the studies. Capric fatty acid (C10:0) content in cow milk fat Ahmad et al. (2013) 2.87%, Mansson (2008) 2.7%, in sheep milk fat Ahmad et al. (2013) averaged 3.0% between 2.95% and 3.5%; an average of 6.75% in goat milk (Saroha et al., 2014), Strazalkowska et al. (2009) 6.54%, Ahmad et al. (2013) determined it as 3.01%. When compared with the literature studies, it was determined that the capric fatty acid ratios found were in line with the results of the studies.

Table 4. Comparison of average fatty acid compositions (%) of Edirne Feta cheese samples with GI and without GI

FATTY ACIDS (%)	with GI	without GI
	AVERAGE (%)	AVERAGE (%)
Butyric acid (C4:0)	2.48±0.12 a	2.16±0.33 b
Caproic acid (C6:0)	1.80±0.07 a	1.90±0.26 a
Caprylic acid (C8:0)	1.22±0.18 b	1.54±0.34 a
Capric acid (C10:0)	2.78±0.18 b	4.16±1.37 a
Lauric acid (C12:0)	3.27±0.16 b	3.56±0.40 a
Myristic acid (C14:0)	11.52±0.50 a	11.12±0.72 a
Palmitic acid (C16:0)	31.40±2.48 a	29.67±3.22 a
Palmitoleic acid (C16:1)	1.30±0.39 a	1.18±0.49 a
Stearic acid (C18:0)	12.46±1.35 a	12.19±1.31 a
Oleic acid (C18:1)	24.95±1.42 a	25.55±4.01 a
Linoleic acid (C18:2)	2.42±0.17 a	2.44±0.30 a
Linolenic acid (C18:3)	0.36±0.14 b	0.52±0.22 a
Total saturated fatty acids Σ SFA	69.41±2.23 a	68.64±3.57 a
Total unsaturated fatty acids Σ UFA	30.59±2.21 a	31.28±3.68 a
Monounsaturated fatty acids Σ MUFA	27.52±1.66 a	27.92±3.41 a
Polyunsaturated fatty acids ΣPUFA	3.07±0.86 b	3.45±0.36 a

a,b (→) The values shown with different letters in each row are statistically from each other at the $p<0.05$ level.

Na, Mg, K, Ca, P, Fe, Cu, Mn, Zn, and Al mineral materials of all cheese samples were determined by ICP-OES and shown in Table 5-6. When determined mineral content of the samples was compared on the basis of having geographical indication or not, a significant difference ($p<0.05$) was found only in the Mn amount of Edirne Feta Cheese and K, P, and Zn amount of Ezine Cheese. On the other hand, when Malkara Aged Kaşar Cheese with geographical indication was compared with other aged kaşar (matured) cheese, a significant difference ($p<0.05$) was found in the mineral amounts except for K, Cu, and A1.

Table 5. Average mineral composition of Malkara aged kaşar cheese with PDO and other aged cheddar cheese (ripened) cheese samples without PDO (mg/100g)

MINERAL	with PDO	without PDO
Na	2316.63±223.21 b	2653.92±95.35 a
Mg	50.87±5.85 b	56.72±3.16 a
K	298.138±42.96 a	304.36±35.22 a
Ca	1243,43±145.89 b	1380.74±102.08 a
P	1081.64±61.84 b	1217.57±72.77 a
Fe	0.364±0.05 b	0.498±0.14 a
Cu	0.089±0.01 a	0.079±0.01 a
Mn	0.047±0.02 a	0.014±0.00 b
Zn	6.29±0.45 b	7.27±0.63 a
Al	0.526±0.07 a	0.573±0.22 a

a,b(→)The values shown with different letters in each row are statistically from each other at the $p<0.05$ level.

Table 6. Average mineral composition of Ezine cheese samples (mg/100g)

MINERAL	with PDO	without PDO
Na	3851.45±1329.49 a	3743.51±986.76 a
Mg	50.43±5.31 a	50.55±12.49 a
K	289.26±26.39 a	258.13±44.79 b
Ca	1166.27±99.45 a	1046.32±222.07 a
P	970.12±86.64 a	852.97±147.60 b
Fe	0.680±0.45 a	1.124±1.00 a
Cu	0.074±0.01 a	0.087±0.02 a
Mn	0.015±0.01 a	0.027±0.02 a
Zn	6.15±0.65 a	4.94±1.30 b
Al	0.650±0.71 a	0.645±0.05 a

a,b (→)The values shown with different letters in each line are different from each other at the $p<0.05$ level.

Table 7. Average mineral composition of Edirne white cheese samples (mg/100g)

MINERAL	with GI	without GI
Na	3188.16±331.59 a	3225.55±444.405 a
Mg	42.21±6.91 a	44.35±4.19 a
K	265.38±37.21 a	268.89±34.44 a
Ca	1027.04±265.49 a	988.91±131.73 a
P	883.45±153.86 a	828.10±86.38 a
Fe	0.579±0.43 a	1.043±1.28 a
Cu	0.076±0.01 a	0.090±0.02 a
Mn	0.033±0.01 a	0.016±0.02 b
Zn	5.19±0.75 a	4.99±0.64 a
Al	0.672±0.2 a	0.687±0.11 a

a,b (→)The values shown with different letters in each line are different from each other at the p<0.05 level.

Table 8. Color values of cheese samples

CODE	L*	a*	b*
MC1	80.16±0.34 a	- 1.87±0.06 a	19.07±0.27 b
MC2	76.38±0.83 b	-1.81±0.02 a	20.56±0.60 a
mean	78.27±2.08 A	-1.84±0.05 A	19.82±0.89 A
M1	69.98±0.30 c	0.61±0.02 a	25.84±1.11 a
M2	70.87±0.32 b,c	-1.59±0.00 c	19.59±0.38 c
M3	72.55±1.41 a,b	-2.59±0.07 d	15.98±1.17 d
M4	73.10±1.72 a	-1.14±0.01 b	23.90±0.03 b
mean	71.63±1.66 B	-1.18±1.19 A	21.34±4.00 A
ÇC1	83.90±0.24 b	1.12±0.01 a	15.29±0.18 b
ÇC2	83.84±1.28 b	- 1.17±0,10 d	11.71±0.77 e
ÇC3	81.25±0.11 c	1.21±0.00 a	18.04±0.02 a
ÇC4	84.61±0.11 b	0.10±0.00 b	15.72±0.07 b
ÇC5	88.47±0.36 a	- 0.30±0.03 c	14.09±0.28 c
ÇC6	67.55±0.20 d	- 0.20±0.07c	12.83±0.29 d
mean	81.60±6.76 A	0.12±0.84 A	14.62±2.10 A
Ç1	85.07±0.14 b	- 0.95±0.00 e	14.94±0.01 a
Ç2	87.78±0.92 a	0.22±0.03 b	12.68±0.48 c
Ç3	79.18±0.31 d	- 1.64±0.00 f	15.49±0.54 a
Ç4	84.09±0.39 b	0.16±0.02 c	10.27±0.21 d
Ç5	84.70±0.39 b	0.78±0.00 a	15.38±0.04 a
Ç6	82.58±0.55 c	- 0.29±0.03 d	13.84±0.11 b
mean	83.90±2.70 A	- 0.29±0.82 B	13.77±1.89 A
EC1	85.40±1.59 b	- 0.32±0.02 d	8.23±0.54 e
EC2	87.68±0.89 a	0.59±0.03 a	12.54±0.14 c
EC3	84.20±0.85 b	- 0.17±0.11 c	9.28±0.39 d
EC4	87.54±0.99 a	0.47±0.06 b	14.05±0.76 b
EC5	85.95±0.12 a,b	- 0.76±0.00 e	15.05±0.02 a
mean	86.15±1.62 A	- 0.04±0.52 B	11.83±2.74 A
E1	87.06±0.51 b	1.48±0.02 a	13.40±0.18 b,c
E2	85.22±0.81 c	0.11±0.04 d	13.46±0.18 b,c
E3	82.87±0.89 d	0.21±0.09 c	13.77±0.31 b
E4	81.89±0.86 d	0.22±0.00 c	13.20±0.00 c
E5	78.19±0.15 e	- 0.49±0.00 e	7.21±0.02 d
E6	89.36 ±0.70 a	0.49±0.02 b	14.95±0.13 a
E7	86.95±1.08 b	0.23±0.03 c	14.66±0.38 a
mean	84.51±3,62 B	0.32±0,55 A	12.95±2.46 A

a, b, c, d, e (↓) Within each cheese group, the values indicated by different letters in each column are different from each other at the p<0.05 level.

A, B(↓) The mean values of each cheese type, indicated by different letters in each column, differ from each other at the p<0.05 level.

When cheese samples were compared for L*, a*, and b* average values on the basis of having geographical indication or not, a significant difference (p<0.05) was found only between the a* value among Ezine cheeses, L* and a* values among Edirne Feta cheeses and L* value among Malkara Aged Kaşar and other aged kaşar (matured) cheeses without the geographical indication. As for the b* values, there was no significant difference (p>0.05) among the cheese groups. The color of the related cheese in the geographical indication registration documents was defined as “from dirty straw yellow to dark

straw yellow", "light yellow color tending to white" or "light yellowish color generating from the local milk fat" with no solid definition (Turk patent, 2007; 2017; 2020).

4. Conclusion

In the Turkish Food Codex Declaration (Anonymous, 2015), the "characteristic specifications" statement is used for all cheese registered with geographical indication. In the registration documents of Ezine Cheese, Edirne Feta Cheese, and Malkara Aged Kaşar Cheese products, fatty acid compositions (%), dry matter (%), salt in dry matter (%), titratable acidity (lactic acid %) and pH values are given. However, whether the given fatty acid % in the Edirne Feta Cheese registration document is based on dry material or not is not stated, and likewise, titratable acidity % and pH values of Malkara Aged Kaşar Cheese are not given in the registration documents. The differences of the mentioned cheese types within their own product group, as well as their discriminating characteristics among the similar cheese types produced in diverse regions, should be stated with much broader and more solid data. Since registered products have characteristic specifications, the sustainability and traceability of their discriminating physical, chemical, sensory and similar properties play a profound role in securing the product quality and identifying product imitations/adulterations. With this study, it was suggested that fatty acid composition, protein content, acidity (free fat acidity %), and color (L*, a*, b*) values should be included in the characteristic specifications in the registration documents. Furthermore, detailed revision of the registration documents of the mentioned cheese types by including aroma-active components to the aroma characterization and scientific data related to texture specifications to the characteristic features through a broader study is advised.

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Research Article

Characterization of African Yam Bean (*Sphenostylis stenocarpa*) Mutant Lines using Phenotypic Markers

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African yam bean lines, Pearson correlation, Phenotypic characterization, Principal component, Seed yield

Abstract: Phenotypic characterization has been recognized as very important for the identification and selection of promising lines in improvement programs. This study aimed at characterizing and selecting early-maturing and high-yielding African yam bean (AYB) mutant lines at M_2 generation. The experiment was carried out at the experimental field of the Institute of Agricultural Research and Training, Nigeria. The experimental design used was a randomized complete block design with three replications. Nineteen promising AYB M_2 mutant lines were selected with their four parents (making 23 lines) and further evaluated for agronomic characters in the field. The results obtained revealed that coefficients of variation ranged from 3.23% (maturity) to 141.81% (seed yield/plant). The M_2 mutant lines flowered and matured earlier than their parents and outyielded their parents by 62.64%. The principal component (PC) showed that the first four PCs accounted for 75.54% of the total variation. The first PC accounted for first flowering, 50% flowering, first podding, and 50% podding. The second PC was responsible for pod yield/plant and seed yield/plant, whereas peduncle length and pod length were associated with the third PC, while the fourth PC was responsible for maturity. The breeding lines were delineated into three heterotic groups with cluster I had three lines; members had the highest pod yield/plant (60.22 g), seed yield/plant (27.33 g), and early-maturing. Cluster II consisted of 17 lines with moderate pod yield/plant, seed yield/plant, and longest pod length. Cluster III contained three mutant lines; exhibited the lowest pod yield/plant, seed yield/plant, and longest peduncle. A highly significant association existed between seed yield/plant and pod yield/plant (r = 0.97**), but negatively correlated with first flowering (r=-0.23*) and 50% first flowering (r=-0.24*). Therefore, AYB lines identified could be utilized by plant breeders/geneticists to develop AYB varieties that are early-maturing and high-yielding in improvement programs.

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1. Introduction

African yam bean (*Sphenostylis stenocarpa*) is an underutilized legume cultivated in Nigeria, Zaire, the Central African Republic, Ethiopia, and Gabon. Except for Africa, no other continent has a record of the crop's origin (Adewale et al., 2008). Although not all African yam bean (AYB) produce tubers, it is considered a dual crop because it has both tubers and seeds (Akinyosoye et al., 2017).

The grain and tuber of the AYB contain around 29 and 19% crude protein, respectively (Oboh et al., 1998). The tubers are richer in protein than Irish potatoes and 10 times that of cassava tubers, and the seeds are palatable like ordinary beans and cowpea (*Vigna unguiculata*) (Onyeike and Omubo-Dede, 2002). Apart from its high nutrient content, the bean has been claimed to be a source of phytochemicals and bioactive substances that provide health advantages to consumers, including the prevention of lifestyle illnesses (Duodu and Apea-Bah, 2017; Soetan et al., 2018). Despite its agronomic and economic benefits, AYB's use, conservation, and management have not been fully exploited (Ojuederie et al., 2015). In Nigeria, there is currently no known released AYB; it is only domiciled with elderly farmers (Adewale et al., 2012).

One of the major constraints affecting the utilization and adoption of AYB is late maturity, low seed yield, and extra agronomic practices like staking (Popoola *et al.*, 2019). The majority of known accessions of AYB are late maturing, with physiological maturity occurring between 6 and 7 months following sowing, depending on genotypes (Akinyosoye et al., 2017). Mutation breeding is a fast, cost-effective, reliable, and proven strategy for creating and selecting novel agronomic characters (FAO/IAEA, 2017), which aids in the identification and release of novel genes that govern the traits of interest (FAO/IAEA, 2017). Mutation has resulted in the development of 3220 mutant cultivars in more than 220 crops, which have been distributed to farmers globally (Bado et al., 2015). Mutants are sources of variation that can be used to introduce new populations with unique and beneficial alleles.

The phenotypic characterization of breeding lines is critical for researchers, breeders, farmers, and local communities to optimize the use of genetic resources (Zannou et al., 2008). Phenotypic characterization also serves as a foundation for the selection of traits and the conservation of genetic resources. Thus, this method is cheap, reliable, and very easy to identify contributing or discriminating characters among breeding lines (Olomitutu *et al.*, 2022). To validate the extent of genetic variability in germplasm, some scientists have used many statistical techniques, among which are principal components (PCA) and cluster analyses. PCA and cluster analyses are statistical methods for the characterization of germplasm based on their origins, genetic constitutions, and contributions of traits to the total variations (Ogunbodede, 1997; Akinyosoye *et al.*, 2017; Okunlola *et al.,* 2020). PCA and cluster analyses have been used to determine the extent of phenotypic variability in germplasm collections, namely maize, mungbean, pea, soybean, alfalfa, and AYB, among others (Matus *et al.,* 1999; Akinyosoye *et al.*, 2017; Okunlola *et al.,* 2020). Also, understanding the relationships among important traits is very useful in indirect selection (Kumar *et al.*, 2015; Sesay *et al.*, 2017). Information on AYB mutation breeding and mutant cultivars in Nigeria is scarce. Phenotypic characterization to assess phenotypic variation is vital for early field-based evaluation and selection. As a result, it is becoming increasingly imperative to develop AYB varieties that are early maturing and high yielding that can adapt to Nigeria's different agro-environments. Therefore, this research work aimed at characterizing and selecting early maturing and high yielding AYB mutant lines in the M_2 generation of AYB cultivars.

2. Materials and Methods

2.1. Genetic materials

The four parents of the mutant lines were obtained from International Institute for Tropical Agriculture, Ibadan, Nigeria, and induced with different concentrations of chemical mutagen (sodium azide) in the previous study (Akinyosoye *et al.*, 2021). Nineteen promising AYB M_2 mutant lines were selected from the M_1 generation, which was previously evaluated in the field for agronomic characters.

2.2. Experimental site

The experiment was carried out during the cropping season between April and October 2021 at the experimental field in Ibadan of the Institute of Agricultural Research and Training, Nigeria. According to the Food and Agriculture Organization classification system, the dominant soil type in Ibadan is Ferric lixisols (Sonneveld 2006). Ibadan is located 200 m above sea level at latitude and longitude of 7°22'N and 3°55'E, respectively, in Nigeria's lowland forest-savanna transition ecology. Rainfall received for the year ranged from 91.44 to 152.40 mm, falling majorly between April and

October with a peak in September. Also, the experimental location received an average rainfall of 107.10 mm and a temperature of 26.23℃ per annum (Table 1).

Table 1. Rainfall and temperature distribution in Ibadan in 2021

Month	Rainfall (mm)	Temperature (℃)
Januarry	7.62	26.32
February	20.32	27.44
March	45.72	28.00
April	91.44	27.44
May	137.16	26.88
June	193.04	25.76
July	187.96	24.64
August	180.34	24.64
September	231.14	25.20
October	152.40	25.76
November	30.48	26.32
December	7.62	26.32
Mean	**107.10**	**26.23**

2.3. Experimental design and crop management

The selected AYB mutant lines, along with their four parents (making 23 lines), were assessed for agronomic characters. The experiment was designed with a randomized complete block design and three replications. Two seeds were sown per hole at 1 m intra-row and 1 m inter-row in a 25 m^2 plot size, resulting in 50 plants per plot. Pest infestations were controlled, particularly during the reproductive stage, with cypermethrin (200 g/l pyrethroids) diluted with a tablespoon of fungicide powder benlate [50% methyl 1-(butylcarbamoyl-2-benzimidazole carbamate)]. Other cultural practices such as plowing and harrowing of land before planting, weed management, and staking were carried out.

2.4. Agronomic data collection

Each plot had ten representative plants sampled, and the average of the values was recorded in a plot. The following data were collected: days to first flowering (number of days from sowing to first flower opening in a plot), days to 50% flowering (number of days from sowing to 50% flower opening in a plot), first podding and 50% podding (number of days from sowing to first pod and 50% pod formation, respectively in a plot), days to 70% physiological maturity (number of days from sowing to when 70% of the plants have reached physiological maturity in a plot), number of seeds per pod (measured as mean of seeds of randomly selected 10 dry pods in a plot), pod length (measured in centimetres as mean of the length of randomly selected 10 dry pods in a plot), pods per peduncle (measured as mean of pods of randomly selected 10 peduncle before harvesting in a plot), peduncle length (measured as mean of peduncle of randomly selected 10 peduncle before harvesting in a plot), pod and seed yield per plant (measured in gramme by dividing total pods and seeds weight in a plot by number of plants at harvest, respectively in a plot).

2.2. Statistical analyses

The obtained data were analysed using analysis of variance (ANOVA). The Duncan Multiple Range Test (DMRT) was used to separate the treatments at 5% and 1% significance levels. Components with Eigenvalues greater than 1.0 were chosen using principal component analysis (PC). Characters with values greater than 0.6 were selected as important for PC (Matus et al.,1999). AYB lines were classified into various clusters based on hierarchical clustering and squared Euclidean distance using Palaeontological Statistics (PAST, version 2.17) software. The data were also subjected to K-means clustering analysis. The Pearson's correlation coefficient between agronomic characters was calculated using the Statistical Tool for Agricultural Research (Version: 2.0.1).

3. Results

3.1. Phenotypic variation and agronomic performance in agronomic characters of AYB lines

The coefficients of variation (CV) revealed significant variability among the AYB lines, where the CV ranged from 3.23% for days to 70% physiological maturity to 141.81% for seed yield per plant and pod length, as well as seed and pod yields per plant, were significant at 5% level of significance (Table 2).

Table 2. Range and coefficient of variation for some agronomic characters of African yam bean lines evaluated

Variable	Min.	Max.	Mean	F-test	SE(0.05)	CV%
First flowering	90.00	104.00	95.68	Ns	0.41	3.55
50% flowering	93.00	108.00	100.88	Ns	0.43	3.57
First podding	95.00	109.00	103.33	Ns	0.43	3.45
50% podding	100.00	116.00	108.32	Ns	0.43	3.29
70% physiological maturity	147.00	164.00	152.68	Ns	0.59	3.23
Pods/peduncle	2.00	4.30	2.47	Ns	0.06	21.13
Peduncle length (cm)	24.00	36.30	31.86	Ns	0.28	7.35
Pod length (cm)	15.00	30.50	22.40	*	0.39	14.39
Pod yield/plant (g)	1.50	155.00	22.46	*	2.88	90.96
Seed yield/plant (g)	0.25	96.00	8.78	*	1.57	141.81

*, ** Significant at ($p < 0.05$) and ($p < 0.01$), respectively level of significance; ns: non-significant; SE(0.05): Standard error; %CV: Coefficient of variation in percentage; Min.: Minimum; Max.: Maximum.

Table 3. Mean performance for agronomic characters of African yam bean lines

SN	LINES	Seed yield/plant (g)	First flowering (g)	50% flowering (g)	First podding	50% podding	70% physiological maturity	Pods/ peduncle	Peduncle length (cm)	Pod length (cm)	Pod yield/plant (g)
Mutant lines											
1	IART-1	33.50a	92.33a	98.00a	99.00a	104.67a	149.33a	2.43a	29.60a	20.57a-d	59.17ab
2	IART-2	24.83ab	93.00a	101.33a	107.67a	112.33a	154.67a	2.20a	32.17a	23.30a-d	64.67a
3	IART-3	23.67ab	95.67a	99.67a	103.67a	109.00a	154.00a	2.23a	29.00a	22.97a-d	56.83ab
4	IART-4	11.78abc	94.67a	102.33a	101.67a	107.00a	153.33	2.43a	30.67a	25.80a	29.78a-d
5	IART-5	11.67abc	94.67a	98.67a	101.33a	107.00a	148.33a	3.20a	33.53a	23.60a-d	38.00a-d
6	IART-6	10.83abc	95.33a	100.33a	102.33a	107.33a	147.00a	2.27a	30.23a	20.87a-d	28.17a-d
7	IART-7	7.83abc	95.67a	102.00a	103.00a	107.67a	151.67a	2.33a	31.10a	21.03a-d	18.06cd
8	IART-8	7.67abc	94.33a	99.00a	104.00a	109.33a	154.00a	2.35a	31.45a	23.50a-d	19.67cd
9	IART-9	7.22abc	94.67a	99.33a	101.67a	106.67a	152.67a	2.35a	31.17a	23.43a-d	18.22cd
10	IART-10	6.67abc	97.33a	102.67a	104.67a	109.33a	156.00a	2.10a	30.33a	19.60cd	20.50bcd
11	IART-11	6.67abc	97.67a	101.67a	105.00a	112.00a	154.33a	2.27a	34.27a	22.87a-d	23.33bcd
12	IART-12	6.44abc	94.00a	99.33a	103.33a	107.67a	153.33a	2.53a	32.17a	24.03ab	14.66d
13	IART-13	5.56abc	95.00a	99.67a	101.33a	106.67a	151.67a	2.53a	33.03a	19.67cd	11.78d
14	IART-14	5.22abc	97.00a	101.33a	103.33a	107.00a	152.67a	2.17a	32.60a	20.10a-d	16.11d
15	IART-15	5.11abc	94.00a	102.33a	100.67a	106.33a	159.67a	2.87a	32.53a	20.50a-d	14.17d
16	IART-16	3.67bc	94.00a	98.67a	99.67a	105.00a	150.33a	2.20a	32.33a	23.33a-d	9.17d
17	IART-17	3.33bc	97.00a	101.67a	104.33a	109.33a	150.33a	3.13a	32.13a	18.47d	10.33d
18	IART-18	3.25bc	95.67a	100.00a	101.33a	106.33a	154.00a	2.77a	32.10a	22.90a-d	9.00d
19	IART-19	1.67c	96.67a	101.33a	105.67a	109.67a	151.67a	2.33a	30.67a	23.00a-d	7.00d
Parents											
20	AYB 94	3.33bc	100.0a	104.00a	107.33a	110.67a	159.33a	2.10a	33.83a	25.53a	7.50d
21	AYB 61	6.67abc	99.00a	104.00a	108.00a	112.00a	153.67a	3.30a	35.50a	26.00a	24.67bcd
22	TSS 79	3.33bc	96.67a	102.33a	104.33a	109.00a	152.33a	2.33a	31.83a	18.17d	8.67d
23	NGB01349	2.00c	96.33a	100.67a	103.33a	109.33a	147.33a	2.43a	30.60a	25.93a	7.17d
	Mean of parents	3.83	98	102.75	105.75	110.25	153.17	2.54	32.94	23.91	12
	Mean of mutants	9.33	90.43	95.47	97.68	102.52	144.95	2.33	30.05	20.98	23.43
	Overall mean	8.78	95.68	100.88	103.33	108.32	152.68	2.47	31.86	22.4	22.46
	SE(0.05)	1.57	0.41	0.43	0.43	0.43	0.59	0.06	0.28	0.39	2.88
	%CV	141.81	3.55	3.57	3.45	3.29	3.23	21.13	7.35	14.39	90.96

Means with the same letter(s) in the same column or row are not significantly different from each other at p=0.05

Agronomic performance of AYB M_2 mutant lines showed that the mutant lines, along with their parents, reached first flowering, 50% flowering, first podding, 50% podding, and 70% physiological

maturity in 90 days after planting (DAP), 93 DAP, 95 DAP, 100 DAP, and 147 DAP, respectively (Table 2). On the other hand, M_2 mutant lines reached first flowering, 50% flowering, first podding, and 70% physiological maturity earlier than their parents and outyielded their parents by 62.64% (Table 3). Significant phenotypic differences were also recorded among the mutant lines, where line IART-1 had the highest seed yield of 33.50 g/plant and reached first flowering, 50% flowering, first podding, and 50% podding earlier than the rest, while parent AYB 94 attained late first flowering, 50% flowering and 70% physiological maturity than the rest (Table 3).

3.2. Principal component analysis for agronomic characters of African yam bean lines

The principal component (PC) of yield and other agronomic characters in AYB lines showed that the first four PCs accounted for 75.54% of the total variation. The selected four PCs had Eigenvalues >1.0 with corresponding contributions of 34.32%, 19.15%, 11.27% and 10.79%, respectively to the total variations (Table 4). The first PC was associated with first flowering, 50% flowering, first podding, and 50% podding. The second PC was responsible for pod and seed yields, whereas peduncle and pod lengths were associated with the third PC, while the fourth PC was responsible for 70% physiological maturity (Table 5).

Table 4. Eigenvalues and variation associated with each component of agronomic characters in African yam bean lines

	PC 1	PC 2	PC 3	PC 4
Eigenvalues	3.43	1.92	1.13	1.08
Percentage of Variance	34.32	19.15	11.27	10.79
Cumulative %	34.32	53.47	64.75	75.54

PC: Principal component.

Table 5. Contributions of some agronomic characters in African yam bean lines based on principal component

Traits	PC 1	PC 2	PC 3	PC 4
First flowering	0.89*	0.14	-0.04	-0.10
50% flowering	0.88*	0.12	-0.04	-0.07
First podding	0.88*	0.25	0.06	0.11
50% podding	0.87*	0.26	0.09	0.05
70% physiological maturity	0.16	-0.17	-0.12	0.68*
Pods/peduncle	-0.07	-0.01	0.49	-0.56
Peduncle length (cm)	-0.08	-0.33	0.51	0.49
Pod length (cm)	0.08	0.06	0.77*	0.10
Pod yield/plant (g)	-0.35	0.91*	0.07	0.14
Seed yield/plant (g)	-0.41	0.89*	0.02	0.13

*Component contributors; PC: Principal component.

3.3. Clustering of African yam bean lines

The goodness of fit of the dendrogram based on cophenetic correlation (rcop) was 0.94. African. AYB M_2 mutant lines and their parents were delineated into three heterotic groups at a rescaled distance of 15 units (Figure 1); cluster I consisted of three lines (IART-1, IART-2, IART-3). The members of this group had the highest pod and seed yields/plant as well as reached first flowering, 50% flowering, and 70% physiological maturity earlier than the rest. Cluster II had 17 lines (including their four parents). The members of this cluster possessed moderate pod and seed yields/plant and the longest pod length. Cluster III was made up of three mutant lines (IART-4, IART-5, IART-6). The members of this cluster had the lowest pod and seed yields/plant and longest peduncle (Figure 1, Table 6, Table 7). According to inter-cluster distance, AYB lines in clusters 1 and 2 had the lowest inter-cluster distance of 16.95 units, whereas clusters 1 and 3 had the highest inter-cluster distance of 52.54 units (Table 8).

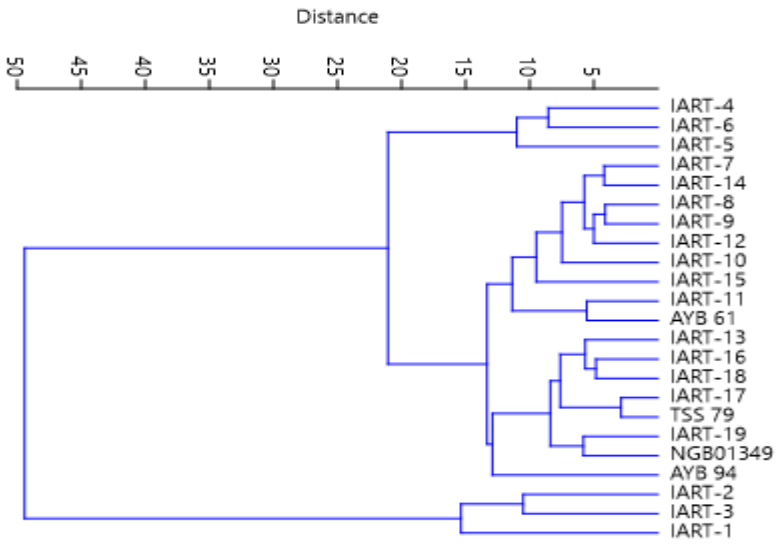

Figure 1. Dendrogram of the 23 African yam bean lines with three heterotic groups at the rescaled distance of 15 units.

Table 6. Means characteristics of African yam bean lines evaluated

Cluster	I	II	III
Member	3	17	3
Days to first flowering	93.67	94.89	96.18
Days to 50% flowering	99.67	100.44	101.18
Days to first podding	103.45	101.78	103.59
Days to 50% podding	108.67	107.11	108.47
Days to 70% physiological maturity	150.67	151.55	153.24
Number of pods per peduncle	2.29	2.63	2.48
Peduncle length (cm)	30.26	31.48	32.21
Pod length (cm)	22.28	23.42	22.24
Pod yield per plant (g)	60.22	31.98	14.12
Seed yield per plant (g)	27.33	11.43	5.04

Table 7. List of the members of each cluster

Cluster	1	2	3
Member	IART-1, IART-2, IART-3	NGB-01349, AYB-94, TSS-79, AYB-61, IART-7, IART-14, IART-8, IART-9, IART-12, IART-10, IART-15, IART-11, IART-13, IART-16, IART-18, IART-17, IART-19	IART-4, IART-5, IART-6

Table 8. Inter-cluster distance, according to K-mean clustering analysis

Cluster	1	2	3
1	-	16.95	52.54
2		-	36.32
3			-

3.4. Pearson correlation between agronomic characters of AYB lines

The association between agronomic characters revealed that seed and pod yields (r= 0.97**) had a significant and positive relationship but negatively correlated with first flowering (r= -0.23*) and 50% flowering (-0.24*). This suggests the increase in pod yield per plant and early flowering contributed to seed yield in AYB mutant lines. Positive and significant associations were obtained between first

flowering with 50% flowering (r= 0.85**), first podding (r= 0.73**) and 50% podding (r= 0.68**). 50% flowering was positively and significantly correlated with first podding (r= 0.69**) and 50% podding (r= 0.68**). Also, there was a strong and positive correlation between the first podding and the 50% podding (r= 0.89**) (Table 9).

Table 9. Pearson correlation between pairs of seed yield and other agronomic characters

	SYPP	DFF	50DF	DFP	50DP	MAT	NPPED	PEDL	PODL	PYPP
SYPP	-	-0.23*	-0.24*	-0.14	-0.16	-0.11	0.00	-0.14	0.00	0.97**
DFF		-	0.85**	0.73**	0.68**	0.09	0.01	-0.13	0.02	-0.19
50DF			-	0.69**	0.68**	0.10	-0.04	-0.14	0.06	-0.19
DFP				-	0.89**	0.11	-0.08	-0.01	0.06	-0.06
50DP					-	0.05	-0.07	-0.08	0.13	-0.06
MAT						-	-0.08	0.09	-0.02	-0.10
NPPED							-	0.02	0.07	0.02
PEDL								-	0.10	-0.12
PODL									-	0.03
PYPP										-

*, ** Significant at ($p < 0.05$) and ($p < 0.01$), respectively.
SYPP: seed yield/plant (g); DFF: first flowering; 50DF: 50% flowering; DFP: first podding; 50DP: 50% podding; MAT: 70% physiological maturity; NPPED: pods/peduncle; PEDL: Peduncle length (cm); PODL: Pod length (cm); PYPP: Pod yield/plant (g).

4. Discussion

In this study, there was significant variability among the agro-morphological traits. The presence of significant variability in pod and seed yields/plant of AYB, as evidenced by coefficients of variation (CV) in this study, had been earlier reported in pod and seed yields per plant of AYB in M_1 generation (Akinyosoye *et al.*, 2021). High CVs (>90%) indicate that significant variation existed in pod and seed yield per plant. Thus, the mutant lines can be adequately distinguished based on pod and seed yields. The low CV values (<22%) obtained in other traits indicate phenotypic uniformity within the lines in this study. This provides an ample opportunity for the selection of promising lines for the traits of interest by the plant breeder for further improvement.

The results obtained for flowering and maturity were lower than the information obtained on them in the M_1 generation of AYB in the previous study, whereas the seed yield obtained in this was higher than that of the M_1 generation in the previous study (Akinyosoye *et al.*, 2021). The M_2 mutant lines reached first flowering, 50% flowering, first podding, and 70% physiological maturity earlier than their parents and outyielded their parents by 62.64%. This phenomenon of induction of early flowering and maturity in mutant lines than their parents had earlier been reported by others in some crops such as AYB (Akinyosoye *et al.*, 2021); Arabidopsis (Onouchi *et al.*, 2000); barley (Matyszczak *et al.*, 2020); wheat (Laghari *et al.*, 2012). Similarly, reports of higher grain yield in mutant lines than their parents had also been reported by others in wheat (Morad *et al.*, 2011; Laghari *et al.*, 2012). Flowering or maturity time is a key factor for adaptation to natural and agricultural settings and directly influences yield (Turner *et al.*, 2005). Also, Nigeria is already plagued with climate change assoicated with biotic (pests and diseases) and abiotic stresses (drought) (Olajide and Adeyinka 2021; Akinyosoye 2022). Thus, the cultivation of early maturing genotypes helps the plants to escape the vagaries of weather due to the short life cycle, especially when there is rain cessation or unexpected drought. The observed phenotypic differences were recorded among the mutant lines. This could be due to the inherent genetic potential of the lines.

The principal component (PC) showed that the first four PCs accounted for 75.54% of the total variation. A similar result was reported by Olomitutu *et al.* (2022) who obtained 70.2% of the variation in the first four principal components in AYB lines. The relative discriminating power of the PCA determines character contribution to the total variation, and its discriminating power is dependent on the strength of the axis as measured by their eigenvalues (Idehen *et al.*, 2016; Akinyosoye *et al.*, 2017). Some traits such as first flowering, 50% flowering, first podding and 50% podding, 70% physiological maturity, pod length, and pod and seed yields had PC values ≥ 0.6 and were adjudged as the most important contributors to the variation in this study. This assertion is corroborated by the findings of Matus *et al.* (1999), who reported that any character having PC greater than or equal to 0.6 is regarded

as the most contributor to the variation. Therefore, identified agronomic characteristics could assist in the effective selection in AYB improvement programmes (Akinyosoye *et al.*, 2017).

The results obtained from cluster analysis indicated that 73.9% of the lines were found in cluster II. This indicates that most of the lines in this cluster were homogenous. Also, the moderate pod and seed yields/plant obtained in cluster II could be due to low variability among the lines in this cluster, whereas members in cluster I were heterogeneous due to high phenotypic variation existing in the cluster with good yield potentials. Adewale *et al.* (2012) reported that selections can be made for promising genotypes exhibiting unique phenotypic characters in a cluster. For instance, all the members in cluster I can be selected for the pod, seed yields/plant, and earliness to flowering and maturity. Few members in cluster II, especially parents AYB 61 and NGB01349 can be selected for longer pod length, whereas all the members in cluster III can only be selected for longer peduncle length. Classification of AYB into different heterotic groups in this study was in line with the findings of Adewale et al. (2012), who opined that breeding for genetic improvement of AYB requires an understanding of the genotype's classification pattern and intra-specific variability. As a result, the presence of genetic variations among AYB genotypes should be taken advantage of in breeding programs.

The significant and positive correlation recorded between pair of seed yield and other agronomic characters suggests that these traits could be governed by similar genes with pleiotropic effect or closely linked genes (Brown and Caligari, 2008). The significant and positive correlation between yield-related traits suggested that the traits could be improved simultaneously (Olomitutu *et al.*, 2022). Hence, any of these characters could be used in indirect selection to increase yield.

Conclusion

This study revealed that M_2 mutant lines flowered and matured earlier than their parents and outyielded their parents. Some traits such as first flowering, 50% flowering, and pod yield/plant were adjudged as the most contributors to the total variation. All the members in cluster I (IART-1, IART-2, IART-3) can be selected for the pod, seed yields/plant, and earliness to flowering and maturity. Few members in cluster II (AYB 61, NGB01349, IART-4, AYB 94, IART 12) can be selected for longer pod length, whereas all the members in cluster III (IART-4, IART-5, IART-6) can only be selected for longer peduncle length. Also, an increase in pod yield per plant and early flowering contributed to seed yield. Therefore, pair of traits that showed a significant and positive association with seed yield could be used for indirect selection to increase yield. AYB breeding lines identified in this study could be utilized by plant breeders/geneticists to develop AYB varieties that are early maturing and high yielding in improvement programs.

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Research Article

Quality Proficiency to Crop, Soil and Irrigation System of Recycled Wastewater from the Van/Edremit Wastewater Treatment Plant

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Abstract: Increasing pressure on water resources in the world has revealed the necessity of using marginal water resources in irrigation. With the use of wastewater, which is one of the marginal water resources, the pressure on freshwater resources is alleviated, the discharge problems of wastewater are solved, and soil and crop productivity increase with the high nutritive effect of wastewater. However, salinity, heavy metals, some harmful chemicals, and the pathogen risks of wastewater should not be ignored. In this context, in this study, the effluent of the wastewater treatment plant located in the central Edremit district of Van province was evaluated in terms of usability in irrigation. Samples were taken from treated wastewater during the vegetation period in 2020 and 2021 and pH, EC, cation and anions, micro elements and heavy metal, total nitrogen and phosphorus, total suspended solids, chemical oxygen demand, biological oxygen demand, fecal coliform, percent sodium, sodium adsorption rate, residual sodium carbonate and Langelier saturation index were determined by analysis and calculations. As a result of the study, the treated wastewater does not pose a risk in terms of pH, EC, cation and anions, micro elements and heavy metal, total suspended solids, percent sodium, sodium adsorption rate, residual sodium carbonate, langelier saturation index and fecal coliform, but attention should be paid to the total nitrogen and phosphorus, chemical and biological oxygen demand contents. It was concluded that the treated wastewater is in compliance with national and international standards, and there is no harm in its use in irrigation and thus treated wastewater can be recommended as a reliable water source for irrigation in the semi-arid province of Van/Edremit. However, in order to ensure safe and sustainable management in irrigation with wastewater, it is necessary to monitor water quality and make necessary inspections of soil, crop and irrigation systems.

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1. Introduction

The increasing pressure on water resources in the world has revealed the necessity of using marginal water resources in the agricultural sector, which is the largest consumer of fresh water. Treated wastewater, which is one of the marginal water resources (Çakmakcı et al., 2016), can be defined as

water that has been polluted as a result of different uses and whose properties have changed completely or slightly. Although the content of typical wastewater varies depending on the source and time, approximately 99% is water and the remainder is composed of colloidal and dissolved solid particles (UN, 2014).

Treated wastewater is a source of water and nutrients that can be used all year round, regardless of seasonality. Irrigation with treated wastewater provides higher yield, especially in arid and semi-arid regions, and also provides the opportunity to grow crops with high economic value (Qadir et al., 2015). Wastewaters are separated from other waters due to their rich organic matter, nitrogen, phosphorus, and potassium contents, and with these aspects, they increase soil and crop productivity with their use in irrigation (Becerra-Castro et al., 2015; Rivas et al., 2017; Yerli et al., 2022). In addition, considering the heavy metal and pathogen risks in the use of wastewater in irrigation, it is of great importance to carry out irrigations (Dogan Demir and Sahin, 2017; Demir, 2021). In a study, it was stated that untreated wastewater contains more macro and micro elements than a chemical fertilizer (Qin and Horvath, 2020). When irrigating 1000 m^3 per hectare with domestic wastewater, an average of 16 to 62 kg of nitrogen, 4 to 24 kg of phosphorus, and 2 to 96 kg of potassium can be added to the soil (Qadir et al., 2007). With the use of treated wastewater in irrigation, the need for synthetic fertilizers decreases, and this contributes to economic production by reducing the chemical fertilizer input as well as the environmental impact. In a study, it was reported that in conditions where wastewater is used in irrigation, approximately 135 dollars per hectare is saved in fertilizer costs (Jimenez et al., 2010). Treated wastewater, which acts as a natural fertilizer with its high organic and inorganic substance contents, improves and develops the soil structure and provides an increase in yield and quality in crop production. In addition, with the use of treated wastewater in irrigation, environmental contributions are provided, and the discharge problems of wastewater are solved.

Wastewaters can meet 30 to 70% of the irrigation water demand according to different regions (Raschid-Sally and Jayakody, 2009). It is known that 20 million hectares of agricultural land in the world are irrigated with wastewater (Koc et al., 2022). Winpenny et al. (2010) reported that approximately 10% of the irrigated agricultural lands around the world are irrigated with wastewater, and a total of 20 million hectares of agricultural land in 50 countries is irrigated with wastewater. Globally, wastewater use in agricultural lands accounts for approximately 11% of all irrigated agricultural land (Thebo et al., 2017). In addition to the insufficient data on the use of wastewater in irrigation in Türkiye, not much progress has been made. However, WHO (2006) stated that the reuse of wastewater would become the most important issue in Türkiye. The annual amount of treated wastewater in Türkiye is approximately 4.2 billion m^3 (TSI, 2018), which is an indicator of an important potential in terms of meeting the irrigation needs of agricultural lands in Türkiye.

Although the use of wastewater in irrigation brings high yield and quality, the risks they create should not be ignored. Wastewaters contain dissolved salts, heavy metals, and some other harmful chemicals and pathogens apart from nutrients (Dogan Demir and Sahin, 2019; Cakmakci and Sahin, 2021). This situation can reveal many negativities in soil quality, crop production, and live health. The sustainability of irrigation with wastewater can only be achieved by managing the effects of the crop, soil, irrigation system, and live health in irrigation with wastewater. In this context, in the use of recycled wastewater in irrigation, water quality must be evaluated, electrical conductivity and heavy metal content must be monitored, and pathogen and chemical risks must be followed. Dogan Demir and Sahin (2017) stated that the heavy metal content of tomato crops irrigated with wastewater increased. Similarly, Demir (2021) stated that heavy metal accumulation in the soil increased under wastewater irrigation conditions, and heavy metal accumulation in the soil should be monitored in irrigation with wastewater.

The following measures can be taken to reduce the risk of salinity caused by irrigation with wastewater; highly resistant to soil salinity; washing the salt from the soil, diluting the salinity in the active root zone by allowing salts to be transported outside the wet front using drip irrigation method; not sowing on the furrow ridge, which may be affected by salinity in seed sowing; ensuring that osmotic pressure remains at low levels by keeping soil moisture close to field capacity with frequent irrigation intervals; reducing the effect of salinity by irrigation cycles with wastewater and freshwater or irrigation by diluting wastewater with fresh water.

Precautions can be taken against the risk of Na accumulation, such as using treated wastewater only on soils with a sandy texture, adding organic matter to the soil (Pagliai et al., 2004), or adding a Ca source to the soil or irrigation water in use in clay soils against the risk of deterioration of the structure. (Hopkins et al., 2007). If there is a precipitated Ca source in the soil, Ca should be dissolved by reducing the pH value of the water in a controlled manner, taking into account the heavy metal mobility (Kanber and Unlu, 2010). Controlled nitrogen fertilizer or humic acid supplementation can be provided to promote leafing in crops against heavy metal risks and toxicity effects (Ding et al., 2019). In order to reduce the risks of pathogens and some harmful chemicals originating from wastewater, drip irrigation methods should be preferred instead of surface and sprinkler irrigation methods, and wastewater should not be preferred for irrigation of raw consumed crops. Filtration, dilute acid, and chlorine treatments are among the main measures to be taken, respectively, against possible physical, chemical, and biological cloggings in irrigation systems, especially drip irrigation, originating from wastewater (Sahin et al., 2005; Eroglu et al., 2009; Eroglu et al., 2012; Dandie et al., 2020; Hashem et al., 2021; Qiu et al., 2022).

According to the "Communique on Technical Procedures for Wastewater Treatment Plants" published in the Official Newspaper No. 27527 on 20.03.2010 in Türkiye, in the evaluation of the use of wastewater in irrigation, the amount of total solids and dissolved substances, electrical conductivity, sodium amount and the ratio of sodium to other cations, boron, heavy metals, and other toxic substances, organic matter load and the amount of floating substances such as oil and grease, pathogens and in some cases the total amount of calcium and magnesium should be examined (Anonymous, 2010). Although Türkiye has not come a long way regarding the use of wastewater in irrigation, studies on this subject are also very limited. In addition, in the literature review, no study was found in which the water quality of a treatment plant for the use of treated wastewater for irrigation was evaluated. In this context, in this study, the evaluation of the effluent of the wastewater treatment plant located in the central Edremit district of Van province in terms of usability in irrigation was determined according to the regulations in Türkiye and other international criteria. In addition, the measures that can be taken against possible problems are also mentioned.

2. Material and Methods

The recycled wastewater was taken from the biological wastewater treatment plant (38°24’53" N and 43°14’09" E) with a daily capacity of 10 400 m^3, located in the central Edremit district of Van province, which started operating in 2013. The inlet water of the treatment plant contains only domestic pollution elements of approximately 130 000 population and does not contain industrial and industrial wastes since there are no large-scale industrial facilities in the region. After entering the facility, the wastewater carried by the sewage line passes through the sand trap, coarse and fine screen, and is transferred to the pre-sedimentation pool and the aeration pool, respectively. After being aerated, the wastewater passing through the bacteria pool is transferred to the final settling unit and then discharged into the Lake Van. There are no farmers in the Van region who use wastewater for irrigation, and also the region has not made much progress in this regard.

The majority of the soils of Edremit district of Van province (38°42’28" N and 43°24’26" E) consist of alluvial soils with high $CaCO_3$ content, rich organic matter, mineral content, and medium textured (Turna and Eski, 2016). During the active crop production period (May-September) the precipitation amount is 92.2 mm, and the average temperature is 18.5ºC (Anonymous, 2021). In the region consisting of approximately 124 000 decares of land, 101 000 decares of land are used as agricultural land, and more than half of this area is irrigated (Anonymous, 2022). The fruit trees and vegetable cultivation such as apples, pears, apricots, plums, walnuts, tomatoes, peppers, cucumbers, beans, cabbage, lettuce, and also alfalfa and silage corn production as forage crops and field crops are cultivated in the central Edremit district of Van province (Turna and Eski, 2016; Anonymous, 2022).

The samples were collected from treated wastewater in 3 periods (July, August, and September) in the vegetation period of 2020 and in 2 periods (July and August) in the vegetation period of 2021. Water samples were taken from the discharge point of the treatment plant. Then pH, electrical conductivity (EC), cations (Ca, Mg, Na, and K) and anions (CO_3, HCO_3, SO_4 , Cl and NO_3), micro elements and heavy metal contents (B, Fe, Cu, Mn, Zn, Pb, Cd, Cr, and Ni), total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD_5), fecal coliform, percent sodium (Na%), sodium adsorption rate (SAR), residual sodium

carbonate (RSC) and Langelier saturation index (LSI) values were determined by analysis and calculations.

pH and EC are directly measured by pH and EC meter (Ayyildiz, 1983). Cations, micro elements and heavy metals; after being prepared for analysis with chemicals, measuring in an ICP-OES (Anonymous, 2007). CO_3 and HCO_3; using phenolphthalein and bromocrocel green indicators (Tuzuner, 1990). TP, SO_4, NO_3 and B; in Hach Lange Dr 5000 UV/VIS spectrophotometer, phosphor reagent no. HACH 8048 (Powder Pillow), SulfaVer 4 no. HACH 8051, Cadmium Reduction-HR no. HACH 8039, HACH 8015 Carmine methods with the help of ready kits (HACH, 2010). TN; Kjeldahl nitrogen determination method (APHA-AWWA-WEF, 1989). Cl; by titration with silver nitrate using potassium chromate indicator (Tuzuner, 1990). TSS; The residue left by the filtered water samples was dried at 105°C and weighed (APHA, 1995). COD; in HACH LCI 400 COD cuvette test with the help of Hach Lange Dr 5000 UV/VIS spectrophotometer and HACH LT 200 thermoreactor using Merck ready-made test kits (HACH, 2005). BOD_5; before the estimated BOD_5 value was obtained by performing COD analysis and the sample volumes determined according to this value were obtained by putting them in the Hach Lange Dr 5000 UV/VIS for 5 days and keeping them in the absorption incubator for 5 days (HACH, 2010). Fecal coliform; after the water sample was passed through the membrane filtration device, it was determined by analysis as the most probable number (MPN) (APHA, 1995).

The Na%, SAR, and RSC values were calculated according to Kanber and Unlu (2010) by considering the ion concentrations with the help of equations 1, 2, and 3, respectively. In order to determine whether lime sediment will occur in drippers due to irrigation, the LSI value was obtained with the help of equation 4 according to Ayers and Westcot (1994). In this equation, pH_a refers to the measured pH value, and pH_c refers to the theoretical pH value when the water reaches equilibrium with lime. This value was determined from ion concentrations according to Kanber and Unlu (2010).

$$Na\% = \frac{Na}{Na + Mg + Ca + K}\ x\ 100 \quad (1)$$

$$SAR = \frac{Na}{\sqrt{(Ca + Mg)/2}} \quad (2)$$

$$RSC = (CO_3 + HCO_3) - (Ca + Mg) \quad (3)$$

$$LSI = pH_a - pH_c \quad (4)$$

The quality of treated wastewater was evaluated according to the quality criteria according to the classes of inland water resources (Anonim, 2008) and irrigation water quality evaluation criteria (Anonim, 2010) and heavy metal contents of treated wastewater were evaluated according to permissible heavy metal amounts in irrigation water (Anonymous, 2010) and the water quality criteria affecting the clogging in the drip irrigation system were evaluated according to the water quality in the evaluation of the clogging in the drip irrigation method (Anonymous, 2010) and also some practical measures that could be taken were mentioned.

3. Results and Discussions

The result of pH, electrical conductivity (EC), cations (Ca, Mg, Na, and K), anions (CO_3, HCO_3, SO_4 , Cl, and NO_3), micro elements, and heavy metal contents (B, Fe, Cu, Mn, Zn, Pb, Cd, Cr, and Ni), total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD_5), fecal coliform, percent sodium (Na%), sodium adsorption rate (SAR), residual sodium carbonate (RSC) and langelier saturation index (LSI) values of the recycled wastewater based on different periods and years are given in Table 1.

In terms of soil quality, the pH of irrigation water should be between 6.5 and 8.4 (Ayers and Westcot, 1994). While EC values of < 0.7 dS m^{-1} in irrigation waters do not pose a problem for crop nutrition and for the crop to benefit from soil water, a value between 0.7 and 3.0 dS m^{-1} may cause the problem to begin, and a value > 3.0 dS m^{-1} may cause the problem to be intensified (Kanber and Unlu, 2010). According to the US Salinity Laboratory System, treated wastewater is classified as C3 (high salt

water for irrigation) (0.75 - 2.25 dS m^{-1}). Wastewater is classified as a no problem or low problem category in terms of pH, EC, infiltration, ion toxicity, and other effects (Ayers and Westcot, 1994). The pH values of the wastewater are included in the I. class water category according to the "Water Pollution Control Regulation" inland water resources classification (Anonymous, 2008). The EC values of the wastewater were evaluated in the category of water with little-moderate damage in use (II. class) according to the "Communique on Technical Procedures for WasteWater Treatment Plants" (Anonymous, 2010). In this case, the wastewater can be easily used for irrigation of crops with medium and high salinity resistance and for light and medium textured soils (Kanber and Unlu, 2010). The fruit trees and vegetable cultivation such as apples, pears, apricots, plums, walnuts, tomatoes, peppers, cucumbers, beans, cabbage, lettuce, and also alfalfa and silage corn production as forage crops and field crops are common in the region (Anonymous, 2022) and this considering that these crops show medium and high resistance to salinity, there will be no risky situation related to salinity in the use of treated wastewater in irrigation. In addition, considering that 65% of the region's soils have a loamy texture (Karaca et al., 2019), the risk of salinity in the use of treated wastewater in irrigation will decrease. However, in the use of treated wastewater in clay textured soils or crops sensitive to salinity, it can be specified as the precautions to be taken for the preservation of soil quality and for a reasonable crop production, performing irrigation with drip irrigation, reducing osmotic pressure by keeping soil moisture close to field capacity in root zone and carrying out washings by following the soil EC in certain periods.

Table 1. Quality analysis results of recycled wastewater

Parameter	July		August		September
	2020	2021	2020	2021	2020
pH	7.49	7.65	7.45	7.79	7.37
EC (dS m^{-1})	1.131	1.143	1.009	1.135	1.184
Ca (me l^{-1})	2.17	2.42	2.05	2.57	2.19
Mg (me l^{-1})	2.98	3.21	3.13	2.78	3.21
Na (me l^{-1})	4.11	4.09	4.19	4.25	4.02
K (me l^{-1})	0.92	1.25	1.01	1.36	0.95
CO_3 (me l^{-1})	-	-	-	-	-
HCO_3 (me l^{-1})	4.91	5.11	5.05	5.24	5.21
Cl (me l^{-1})	1.94	1.84	2.11	2.13	2.07
SO_4 (me l^{-1})	1.68	1.35	1.74	1.67	1.81
NO_3 (me l^{-1})	1.54	1.45	1.67	1.68	1.52
B (mg l^{-1})	0.51	0.42	0.59	0.49	0.56
Fe (mg l^{-1})	0.397	0.425	0.429	0.412	0.411
Cu (mg l^{-1})	0.011	0.011	0.012	0.010	0.010
Mn (mg l^{-1})	0.081	0.099	0.072	0.091	0.061
Zn (mg l^{-1})	0.015	0.015	0.015	0.015	0.016
Pb (mg l^{-1})	0.001	0.002	0.001	0.001	0.002
Cd (mg l^{-1})	0.002	-	0.002	-	-
Cr (mg l^{-1})	-	-	0.001	0.001	0.001
Ni (mg l^{-1})	0.036	0.045	0.038	0.049	0.041
TN (mg l^{-1})	12.65	10.35	9.93	11.27	10.27
TP (mg l^{-1})	1.51	1.15	1.87	1.21	1.69
TSS (mg l^{-1})	19.3	31.2	21.8	28.5	24.5
COD (mg l^{-1})	36.5	41.9	35.2	35.5	37.1
BOD_5 (mg l^{-1})	22.4	25.8	21.1	22.8	22.6
SAR	2.56	2.43	2.60	2.59	2.45
RSC (me l^{-1})	-0.24	-0.52	-0.13	-0.11	-0.19
Na%	40.4	37.3	40.4	38.8	38.8
LSI	0.324	0.599	0.299	0.664	0.247
Fecal coliform (MPN 100 ml^{-1})	145	167	121	149	139

''-'': Not detected.

When the values of Na, which has a high toxic effect, are examined, according to the "Communique on Technical Procedures for Wastewater Treatment Plants" (Anonymous, 2010), wastewater II. class belongs to the group. Thus, the level of damage to be caused by the use of wastewater in irrigation is considered as low-moderate. In addition, Na% and SAR values are calculated in order to determine the negative effect of Na on the soil structure (Pacci et al., 2022). Considering the

Na% and SAR values of the wastewater, it is seen that it is within the usable limits for irrigation (Kanber and Unlu, 2010). Another parameter evaluating the negative impact on soils is RSC. The RSC value in waters should be lower than 2.5 me l^{-1} (Kanber and Unlu, 2010). All RSC values in wastewater are negative and show that it does not pose a risk.

Wastewater is in the class of water that does not pose any risk in irrigation in terms of SO_4 content. Considering the quality criteria according to inland water resources classification (Anonymous, 2008), the wastewater is evaluated in the category of I. class waters in terms of SO_4. SO_4 content in irrigation waters is lower than 4 me l^{-1} in Scofield-1936 classification shows that the irrigation water class is very good, and there will be no problem with its use in irrigation (Kanber and Unlu, 2010). High concentration of HCO_3 causes Ca to precipitate and Na to become dominant, increasing Na damage (Kanber and Unlu, 2010). Ayers and Westcot (1994) reported that severe problems would occur if the HCO_3 content was > 8.5 me l^{-1}. In order to reduce the risk of Ca precipitate, it may be recommended to reduce the pH of the treated wastewater and thus to perform irrigations or to add Ca to the soil to prevent Na from becoming dominant. In addition, organic matter additives can also be effective. However, high organic matter (BOD_5) entry into the soil with treated wastewater will also reduce the negative effect of Na (Kanber and Unlu, 2010). The high organic matter contribution of the treated wastewater used in the study also eliminates this situation. Considering the toxicity effect of Na, the N supplied to the soil with the treated wastewater will reduce the negative effect of Na by promoting the growth and leafing of the crop (Ibrahim et al., 2018). Singh et al. (2016) reported that the negative effect of Na on the crop improved with the addition of N. However, in this study, the HCO_3 content was found below the limit values.

Cl ion, which has a high amount of toxic effect, is in the category of I. class waters that do not cause any problems in irrigation according to the “Communique on Technical Procedures for Wastewater Treatment Plants" (Anonymous, 2010). In addition, in terms of Cl ion, the wastewater was evaluated in I. class waters in Scofield-1936 (< 4 me l^{-1}), Doneen-1954 (< 5 me l^{-1}) and Christiansen-1977 (< 3 me l^{-1}) classifications (Kanber and Unlu, 2010). However, considering the "Water Pollution Control Regulation" inland water resources classification (Anonymous, 2008) in terms of Cl content, wastewater in the II. category of quality. According to the same regulation, wastewater in terms of NO_3 content is classified as IV. water quality. In this context, the pollution load of NO_3 should be monitored, and it would be beneficial to use the drip irrigation method with high efficiency in order to prevent leakage into groundwater in the use of treated wastewater in irrigation.

B is an essential element for crop growth in low amounts but is a special ion that has high toxicity in even low amounts (Yerli et al., 2020). When the concentrations of B in wastewater are examined, it has been evaluated in the class of water that does not pose any inconvenience to use, according to the "Communique on Technical Procedures for Wastewater Treatment Plants" (Anonymous, 2010). According to FAO (Ayers and Westcot, 1994) and the US Environmental Protection Agency (EPA, 2004), the amount of B in irrigation waters lower than 0.75 mg l^{-1} does not pose a problem in even long-term irrigation. However, attention should be paid to the sensitivity of the crop irrigated with wastewater to B toxicity. Stone fruits are more easily damaged by B than pome fruits (Kanber and Unlu, 2010). Considering that the resistance to B toxicity of apples, pears, apricots, plums, walnuts, cucumbers, and beans grown in the region is sensitive or semi-sensitive, precautions should be taken against B toxicity in irrigation with treated wastewater. In this context, the growth and leafing of the crop should be encouraged by supplementing with N from the outside, and its resistance to stress should be increased (Ding et al., 2019). However, since N already obtained from wastewater can provide this benefit, there is no need to give N from outside.

The Mn, Cu, Fe, Zn, Cr, Cd, Ni, and Pb contents of treated wastewater are below the maximum allowable values permitted by the "Communique on Technical Procedures for Wastewater Treatment Plants" (Anonymous, 2010) and the US Environmental Protection Agency (EPA, 2004). In addition, according to the "Water Pollution Control Regulation" inland water resources classification (Anonymous, 2008), treated wastewater does not pose a risk in its use in irrigation since it is in the class of water of good quality. However, in the long-term use of wastewater in the same region, possible accumulations in the soil and crops should be followed up. In addition, since some crops such as corn cultivated in the region absorb heavy metal more and accumulate it in their bodies (Ab Rhaman et al., 2021), it may be recommended to irrigate these crops with wastewater by diluting them with fresh water instead of directly treated wastewater. In long-term irrigation with wastewater, heavy metal risks can be

reduced by using phytoremediation techniques with accumulator crops (Bhargava et al., 2012; Sharma et al., 2018).

N and P contained in wastewater have a positive effect on soil and crop growth. TN and TP contents of treated wastewater are listed as the lowest grade IV. category of class waters in the "Water Pollution Control Regulation" inland water resources classification (Anonymous, 2008). When the Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD_5) of the wastewater are examined, according to the "Water Pollution Control Regulation" inland water resources classification (Anonymous, 2008), COD and BOD_5 are evaluated in the II. and IV. water categories, respectively. The excess of BOD_5 is considered as the input of dissolved organic matter into the soil. This improves the structure of the soil. Especially for agricultural lands where crop production is widespread and soil structure is deteriorated, the structure of the soil will be improved with the use of treated wastewater and it will become an alternative water source (Barbera et al., 2013; Çakmakcı and Sahin, 2019;2020).

Fecal coliforms contained in wastewater are the main source of many diseases in terms of health as a microbiological parameter. In general, its amount in water is expressed as the most probable number in 100 ml (MPN 100 ml^{-1}). According to the fecal coliform values of the treated wastewater, the quality of the wastewater is classified as II. class and is considered as water that has no objection to use in "Water Pollution Control Regulation" inland water resources classification (Anonymous, 2008). In addition, the limit values (1000 EMS 100 ml^{-1}) determined by the US Environmental Protection Agency (EPA, 2004) show that it will not cause any problems in using it in irrigation. However, in order to reduce possible risks, it may be recommended to prefer drip irrigation instead of surface and sprinkler irrigation methods in irrigation with treated wastewater and not to use treated wastewater for irrigation of raw consumed crops.

Although crops need cations in terms of development and growth, especially in conditions where the pH is > 8.0 in water, also the sum of Ca and Mg content is > 3, causing clogging in the irrigation system (Kanber and Unlu, 2010). Considering that the pH values of the wastewater are below 8 and the salinity values are < 2000 mg l^{-1}, the risk of clogging in the irrigation system has decreased. Another factor affecting clogging in the drip irrigation system is the amount of total suspended solids. According to the "Communique on Technical Procedures for Wastewater Treatment Plants" (Anonymous, 2010), the upper limit of the amount of total suspended solids that can be used in the drip irrigation system is 50 mg l^{-1}, thus reducing the risk of clogging in the use of wastewater in irrigation. According to the water quality criteria affecting the clogging in the drip irrigation system in the same communique, the wastewater was also included in the category of waters in which the risk of clogging is low or low-moderate in terms of Mn and Fe. In addition, the LSI values of the wastewater are positive and close to zero in the study indicating that there will be no risk in terms of lime accumulation. Dilute acid treatments are one of the precautions to be taken against possible clogging risks. Although strong acids prevent lime accumulation in drippers by lowering the pH of the water, crop nutrients may have an antagonistic or synergistic effect with each other depending on the decreasing pH. In addition, organic acids are also used in lime (Sahin et al., 2005; Eroglu et al., 2009; Eroglu et al., 2012; Dandie et al., 2020; Hashem et al., 2021; Qiu et al., 2022).

Conclusion

According to the analyzes of the wastewater evaluated during the irrigation seasons, it was seen that there were similarities in terms of quality criteria in both years of the study (2020 and 2021) and values close to each other were obtained. As a result of the evaluation of wastewater according to the "Water Pollution Control Regulation" inland water resources classification (Anonymous, 2008), "Communique on Technical Procedures for Wastewater Treatment Plants" (Anonymous, 2010), and other international criteria, it was concluded that there is generally no harm in its use in irrigation. In this context, treated wastewater was found to be recommendable as a reliable water source for agricultural irrigation in the Van/Edremit region. However, in regions where wastewater is irrigated for a long time, a quality control by matching the results obtained by keeping records by making follow ups in soil, crop, and drip irrigation systems with the quality parameters of treated wastewater will be important in terms of ensuring sustainability and environmental safety in wastewater irrigated agriculture in the region.

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Research Article

Determination of the Relationship between Rice Suitability Classes and Satellite Images with Different Time Series for Yeşil Küre Farm Lands

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RE-OSAVI,
Rice

Abstract: In this study, rice land designated for agricultural land suitability indices belonging to the enterprise Yeşil Küre Farm Land with different time series Sentinel-2A satellite images calculated utilizing spectral vegetation index, which are Normalized Difference Vegetation Index and Red Edge Optimized Soil Adjusted Vegetation Index values by statistical comparison of the relationship between rice for monitoring and estimation of potential productivity is presented a different perspective. Firstly, according to the rice suitability assessment for the study area, the area of 5488.9 ha was determined to be suitable for rice cultivation at the S1 and S2 levels, whereas the area of 588.9 ha was determined to be unsuitable. In this study, it was determined that the most successful results for each land conformity class were obtained using the NDVI. In particular, it was determined that August received the highest r^2 value (NDVI; 0.8580 and RE-OSAVI; 0.8465) in both vegetation index models at the S1 level, and on the other hand, a higher r^2 value was obtained with NDVI.

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1. Introduction

Soil is a limited and non-renewable natural resource for the needs of the increasing human population, as well as being the main element in meeting the nutritional and shelter needs of the terrestrial ecosystem (Blum, 2006). As a matter of fact, it is predicted that the world population will grow by 19.7% until 2050 and reach 9.6 billion. The increase in the world population and the increase in the demand for food that it will bring with it causes the intensification of agricultural activities and an increase in the pressure on the limited land resources (FAO, 2009; Liniger, et al., 2011; Dumanski and Peiretti, 2013). Therefore, food security, demand for energy and water, climate change, and biodiversity in relation to sustainable land use are among the important global problems of the 21st century (Lal, 2008; 2009; Jones, et al., 2009; Lichtfouse, et al., 2009). The continuity of the functionality of the ecosystem depends on using the soil in the best way to get high productivity (Lal, 2009; Walter and Stützel, 2009).

Determination of soil quality is the basis of common and reliable approaches in the evaluation of soil fertility (Mueller et al., 2010; Ahmed et al., 2016; Xue et al., 2019; Dengiz, 2020). Soil quality

is defined as the capacity of soil to perform its functions within the boundaries of natural or managed ecosystems (Larson and Pierce, 1991; Doran and Parkin, 1994; Karlen, et al., 1997). Soil quality, like air and water, has a profound impact on the health and productivity of a particular ecosystem and its associated environments. Soil fertility is also the basic elements of a high input-based agricultural production system. For this reason, it is essential to evaluate soil fertility with parametric approaches in a periodic process (Rogowski and Wolf, 1994; Dengiz and Sağlam, 2012; Askari et al., 2015).

Today, Geographic Information Systems (GIS) and Remote Sensing (RS) technologies are useful tools used in similar monitoring issues encountered in the agricultural sector. Saving time and providing reliable, cost-effective, and repetitive information are among the advantages of GIS and UA to the agricultural sector. GIS and UA technologies are used in many areas, such as monitoring plant development process, evaluation of soil quality, crop yield estimation, and spatial analysis required in modern agriculture. As a matter of fact, the use of visible near infrared (VNIR) spectroscopy and GIS technologies in the evaluation of soil fertility by integrating agricultural practices and expert opinions makes it practical and economical to monitor agricultural areas locally (Moran, et al., 1996; Malczewski, 2006; Vohland, et al., 2014; Askari, et al., 2015). For this purpose, spectral reflections at different wavelengths obtained from earth observation satellites have been used to determine measurable plant vegetation characteristics such as plant biomass and active photosynthetic radiation since the launch of the Landsat-1 satellite in 1972 and to monitor the phenological changes of plants (Jackson, 1986). However, developing spectral sensors and band combination techniques have brought along the concept of vegetation index and have been widely used in the evaluation of cultivars grown in large-scale areas (Shou, et al., 2007; Jia, et al., 2011; Savasli, et al., 2021). It is known that especially the red (Red), red edge (Red-Edge), and near infrared (NIR) bands of the electromagnetic spectrum play an active role in monitoring the agricultural ecosystem (Liu, et al., 2004). Indeed, the relevant spectral regions are closely related to plant biophysical variables (height, density, and percentage of coverage), and it is known that the ratio of near-infrared bands to red edge spectral bands provides a high correlation with crop development in different growth periods. Today, many spectral indices, defined as unitless radiometric measurements, have been developed to obtain information about the biophysical properties of green leafy plants. The working principle of vegetation indices is basically based on the presence of chlorophyll, which is directly related to green leaf area and plant biomass and its response to biotic/abiotic factors (Kokaly and Clark, 1999; Li, et al., 2015). For this purpose, Normalized Difference Vegetation Index (NDVI), Effective Leaf Area Index, Chlorophyll Absorption Index at Modified Reflection Rate (Red-Edge Modified Chlorophyll Absorption in Reflectance Index), Red Edge Optimized Soildable Vegetation Index Red-Edge Optimized Soil Adjusted Vegetation Index, Green Normalized Difference Vegetation Index, Green Normalized Difference Vegetation, Health Index and Leaf Area Index are used (Fitzgerald, et al., 2010; Bagheri, et al., 2012; Wójtowicz, et al., 2016; Demir and Başayiğit, 2021). However, while studies have shown that vegetation indices derived from multi-spectral satellite images give successful results in early yield estimations, similar spectral index approaches that can directly evaluate the yield potential of soils could not evolve with a pragmatic model due to the complex nature of soils (Barnes et al., 2000). For this reason, the vegetation indices of the plants grown in agricultural lands and the biomass ratios determined at varying levels provide an indirect benefit to the researchers in the evaluation of soil fertility. In this way, the productivity of the soil where strategically important crops based on crop yield are grown under an agricultural management can be estimated experimentally (Gupta, et al., 2003; Zand and Matinfar, 2012; Mezera, et al., 2017; Sharabiani, et al., 2019). As a matter of fact, the factors affecting plant spectral reflections and indices values may be due to differences in management practices, as well as related to soil fertility (Zhou, et al., 2018; Dedeoğlu, et al., 2020). With this study, it is aimed to establish the land suitability classes of rice lands of Yeşil Küre Agricultural Enterprise and to reveal the relationship between NDVI and RE-OSAVI vegetation index values produced from Sentinel-2A satellite images with different time series and rice land suitability classes.

2. Material and Methods

Yeşil Küre Agricultural Enterprise (Center) is located at the 40th km on the Samsun-Bafra highway and is between 249000-254000 East and 4599200 - 4602400 North (WGS84, Zone 37, UTM-m) coordinates. Total land asset is 9239.8 da. Düden, which is the other part of the operation area where

the Black Sea is located in the east, Bünyan Mountain in the south, and the Bafra Plain in the west and north, is adjacent to the western shore of Balık Lake in the Kızılırmak Delta.

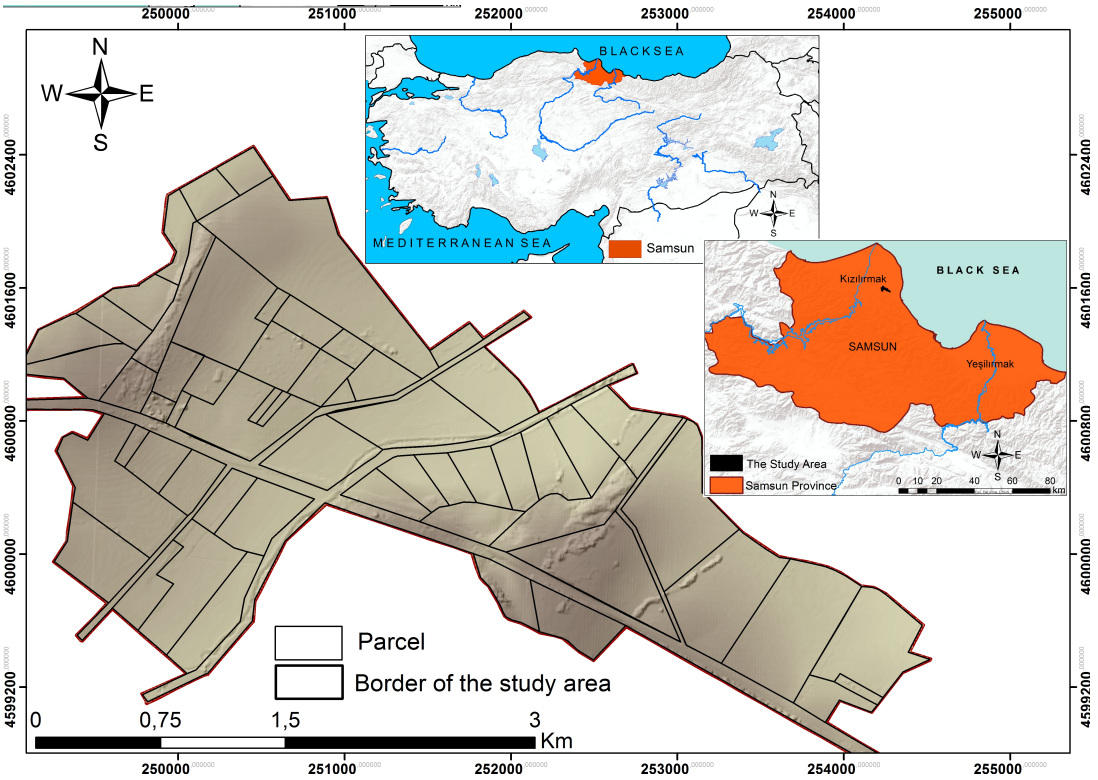

Figure 1. Location map of Yeşil Küre agricultural enterprise.

The height of the operation lands (Center and Düden) above sea level varies between 5 and 74 m. While the northwest and southeast parts of the land are areas where moderately steep and steep slopes are distributed in terms of slope, generally, the middle and northwest parts of the land constitute areas with 0-4%, nearly flat and mild slopes (Figure 2).

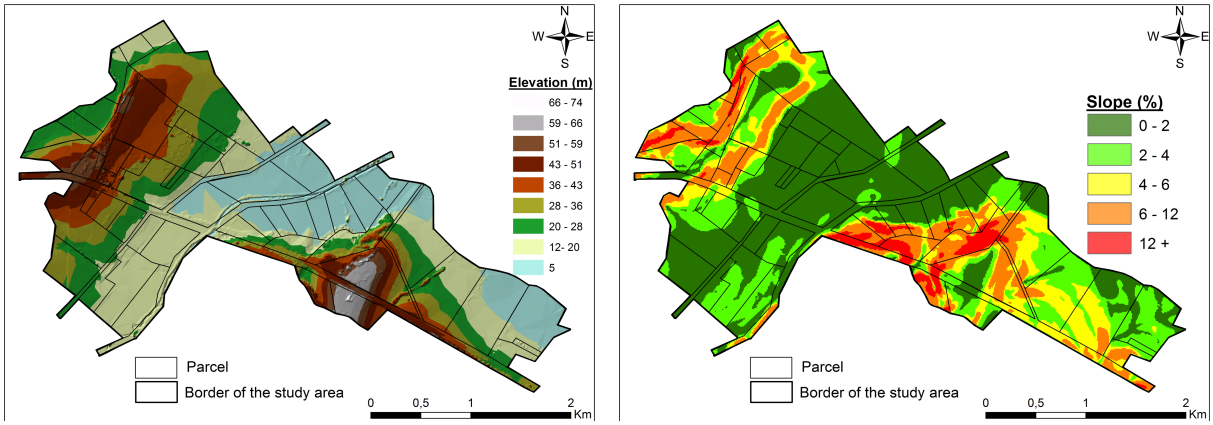

Figure 2. Elevation and slope map of the study area.

The widest coastal plains of the Black Sea Region are Çarşamba and Bafra plains. Yeşil Küre Agricultural Enterprise is also located on the Bafra Plain. The Black Sea climate, which is rainy in all seasons, cool in summers and warm in winters, is active on the coastline of the Black Sea Region. This effect of the climate extends to the inner parts depending on the landforms in the Central Black Sea Region. The annual average temperature of Yeşil Küre Agricultural Enterprise was 14.3˚C, the highest average air temperature was 18˚C and the lowest average temperature was 10.7˚C. The annual

precipitation average of the enterprise is around 710.0 mm. Snowfall in this region is less and does not last long. Snow does not stay for long in the coastal part. The coldest months in Bafra district are January, February and the hottest month is August.

The Bafra Plain, where the Yeşil Küre Agricultural Enterprise lands are located, is a wide delta plain formed by the rich alluvial soils brought by the Kızılırmak river. Bafra Plain is the largest plain of the Black Sea Region. Kızılırmak has formed many lakes near the sea. The area where the Agricultural Enterprise is located is covered by new Holocene alluvial deposits. In the central enterprise, there are high lands that emerged as a result of the erosion of these alluviums and carrying the eroded material (MTA, 1974). In addition, another series was added to the soil profiles in the updated study carried out in 2021 for the series classified as vertisol, mollisol, and entisol in the soil mapping study of the area carried out in 2005 by the General Directorate of Agricultural Enterprises, and the Yörükler series, which was classified as mollisol, was included in the entisol order due to losing its mollic feature as a result of intensive agricultural applications. (Figure 3).

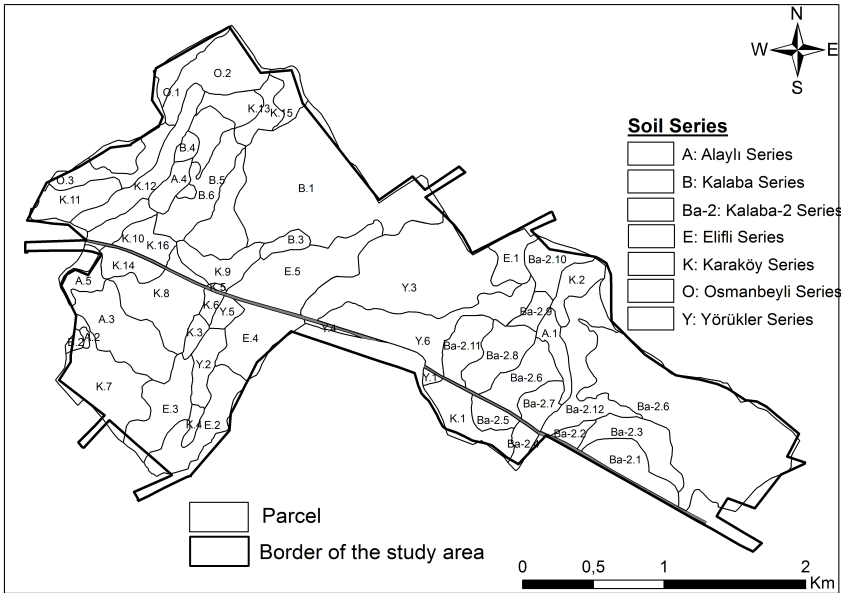

Figure 3. Updated soil map of the study area.

Almost all of the central part of the study area, called "Hara", has been cultured. Corn, wheat, alfalfa, and garlic are grown extensively. In addition, hazelnut and blackberry are planted. Rice farming is carried out in the farm area called Düden, located around Balık Lake in the Bafra delta. There are also oak, pine, poplar, maple, and elm trees, as well as ash, which are naturally found among dense rice fields. In Düden, natural living conditions prevail in the lower parts of the sea, where rice farming is not done. These regions are used as seasonal pastures by the local people. However, in periods when the rainfall is not heavy, cattle can be found in this region for grazing and sheltering.

Table 1. Characteristics of the Sentinel-2 satellite sensor

Band	Name	Wavelength (nm)	Band width (nm)	Spatial Resolution (m)
B1	**Coastal Aerosol**	443	20	60
B2	**Blue**	490	65	10
B3	**Green**	560	35	10
B4	**Red**	665	30	10
B5	**Vegetation Red-Edge**	705	15	20
B6	**Vegetation Red-Edge**	740	15	20
B7	**Vegetation Red-Edge**	783	20	20
B8	**Infrared**	842	115	10
B8A	**Vegetation Red-Edge**	865	20	20
B9	**Water vapor**	945	20	60
B10	**Cirrus**	1380	30	60
B11	**SWIR1**	1610	90	20
B12	**SWIR2**	2190	180	20

In this study, a total of 4 Sentinel-2 satellites images (with WGS84 datum) dated May, June, July and August 2021 of the Yeşil Küre Agricultural Enterprise lands on which various cultural plants are grown were used (Figure 4). Satellite images were obtained from the United States Geological Survey (USGS) open access center (https://earthexplorer.usgs.gov/). Multispectral Sentinel-2 satellite images have a spatial resolution of 10 m in 4 bands, 20 m in 6 bands, and 60 m in the other 3 bands (Table 1).

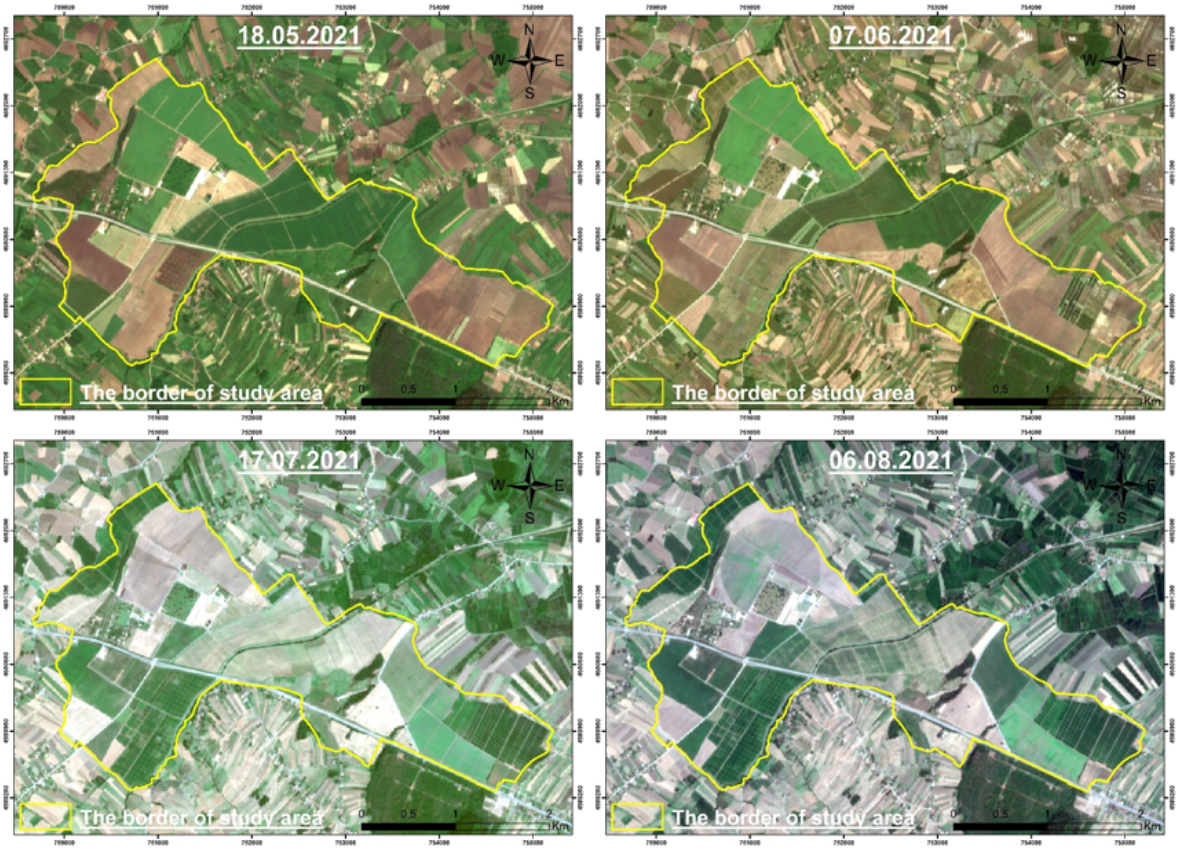

Figure 4. Sentenal-2 satellite images of different months.

2.1. Method

2.1.1. Creation of rice land suitability classes and index values

Soil series and their important phases were used as the mapping unit in the creation of the 1:25,000 scale basic soil map of the Yeşil Küre Agricultural Enterprise. Soil Survey Staff (1962) and Özbek et al., (1974) were used to classify important criteria such as depth, slope, salinity, alkalinity, drainage, and stoniness observed in the separation of soils into phases. Then, these criteria of the study area and topographic data were used to create the rice land suitability classes and index values. The determination of rice land suitability index values in terms of topographic and soil physico-chemical criteria has been handled by many researchers (FAO, 1983 and 1985; Sys et al., 1993; Yamada et al., 1995; Sönmez, 2003; Özcan, 2004; Mongkolsawat et al., 2002; Dengiz, 2013). These criteria used to determine the rice land suitability indices were obtained by multiplying the quality index value (SQI) (Equation 1) of the rice land soils belonging to each soil series with the nutrient availability index value (NAI) (Equation 2) and (Equation 3).

$$SQI: R * T * D * F * Y * P * G * S * K * H \quad (1)$$

R: Drainage, T: Texture, D: Depth, T: Topography, Y: Surface stony, P: Cream layer, G: Hydraulic conductivity, S: Salinity, K: Lime, H: Soil reaction.

$$NAI = N * P * K * Zn \quad (2)$$

NAI: Nutrient availability index.

$$LSC = NAI\ SQI \quad (3)$$

LSC: Land suitability class, NAI: Nutrient availability index, SQI*: Soil quality index.

Table 2. Rice suitability classes and index values

Statement	Suitability Classes	Index Value
Very suitable	S1	1.00-0.250
Moderate Suitable	S2	0.250-0.100
Low Suitable	S3	0.100-0.025
Not Suitable	N	< 0.025

2.1.2. Vegetation indexes

In this study, the vegetation indices of NDVI and RE-OSAVI, which are chlorophyll sensitive indices, were applied on the different time series Sentinel-2 satellite images of the rice fields of the Yeşil Küre Agricultural Enterprise by using the SNAP 8.0 tool and ArcGIS 10.7 environment. Information on both indexes is presented below.

2.1.2.1. Normalized difference vegetation index (NDVI)

There are many methods used in the identification of herbal product phenology. NDVI is the most widely used among these methods (Matton, et al., 2015). There have been many studies emphasizing the importance (Pettorelli et al., 2005a) of the role of satellite images in ecology, especially on the Normalized Difference Vegetation Index (NDVI) (Kerr and Ostrovsky, 2003; Damian, et al., 2019; Vorobiova and Chernov, 2017). As a matter of fact, Sahararini, et al. (2020) reported that the NDVI algorithm can be used as a base for processes such as image processing in Sentinel-2 satellite data, determination of rice phenology, estimation of rice harvest time. NDVI is used by many national and international organizations in many countries as an indicator of product yield. NDVI provides information with values ranging from -1 to +1 on subjects such as the spatial and temporal distribution of plant communities, plant biomass, CO_2 flows, pasture quality, and land degradation in various ecosystems (Salinas-Zavala et al., 2002; Al-Bakri et al., 2004; Pettorelli et al., 2005; Jiang et al., 2021).

NDVI values approaching -1 on the specified scale correspond to water bodies, while values approaching 0 indicate the presence of bare lands, rocky areas, sand, snow, or residential areas. NDVI values ranging from 0.2 to 0.4 correspond to bush or pasture areas, while this value approaching +1 indicates the presence of temperate regions, tropical rainforests, or areas with healthy and dense vegetation. With NDVI, it helps users in subjects such as the absorption of red wavelength energy within the visible region on the electromagnetic spectrum by the chlorophyll of the plant and the reflection of infrared (IR) energy by the plant cell structure, determination of biomass, and monitoring of changes in product development and production. By using NDVI in different time series satellite images, different growth periods of the plant, such as tillering, rooting, and harvesting, can be observed (Hufkens et al., 2019). In the Sentinel-2 satellite image, NDVI is calculated by using the red (B4) and infrared (B8) bands (Equation 4). The NDVI class values used in the estimation of the amount of product to be obtained from rice plants are given in Table 3.

$$NDVI = \frac{NIR - RED}{NIR + RED} \quad (4)$$

Table 3. NDVI values used in the definition of rice phenology (Pradipta, 2012).

NDVI values	Vegetation density	Age of Rice Plant (number of weeks after planting)
-0.096-0.036	No vegetation/bare/water	<3
0.036-0.240	Very low	3 - 4
0.240-0.456	low	4-6
0.456-0.652	Middle	6-8
0.652-0.884	high	8-13

2.1.2.2. Rededge optimized soil adjusted vegetation index (RE-OSAVI)

RE-OSAVI is an updated version of the Soil Vegetation Index (SAVI) family developed by Rondeaux et, al., (1996). The Optimized SAVI (OSAVI) model (RE-OSAVI) has been updated with the addition of the red edge (705 nm) band instead of the red band (670 nm) to minimize the effect of the submass on the red wavelength spectral reflections and make it more sensitive to the green field. It has been reported that this index can be used especially in periods when the vegetation density of plants is low (Wu et al., 2008). RE-OSAVI is calculated using the following formula (Equation 5).

$$\mathrm{RE-OSAVI} = (1 + 0.16) \times [(\mathrm{NIR} - \mathrm{REdge})/(\mathrm{NIR} + \mathrm{REdge} + 0.16)] \quad (5)$$

Vegetation indices (NDVI, RE-OSAVI) were calculated using the ESA-SNAP 8.0 tool in Sentinel-2 satellite images of the rice fields of the Yeşi Küre Farm Land.

2.1.3. Production of thematic maps and spatial statistical analysis in GIS environment

NDVI and RE-OSAVI vegetation index values were derived from each satellite image on a pixel basis, and maximum (S1), average (S2, N), and minimum (S3) NDVI and RE-OSAVI values were obtained for each rice suitability class. Thus, index values representing each parcel belonging to different rice land suitability classes were created. ArcGIS 10.7v was developed by ESRI (2010), for this purpose. Using the Zonal Statistics tool in the Spatial Analysis extension of the software, the index values reserved for different plots were extracted and transferred to MS Excel. Then, all indices' values were statistically compared with rice land suitability index (LSI) values, and regression relations (r^2) were determined.

3. Results and Discussion

3.1. Distribution of rice suitable areas

The areal and proportional distributions of rice suitability classes in the study area are given in Table 4, and its map is given in Figure 5. It was determined that 7205.5 da area, which is more than half of the study area (69.6%), is suitable for rice cultivation (S1 and S2), while 588.9 decares, which corresponds to 7.8% of the area, were found to be unsuitable for rice cultivation. In addition, depending on the limiting factor (slope, soil depth, texture, etc.) and grade of rice cultivation, less suitable (S3) classes cover 23.0% of the area.

Table 4. Spatial-proportional distributions of rice suitability classes

Statement	Suitability Classes	Area (da)	Ratio (%)
Suitable	S1	2964.4	37.6
Moderate Suitable	S2	2524.5	32.0
Low Suitable	S3	1816.3	23.0
Not Suitable	N	588.9	7.4
Total	-	7894.1	100.0

Areas that are not suitable for rice cultivation in the study area are generally the K12 and K11 mapping units of the Karaköy series, located in the northwest of the study area, and the B7, B8, B9, and B11 mapping units of the Kalaba series, which are distributed in the southeastern parts of the study area, especially the height and elevation in the slope grade. It has been determined that unsuitable areas are formed due to factors such as soil shallowness.

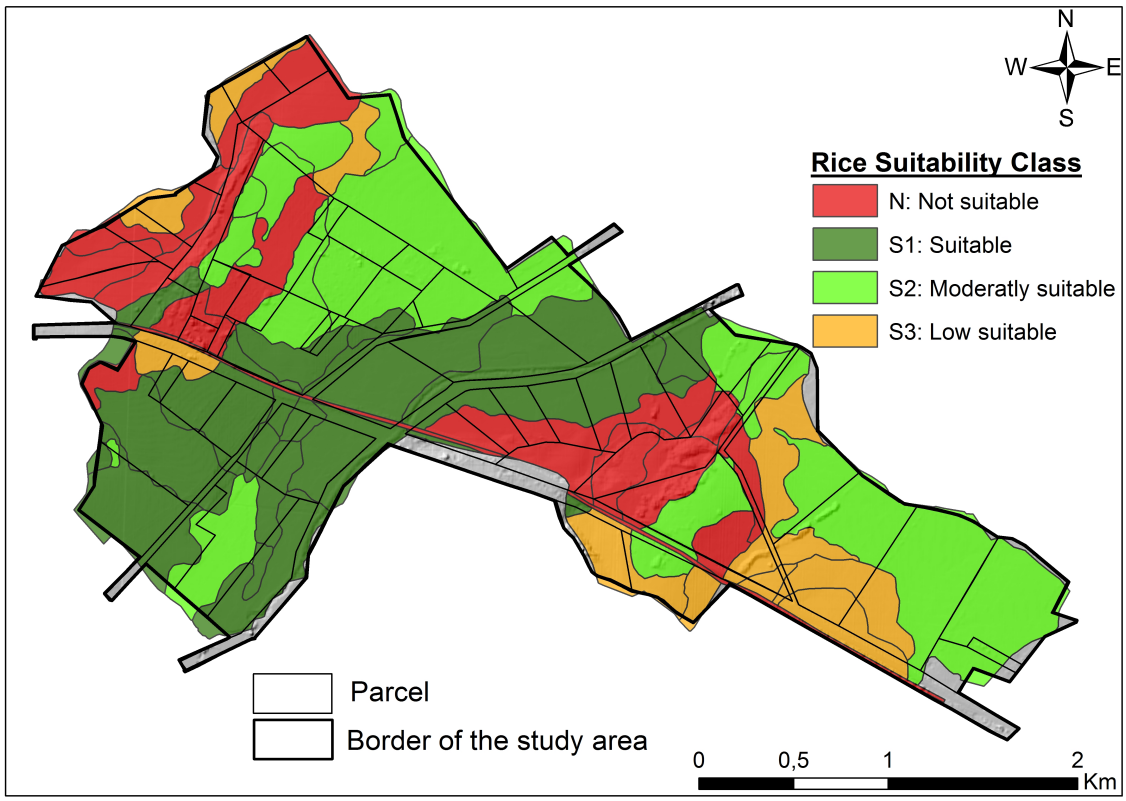

Figure 5. Rice suitability distribution map of the study area.

3.2. The relationship between rice suitability classes and vegetation indices

The land suitability index values for each land suitability class (S1, S2, S3, and N) of the rice fields obtained from the satellite images during May, June, July, and August dated May, June, July, and August of 2021, and the land suitability index values of the Sentinel-2A satellite images with different time series. The NDVI values and the r^2 values between which the statistical relationship was established are given in Table 5 and Figure 6, respectively. When the statistical relationship between the NDVI values obtained from the satellite image of May and the rice suitability index classes (S1, S2, S3, and N) is examined, the r^2 values are 0.7583, 0.8878, 0.8212, and 0.992, respectively; The r^2 values of the statistical relationship between the NDVI values obtained from the satellite image of June and the rice suitability index classes (S1, S2, S3, and N) were determined as 0.795, 0.873, 0.4725 and 0.9274, respectively.

In addition, the r^2 values of the statistical relationship between the NDVI values obtained from the July satellite image and the rice suitability index classes (S1, S2, S3, and N) were determined as 0.8396, 0.8833, 0.8879, and 0.9135, respectively, and finally, obtained from the August satellite image. When the statistical relationship between the obtained NDVI values and the rice suitability index classes (S1, S2, S3, and N) was examined, the r^2 values were found to be 0.858, 0.898, 0.8394, and 0.8924, respectively.

It should not be ignored that data variability may be caused by different cultural practices (fertilization, irrigation, soil cultivation, hoeing, etc.), such as the r^2 values of which the statistical relationship between the individual NDVI values of the land suitability classes and the ones belonging to the S3 suitability class are higher than the r^2 values of the S1 suitability class. (Dedeoğlu, et al., 2020).

Table 5. Each land suitability index values of rice fields and NDVI values of Sentinel-2A satellite images with different time series

Mapping Unit	Land Suitability Index	Land Suitability Class	NDVI Values			
			18.05.2021 Sentinel-2A	07.06.2021 Sentinel-2A	17.07.2021 Sentinel-2A	06.08.2021 Sentinel-2A
B11	0.02	N	0.6305	0.3091	0.2132	0.1530
B7	0.02	N	0.7319	0.5377	0.5690	0.4742
B8	0.016	N	0.6916	0.2862	0.4149	0.4987
B9	0.02	N	0.7359	0.7263	0.3275	0.3910
K11	0.016	N	0.5512	0.4934	0.3717	0.4185
K12	0.02	N	0.7368	0.5280	0.5277	0.4237
YOL	--	--	--	--	--	--
A1	0.4	S1	0.8119	0.8233	0.8135	0.8203
Ba.2	0.32	S1	0.7416	0.7217	0.6324	0.5041
Ba.4-A4	1	S1	0.6086	0.6348	0.7634	0.8094
Ba3	0.64	S1	0.8059	0.7391	0.6923	0.7842
E2	0.64	S1	0.5477	0.5507	0.6835	0.7711
E4-Y3	1	S1	0.7583	0.7677	0.7614	0.7795
E5	0.64	S1	0.7501	0.7662	0.7530	0.7753
K10	0.256	S1	0.7043	0.7114	0.8114	0.8093
K3	0.256	S1	0.7723	0.8009	0.8109	0.8050
K3	0.625	S1	0.7828	0.7541	0.8081	0.8037
K6	0.4	S1	0.8459	0.8108	0.7922	0.7317
K7	0.32	S1	0.7860	0.7860	0.7455	0.7968
K8	0.5	S1	0.7195	0.7766	0.7615	0.7950
K8	0.5	S1	0.8282	0.8011	0.7897	0.8043
K9	0.512	S1	0.8277	0.7542	0.7146	0.7198
Y1-E1	0.25	S1	0.8435	0.8053	0.7691	0.7305
Y2	0.4	S1	0.8481	0.8136	0.7547	0.7351
Y4-A3	0.205	S2	0.2462	0.3331	0.5227	0.6367
Y5	0.16	S2	0.4133	0.3386	0.2319	0.2064
A2	0.18	S2	0.6273	0.4220	0.2983	0.2493
A4	0.2	S2	0.5202	0.2388	0.5191	0.6134
A4	0.16	S2	0.7707	0.5536	0.2460	0.3673
A4	0.18	S2	0.7244	0.7342	0.2395	0.3238
B10	0.22	S2	0.7302	0.6552	0.1863	0.3435
B5-B9	0.16	S2	0.6897	0.5321	0.3012	0.3566
B6	0.25	S2	0.6195	0.5511	0.3192	0.3295
Ba.1	0.18	S2	0.7500	0.7136	0.1442	0.3505
Ba.4	0.2	S2	0.5989	0.5520	0.3741	0.4768
E3	0.14	S2	0.7540	0.7198	0.1325	0.4039
E3	0.25	S2	0.5630	0.5738	0.3178	0.3148
K2	0.128	S2	0.7534	0.7182	0.1519	0.3046
K2	0.1	S3	0.1545	0.1746	0.2079	0.1565
K2	0.08	S3	0.1216	0.1179	0.0786	0.0579
B1	0.08	S3	0.1430	0.1403	0.1470	0.1236
B2	0.054	S3	0.1237	0.1312	0.1880	0.1586
B3	0.042	S3	0.1402	0.1308	0.1027	0.1011
K1	0.05	S3	-0.0218	-0.2451	0.1350	0.1657
O1	0.051	S3	0.1344	0.1119	0.1129	0.1728
O2	0.09	S3	0.1067	0.1851	0.1123	0.1321
B12	0.05	S3	0.1492	0.1322	0.0876	0.0906

A*: Alaylı, B*: Kalaba, E*: Elifli, K*: Karaköy, O*: Osmanbeyli, Y*: Yörükler.

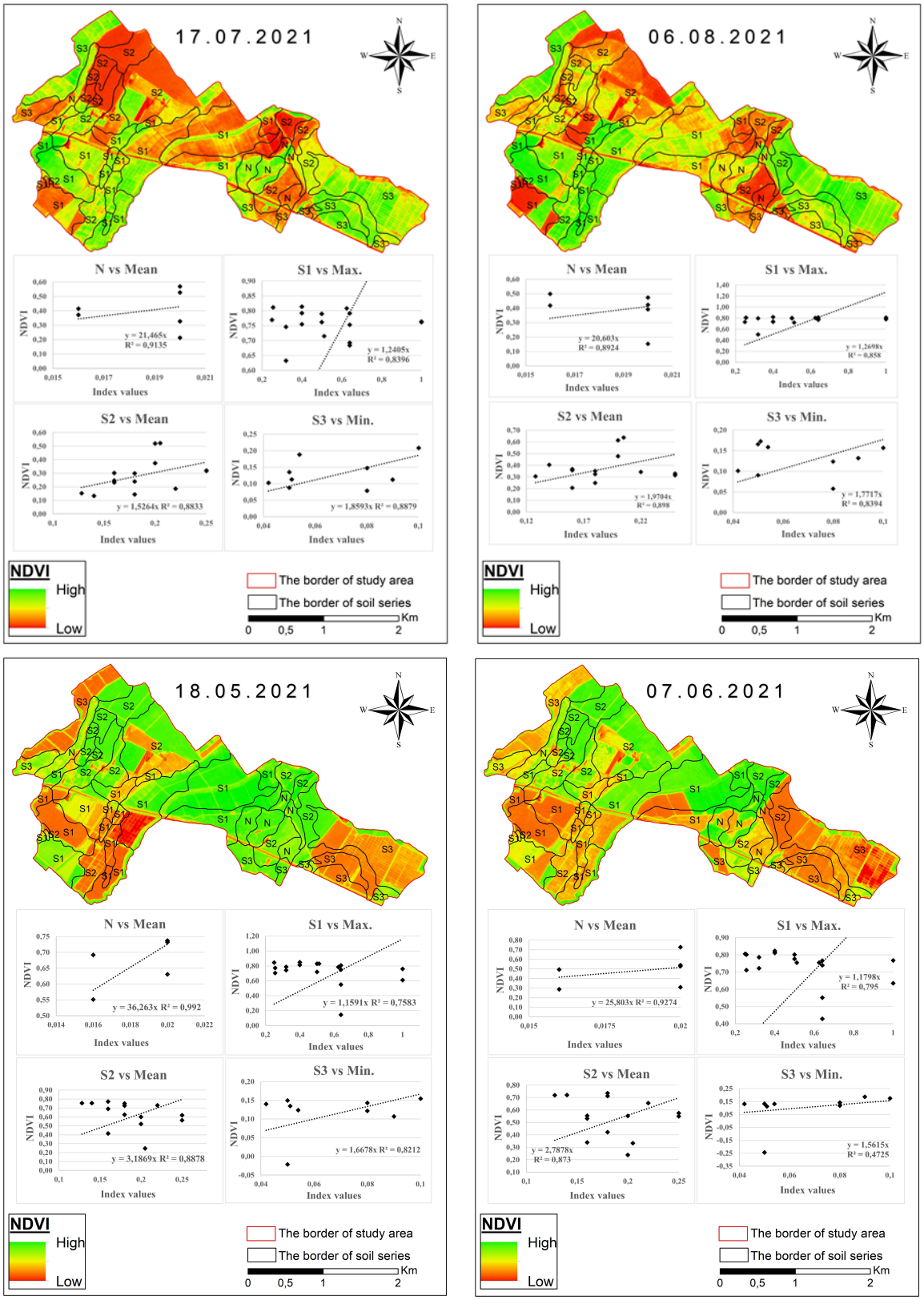

Figure 6. r2 values of each land suitability index value of rice fields and NDVI values obtained from Sentinel-2A satellite images with different time series.

The r^2 values, in which the statistical relationship is established between the NDVI values obtained from the May, June, July, and August satellite images of the Yeşil Küre Farm Land Enterprise, dated 2021, without evaluating the land suitability classes (S1, S2, S3, and N) separately, are shown in

Figure 7 and Table 6, respectively. In the evaluation of the statistical relationship between the land suitability classes (S1, S2, S3, and N) and the NDVI values without evaluating them separately, the r^2 values for July and August, which were determined to be high, were determined as 0.5707 and 0.6321, respectively, according to the r^2 results discussed. According to the r^2 results, which were considered in the evaluation of the statistical relationship between the NDVI values without evaluating the land suitability classes (S1, S2, S3, and N) separately, the r^2 values for the months of May and June, which were determined as low, were determined as 0.3341 and 0.4297, respectively.

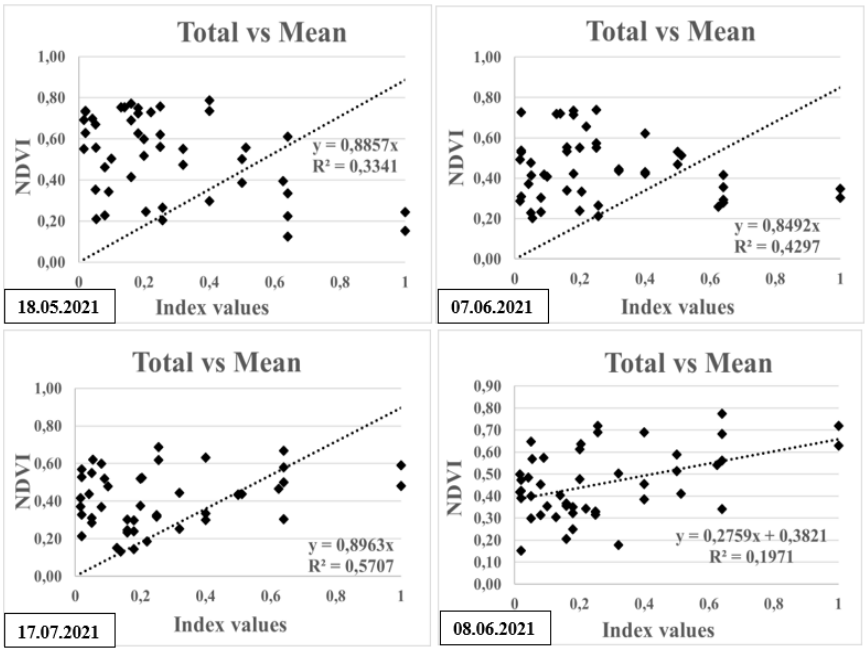

Figure 7. Land suitability index values of rice fields and r^2 values of NDVI values obtained from Sentinel-2A satellite images with different time series.

The highest r^2 values for NDVI were obtained in satellite images of July and August. This situation was associated with the fact that the plant vegetation density of the rice fields reached its maximum value within a month from mid-July. The high NDVI values of the July and August satellite images may also be associated with the shade forming feature of the rice plant (structural or vegetation cover percentage). The abundance of the stem and leaves of the rice plant reduces the reflectance effects of the plant substrate, and thus the enhanced vegetation of the rice allows more reliable estimates of the NDVI reflectance values, an index based on slope (Panda et al., 2010; Mirasi et al., 2019). As a matter of fact, Verhulst et al. (2009) reported that NDVI vegetation indices have a strong correlation with plant physiological parameters, crop yield and biomass.

The lowest r^2 values for NDVI were obtained in satellite images of May and June. In this period (May-June), the rice plant is in tillering period and tillering is one of the basic rice growth stages that determines the final yield. During the first six months after the start of the rice season (until the end of June), it is known that there is almost no correlation between the spectral reflectance values and the AUI values, considering the short height of the rice plant and the fact that it is a plant that grows constantly under water (Franch et al., 2021). This is considered as an important reason why the relationship between NDVI values and AUI values of satellite images in May and June is lower than in July and August.

Table 6. Land suitability index values of rice lands of Yeşil Küre Agricultural Enterprise and NDVI values of different time series satellite images

		NDVI Values			
Mapping Unit	Land Suitability Index	18.05.2021 Sentinel-2A	07.06.2021 Sentinel-2A	17.07.2021 Sentinel-2A	06.08.2021 Sentinel-2A
B11	0.02	0.6305	0.3091	0.2132	0.1530
B7	0.02	0.7319	0.5377	0.5690	0.4742
B8	0.016	0.6916	0.2862	0.4149	0.4987
B9	0.02	0.7359	0.7263	0.3275	0.3910
K11	0.016	0.5512	0.4934	0.3717	0.4185
K12	0.02	0.7368	0.5280	0.5277	0.4237
YOL	--	--	--	--	--
A1	0.4	0.8119	0.8233	0.8135	0.8203
Ba.2	0.32	0.7416	0.7217	0.6324	0.5041
Ba.4-A4	1	0.6086	0.6348	0.7634	0.8094
Ba3	0.64	0.8059	0.7391	0.6923	0.7842
E2	0.64	0.5477	0.5507	0.6835	0.7711
E4-Y3	1	0.7583	0.7677	0.7614	0.7795
E5	0.64	0.7501	0.7662	0.7530	0.7753
K10	0.256	0.7043	0.7114	0.8114	0.8093
K3	0.256	0.7723	0.8009	0.8109	0.8050
K3	0.625	0.7828	0.7541	0.8081	0.8037
K6	0.4	0.8459	0.8108	0.7922	0.7317
K7	0.32	0.7860	0.7860	0.7455	0.7968
K8	0.5	0.7195	0.7766	0.7615	0.7950
K8	0.5	0.8282	0.8011	0.7897	0.8043
K9	0.512	0.8277	0.7542	0.7146	0.7198
Y1-E1	0.25	0.8435	0.8053	0.7691	0.7305
Y2	0.4	0.8481	0.8136	0.7547	0.7351
Y4-A3	0.205	0.2462	0.3331	0.5227	0.6367
Y5	0.16	0.4133	0.3386	0.2319	0.2064
A2	0.18	0.6273	0.4220	0.2983	0.2493
A4	0.2	0.5202	0.2388	0.5191	0.6134
A4	0.16	0.7707	0.5536	0.2460	0.3673
A4	0.18	0.7244	0.7342	0.2395	0.3238
B10	0.22	0.7302	0.6552	0.1863	0.3435
B5-B9	0.16	0.6897	0.5321	0.3012	0.3566
B6	0.25	0.6195	0.5511	0.3192	0.3295
Ba.1	0.18	0.7500	0.7136	0.1442	0.3505
Ba.4-A4	0.2	0.5989	0.5520	0.3741	0.4768
E3	0.14	0.7540	0.7198	0.1325	0.4039
E3	0.25	0.5630	0.5738	0.3178	0.3148
K2	0.128	0.7534	0.7182	0.1519	0.3046
K2	0.1	0.1545	0.1746	0.2079	0.1565
K2	0.08	0.1216	0.1179	0.0786	0.0579
B1	0.08	0.1430	0.1403	0.1470	0.1236
B2	0.054	0.1237	0.1312	0.1880	0.1586
B3	0.042	0.1402	0.1308	0.1027	0.1011
K1	0.05	-0.0218	-0.2451	0.1350	0.1657
O1	0.051	0.1344	0.1119	0.1129	0.1728
O	0.09	0.1067	0.1851	0.1123	0.1321
B	0.05	0.1492	0.1322	0.0876	0.0906

A*: Alaylı, B*: Kalaba, E*: Elifli, K*: Karaköy, O*: Osmanbeyli, Y*: Yörükler.

RE-OSAVI is a plant index that functions with the Red-Edge spectral reflectance band, which is stated to be able to reduce the spectral reflectance values of plants and the substrate reflection effects (Feng et al., 2014), and has a widespread and reliable use in the determination of plant biomass (Fitzgerald et al. et al., 2010). There is a strong correlation between RE-OSAVI and plant chlorophyll content, and the RE-OSAVI index is used in plant yield potential estimation studies (Huang et al., 2017).

The land suitability index values for each land suitability class (S1, S2, S3, and N) of the rice fields obtained from the satellite images of May, June, July, and August dated May, June, July, and August 2021 of Yeşil Küre Agricultural Enterprise and different time series Sentinel-2A satellite images were obtained. The RE-OSAVI values obtained and the r^2 values with which the statistical relationship was established are given in Table 7 and Figure 8, respectively. When the statistical relationship between the RE-OSAVI values obtained from the satellite image of May and the rice suitability index classes (S1, S2, S3, and N) was examined, the r^2 values were found to be 0.7167, 0.9135, 0.0265 and 0.9877, respectively, while the RE-OSAVI values obtained from the satellite image of June were examined. - The r^2 values determined as a result of the statistical relationship between the OSAVI values and the

rice suitability index classes (S1, S2, S3, and N) were determined to be 0.795, 0.873, 0.4725, and 0.9274, respectively.

In addition, the r^2 values obtained as a result of the statistical relationship between the RE-OSAVI values obtained from the satellite image of July and the rice suitability index classes (S1, S2, S3, and N) were 0.8465, 0.915, 0.789, and 0.8872, respectively. When the statistical relationship between the RE-OSAVI values obtained from the satellite image of the rice plant and the rice suitability index classes (S1, S2, S3, and N) was examined, the r^2 values were determined as 0.8162, 0.8907, 0.7987 and 0.9166, respectively.

Table 7. Each land suitability index values of rice fields and RE-OSAVI values of Sentinel-2A satellite images with different time series

Mapping Unit	Land Suitability Index	Land Suitability Class	RE-OSAVI Values			
			18.05.2021 Sentinel-2A	07.06.2021 Sentinel-2A	17.07.2021 Sentinel-2A	06.08.2021 Sentinel-2A
B11	0.02	N	0.5221	0.2285	0.1982	0.1449
B7	0.02	N	0.6483	0.4863	0.5277	0.4361
B8	0.016	N	0.6105	0.2657	0.3853	0.4832
B9	0.02	N	0.6705	0.6843	0.3060	0.3644
K11	0.016	N	0.4682	0.3984	0.3331	0.3746
K12	0.02	N	0.6448	0.4629	0.4619	0.3645
YOL	--	--	--	--	--	--
A1	0.4	S1	0.7384	0.7729	0.7637	0.8375
Ba.2	0.32	S1	0.6684	0.6188	0.6092	0.4404
Ba.4-A4	1	S1	0.5071	0.5591	0.7191	0.8104
Ba3	0.64	S1	0.7445	0.6640	0.6096	0.8017
E2	0.64	S1	0.4385	0.4156	0.5915	0.7111
E4-Y3	1	S1	0.5760	0.6352	0.6285	0.7441
E5	0.64	S1	0.5945	0.6507	0.6071	0.7379
K10	0.256	S1	0.6130	0.5823	0.7785	0.8278
K3	0.256	S1	0.7377	0.7229	0.7868	0.8221
K3	0.625	S1	0.7234	0.6704	0.7734	0.8124
K6	0.4	S1	0.8327	0.7623	0.7033	0.7180
K7	0.32	S1	0.6841	0.7069	0.7229	0.8297
K8	0.5	S1	0.5838	0.7057	0.7466	0.8138
K8	0.5	S1	0.8099	0.7644	0.7675	0.8231
K9	0.512	S1	0.7576	0.6745	0.6849	0.6458
Y1-E1	0.25	S1	0.8308	0.7586	0.7459	0.7176
Y2	0.4	S1	0.8379	0.7521	0.6801	0.7212
Y4-A3	0.205	S2	0.2286	0.2368	0.4463	0.5875
Y5	0.16	S2	0.3517	0.2639	0.1922	0.2002
A2	0.18	S2	0.5489	0.3448	0.2783	0.2354
A4	0.2	S2	0.4573	0.1898	0.4607	0.5657
A4	0.16	S2	0.7066	0.5228	0.2296	0.3397
A4	0.18	S2	0.6465	0.6719	0.2332	0.3003
B10	0.22	S2	0.6243	0.5178	0.1776	0.3186
B5-B9	0.16	S2	0.5771	0.4203	0.2798	0.3265
B6	0.25	S2	0.5282	0.4482	0.2896	0.3067
Ba.1	0.18	S2	0.6251	0.5459	0.1576	0.3322
Ba.4-A4	0.2	S2	0.5132	0.4543	0.3497	0.4378
E3	0.14	S2	0.6186	0.5460	0.1361	0.3606
E3	0.25	S2	0.4915	0.4749	0.2878	0.2954
K2	0.128	S2	0.6493	0.5729	0.1495	0.2804
K2	0.1	S3	0.1098	0.1679	0.1781	0.1727
K2	0.08	S3	-0.0444	0.1081	0.1303	0.1106
B1	0.08	S3	0.1252	0.1149	0.1291	0.1524
B2	0.054	S3	-0.0394	-0.0505	0.1930	0.1510
B3	0.042	S3	0.1322	0.0605	0.0972	0.1057
K1	0.05	S3	-0.0917	-0.3849	0.0779	0.1583
O1	0.051	S3	0.0991	0.0773	0.1214	0.1965
O2	0.09	S3	0.0479	0.0108	0.0840	0.1268
B12	0.05	S3	0.1393	0.1171	0.0863	0.0803

A*: Alaylı, B*: Kalaba, E*: Elifli, K*: Karaköy, O*: Osmanbeyli, Y*: Yörükler.

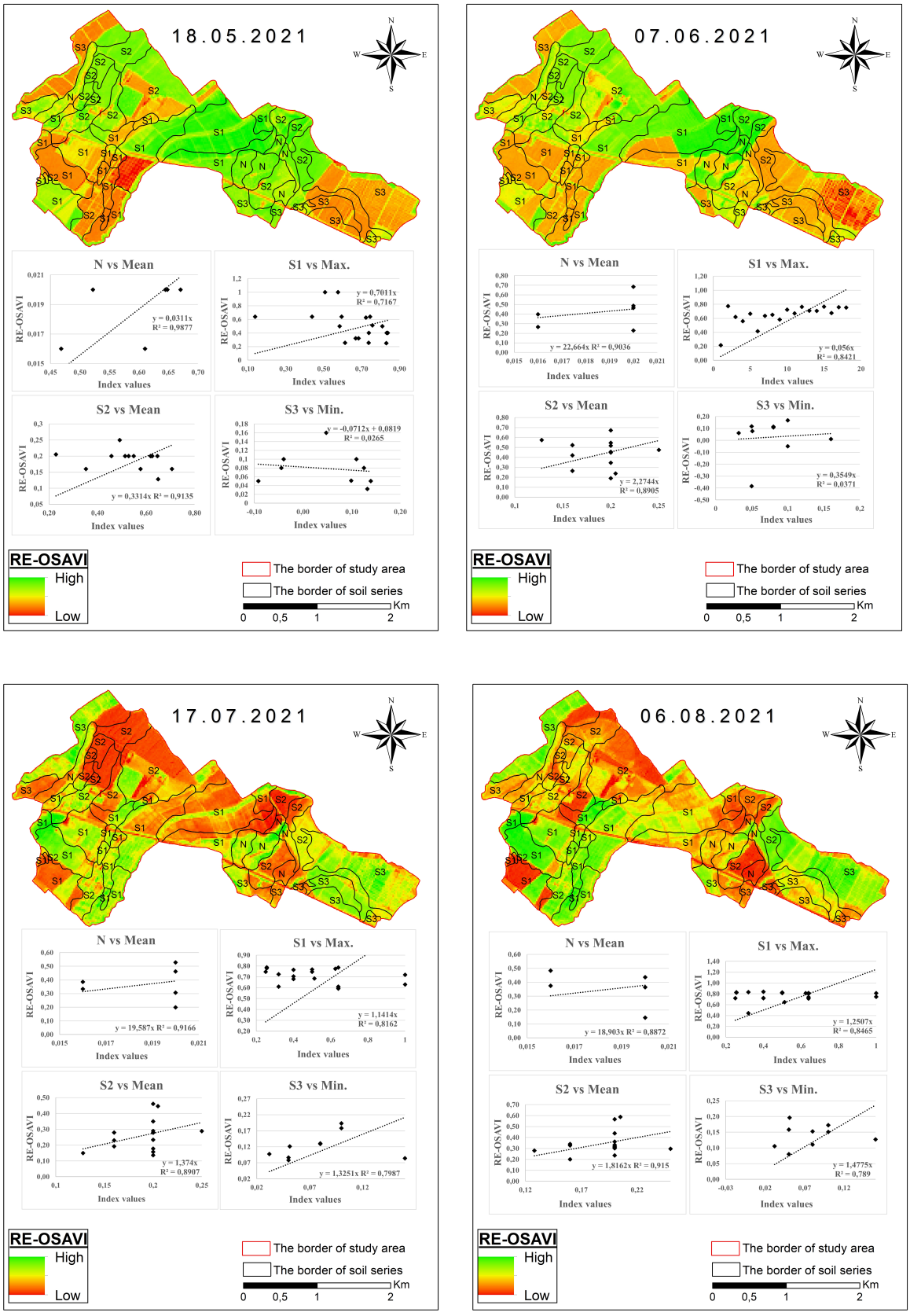

Figure 8. The r^2 values of each land suitability index value of rice fields and RE-OSAVI values obtained from Sentinel-2A satellite images with different time series.

It is thought that the low results of RE-OSAVI values in May and June are due to the fact that this index was calculated to represent the soil series instead of establishing a relationship with the rice land suitability index values. It is also predicted that the effects of climatic and atmospheric conditions may have reduced the vegetation index prediction values. Such problems show the fragile side of using remote sensing for agricultural purposes, especially when the database is limited. For this reason, it is predicted that in future similar studies with spectral vegetation indices, more successful results will be obtained when soil series and reference plant plots of similar size are used (Dedeoğlu et al., 2020).

The r^2 values, in which the statistical relationship is established between the RE-OSAVI values obtained from the satellite images of May, June, July, and August of 2021 dated May, June, July, and August belonging to the Yeşil Küre Farm Land and the land suitability classes (S1, S2, S3, and N) without evaluating them separately, are shown in Figure 9, respectively, and are given in Table 8. According to the r^2 results considered in the statistical evaluation between the RE-OSAVI values without evaluating the land suitability classes separately, the relationship between the RE-OSAVI values obtained from the satellite images of July and August, which were determined to be high, and the suitability index classes (S1, S2, S3, and N). The r^2 values were determined as 0.5732 and 0.6508, respectively. According to the r^2 results, in which the statistical relationship between the land suitability classes (S1, S2, S3, and N) and the RE-OSAVI values was evaluated without evaluating them separately, the r^2 values for the months of May and June, which were determined to be low, were determined as 0.3433 and 0.4012, respectively. According to these results, it was seen that the use of RE-OSAVI index of rice plants in late vegetation periods is suitable for estimating soil fertility potential, but not in early vegetation periods.

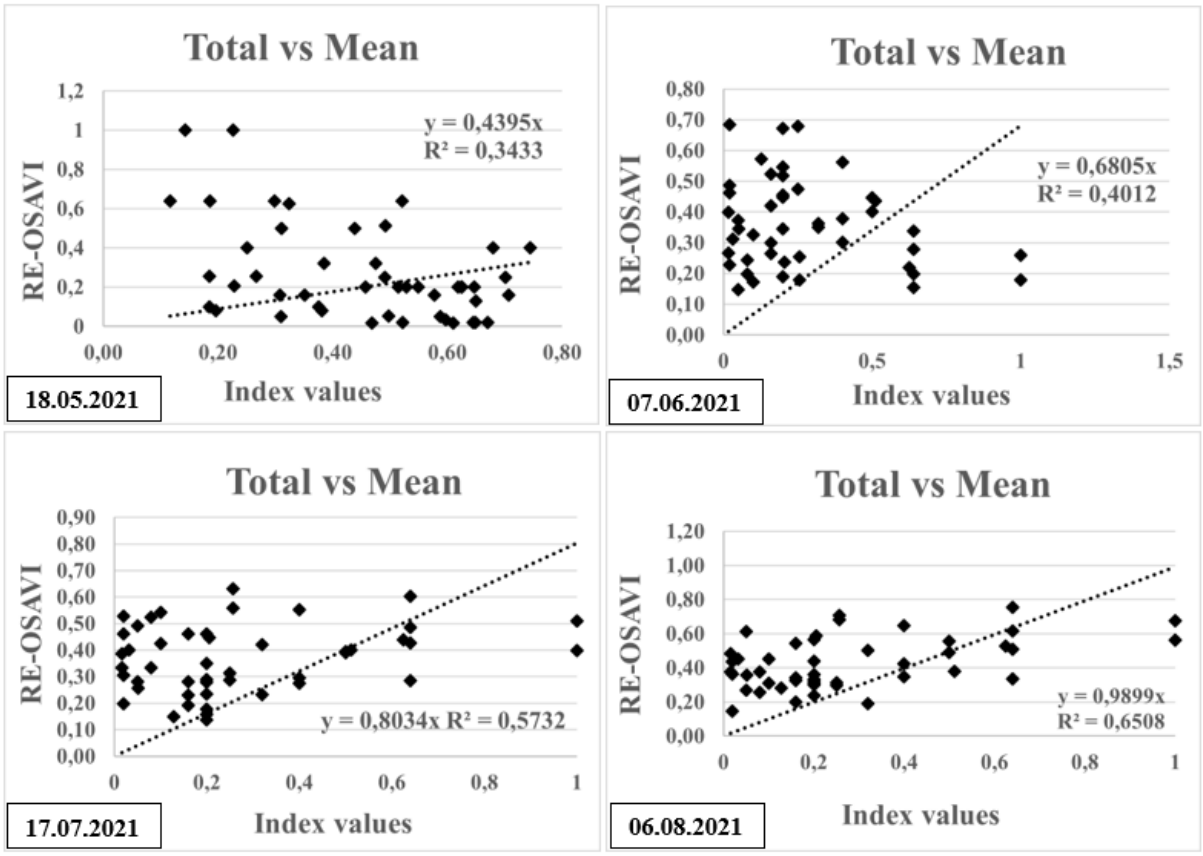

Figure 9. Land suitability index values of rice fields and r^2 values of RE-OSAVI values obtained from Sentinel-2A satellite images with different time series

Table 8. Land suitability index values of rice fields and RE-OSAVI values of different time series satellite images

Mapping Unit	Land Suitability Index	RE-OSAVI Values			
		18.05.2021 Sentinel-2A	07.06.2021 Sentinel-2A	17.07.2021 Sentinel-2A	06.08.2021 Sentinel-2A
B11	0.02	0.5221	0.2285	0.1982	0.1449
B7	0.02	0.6483	0.4863	0.5277	0.4361
B8	0.016	0.6105	0.2657	0.3853	0.4832
B9	0.02	0.6705	0.6843	0.3060	0.3644
K11	0.016	0.4682	0.3984	0.3331	0.3746
K12	0.02	0.6448	0.4629	0.4619	0.3645
YOL	0.64	0.1168	0.1538	0.6020	0.7552
YOL-A1	0.4	0.2509	0.3008	0.5523	0.6456
Ba.2	0.32	0.4750	0.3618	0.2315	0.1909
Ba.4-A4-Ba	1	0.1422	0.1780	0.5091	0.6747
Ba3	0.64	0.5209	0.3372	0.2846	0.3347
E2	0.64	0.1858	0.1985	0.4855	0.6155
E4-Y3	1	0.2269	0.2587	0.3978	0.5633
E5	0.64	0.2985	0.2786	0.4270	0.5084
K10	0.256	0.1849	0.1792	0.5578	0.6803
K3	0.256	0.2660	0.2547	0.6306	0.7064
K3	0.625	0.3239	0.2196	0.4388	0.5260
K6	0.4	0.6785	0.3789	0.2955	0.3482
K7	0.32	0.3849	0.3495	0.4209	0.5028
K8	0.5	0.3114	0.4007	0.3946	0.4903
K8	0.5	0.4389	0.4463	0.3908	0.5557
K9	0.512	0.4920	0.4367	0.3999	0.3794
Y1-E1	0.25	0.7007	0.6792	0.3129	0.3143
Y2	0.4	0.7449	0.5619	0.2740	0.4230
Y4-A3	0.205	0.2286	0.2368	0.4463	0.5875
Y5	0.16	0.3517	0.2639	0.1922	0.2002
A2	0.18	0.5489	0.3448	0.2783	0.2354
A4	0.2	0.4573	0.1898	0.4607	0.5657
A4	0.16	0.7066	0.5228	0.2296	0.3397
A4	0.18	0.6465	0.6719	0.2332	0.3003
B10	0.22	0.6243	0.5178	0.1776	0.3186
B5-B9	0.16	0.5771	0.4203	0.2798	0.3265
B6	0.25	0.5282	0.4482	0.2896	0.3067
Ba.1	0.18	0.6251	0.5459	0.1576	0.3322
Ba.4-A4-Ba	0.2	0.5132	0.4543	0.3497	0.4378
E3	0.14	0.6186	0.5460	0.1361	0.3606
E3	0.25	0.4915	0.4749	0.2878	0.2954
K2	0.128	0.6493	0.5729	0.1495	0.2804
K2	0.1	0.3755	0.3254	0.4239	0.3101
K2	0.08	0.1964	0.1973	0.5238	0.3752
B1	0.08	0.3802	0.2426	0.3338	0.2559
B2	0.054	0.1853	0.1723	0.5429	0.4509
B3	0.042	0.5964	0.3112	0.4003	0.4499
K1	0.05	0.3103	0.1475	0.4905	0.6109
O1	0.051	0.4975	0.3452	0.2557	0.3566
O	0.09	0.3069	0.2999	0.4610	0.5423
B	0.05	0.5876	0.3734	0.2812	0.2685

A*: Alaylı, B*: Kalaba, E*: Elifli, K*: Karaköy, O*: Osmanbeyli, Y*: Yörükler.

Conclusion

In this study, the statistical relationship between the land suitability indices determined for rice lands belonging to Yeşil Küre Agricultural Enterprise and the spectral vegetation index (NDVI and RE-OSAVI) values calculated by using different time series Sentinel-2A satellite images, and the estimation of the rice productivity potential. A different perspective is presented for viewing and monitoring. First of all, according to the rice conformity assessment study of the farm lands, 6488.9 ha area was determined to be suitable for rice cultivation at S1 and S2 levels, while the 588.9 ha area was determined to be unsuitable. In this study, it was observed that the most successful results for each land suitability class were obtained by using the NDVI index. It has been determined that the RE-OSAVI index gives more accurate results in determining the sparse vegetation, and provides a limited opportunity to monitor cultivars grown in water-saturated land conditions.

The r^2 values, in which the statistical relationship between the rice land suitability index values values were revealed without evaluating the land suitability classes separately, showed that both indices

were not suitable for soil fertility potential estimations in early vegetation periods, but were suitable for soil fertility potential estimations in medium to long vegetation periods. It was concluded that the vegetation indices obtained from the satellite images of the different growth stages of the rice plant can be used as an alternative to determine the critical growth stages of the rice plant. With this study, it is predicted that the use of vegetation indices can help to monitor the rice plant at critical time stages and to intervene in time to possible problems, as well as to ensure the continuity of food safety by getting more yield from the rice plant with these interventions. In addition, this study showed that the spectral capabilities of Sentinel-2A satellite images can be used in similar studies to be done in the future with the ESA-SNAP image processing tool. As a result, this study using vegetation index values obtained from different time series satellite images covering different development stages of rice plants has created an awareness for future research in determining the potentials for the relationships between index values and rice soil quality.

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Research article

Plant defense elicitor, 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl} phenol (DPMP) and its mode of action against fungal pathogen *Alternaria solani* in tomato (*Solanum lycopersicum* L.)

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Abstract: Biotic stress factors are one of the major constraints plants face, and they significantly affect production and yield. There are multiple ways to cope with stress factors, including genetic enhancement. When they cannot provide sufficient protection, pesticides are commonly applied. Plant defense elicitors are a new approach for boosting plants' natural immune responses and tolerance levels. The newly identified promising plant defense elicitor; 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl} phenol (DPMP) was previously studied against the oomycete *Hyaloperonospora arabidopsidis*, the bacterial pathogens *Pseudomonas syringae* and *Clavibacter michiganensis* ssp *michiganensis* and found to induce disease resistance against these phytopathogens. However, it was not tested against fungal pathogens. Here for the first time, DPMP was evaluated against one of the most destructive fungal pathogens, *Alternaria solani*. Disease severity and plant development were evaluated. The results revealed that DPMP neither inhibited nor enhanced the disease severity of *A. solani*. Gene expression of several salicylic acid, jasmonic acid, and ethylene pathway-related genes (*Pti4, TPK1b, Pto kinase, PRB1-2, SABP2*, and *PR3*) were also analyzed. According to the results, while DPMP induces *PRB1-2, TPK1b*, and *Pto kinase* gene expressions, the protection against *A. solani* does not occur via these genes. *PR3* is one of the most important genes for defense responses against necrotrophic pathogens, and DPMP downregulated gene expression of *PR*3. These results demonstrated that DPMP mostly takes a role through the SA-related defense pathway and was effective against biotrophic and hemibiotrophic pathogens. However, it is not suitable for protection against the necrotrophic pathogen *A. solani*. Further research may pinpoint the activity of DPMP on the defense pathway and provide a better understanding of the mode of action for DPMP and other plant elicitors for specific plant protection solutions.

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1. Introduction

Plants provide a rich source of nutrients for heterotrophic microorganisms and are, therefore, subject to attack by many pathogens. These attacks cause significant yield and quality losses in agriculture (Onaga and Wydra, 2016). Chemical substances called 'pesticides' are used against various pests to protect plants against pathogens and reverse the yield loss caused by biotic stress. Pesticides

can be classified as acaricides, insecticides, fungicides, herbicides, etc., according to the organism they target (Nicolopoulou-Stamati et al., 2016). Pesticides act directly against the target organism and aim to kill or stop the pest from spreading. Today, pesticides are used intensively and unconsciously in many agricultural fields. As a result of the intensive use of pesticides, the pesticide itself or its transformation products can remain in the food, soil, water, and air, which endangers all living organisms, especially humans (Pretty, 2008; Nicolopoulou-Stamati et al., 2016). The negative effects of pesticides on humans and the environment have been revealed extensively (Nicolopoulou-Stamati et al., 2016). The health problems and environmental pollution caused by the use of pesticides have forced many countries, especially the United Nations and non-governmental organizations, to take some precautions (Skevas et al., 2013). The high level of unwanted side effects of pesticides has led researchers to use plant activators as an alternative method for pest control (Walters et al., 2013; Villaverde et al., 2014).

Plant activators are stimulants that are given to the plant from the outside and strengthen the plant's natural immune system by stimulating and making it more resistant or tolerant against plant pests. While stimulants trigger the plant defense system, making the plant stronger against the pathogen, they do not have direct toxicity against the pathogen or other organisms (Bektas and Eulgem, 2015). For this reason, plant activators are used as an alternative to pesticides to eliminate the side effects of pesticides on the environment and other organisms. The "good agricultural practices" targets of the Ministry of Agriculture and Forestry of Turkey also support the use and formulation of products within this framework (Ministry of Agriculture and Forestry 2018-2022 Strategic Plan, 2018).

Various studies have been carried out since the 1970s regarding plant activators that stimulate the plant immune system, and some chemicals have been shown to stimulate it. Polyacrylamide acid compounds were tested against Tobacco Mosaic Virus (TMV) as plant activators and it was found to increase *PR1* gene expression (Gianinazzi and Kassanis, 1974). Since this first study, other synthetic chemicals have been shown to trigger the plant immune system over the years (Langcake and Wickins, 1975; Watanabe et al., 1977). Probenazole (PBZ), discovered in 1977, increased the defense response against *Magnaporthe oryzae* by activating enzymes related to plant immunity (Watanabe et al., 1977). In a study conducted by Ciba-Geigy (Syngenta) company in 1987, the contributions of 2,6-dichloro-isonicotinic acid (INA) and the later discovered acibenzolar-S-methyl (ASM/BTH) to plant protection were demonstrated (Metraux et al., 1990 and 1991; Ward et al., 1991; Uknes et al., 1992). BTH Bion® has been used as a pesticide for many years in many countries with this trademark. While plant activators can be synthetic, various studies have shown that pathogen-derived or plant-derived products have effects on the plant immune system (Kishimoto et al., 2006; Serrano et al., 2010).

Tomato (*Solanum lycopersicum* L.), a nutritious and delicious vegetable, is widely consumed in fresh or canned form and constitutes an important part of vegetable production. Pathogens emerging in tomato production areas can cause significant yield losses. As a result of these diseases and pests, growth retardation in the plant, deterioration of product quality, and death may occur (Foolad and Panthee, 2012). *Alternaria solani*, which is a necrotrophic fungal pathogen, causes early blight in tomatoes. It is an important disease agent that causes root rot and root collar blight, as well as early leaf blight (Rao et al., 2007; Ray et al., 2015). Early blight (EB) causes more than 10% yield loss in the world and Turkey (Boyno et al., 2020; Çevik et al., 2021). *A. solani* infects almost all Solanaceae members, including tomatoes and potatoes mostly through dead plant tissues. It has also been reported to infect vegetables, ornamental plants, and fruit species (apple, orange) (Foolad et al., 2008). EB is seen on the leaves, stems, and fruits of the tomatoes and causes severe damage throughout the season. The disease first appears in the field as small, irregular, brownish-black spots on old leaves. The spots take a round or elongated shape over time and look like intertwined rings, with the central part open (Adhikari et al., 2017). Diseased spots can spread to the whole fruit, and infected fruits are shed over time. Due to the ability of disease agents to survive under adverse conditions, there are difficulties in their control (Jindo et al., 2021). While cultural practices are important for the control of *A. solani*, prominent control is provided by chemical applications (Adhikari, et al., 2017).

Plant activators are powerful alternatives to the use of pesticides in agriculture. The introduction of plant activators in agriculture may contribute to the reduction of environmental pollution caused by the use of pesticides and their toxic effects on human health and will also be compatible with environmentally friendly agricultural policies implemented in the world and our country (Skevas et al., 2013; Nicolopoulou-Stamati et al., 2016; Ministry of Agriculture and Forestry 2018-2022 Strategic Plan, 2018). Even though there have been some scientific studies on plant activators, very few synthetic

stimulants have been identified, and new research in this area is essential. Developments in today's technology and molecular biology may enable more plant activators to be found and defined and their mechanisms of action to be revealed in this field. Recently 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl}e phenol (DPMP) is described as a novel synthetic elicitor. Recent studies showed its activity against some pathogens, including the biotrophic pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) on *Arabidopsis thaliana* (Bektas et al., 2016). Also, its activity against two distinct bacterial pathogens*; Pseudomonas syringae* pv *tomato (Pst)* and *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) were revealed (Bektas et al., 2016; Bektas, 2021) with significant potential as a plant protection agent. While this research showed that DPMP is a robust synthetic elicitor against some tested biotrophic and hemibiotrophic pathogens, its activity against necrotrophic pathogens was not revealed. Therefore the goal of this study was the elicit the activity of DPMP against early blight caused by *A. solani* on the molecular level. Understanding the effect of DPMP on necrotrophic disease response can give us clues about DPMP's mode of action on the plant defense induction pathway.

2. Material and Methods

2.1. Plant material and growth conditions

The study was conducted under controlled conditions in the Department of Agricultural biotechnology, Siirt University, Siirt, Turkey. "Moneymaker" Tomato (*Solanum lycopersicum* L.) cultivar was used as plant material. Seeds were surface sterilized with 5 % sodium hypochlorite (NaOCl), and 70% ethanol, followed by rinsing under sterile water excessively. Sterilized seeds were germinated in Petri dishes at 25-27 °C and 16 h/8 h light/dark regimes. Seedlings were transplanted into pots containing peat and perlite mixture with a ratio of 2:1. Relative humidity and mean temperatures of the growth environment ranged between 60-70% and 25-27 °C, respectively. Chemical applications were initiated when plants reached three to the four-leaf stage in the 5th week.

2.2. Fungal material, disease assessments, and growth measurements

One of the most commonly found disease agents, *A. solani* was used for disease assessment. The *A. solani EAb 1* isolate (Boyno et al., 2020) was obtained from Dr. Semra Demir, Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yıl University, Turkey. *A. solani* isolates were grown on potato dextrose agar (PDA), and a sterilized water solution with 5 × 105 conidia mL^{-1} for the foliar spray was prepared. 24 hours after the second (last) application of the DPMP or control treatment, Each plant (5 weeks old) was sprayed with the stated concentration using a manual hand sprayer.

The disease severity (DS) of the infected plants was evaluated on the 18th, 23rd, 28^{th,} and 33rd days after the experiment started. A commonly applied 0-5 scale was used for disease severity scoring (Pandey et al., 2003). The percentage of the necrotic lesions on the leaf surface is used for the scoring. With this scale, 0 equals no symptoms, 1 equal 1-11%, 2 equals 11-25%, 3 equals 26-50%, 4 equals 51-75%, and 5 equals 76-100% symptoms on the leaves. The disease progress curve (AUDPC) value was calculated according to Pandey et al. (2003). Plant growth parameters, plant height (PH), shoot fresh weights (SFW), and shoot dry weights (SDW) were collected to obtain plants development under disease and chemical applied conditions. PH was measured manually with a ruler, SFW was determined with a precision scale (Weightlab instruments), while SDW was obtained after drying samples at 70 ˚C for 48 h in an oven (Nüve, TR).

2.3. Plant defense elicitor treatments

DPMP was used as a plant elicitor against *A. solani* inoculation. DPMP was generously provided by Dr. Thomas Eulgem, University of California, Riverside, USA. Since DPMP needs to be dissolved in a specific solvent, DMSO (100%) is used to dissolve DPMP. Stock DPMP solution was diluted to 10 µM with 0.2% DMSO in it. DMSO at 0.2% without DPMP is also applied to control plants to observe any possible effects of the solvent. Final concentrations of DPMP (10 µM) and DMSO (0.2%) were applied to plants one week and one day before pathogen application. Twenty-four hours after the second application of the DPMP or DMSO, the pathogen application was assessed. Each experiment was replicated three times with three plants per replication.

2.4. Gene expression analysis

Samples for gene expression analysis were collected 24 hours after the second chemical application, before pathogen application. A total of nine leaves per plant were collected and grounded in liquid nitrogen. Total RNA was isolated using PureLink RNA Mini Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA concentration and purity were determined using a Multiskan GO spectrophotometer (Thermo Scientific). RNase-Free DNase I (Thermo Scientific) was used to remove any DNA contamination. RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was used for cDNA synthesis. Selected genes listed in Table 1 were used for the gene expression analysis. The experiments were repeated with three different biological and three different technical replicates. Real-time reverse transcription-quantitative PCR (RT-qPCR) was used for quantification of gene expression patterns using the PicoReal Real-Time PCR system (Thermo Scientific). Average Ct values were normalized to *Actin* for each gene of interest, and relative transcript levels were calculated according to Livak and Schmittgen (2001).

Table 1. Plant defense-related genes, forward and reverse primers, and references

Gene	Forward	Reverse	Reference
Pti4	CAACAGTTACCACCGACGAAC	GACCAATAGTTGATGGACACC TG	(Rasool et al., 2021)
PRB1-2	CGGTGAACACTGGAAATGTG	GGAGCATCGCCATTAATCAT	(Nehela et al., 2021)
SABP2	AACGGACACCAGCAGAGAAT	TGGCCTTTGACAAATCTTCC	(Nehela et al., 2021)
Pto kinase	AGATTGAACCATGGCAGACC	GATACTCTCACGCCGTAGCC	(Khan et al.,2012)
PR3	CAATTCGTTTCCAGGTTTTG	ACTTTCCGCTGCAGTATTTG	(Khan et al., 2012)
TPK1b	ATGGGGATATGTTTGAGTGCTA GAA	GAACGTGTTCTCGTCGATCCA CCCT	(Ray et al., 2015)
Actin	TGTCCCTATTTACGAGGGTTATG C	CAGTTAAATCACGACCAGCAA GAT	(Zhou et al., 2015)

2.5. Statistical analysis

Data collected in the study were analyzed according to Analysis of Variance (ANOVA). Means were compared following Tukey's Honest Significant Difference (HSD) test. Significance (*$p<0.05$) in each figure and table was indicated with different letters. Statistics software V10 (Analytical Software, Tallahassee, FL) was used for the statistical analyses.

3. Results

3.1. Evaluation of the Effects of DPMP on *A. solani* Disease Severity and Progress

Tomato plants at 5 weeks were subjected to foliar spraying of DPMP two times (1 and 7 days before inoculation (dbi)), to elaborate their responses under *A. solani* infected conditions. The results of four consecutive disease scoring clearly indicated that DPMP did not reduce the disease severity of *A. solani* in tomato (Figure 1a). A similar outcome was obtained in AUDPC values, for which DPMP and DMSO had similar AUDPC values (Figure 1b).

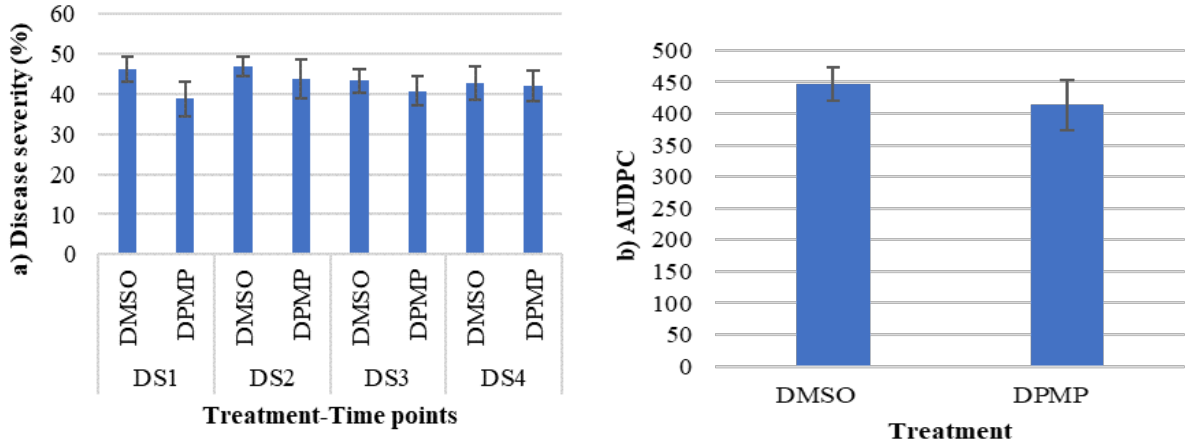

Figure 1. The effects of DPMP on *Alternaria solani* disease severity and progress. DPMP or DMSO was applied before pathogen inoculation. The AUDPC value was obtained with disease severity scores (%) at 4-time points. Three independent trials were analyzed using ANOVA and the means were separated according to Tukey's HSD multiple comparison test. Significant differences ($p < 0.05$) within each group are shown with different letters, otherwise, the data was Non-Significant (NS).

To make a precise comparison of DMSO, DPMP, and negative control (NK), four different morphological traits were evaluated. Plant height (PH), number of leaves, and plant fresh and dry weights were compared. Plant height was the tallest ($p<0.05$) in NK, while DPMP and DMSO had similar PH values and DMSO applied plants had slightly taller statures (Figure 2a). The number of leaves was also the highest in NK, followed by DMSO and DPMP, but the difference was not significant. Plant fresh (PFW) and dry (PDW) weights were measured to obtain the biomass potential of each application. Accordingly, NK had the highest PFW and PDW values that were predicted (Figures 2c and d). Since DPMP did not provide any protection against *A. solani*, it did not cause any change in the negative effect of pathogens on plant development as well.

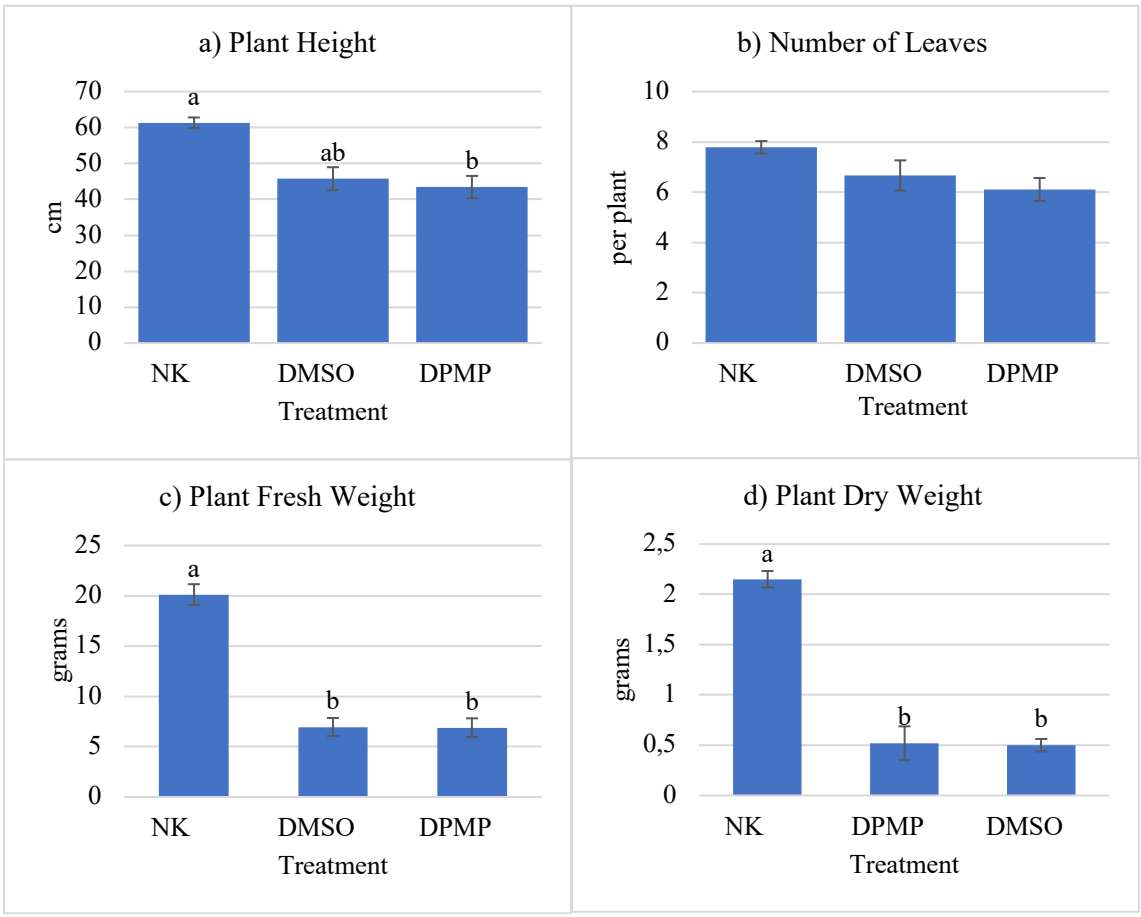

Figure 2. The effects of DPMP on a) Plant height, b) Number of leaves, c) Plant fresh weight, and d) Plant dry weight on *Alternaria solani* infected or uninfected (negative control (NK)) tomato plants. Significant differences within each group are shown with different letters according to Tukey's HSD test ($p < 0.05$).

3.2. Gene expression profiles of tomato plants sprayed with DPMP and DMSO (Control) under *A. solani* inoculated conditions

In the first part of this study, the effectiveness of DPMP against *A. solani* was evaluated with morphological observations and disease severity scoring in comparison with DMSO (inoculated control), and NK (non-inoculated, negative control). Since DPMP did not reduce disease severity under any of the four data points, we aimed to see the molecular mechanism of the disease response induction by monitoring selected marker defense response genes. Six different plant disease response-related genes, *Pti4, TPK1b, Pto kinase, PRB1-2, SABP2,* and *PR3*, were compared with RT-qPCR between DMSO and DPMP sprayed plants. Gene expression profiles of DPMP and DMSO sprayed plants were compared and normalized with *Actin.* According to the results, DPMP application down-regulated the expression of *Pti4* and *PR3,* on the other hand, overexpressed the activity of *PRB1-2, TPK1b,* and *Pto kinase.* The gene expression level of *SABP2* remained the same though (Figure 3). These results showed that DPMP had a remarkable effect on the gene activity of *PRB1-2, TPK1b,* and *Pto kinase* that was induced 3-9 fold compared to control, however, the induction of these genes did not affect the plant protection against *A. solani*.

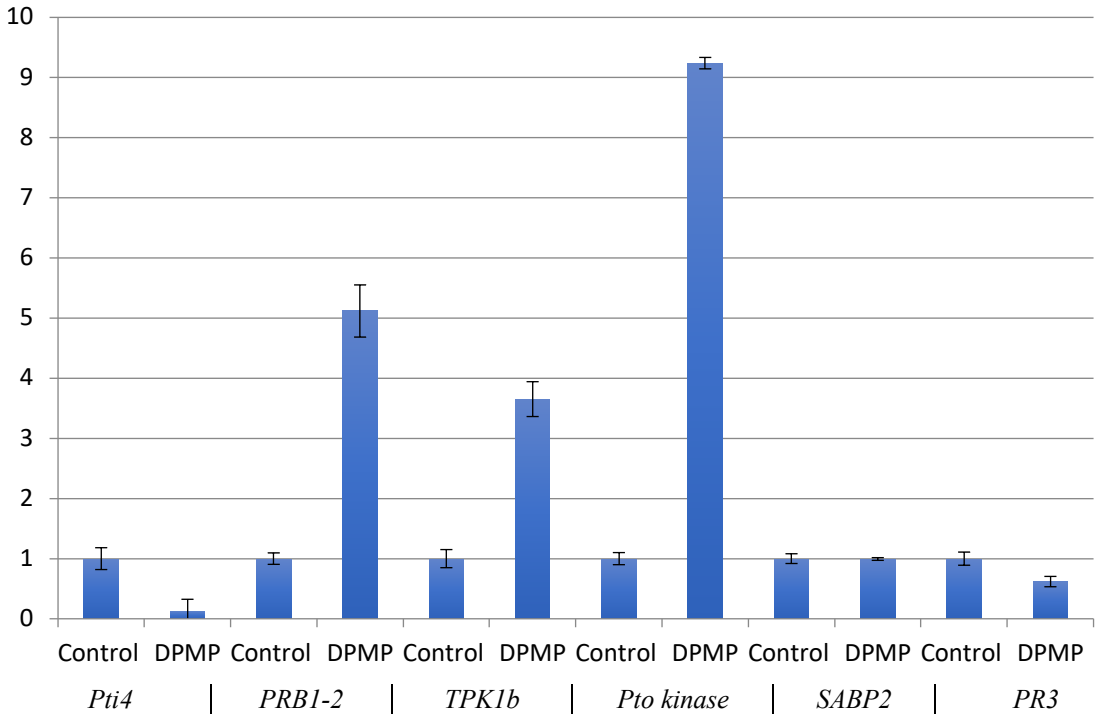

Figure 3. Transcriptional regulation of defense-related genes with the application of DPMP on tomato. Analysis of *Pti4*, *PRB1-2, TPK1b*, *Pto kinase*, *SABP2,* and *PR3* genes after DPMP or control (DMSO) applications that were normalized to *Actin*. Values presented mean ± SE of 3 biological replicates per treatment.

4. Discussion

Biotic stress factors are one of the major constraints in plant production and sustainability. One of the many ways to cope with stress factors is to improve disease tolerance or resistance genetically (Foolad et al., 2008; Singh et al., 2017). If plants do not have a natural allelic structure for disease resistance, exogenous applications, such as pesticides, are commonly used for plant protection (Nicolopoulou-Stamati et al., 2016). Even though pesticides are the most common way to fight disease agents, alternative ways are emerging with the new developments in agriculture and biotechnology. Plant defense elicitors are relatively new substances that can be obtained synthetically or organically (Bektas and Eulgem, 2015; Cohen et al., 2016). DPMP is a new plant defense elicitor with significant promise against *Pst* and *Cmm,* and *Hpa* on tomatoes and *Arabidopsis* (Bektas et al., 2016; Bektas, 2021). However, It has not been tested against many different pathogens, and the mode of action and effectiveness range is not fully known yet. *A. solani* (Early blight disease of Solanaceae family: EB), a necrotrophic fungal pathogen, causes a significant economic impact on tomato production worldwide (Adhikari et al., 2017). Even though there are pesticides commonly used to fight EB, they are not the best choice due to environmental and health-related side effects. These constraints force researchers and farmers to find new-novel approaches that are more environmentally friendly and cost-effective (Gerage et al., 2017; Nicolopoulou-Stamati et al., 2016). A novel approach is the enhancement of a plant defense system with an exogenous application, and DPMP is one of the candidates for this new approach (Bektas and Eulgem, 2015). Here, to elucidate possible roles of DPMP against *A. solani*, 5 weeks old tomato plants (cv. Moneymaker) were sprayed with DPMP, and the disease severity of *A. solani* was analyzed. Also, the molecular basis of the plant defense induction was elicited with relative expression levels of six different defense-related genes.

DPMP with a concentration of 10 µM was applied to plants before *A. solani* inoculation. It has been shown that DPMP did not reduce the disease severity of the pathogen (Figure 1). Compared to the control group, DPMP-applied plants also showed a similar defense response against pathogens and did not show any significant induction on any disease severity evaluation time points (Figure 1a). Correlated with that, the AUDPC values were not significantly different compared to the control (Figure 1b). On the other hand, previous research showed that DPMP induced plants' defense mechanism

against *Pst* (Bektas et al., 2016) and *Cmm* (Bektas, 2021*).* These indicated bacterial pathogens are hemibiotroph, but *A. solani* is a necrotrophic pathogen (Foolad et al., 2008). Previous research also provides information about the protective effect of DPMP against biotrophic oomycetes (Bektas et al., 2016). Based on these results, DPMP may induce defense responses related to biotrophic and hemibiotrophic pathogens, but plant defense response against necrotrophic pathogens might be through different pathways (Glazebrook, 2005; Lai and Mengiste, 2013). Previous findings demonstrated that the salicylic acid (SA) pathway is one of the central elements of plant defense induction against biotrophic and hemibiotrophic pathogens, and the jasmonic acid pathway is most crucial for necrotrophic pathogens (Bektas and Eulgem, 2015; Brouwer et al., 2020; Glazebrook, 2005; Vernooij et al., 1995). However, recent studies have provided controversial information to argue that SA-, ET-, and JA-related defense responses involve extensive transcriptional reprogramming against *A. solani* (Brouwer et al., 2020; Nehela et al., 2021; Spletzer and Enyedi, 1999).

To understand why the DPMP did not have any effect on *A. solani*, we tried to elicit some marker defense-related genes from different defense pathways, including salicylic acid, jasmonic acid, and ethylene-related defense responses. Six genes were evaluated with RT-qPCR to see which pathways are active under the current scenario. Of these, *Pto kinase* is a gene that encodes serine/threonine kinase. Previous research revealed that its overexpression activity inhibits *Pst* infection through effector-triggered immunity (ETI) (Oh and Martin, 2011). In this study, the relative gene expression level of *Pto kinase* was up-regulated more than 9 folds in response to DPMP spraying; however, it did not provide any protection against *A. solani*. This finding is consistent with previous research that suggested that *Pto kinase* is important in ETI and coordinated with SA-related pathways (Glazebrook, 2005; Oh and Martin, 2011). As a result, *Pto kinase* is not required for plant protection against necrotrophic pathogen *A. solani. Tomato protein kinase 1* (*TPK1b)* was another gene that was over-expressed through the application of DPMP. *TPK1b* encodes receptor-like cytoplasmic kinase and is known to be induced by infection, wounding, and oxidative stress (Smith et al., 2014). DPMP application induced *TPK1b* gene expression in about 3 to 4 folds (Figure 3). This finding demonstrated that *TPK1b* is not a key element for plant protection against early blight.

JA and ethylene-induced defense mechanisms are more active when the plant is infected with necrotrophic pathogens (Glazebrook, 2005; Rasool et al., 2021). *Pti4* encodes a transcription factor in the ethylene-responsive element-binding factor (ERF) family of proteins (Gu et al., 2000). So, we analyzed the gene expression of *Pti4* after DPMP application. Relative gene expression of *Pti4* was slightly downregulated with the DPMP spraying compared to DMSO (control), suggesting that *Pti4* may not be the key element for *A. solani* defense responses. Accordingly, previous reports suggest that *Pti4* takes a role in the activation of GCC-box *PR* genes against hemibiotroph pathogen *Pst* in tomatoes (Gu et al., 2000 and 2002; Wang et al., 2021) and does not involve in the disease response against this pathogen.

It was previously reported that benzoic acid and its hydroxylated derivatives increase *SABP2* and *pathogenesis-related protein* (*PRB1*-2) gene expressions and reduce the severity of *A. solani* (Nehela et al., 2021). Thus, we evaluated the relative expression levels of these genes. *SABP2* plays a role in the transformation of methyl salicylic acid (MeSA) into salicylic acid (SA) and induces systemic acquired resistance (SAR) (Tripathi et al., 2010). Here, DPMP did not affect the gene expression level of *SABP2.* On the contrary, DPMP applications up-regulated *PRB1-2* expression (Figure 3), but *PRB1-2* over-expression did not protect plants against the *A. solani*. The controversies of *PRB1*-2 activity against *A. solani* might be due to downstream activity of *PRB1*-2 gene activation.

To make a diverse comparison, we also evaluated *PR3,* which is one of the most important players in the plant defense system for necrotrophic pathogens. *PR3* is one of the pathogenesis-related genes (PR genes) (Edreva, 2005; Sinha et al., 2014). PR genes may differ in structure, mechanisms of action, and pathogen specificity (Anisimova et al., 2021). Some are hydrolytic enzymes (like chitinases (*PR3*)), others are antimicrobial proteins (like defensins), phytoalexins, anti-fungal proteins, etc. (Anisimova et al., 2021; Edreva, 2005). Biotrophic pathogens activate the SA pathway and related PR genes (*PR1, PR2* & *PR5*), while necrotrophic pathogens stimulate the JA pathway and activate specific PR genes (*PR3, PR4* & *PR12*) (Ali et al., 2018). Here, *PR3* (Chitinase/Chi3) encoding chitinase involved in the ethylene/jasmonic acid-mediated signaling pathway was evaluated with DPMP treatment. Accordingly, *PR3* gene expression was neither up nor down-regulated with DPMP application (Figure 3) and therefore did not contribute any protection against early blight. Previous research showed that DPMP induces *PR1* gene expression and provides significant protection against *Cmm*. All of these results showed that DPMP induces plant defense responses against biotrophic and hemibiotrophic pathogens through SA-related defense responses but not necrotrophic pathogens.

5. Conclusions

Early blight disease (*A. solani*), causes significant yield losses in tomato production. Plant protection against the disease is provided by Fungicides, but their side effect on the environment and living organisms forced researchers to find alternative ways. In this study, the promising plant defense elicitor DPMP as an alternative to pesticides was evaluated at disease severity and molecular levels. In our research, for the first time, we provided a report that DPMP-regulated defense activation did not provide effective protection against necrotrophic pathogen *A. solani*. On the contrary, it provides significant protection against some biotrophic and hemibiotrophic pathogens. This finding provides us some foresight about what is the mode of action of DPMP as a plant defense elicitor. These findings contribute valuable information for a researcher to come up with molecular mechanisms of defense activation against distinct pathogens.

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Research Article

Determination of Antioxidant, Antimicrobial Properties with Evaluation of Biochemicals and Phytochemicals Present in Oscillatoria limosa of District Jamshoro, Pakistan

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Abstract: *Oscillatoria limosa* is a well-known member of blue-green algae, usually found in open water reservoirs. Ecologically it plays very important roles, like releasing Oxygen and being a supportive alternative source of food for aqua fauna. In research, it is being investigated as a medicinal organism for cancer and infectious diseases. In the current study, we have determined the medicinal and ecological importance of *Oscillatoria limosa* particularly found in the vicinity of district Jamshoro 76080, Sindh, Pakistan. For this purpose, four different solvent extracts of 20% (w/v) dried powder of organism were used to determine the presence of bioactive compounds, phytochemicals, antimicrobial activities, and antioxidant properties through previously well-reported methods. The obtained results of this study prove the presence of phenolic acid, flavonoids, total proteins, total sugar, reducing sugar and one free amino acid, and phytochemicals like alkaloids, phytosterol, tannin, terpenoids, glycosides, and saponin in samples. In this study, remarkable antioxidant properties ranging from 0.248 to 1.080 mg ml^{-1} were observed in all the samples. The antibacterial activities against *S. aureus*, *A. tumefaciens*, *K. aureus*, *E.coli*, and *P.aeruginosa*, and antifungal activities against *A. niger*, *P. notatum,* and *Rhizopus* were observed, which proves it a good antimicrobial organism. It may be concluded from this study that *Oscillatoria limosa* of local vicinity is a potential organism of interest for biotechnological and pharmaceutical industries as an antitumor, antimutagen, free radical scavenger, and possess antimicrobial properties against various kind of bacteria and fungi.

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1. Introduction

Oscillatoria limosa is the filamentous blue-green algae, which is widely distributed in all convincible aquatic habitats on earth and possesses its particular ecological importance of being oxygenic phototrophs and aquatic food of aqua fauna, although some of its species are reported toxic for certain organisms (Abed et al., 2009; Luu et al., 2019). The phycologists can easily identify the freshwater *Oscillatoria limosa* in freshwater reservoirs by its exceptional morphological characteristics like blue-green long, straight, and without mucous membrane filamentous colonies (Luu et al., 2019). Taxonomically *Oscillatoria limosa* belongs to the diversified group of cyanobacteria, which has gained

a lot of attention in recent years, particularly in the field of biotechnology, food sciences, and pharmacological industries.

Currently, many members of cyanobacteria attracted many researchers and scientists worldwide to search it, as these organisms are a rich source of biochemical compounds, phytochemicals, as well as antibiotic and antiviral compounds; they are immunosuppressive agents, anticancer, antiplasmodial, and algicide organisms(Patterson et al., 1994; Papke et al., 1997; Papendorf et al., 1998; Kajiyama et al., 1998; Dahms et al., 2006; Abed et al., 2009). Cyanobacteria, through research, are also being tried to be used as food, biofertilizer, and medicinal organism (Lem & Glick, 1985). Throughout history, many members of algae, fungi, and plants have been widely used in particular to promote and maintain good health, fight against sickness, provide relief from pain, and for treatment of diseases since times immemorial (Hemavani et al., 2012; Charan et al., 2021). These all organisms and their derivatives which are being used as medicines are counted as parts or members of traditional medicines and practices through the centuries, particularly in the countries of the continent of Asia like India, China, Japan, Thailand, Pakistan, etc. (Das, 2016; Piwowar & Harasym, 2020; Wells et al., 2017). Throughout the study of various species of *Oscillatoria,* many researchers have reported various important findings like *Oscillatoria raoi to* possess an antiviral bioactive compound, Acetylated sulfoglyco-lipids (V et al., 1997), *Oscillatory Sp.,* used as food as well as medicinal organism, and further, it has been tested for various purposes in research like for removal of heavy metals, activation of monocytes and B-cell of blood and as antibiotics (Azizi et al., 2012; Swanson-Mungerson et al., 2017; Swanson-Mungerson et al., 2018). However, on the other hand, the scientific study on *Oscillatoria limosa* from district Jamshoro, Pakistan, like other species of cyanobacteria, is still limited, particularly on its bioactive components, antimicrobial agents, and antioxidant properties.

So, the present study aimed to explore the biological activities of *Oscillatoria limosa* found in the vicinity of the district Jamshoro 76080, Pakistan, like antioxidant, antifungal, antibacterial, and cytotoxicity. And to investigate the feature benefits of this organism as an alternative source of food and medicines. The obtained results of the present study confirm the antioxidant, antimicrobial and antifungal activity and the presence of various known and unknown biochemicals and phytochemicals in *Oscillartoria limosa* collected from the said local regions.

2. Material and Methods

2.1. Materials

Formalin, peptone, China grass**,** acetone, dextrose, agar, methanol, ethanol, H_2SO_4, glucose, 80 % phenol, 28 mM sodium phosphate, purchased from Sigma Aldrich Roche, and Yeast extract was obtained from IBGE, ferric chloride, NaCl, sodium hydroxide, acetic acid, alkaline sodium carbonate, copper sulphate-potassium sodium tartrate, alkaline solution, folin-ciocalteu parched from Merk, 10% aluminum chloride, 5% sodium nitrate, 0.1% ferric chloride ($FeCl_3$), 0.2 M phosphate buffer (Ph 6.6), acetic anhydride, all parched from Merck. Dinitrosalicyclic acid (DNS) from Bio Basic INC, 4mM Ammonium Molybdate BDH, sodium acetate, 1% potassium ferricyanide, 10% trichloroacetic acid (TCA), 0.008 M Potassium ferric cyanide and chloroform provided by IBGE. All the reagents and chemicals used were of analytical grade.

2.2. Methodology

2.2.1 Collection or ısolation of algal strains

Oscillatoria limosa algae were collected in plastic bags from natural freshwater reservoirs. Environmental and water temperature were noted along with water pH at the time of sample collection. All samples were collected and stored in black plastic bottles. Then in the laboratory, all the samples were washed twice in tap water and dried at room temperature in the shade. After that, all the collected algal species were stored in 4% formalin (commercial) for further taxonomical or morphological studies.

The collected specimen material was taxonomically verified as *Oscillatoria limosa* with the help of a microscope by a phycological expert from the institute of plant science.

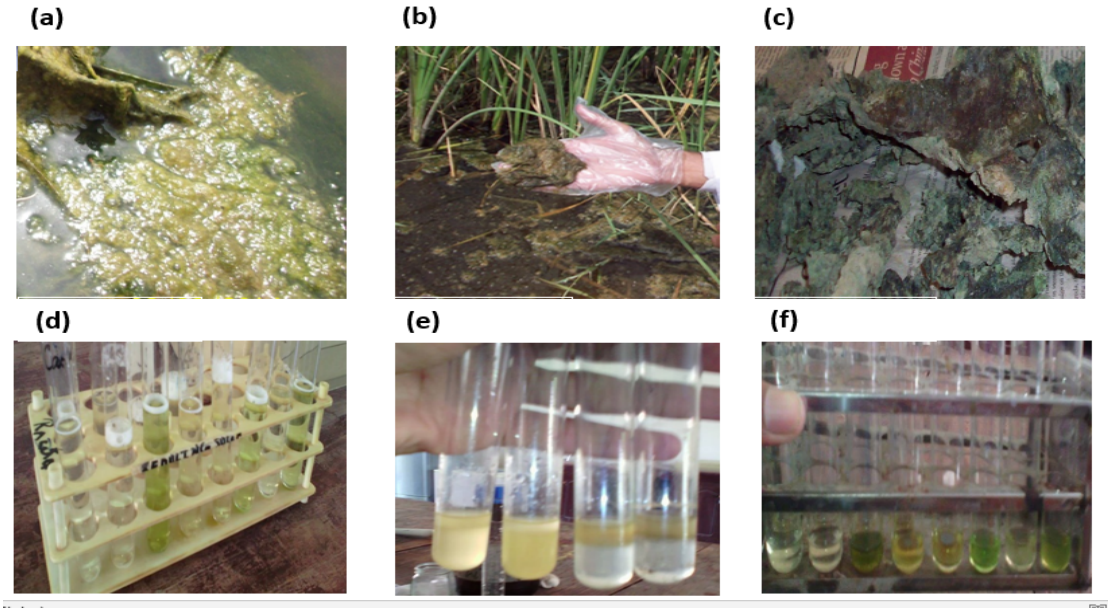

Figure 1. (a), (b) floating specimen at the water surface, (c) dried specimen at the laboratory, (d), (e), (f) various test tubes of 20% *Oscillatoria limosa* extracts at different laboratory testings.

2.2.2. Preparation of 20% extracts

Prepared 20% extracts through the method reported by Habib Naqvi et al., 2011. Briefly, for the preparation of 20% extracts of water, ethanol, methanol, and acetone, the dried material (coarse powder) of *Oscillatoria limosa* was dissolved in all the solvents at the ratio of 5g ml^{-25}, while the purity of all the organic solvents were 70%. For further proper extraction, all the solutions were centrifuged at 6000 rpm for 30 minutes, then stand stored at -40°C before any procedure of experiments or tests.

2.2.3. Determination of total and reducing sugar

Total and reducing sugar contents, analyzed from the extracts of *Oscillatoria limosa,* followed by the reported method of Miller (2002) and Montgomery (1961). Briefly, a single sample of every extract of 0.5 ml was mixed with concentrated H_2SO_4 and 50 µl of 80% phenol solution in separate Eppendorf tubes and rest them at room temperature for complete mixing with each other. Then, the solutions of Eppendorf tubes were taken for determination of total sugar in UV-spectrophotometer at 485 nm, as put up in R, Montgomery's method. Whilst, the test solution of 2.0 ml from every extract was taken in other Eppendorf tubes, mixed with 2.0 ml of dinitrosalicyclic acid for estimating the presence of reducing sugar. Finally, observed the absorbance at 540 nm of samples for verification of the presence of compounds in samples as followed previously reported method of Miller. All the experiments were repeated thrice for further confirmation of the results.

2.2.4. Determination of total protein

Total protein, determined by the method of Lowry et.al, with some variations (Lowry et al., 1951). Briefly, test samples of 0.5 ml of each extract were taken in Eppendorf tubes, then 2.5 ml of alkaline copper reagent was added with folin ciocalteus reagent (1:1 v/v with water) in each tube after that, tubes were left at room temperature for 30 minutes. The results were developed as described in Lowry et al.'s method. The total protein from samples of Eppendorf tubes was read against the blank of bovine serum calibrated a standard curve at 750 nm in UV-spectrophotometer.

2.2.5. Determination of total antioxidant, total phenolic, and total flavonoids

Total antioxidant, total phenolic, and total flavonoid of *Osillatoria limosa* were determined by applying the relevant method of Prieto et al. (1999), Yasoubi et al. (2007) and Djeridane et al. (2008)

with minor changes. Briefly, for total antioxidant activity, samples of 0.2 ml of each extract were mixed separately with 2 ml of reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid, and 4 mM ammonium molybdate) in Eppendorf tubes. All Eppendorf tubes were incubated at 95°C for 90 minutes, then kept at room temperature to cool down. The absorbance of each sample was measured at 695 nm against a blank standard curve of α-tocopherol and ascorbic acid, respectively.

Whilst, the 0.2 ml test sample of each extract was mixed with 1 ml of 10-fold diluted Folin-ciocalteu, and 0.8 ml $NaCO_3$ in an Eppendorf tube for quantification of phenolic contents. The result was read against the blank of Gallic acid calibrated a standard curve at 765 nm in UV-spectrophotometer.

The quantity of flavonoid was estimated through flavonoid–aluminum complex solution treated with 0.1 ml of every extract in separate Eppendorf tubes. Here Rutin was used for a standard calibration curve at the absorbance of 430 nm in a UV-spectrophotometer, compared with prepared samples of Eppendorf tubes for total flavonoids.

2.2.6. Antimicrobial activity

Antimicrobial activity of *Oscillatoria limosa* from its extracts in water, ethanol, methanol, and acetone was tested against common pathogenic fungi like *Aspergillus niger*, *Penicillium notatum*, and *Rhizopus spp*. While bacterial species like *Escherichia Coli*, *Agrobacterium tumefaciens*, *Staphylococcus aureus*, *Klebsiella aureus*, and *Pseudomonas aeruginosa*, were chosen for analyzing antibacterial activity. Through the agar-well-diffusion method, previously reported by Mothana and Lindequist (2005). İnitially, all the samples of bacteria and fungi were obtained from the microbiology laboratory of the Institute of Biotechnology and Genetic Engineering, University of Sindh.

2.2.7. Identification of free amino acids through thin layer chromatography (TLC)

Free amino acids were identified by the method of Thin Layer Chromatography (TLC) previously reported by Hudaib et al. (2016). Briefly, silica gel G-60 as stationary phase and butanol: acetic acid: water (5:1:4 v/v and 4:1:5 v/v) as the mobile phase was used. Thin layer gel plates were prepared and activated as described in the earlier method before applying the samples of extracts. The following amino acids, glycine, serine, leucine, cysteine, valine, aspartic acid, tryptophan, tyrosine, threonine, histidine, proline, glutamic acid, cystine, arginine, alanine, glutamine, isoleucine, asparagine, methionine, phenylalanine, hydroxyproline, and lysine were applied as standard amino acids, and their R_F noted through TLC spots, after preparing 2% (w/v) of aqueous amino acid samples.

3. Results and Discussion

The temperatures of water and environment at the spot of the collection of the *Oscillatoria limosa*, were 30 oC and 39 °C, respectively, while the pH of the pond water was 8.5. The pH of dried filamentous extracts was observed in acetone 8.9 pH, water 6.3 pH, methanol 7.1 pH, and ethanol 6.5 pH. The results are presented in Figures 2. (a) and (b).

3.1. Total sugar and reducing sugar

Carbohydrate is the main component of algal organisms which is considered a helpful source of health for humans in the form of nutrients, antioxidants, anticoagulants, and antiviral. Different types of carbohydrates are found abundantly in various algal species (Chennubhotla, 1996). In the present study, the total sugar concentration of *Oscillatoria limosa* was analyzed from extracts of four different solvents (water, acetone, methanol, and ethanol). Our results indicate that acetone extract has a higher concentration of about 9.531 mg ml^{-1} as compared with other extracts such as water, methanol, and ethanol with 5.40, 4.875, and 2.06 mg $ml^{-1,}$ respectively. While the reducing sugar concentration was determined in four extracts, the maximum concentration recorded in acetone was 4.689 mg ml^{-1}, while other samples from methanol, water, and ethanol extracts showed 4.65, 3.67, and 1.93 mg $ml^{-1,}$ respectively.

3.2. Total protein

Total protein concentration was measured from four extracts, the higher concentration of 3.34 mg ml^{-1} was noted in water extract as compared with methanol, acetone, and ethanol having a quantity of 2.86, 2.09, and 1.95 mg $ml^{-1,}$ respectively, by applying bovine serum as standard. All the results of total sugar, reducing sugar, and total protein are presented through graphs in Figure 2 (d).

3.3. Qualitative analysis of phytochemical

Phytochemicals are very important compounds present in plants and algae, generally, humans use them as antioxidants, antitumors, antimutagens, enzyme modulators, free radical scavengers while as well as antimicrobial agents (Rutikanga et al., 2017). Hereby this study, various important phytochemicals of *Oscillatoria Limosa,* like terpenoids, alkaloids, phenolic, flavonoids, phytosterol, glycosides, tannin, saponin, etc., were observed in extracts of solvents (ethanol, methanol, acetone, and water). Briefly, 32 tests were conducted to analyze various phytochemicals; however, we found 25 positive and 7 negative results. The detailed results are prescribed in Table 1.

Table 1. Qualitative analysis of different bioactive compounds from collected algal specie O*scillatoria limosa*

Phytochemicals	Ethanol	Acetone	Methanol	Water
Alkaloids	++	++	+++	+
Phytosterol	++	+	++	++
Phenolic	-	++	+	+
Flavonoids	-	+	+	+
Tannin	++	++	-	-
Terpenoids	+	++	-	-
Glycosides	++	++	-	+
Saponin	+	+++	+++	+

The + and - sign respectively used for the presence and absence of phytochemicals.

3.4.Total flavonoids

Total phenolic acid, flavonoids, and tannins are important biological chemicals present in medicinal plants which develop antioxidant, anti-carcinogenic, anti-atherosclerotic, and anti-inflammatory activities, etc. (Hemalatha et al., 2013). So, the presence of these compounds in any organism authenticates the medicinal significance of that organism. Here in this study, Rutin was used as standard, and the quantification of total flavonoid contents showed the highest result of 8.268 mg ml^{-1} observed in methanolic extract, while samples of ethanol, water, and acetone extracts contained 7.30, 6.99, and 2.35 mg ml^{-1}, respectively.

3.5. Phenolic quantity

For this study, gallic acid was applied as standard, and the maximum concentration of phenolic quantity noted in extracts from acetone of 2.937 mg ml^{-1}, while in water, methanol, and ethanol extracts showed 2.01, 0.33, and 0.26 mg ml^{-1} of phenolic quantity respectively.

3.6. Antioxidant

Total antioxidant activity was checked in all extracts of the organism. The result was noted in mg ml^{-1} by using α-tocopherol standard. The highest antioxidant activity 1.080 mg ml^{-1} was noted in methanolic extracts, followed by ethanol, water, and acetone of 0.559, 0.261, and 0.248 mg ml^{-1}, respectively. The results are presented through graphs in figure 2 (c)

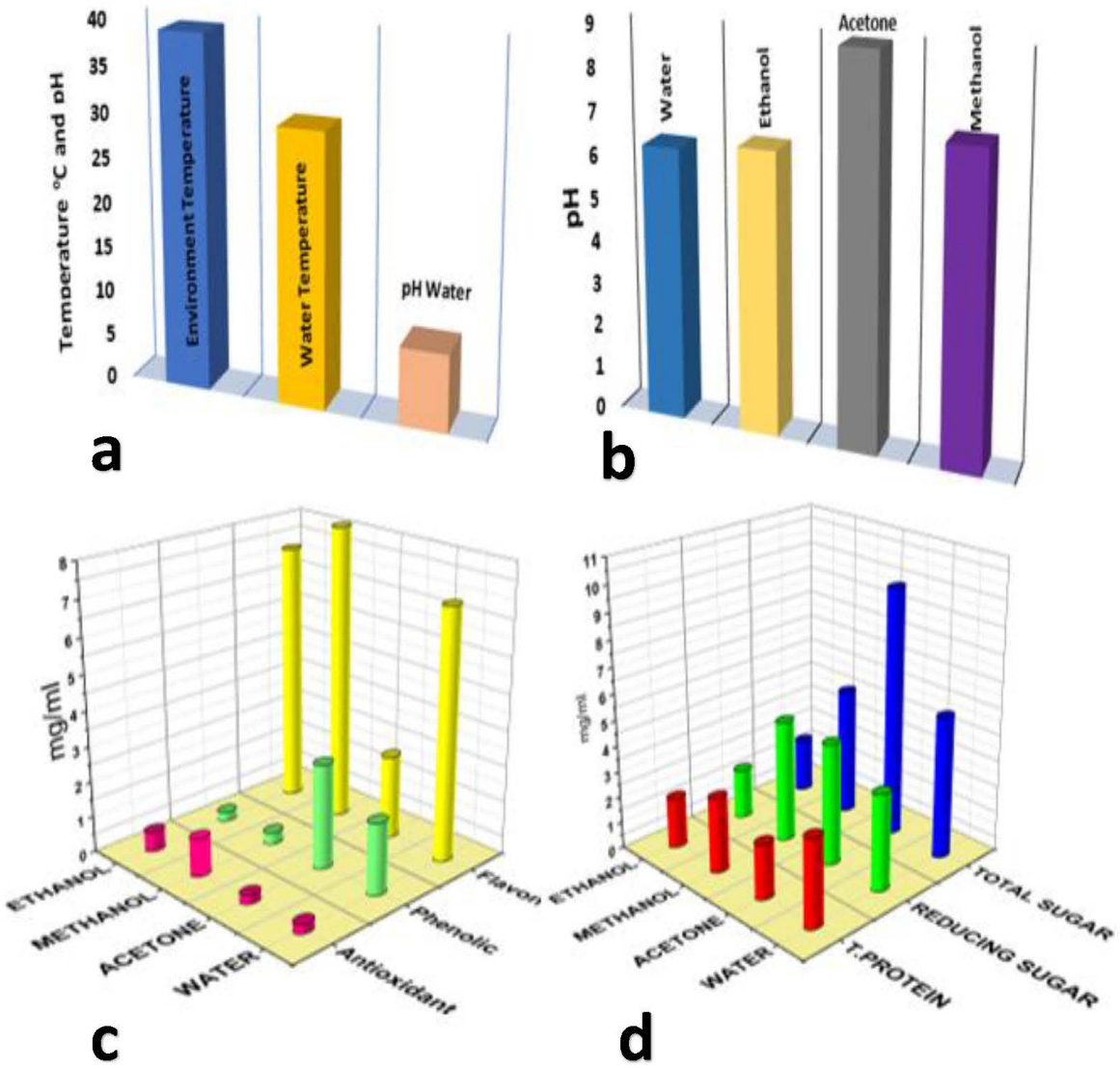

Figure 2. (a) Temperature and pH at the time of sample collection, (b) pH of different extracts of 20% *Oscillatoria limosa* floating specimen at the water surface, (c) antioxidant, phenolic, and flavonoids contents in extracts of different solvents (d) total sugar, reducing sugar and total proteins contents in extracts of different solvents.

3.7. Antimicrobial activity

Antibacterial activity of *Oscillatoria limosa* extracts was examined over five different bacterial species, *E.coli*, *A.tumifaciens*, *S.aerus*, *K.aerus,* and *P.aeruginosa.* The highest zone of inhibitions 31.2 mm was observed from methanol extract. While 10 samples were checked positive from all of the extract's samples, ethanol and *acetone* extracts showed activity against only *S. aureus* and *A.tumifacians,* while methanol extract showed activity against *K. aureus and E.Coli*, while the water extracts showed activity against four bacterial species, *S.areus*, *A.tumifacians, E.Coli,* and *P.aeruginosa.*

Antifungal activity of *Oscillatoria limosa,* over the pathogenic fungi like *A.niger*, *Rhizopus spp,* and *P.notatum* were observed using the agar well diffusion method. Methanol and aqueous extracts strongly inhibited the growth of *Rhizopus* spp and *P.notatum* 24.66 mm. While the minimum inhibition zone was observed from aqueous extracts of 9.0 mm against *P.notatum*. The acetone and methanolic extracts had potential against *A.niger* 11.333mm, 17.66 mm, respectively. In the current study, antifungal activity was checked over 12 samples of *O.limosa* (water, acetone, methanol, and ethanol). However, Six negative results were noted from samples of different extracts, those did not show any inhibition against specific fungus species. The detailed results of antimicrobial activities of *Oscillatoria limosa* are prescribed in Tables 2 and 3.

Table 2. Antibacterial Activity from 20 % algal extract of *Oscillatoria limosa* specie

	Antibacterial activity from 20% *Oscillatoria limosa* algal extracts			
	Ethanol	**Acetone**	**Methanol**	**Water**
Styphylococus aureus	12 ± 3*	14 ± 3*	Negative	26.33 ± 3.21*
A.tumifacians	10.33 ± 1.73*	15 ± 3*	Negative	12 ± 3*
Klebsiella aureus	Negative	Negative	30.4 ± 1.3*	Negative
Escherichia. Coli	Negative	Negative	31.2 ± 0.9*	10 ± 2.73*
Pseudomonas aeruginosa	Negative	Negative	Negative	10.33 ± 3.08*
Control	Negative	Negative	Negative	Negative

Zone of inhibition was measured in mm and ± standard deviation.

Table 3. Antifungal activity from 20% algal extract of *Oscillatoria limosa* specie

	Antifungal activity from 20% *Oscillatoria limosa* algal extracts			
	Ethanol	**Acetone**	**Methanol**	**Water**
Aspergillus Niger	Negative	11.33± 1.52*	17.66± 3.51*	Negative
Rhizopus sp	Negative	Negative	24 ± 2.64*	Negative
Penicillium notatum	Negative	Negative	11.66 ± 1.15*	9 ± 4.35*
Control	Negative	Negative	Negative	Negative

Zone of inhibition was measured in mm and ± standard deviation.

3.8. Free amino acids and free sugar

Free amino acids are generally recognized to serve as the principal currency of protein metabolism in the multicellular organism, and their concentrations are low compared with the quantities present in the protein-bound form. Free amino acid analysis determines the amount of each unbound individual amino acid i.e. not bound in a protein (Christensen, 1964). The free amino acid from each extract (acetone, ethanol, methanol, and water) of the algal species, *Oscillatoria Limosa,* was identified through the Thin-layer chromatography (TLC) method. In the present study, the R_F value of subjected amino acids was noted through TLC as standard. Their R_F values were recorded for comparing and matching with our results (R_F values of standards summarized in Table 4). However, only Leucine was found in the methanolic extract, showed in Table 5.

The standard R_F value of lactose, glucose, fructose, ribose and maltose were compared with R_F of *Oscillatoria Limosa's* extracts for identification of the presence of free Sugars through TLC. Only one R_F value of ethanol extract was matched with lactose standard R_F value, and one unknown R_F value of sugar was noted. These results are presented in Table 6.

Table 4. list of R_F values of various amino acids find out through TLC, used as standards

	Name of Amino acid	***R_F* Value of Standard Amino acids**		**Name of Amino acid**	***R_F* Value of Standard Amino acids**
1	**Aspartic acid**	0.001	12	**Hydroxyproline**	0.312
2	**Threonine**	0.279	13	**Tyrosine**	0.400
3	**Phenylalanine**	0.628	14	**Cysteine**	0.466
4	**Lucien**	0.496	15	**Cystine**	0.439
5	**Glycine**	0.406	16	**Arginine**	0.320
6	**Glutamic acid**	0.076	17	**Valine**	0.409
7	**Alanine**	0.304	18	**Serine**	0.252
8	**Proline**	0.545	19	**Glutamine**	0.426
9	**Lysine**	0.207	20	**Isoleucine**	0.433
10	**Tryptophan**	0.521	21	**Histidine**	0.404
11	**Asparagine's**	0.363	22		

Table 5. Result for free amino acid by TLC, only Lucine was found, with two unknown readings

Extracts	R_F values of Sample	Amino acid
Ethanol	0.91	Unknown
Acetone	No	
Methanol	0.50	Lucine
Water	0.94	Unknown

Table 6. Free sugars from different algal species by thin-layer chromatography (TLC) method

Name of Sugar	R_F Value of Standard Sugar	Extracts	R_F value of samples	Identification of Sugar
Lactose	0.849	Ethanol	0.725	Unknown
Glucose	0.938	Acetone	0.795	Unknown
Fructose	0.938	Methanol	No	
Ribose	0.319	water	No	
Maltose	0.885			

Conclusion

In the current study, we have analyzed bioactive compounds, phytochemicals, antifungal, antibacterial, and antioxidant activities of *Oscillatoria limosa* a species of cyanobacteria found around in the vicinity of district Jamshoro, Pakistan. We concluded that this organism possesses a significant amount of total sugar, reducing sugar, total proteins, and one free amino acid leucine, so it may serve as a better nutritional source for aquatic organisms. We have also observed the remarkable antioxidant activity, and the presence of a variety of phytochemicals, like phenolic acid, flavonoid, tannins, and others, so this organism can be used in pharmaceutical industries as an antitumor, antimutagen, enzyme modulator, and free radical scavenger. We have also observed that this specie can work as a powerful natural antimicrobial agent against a variety of bacteria and fungi.

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Research Article

Effect of Brassinosteroid Applications on Flower Sex Distribution of 'Chandler' Walnut Cultivar

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Abstract: In this study, the effects of brassinosteroid (BR) group compounds, 24-epibrassinolide (EBr) and 22(S), 23(S)-homobrassinolide (HBr), on flower sex distribution were studied. The study was carried out on the 'Chandler' walnut (*Juglans regia* L.) cultivar between 2016-2018 at the Department of Horticulture Faculty of Agriculture, Çanakkale Onsekiz Mart University. 'Chandler' grafted on a seedling rootstock were planted into 70-liter pots containing soil: peat: perlite (2:1:1) medium. HBr and EBr were applied twice at a concentration of 1 mg L^{-1} for two consecutive years in the dormant season and at bud burst, using a hand sprayer. The results show that BR applications could alter the flower sex distribution in walnuts. EBr and HBr applications significantly increased the number of females, catkin (male), and total flowers per plant. The highest number of female flowers (5.2) was observed in the plant treated with HBr. It was determined that the annual growth of the plant and the increase in the numbers of the female flower, catkin, and total flowers were statistically positively related. It is seen that the proportional relationship between male and female flowers is independent of BR applications and the growth of the plant.

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Brassinosteroid Uygulamalarının 'Chandler' Ceviz Çeşidinin Çiçek Cinsiyet Dağılımına Etkisi

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Anahtar kelimeler

Brassinosteroid,
Kedicik,
Chandler,
Dişi Çiçek,
Juglans regia

Öz: Araştırmada brassinosteroid (BR) grubu bileşiklerinden 24-epibrassinolid (EBr) ve 22(S), 23(S)-homobrassinolid (HBr)'in çiçek çinsiyet dağılımına etkileri çalışılmıştır. Çalışma Çanakkale Onsekiz Mart Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü'nde 2016-2018 yılları arasında 'Chandler' ceviz (*Juglans regia* L.) çeşidi üzerinde yürütülmüştür. Çöğür anaç üzerine 'Chandler' ceviz çeşidi aşılanarak toprak: torf: perlit (2:1:1) yetiştirme ortamı ile doldurulmuş 70 litrelik saksılarda yetiştirilmiştir. HBr ve EBr 1 mg L^{-1} konsantrasyonunda birbirini takip eden iki yıl boyunca kış dinlenme ve tomurcukların uyandığı dönemde iki kez el pülverizatörü kullanarak uygulanmıştır. Elde edilen sonuçlar, BR uygulamalarının cevizlerdeki çiçek cinsiyet dağılımını değiştirebileceğini göstermektedir. EBr ve HBr uygulamaları fidan başına dişi, kedicik (erkek) ve toplam çiçek sayısını önemli ölçüde artırmıştır. En fazla dişi çiçek (5.2) HBr uygulanan fidanlarda saptanmıştır. Fidanların yıllık gelişmesi ile dişi çiçek, kedicik ve toplam çiçek sayılarındaki artışın istaistiksel olarak pozitif ilişkili olduğu belirlenmiştir.

Kedicik ve dişi çiçekler arasındaki oransal ilişkinin BR uygulamaları ve fidanların gelişiminden bağımsız olduğu görülmektedir.

1. Giriş

Cinsiyet dağılımı başka bir ifade ile meyve ağaçların üzerinde bulunan erselik, erkek ve dişi çiçeklerin oranı, verimliliği etkileyen en önemli faktörlerden biridir. Bu oran çevresel, hormonal ve genetik faktörlerden etkilenir (Buide ve ark., 2018; Engin, 2020). Ceviz ağaçları monoik (tek evcikli) bitkilerdir. Bu nedenle erkek ve dişi çiçekler aynı ağacın farklı yerlerinde bulunur ve dikogami özelliğinden dolayı farklı zamanlarda olgunlaşırlar (Krueger, 2000). Kedicik olarak da isimlendirilen erkek çiçeklerin oluşturduğu püsküllerin bir tanesi iki milyon çiçektozu tanesini üretebilmektedir (Krueger, 2000). Dişi çiçekler ise tozlanma ve döllenme aşamalarından sonra gelişmelerine devam ederek meyve bağlar. Bu durum ceviz ağaçlarında verimliliğe etki etmektedir. Dişi çiçekler 20 çiçeğin bir arada bulunduğu salkım ceviz oluşturan tipler hariç, genellikle tekli, ikili veya üçlü olarak bulunmaktadır. Dişi çeviz çiçeklerinde taç yaprakları bulunmamasına rağmen epidermis hücrelerinden oluşan uzantılara sahip büyük bir stigma vardır. Ceviz ağaçları rüzgar ile tozlanmakta ve dikogami özelliğinden dolayı polen yayılma dönemi dişi çiçeklerin stigmalarının polen kabul etme dönemi ile geçici olarak örtüşmemektedir (Golzari ve ark., 2016). Özcan ve Sütyemez (2019) farklı melez genotiplere ait polenlerin yüksek çimlenme yeteneğine sahip olduğunu belirtmiştir. Cevizlerde döllenmenin çiçektozunun stigma üzerinde çimlenmesinden yaklaşık 7 gün sonra olduğu ve tek bir çiçektozunun tek bir yumurtayı döllemek için yeterli olduğu ifade edilmektedir (Hassankhah ve ark., 2018). Ceviz ağaçlarında çiçektozu meyve tutumu için gerekli olmasına rağmen kullanılacak tozlayıcı çeşitlerin yoğunluğu tam olarak belirgin değildir. Genelde bahçelerdeki ağaçların % 10'nu tozlayıcı olarak kabul edilir. Döllenmeyi sağlayabilmek için ceviz çeşitlerinden birinin dişi çiçek reseptif zamanı diğerinin erkek çiçek polen yayım zamanı ile örtüşücek şekilde seçilmelidir. İyi bir meyve tutumu ceviz ağaçları üzerindeki dişi çiçeklerin miktarındaki artışlarla doğru orantılı olduğu gibi, çiçek tozu kalitesi ve artan püskül sayılarıyla da desteklenebilir.

Birçok türde çiçeklerin cinsiyeti, çevresel faktörlerden (yüksek veya düşük sıcaklık, kısa veya uzun gün) etkilenmektedir. Bununla birlikte son yıllarda yapılan araştırmalar, bitki türlerinin çiçek cinsiyetlerinin bitki büyüme düzenleyiciler tarafından etkilendiğinide ortaya koymuştur (Khryanin, 2002; Ghani ve ark., 2013). Genel olarak, büyümeyi düzenleyici maddelerin çiçeklerin cinsiyet dağılımları üzerine olan etkileri değişkenlik göstermekle birlikte gibberellin (GA), brassinosteroid ve oksin grubunun narlarda (*Punica granatum L.*) erkek çiçek oluşumunu teşvik ettiği (Engin ve Gökbayrak, 2019), etilen, absisik asit ve sitokinin grubunun ise hıyarlarda (*Cucumis sativus*) dişi çiçek oluşumunu engellendiği belirtilmiştir (Ueguchi ve Matsuoka, 2010). Farklı durumlarda mısırda (*Zea mays*) bitkisinin dişi çiçek sayısı artışı üzerine GA ve sitokinin olumlu etkileri tespit edilmiştir (Young ve ark., 2004).

Brassinosteroid (BR), öncü maddesi fitosterol olan steroid yapıdaki bitki büyüme düzenleyici olduğu ve Kolza (*Brassica napus* L.) bitkisinin çiçek tozlarından elde edilmiştir (Grove ve ark., 1979) Yapay ortamda sentezi gerçekleşmiş en aktif formu brassinoliddir (Khripach ve ark., 2000). Daha sonraki yıllarda onlarca farklı bitki türünde tespit edilen brassinosteroidlerin çok düşük konsantrasyonda etkili oldukları ifade edilmiştir (Rao ve ark., 2003). BR'nin birçok bitki türünde büyüme, gelişme, çiçek tozu çimlenmesi, olgunlaşma ve çiçeklenme üzerinde rol oynadığı tespit edilmiştir (Manzano ve ark., 2011; Gökbayrak ve Engin, 2016; Engin ve Gökbayrak, 2019). BR'nin çiçek oluşum ve farklılaşmasına etki ederek yazlık kabaklarda (*Cucurbita pepo*) gelişen çiçeklerin cinsiyet dağılımını etkilediği bildirilmektedir (Manzano ve ark., 2011).

Bu çalışmada, brassinosteroid grubu bileşiklerden 24-epibrassinolid (EBr) ve 22(S), 23(S)-homobrassinolid (HBr) uygulamalarının 'Chandler' ceviz çeşidinin çiçek çinsiyet dağılımına üzerine olan etkilerinin belirlenmesi hedeflenmiştir.

2. Materyal ve Yöntem

2.1. Bitki materyali

Araştırma Çanakkale Onsekiz Mart Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümünde ceviz çöğürü (*Juglans regia* L.) üzerine aşılı 'Chandler' çeşidinde 2016-2018 yılları arasında yürütülmüştür. 'Chandler' çeşidi Kaliforniya Üniversitesi ıslah programı kapsamında elde edilen 'Pedro' (Serr ve Fonde, 1968) ile '56-224'ün melezidir. 2016 yılında özel bir fidanlıkta yapılan aşılama sonrasında araştırmada kullanılacak fidanlar elde edilmiştir. Kasım ayına kadar fidanlıkta gelişmesi sağlanan chandler fidanları 70 litrelik saksılarda toprak: torf: perlit (2:1:1) ortamına alınarak yetiştirilmiştir (Şekil 1a).

2.2. Brassinosteroid uygulamaları

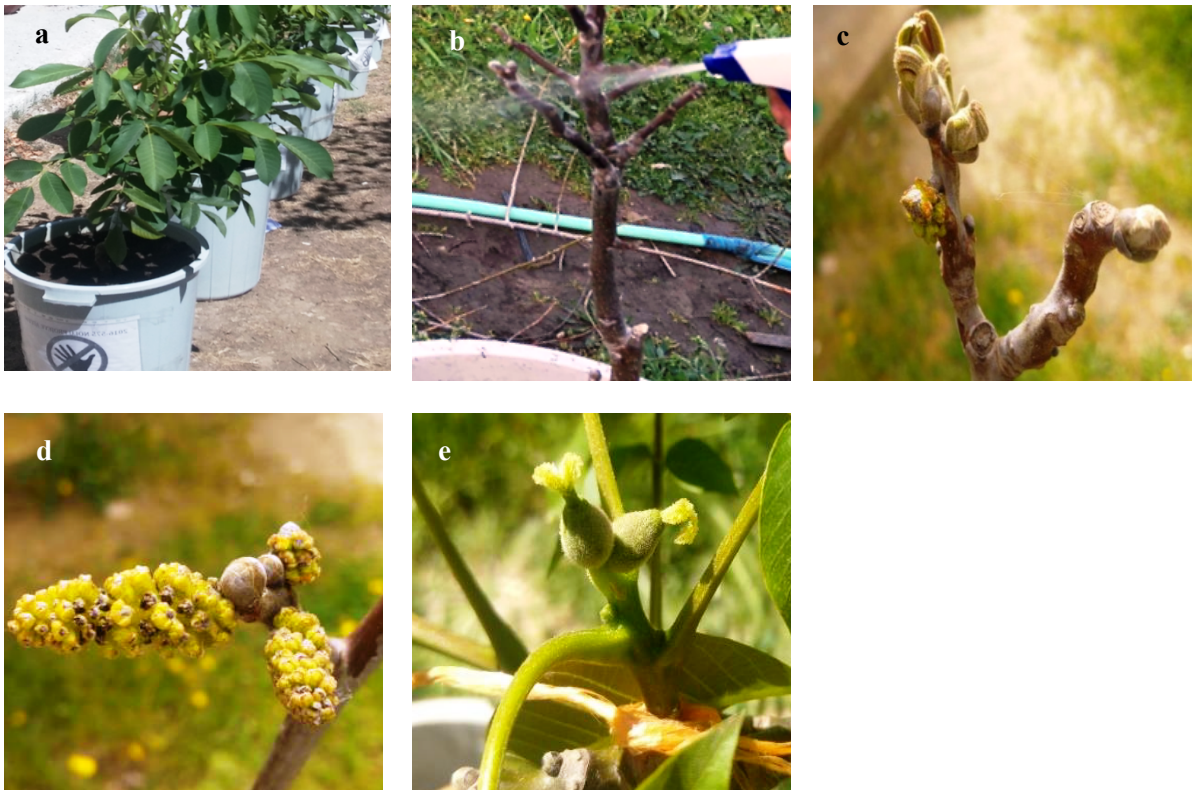
24-epibrassinolid (EBr) ve 22(S), 23(S)-homobrassinolid (HBr) olmak üzere farklı iki brassinosteroid (BR) kullanılmıştır. Her iki BR 1 mg L^{-1} konsantrasyonunda kış dinlenme (Şekil 1b) ve tomurcukların uyandığı (Şekil 1c) dönemde iki kez el pülverizatörü kullanarak uygulanmıştır. BR uygulamaları birbirini takip eden iki yıl boyunca aynı dönemlerde tekrar edilmiştir. Kontrol uygulamasındaki fidanlara aynı uygulama zamanlarında sadece saf su uygulanmıştır. Kimyasal madde uygulamaları fidanların gövde, dal ve yan dalları tamamen ıslanıncaya kadar püskürtülerek yapılmıştır (50-100 ml).

2.3. Çiçek cinsiyet dağılımının belirlenmesi

Araştırmada yer alan her bir fidanın üzerindeki çiçeklerin tamamı birbirini takip eden iki yıl boyunca sayılarak çiçek cinsiyet durumları belirlenmiştir. Uygulama kapsamında yer alan tüm fidanlar üzerindeki püskül (Şekil 1d) ve dişi çiçek (Şekil 1e) sayıları ve püskül/dişi çiçek oranı tespit edilmiştir.

2.4. İstatistik analizi

Araştırma tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak yürütülmüştür. Her tekerrürde 4 fidan kullanılmıştır. Verilerin normal dağılım göstermediği belirlenmiş olup transformasyonlara tabi tutularak Minitab istatistik programı ile ANOVA varyans analizine tabi tutulmuştur. Değerler arasındaki farklılıklar Tukey çoklu karşılaştırma testi ile $p<0,05$ düzeyinde belirlenmiştir. Sayılarak elde edilen verilerde karekök transformasyonu (değerleri 10'dan küçük olduğu için ($\sqrt{X+0.5}$) şeklinde hesaplanmıştır) kullanılmıştır. Sayıldıktan sonra oran veya yüzde olarak ifade edilen verilerde sinüs açı transformasyonu kullanılmıştır.



Şekil 1. 'Chandler' cevizi, brassinosteroid uygulama dönemleri ve çiçekler. a) 70 litrelik saksılarda toprak: torf: perlit ortamında Chandler fidanları; b) birinci BR uygulama dönemi (dinlenme); c) ikinci BR uygulama dönemi (tomurcuk uyanması); d) püskül ve e) dişi çiçek.

3. Bulgular

İstatistik analizi sonuçlarına göre 'Chandler' çeşidinde çiçek cinsiyet dağılımı üzerine yıl ve BR uygulamalarının etkisinin önemli, buna karşılık interaksiyonun önemsiz olduğu tespit edilmiştir (Çizelge 1). İncelenen tüm parametrelerde, püskül/dişi çiçek oranı hariç, ikinci yılda önemli artışlar görülmüştür. Bir fidan üzerindeki püsküler ile dişi çiçekler arasındaki oransal ilişkinin uygulamalar ve uygulamaların yapıldığı yıllardan bağımsız olduğu görülmektedir. Püsküllerin dişi çiçeklere oranı 0.4 ile 0.5 bandında değişim göstermektedir. Başka bir ifade ile teşekkül eden bir püsküle karşılık yaklaşık iki dişi çiçek şekillenmektedir.

Çizelge 1. 'Chandler' ceviz çeşidinde çiçek cinsiyet dağılımı üzerine brassinosteroid uygulamalarının ve yılların etkisi (ortalama±standart hata)

Çiçek sayısı ve oranları	Yıllar		Uygulamalar		
	2017	2018	EBr	HBr	Kontrol
Dişi çiçek sayısı	3.63 ± 0.27 b*	4.89 ± 0.32 a	4.83 ± 0.28 A	5.22 ± 0.37 A	2.72 ± 0.21 B
Püskül sayısı	1.52 ± 0.10 b	2.56 ± 0.17 a	2.44 ± 0.20 A	2.22 ± 0.19 A	1.44 ± 0.17 B
Püskül /dişi çiçek oranı	0.48 ± 0.04 a	0.54 ± 0.03 a	0.53 ± 0.04 A	0.44 ± 0.04 A	0.56 ± 0.05 A
Toplam çiçek sayısı	5.15 ± 0.34 b	7.45 ± 0.43 a	7.51 ± 0.41 A	7.44 ± 0.49 A	4.17 ± 0.34 B

*Yıllar bazında aynı satırda küçük benzer harf ile ve uygulamalar bazında aynı satırda büyük benzer harf ile ifade edilen değerler arasında Tukey çoklu karşılaştırma testine göre istatistiki açıdan fark yoktur ($p<0.05$).

'Chandler' çeşidinde farklı BR uygulamalarının çiçeklerin cinsiyet dağılıma etkileri benzerlik göstermiş ve kontrol değerlerine göre önemli derecede etkiye sahip olmuştur. En fazla dişi çiçek (5.2 adet) HBr uygulamasında görülmüştür. EBr uygulamasında ise fidan başına 4.8 adet dişi çiçek tespit edilmiştir. En az dişi çiçek (2.7 adet) uygulama yapılmayan kontrol fidanlarında saptanmıştır.

Püskül sayıları incelendiğinde, HBr (2.2 adet) ve EBr (2.4 adet) uygulamalarında birbirine çok yakın değerlerin olduğu görülmektedir. Fidan başına en az püskül (1.4 adet) uygulama yapılmayan kontrol fidanlarında tespit edilmiştir.

Uygulamaların toplam çiçek sayısı üzerine etkisi kontrol uygulamasına göre önemli olmasına rağmen, EBr ve HBr'nin benzer etkiyi gösterdiği belirlenmiştir. HBr ve EBr uygulaması yapılan fidanların çiçek sayıları arasında sadece %1'lik bir fark söz konusu olmuştur.

4. Tartışma

Genel olarak, BR uygulamaları 'Chandler' çeşidinde çiçeklerin cinsiyet dağılımını değiştirmiştir. BR'lerin çiçeklenme üzerindeki etkileri nar (Engin ve Gökbayrak, 2019), kavun ve kabak (Papadopoulou ve ark., 2005) üzerine yapılan çalışmalarla ortaya konulmuş ve çiçeklenme üzerindeki etkisi bitki türlerine göre farklılık göstermiştir. Uzun gün bitkilerinde BR'ler çiçeklenmeyi teşvik ederken, erselik ve erkek çiçeklerin değişik oranlarda bulunduğu (andromonoik) bitkilerde çiçeklenmeyi engellemektedir (Manzano ve ark., 2011; Abubakar ve ark., 2012). BR'lerin çiçek çinsiyetleri üzerine etkisi monoik ve dioik bitkilerde karmaşık bir durum göstermektedir. BR'lerin monoik bitkilerde erkek çiçek sayısını azaltığı bildirilmektedir. Örneğin hıyarlarda (*Cucumis sativus*) erkek çiçek sayısını azaltarak dişi çiçek oluşumunu teşvik etmektedir (Papadopoulou ve ark., 2005). Meyve ağaçlarında cinsiyet durumu, meydana getirdikleri çiçeklerin biyolojik yapıları göz önüne alındığında daha da karmaşık bir hal almaktadır. Çiçeklerinin özelliklerine göre ortaya çıkan farklı cinsiyet durumları ve ağaçlarda erselik, erkek ve dişi çiçeklerin değişik oranlarda bulunması, BR'lerin çiçek cinsiyetleri üzerine etkisinin yorumlanmasını zorlaştırmaktadır.

Çalışmada her iki BR uygulaması da fidan başına düşen püskül, dişi ve toplam çiçek sayılarını artırmıştır. Brassinosteroid grubu bileşiklerden HBr ve EBr uygulamalarının çiçek cinsiyet dağılımına etkileri benzerdir. Ceviz ağaçlarında verim, ağaç başına düşen dişi ve erkek çiçekler ile bunların oranlarıyla yakından ilişkilidir. Fakat erkek çiçeklerin tamamı, anterleri açılıp çiçektozlarının yayılmasından sonra dökülür. Sadece dişi çiçeklerden meyve elde edilebilir. BR uygulamaları 'Chandler' çeşidinde fidan başına dişi çiçek sayılarını dikkate değer şekilde artırmıştır. Özellikle, HBr uygulamasında bu artış yaklaşık olarak %50'dir. Sladky (1972) cevizdeki dişi çiçeklerin farklılaşmasının oksin benzeri bileşiklerin seviyesinde önemli bir artış ve yüksek seviyelerde inhibitör ile ilişkili olduğunu bildirmiştir. Vejetasyon döneminde ceviz dallarında içsel hormonların birlikte etkileri söz konusudur ((Muradoğlu ve ark., 2010) ve bu çalışma brassinosteroidlerin de bu kolektif etkiye dahil olabileceğini göstermiştir.

Büyümeyi düzenleyici maddelerin çiçeklerin farklılaşmasına etkileri üzerine yapılan araştırmalar değişkenlik göstermektedir. Araştırma bulgularına benzer şekilde brassinosteroid ve gibberellin (GA) narlarda (*Punica granatum L.*) erkek çiçek oluşumunu üzerine etkilidir (Engin ve Gökbayrak, 2019). GA uygulamasının dişi çiçek sayısını, toplam çiçek sayısını ve meyve verimini kurkasta (*Jatropha curcas*) önemli ölçüde artırdığını bildirilmiştir (Makwana ve Robin, 2013). Sitokinin uygulamasının mısırda (*Zea mays*) dişi çiçek sayısını artırdığı ifade edilmektedir (Young ve ark., 2004). Bu sonuçların aksine, çin kestanesinde (*Castanea mollissima* BL) etilen uygulaması erkek çiçek salkımlarının sayısında önemli bir azalmaya neden olmuştur (Qiguang ve ark., 1985).

24-epibrassinolid ve 22(S), 23(S)-homobrassinolidin çiçeklenme öncesi dönemde uygulaması 'Chandler' çeşidinin dişi ve erkek çiçeklerine etki ederek çiçek cinsiyet dağılımını değiştirmiştir. Büyümeyi düzenleyici maddelerin uygulama zamanlarının çiçeklerin farklılaşmasına etkileri uygulama dönemlerine göre farklılık gösterebilir. Örneğin 'Şebin' ceviz çeşidinde döllenme sırasında gibberellik asit uygulaması etilen sentezini engellemekte ve çiçeklerin dökülmesini azaltarak meyve tutumunu artırmaktadır (Akça ve ark., 2012). Erkek, dişi ve toplam çiçek sayılarının artışı üzerine fidanların yıllık gelişiminin de etkili olduğu görülmektedir. Çalışmanın ikinci yılında fidanlar üzerinde bulunan erkek ve dişi çiçeklerin sayıların da dikkate değer bir artış söz konusudur. Bir yıllık gelişme, fidan başına şekillenen çiçek oranını yaklaşık olarak %50 artırmıştır. Bu artışta anaç ve kalemin her ikisinin de yıllık gelişimi etkilidir. Ceviz ağaçlarının yıllık gelişimleri üzerine 1996 yılında yapılan bir araştırmada anaç ve kalemin enine büyümesi ile çiçek sayıları arasında pozitif ve güçlü bir ilişki olduğunu ifade edilmektedir (Forde ve McGranahan, 1996).

'Chandler' çeşidinde brassinosteroid uygulamaları dişi ve erkek çiçek sayılarını artırmıştır. Ceviz ağaçlarında dişi çiçekler artırılarak verim artırılabileceği gibi erkek çiçekler sayısı yükseltilerek

tozlayıcı ağaç ihtiyacı azaltılabilir. Bu hipotezi geliştirmek için kapama meyve bahçelerinde BR uygulamaları üzerine daha fazla çalışmaya ihtiyaç vardır.

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Research Article

Potassium and Salicylic Acid Fertilization Effects on Productive and Qualitative Traits of *Cyamopsis Tetragonoloba* Under Drought Stress

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Guar,
Grain,
Protein,
Yield

Abstract: Drought is one of the main factors of abiotic stress in the agricultural world. The purpose of this study was to assess the impact of drought on Guar (*Cyamopsis tetragonoloba* (L.) Taub.) Plant and finally improve the productivity of the grain yield and the qualitative characteristics in case of high dryness by spraying salicylic acid and potassium. This experiment was performed as a plot divided into strips in a randomized complete block with three replicates over two years in Kerman, Iran. Experimental treatments include drought stress at three levels, salicylic acid (three levels) by foliar application, and potassium (two levels). Guar is resistant to high drought stress and has had a significantly improved yield. Applying 100 kg/ha of potassium in combination with a foliar spray with salicylic acid produced the highest potassium and cereal protein content. The results clearly demonstrated that potassium and salicylic acid application at all levels of drought stress and dry conditions had a positive effect on cereal yield and quality features.

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Footnote: Effect of potassium and salicylic acid on yield and its attributes some agronomic and morpho-physiology traits under drought stress. (PhD), Islamic Azad University, Agricultural Faculty, Jiroft Branch, Jiroft, Iran.

1. Introduction

Drought is one of the most serious challenges in regions where there is a lack of water, such as in Iran. The consequence of drought on vegetation is known to limit plant productivity and growth (Cevik, 2021). Over 80% of Iran is located in arid and semi-arid zones, and deals with extreme dry periods without precipitation and high evapotranspiration (Nouri et al., 2020.a). Drought conditions become more severe, longer, and more frequent, resulting in reduced plant growth and productivity. (Essahibi et al., 2018; Demirhan and Özyazıcı, 2019).

Significant expansion of areas under water stress requires planting crops with adaptability and drought tolerance characteristics (Maqbool et al., 2017). Guar is considered due to its high resistance to drought stress, rocky soils with intense light radiation, and compliance with the climatic conditions of southern Iran, as well as its industrial aspect for the economic growth and prosperity of farmers in the region. This plant is one of the most important economic products belonging to the legume family, which is mainly cultivated in semi-arid regions of the world, such as India, Pakistan, and to a lesser extent, the USA and South Africa (Ghorbani et al., 2019). Guar is used fresh for human consumption, as fodder for animals, and as seeds for gum production. It has a high protein content, around 50% (Chiofalo et al., 2018). Guar seed endosperm is the main source of galactomannans which are used mostly for industrial applications (Mathur, 2012).

Fertilizer plays an important role in supporting plant stress (Azmatet et al., 2020). According to its biochemical properties, any kind of nutrient can influence plant growth and yield (Nouri et al., 2020.b).. Salicylic acid (SA) is a phenol-based phytohormone and regulates vital plants' physiological processes, particularly water uptake and ion transport, transpiration, and photosynthesis (Klessig et al., 2018). SA interacts with other signaling molecules and thus regulates various physiological and morphological responses in plants (Iqbal et al., 2021) and increases protection against abiotic stress (Özyazici and Açikbaş, 2021). SA has been reported to increase calcium, magnesium, and potassium levels in shoots and roots in dry conditions, which can reduce the harmful effects of drought stress on plants (El-Tayeb and Ahmed, 2010). Potassium (K) is a key component of plant growth in terms of physiological and biochemical functions. It is required to activate starch synthesis enzymes. (Fathy et al., 2009). Under stressful conditions, K is capable of reducing the rate of perspiration and increasing the absorption of water, which leads to an increase in yield. Also, this element increases cell turbulence under stress conditions and reduces the adverse effect of reactive oxygen species (Aslam et al., 2021). It was reported that applying K significantly improved the morphological and physiological parameters of plants. (Raza et al., 2018). Studies show that the amount of plant proteins capable of regulating different plant activities under stressful conditions increases as a result of the application of K (Cui et al., 2019).

The objectives of this study were to assess the negative effects of drought on Guar plants and, in the long term, to improve the productivity of cereal yield and qualitative characteristics under high drought by spraying salicylic acid and potassium.

2. Material and Methods

2.1. Site description and planting

This experiment was performed as a strip split-plot in a randomized complete block design with three replications for two years (2019 and 2020) in the Agricultural Research Center of southern Kerman, Iran (28.54 degrees north and 57.85 degrees east).

Experimental treatments include drought stress at three levels (no drought stress: 100% irrigation, moderate drought stress: 80% full irrigation, and high drought stress: 60% full irrigation), salicylic acid solution according to the doses (0, 0.1 mM: 138.12 mg l^{-1}, and 0.5 mM: 690.6 mg l^{-1}) were applied by foliar application and potassium (non-application and application of 100 kg/ha). Drought stress was determined by determining the irrigation cycle and duration based on KC coefficient, plant evapotranspiration, and 10-year meteorological statistics. The exact duration of discharge was calculated based on the formula of water requirement = evapotranspiration/time.

The planting date in both years was 20 July and the growth period was approximately between 100 to 120 days, and irrigation was applied mechanized and drip. The project consisted of 18 plots (treatments) in three replications (54 treatments in total on land 60 meters long and 15 meters wide (900 square meters)). The dimensions of each plot were 2 × 2 square meters (4 square meters), and the distance of each plot was 1.5 meters, and the distance between replicates was 2 meters. Each plot consisted of 6 rows, and the row spacing was 30 cm, and the seed spacing on the row was 10 cm.

2.2. Laboratory analyses

Guar galactomannan gum (Gum) content flour was prepared from guar seeds by milling endosperm splits to a fine powder after the removal of the seed (testa) and germ (Das et al., 1977). Grain

yield (GY), grain endosperm content (Ando), harvest index (HI), and biological yield (BY) were measured by Das method (Das et al., 1977). The fresh leaves were dried at 70 ° C for 48 hours then it was milled. Protein (Pro) content by Kjeldahl apparatus and leaf potassium (K leaf) content by Swift and Sparks method (1996) were measured.

2.3. Statistical analysis

All parameters were tested using variance analysis (ANOVA). Bidirectional ANOVA was used to determine the effect of two levels of potassium and salicylic acid on Guar under different levels of irrigation (three levels). Furthermore, parameter correlation analyses were performed using a linear regression model and the PCR. Some of the datasets have been modified in terms of logarithm to satisfy the ANOVA requirement in terms of normality and homogeneity of variance. Multiple comparisons were made across partial datasets using the Duncan test. All statistical analyses have been done in software R (4.3.19).

3. Results

The analysis of variance of related-yield traits indicated that drought stress significantly affected all parameters (Table1). K fertilization, as well as a foliar spray with SA, caused significant effects on all parameters. The interactions between the stress, year, K, and SA were statistically significant for most parameters (Table1).

Table 1. Two-way ANOVA results of variables of at different years of C. tetragonoloba under different irrigation and fertilization levels

Variables	df	Grain yield (Ton/ha)	Biological yield (Ton/ha)	Harvest index	Grain endosperm (%)	Grain gum (%)	Grain protein (%)	Leaf potassium (mg/g)
Year (A)	1	0.95 **	22.6**	29 ns	27.5**	20.4**	**21.4****	**0.42****
Replication (Year)	4	0.003 ns	0.36**	21.7 ns	0.007 ns	0.04**	**0.24 ns**	**0.02 ns**
Drought stress (B)	2	4.05 **	481.8**	1332.6**	730**	309**	**710.3****	**30.5****
A×B	2	0.002 ns	0.58**	16.3 ns	0.01 ns	0.29**	**0.68***	**0.05***
Error a	8	0.04	0.18	38	0.01	0.002	**0.2**	**0.01**
Potassium (C)	1	15.2**	33**	923.6**	80.3**	53.6**	**65****	**0.78****
A×C	1	0.00001ns	0.02ns	10.8ns	0.008ns	0.39**	**0.04ns**	**0.03ns**
B×C	2	0.05ns	9.5**	53.8*	60**	0.36**	**0.06ns**	**3****
Salicylic acid (D)	2	10.7**	2.2**	1227**	16.3**	10.6**	**69.2****	**0.55****
A×D	2	0.0008ns	0.03ns	21.2ns	0.01ns	0.09**	**0.07ns**	**0.06***
B×D	4	0.08*	0.34**	63**	1.4**	0.06**	**0.09ns**	**0.08****
C×D	2	1.6**	0.51**	212.6**	1**	0.26**	**0.79****	**0.22****
B×C×D	4	0.07*	0.3*	19.7ns	1**	0.06**	**0.72****	**0.02ns**
A×B×C×D	12	0.002ns	0.11ns	11ns	0.01ns	0.12**	**0.71****	**0.05****
Error b	60	0.02	0.08	16	0.01	0.007	**0.17**	**0.01**
CV (%)	-	7	3.2	16	0.27	0.22	**1.3**	**5.6**

**,*, ns: respectively, significant at the level of 1, 5%, and no-significant.

The highest grain protein content and leaf potassium were found in the high drought stress under high application of potassium and salicylic acid in two years (Figure 1). Moreover, the lowest leaf potassium content was observed in the no drought stress and salicylic acid.

At all drought stress levels, their maximum grain yield was found in the high application of potassium and salicylic acid (Figure 2). The lowest biological yield was recorded in high drought stress under no application of potassium and salicylic acid, whereas the highest content of it exists in no drought at all levels of salicylic acid and high potassium.

The percentage of grain and gum endosperm followed similar trends under various drought stress conditions (Figure 3). There was a general decline in grain and gum endosperm with increasing

drought. The highest harvest index was found in the high drought stress under high application of potassium and salicylic acid in two years (Figure 4).

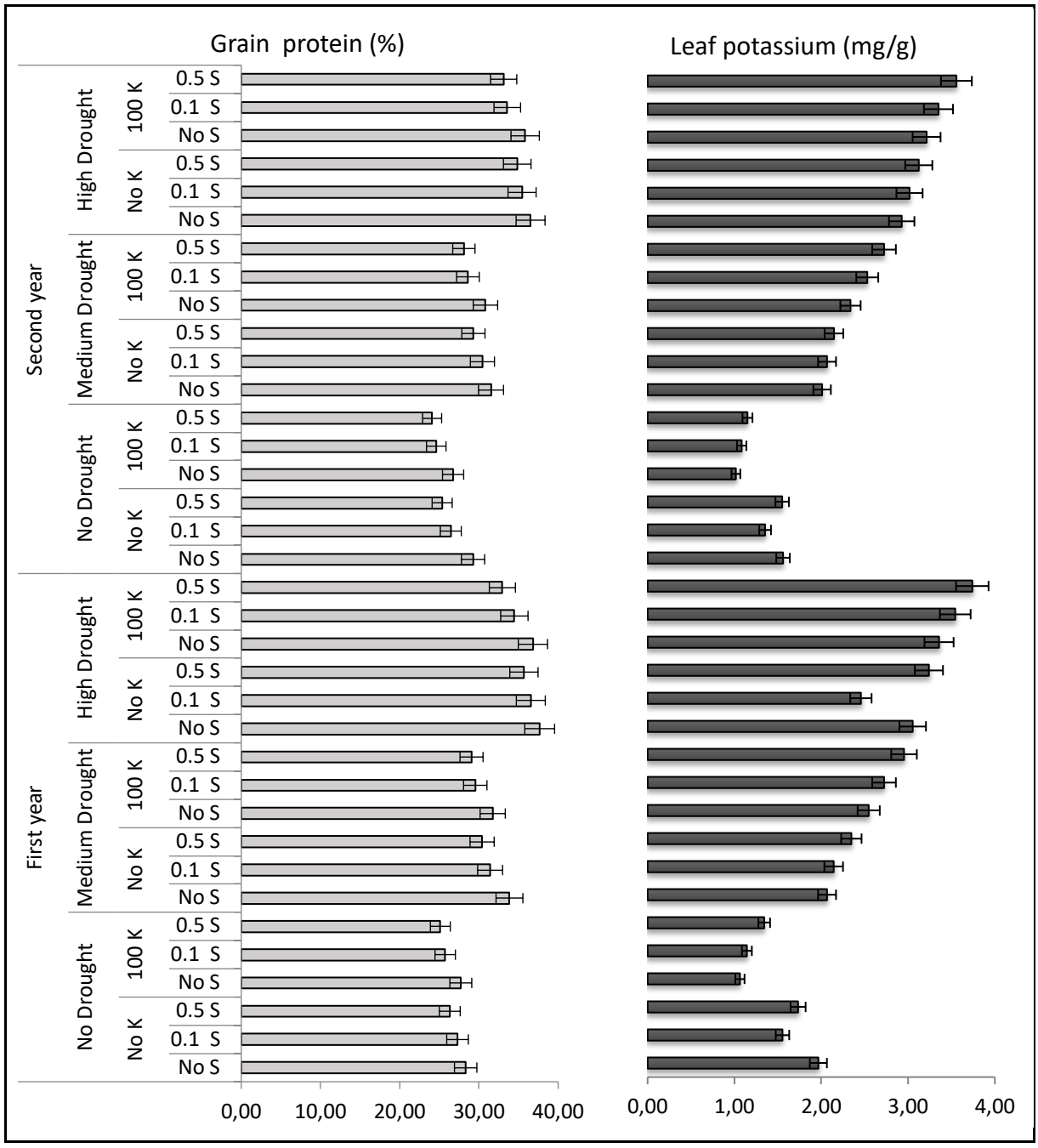

Figure 1. Average comparison of the three effects of drought stress, salicylic acid and potassium treatment on guar in two years on Nutrient factors.

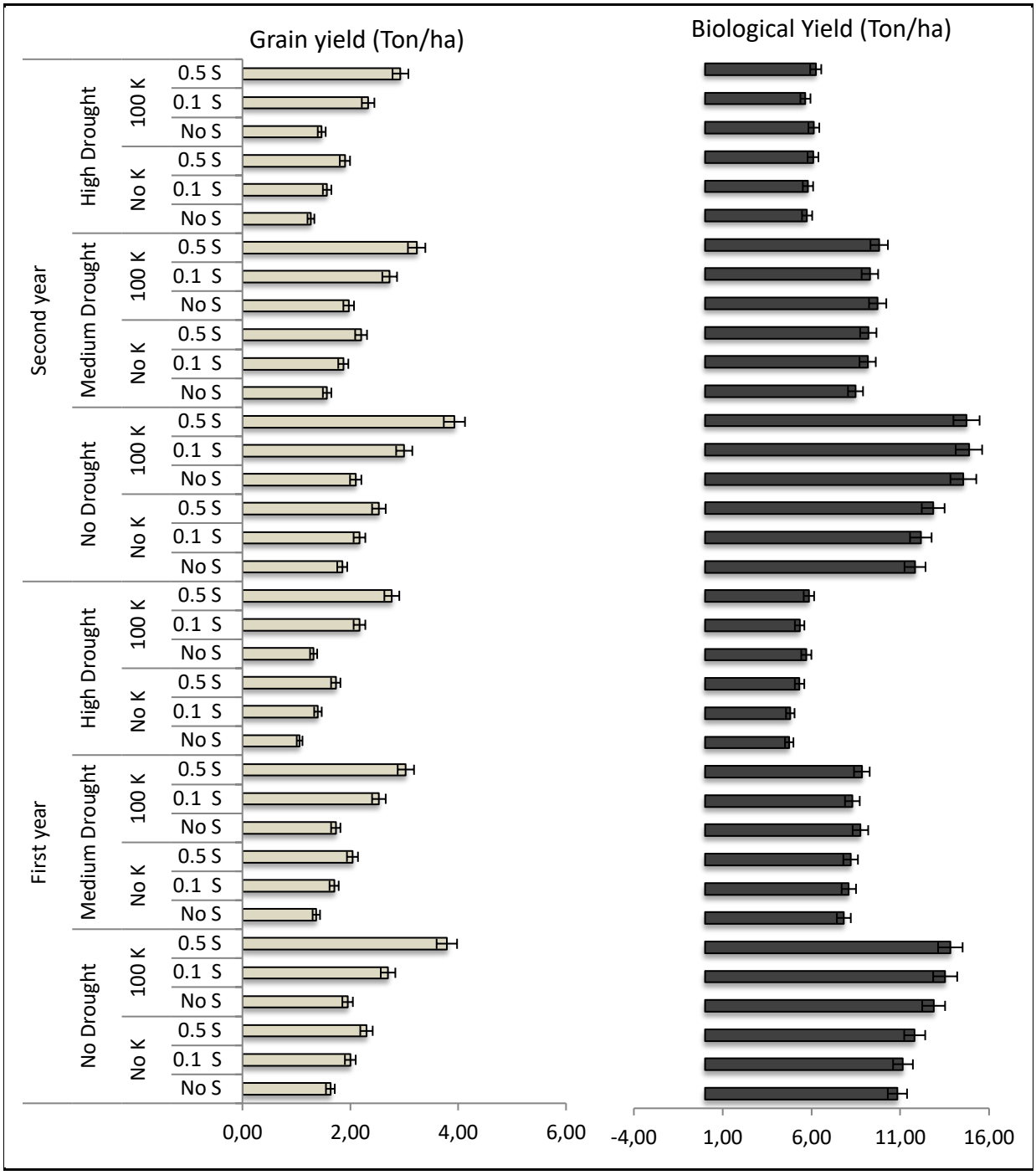

Figure 2. Average comparison of the three effects of drought stress, salicylic acid and potassium treatment on guar in two years on yeild factors.

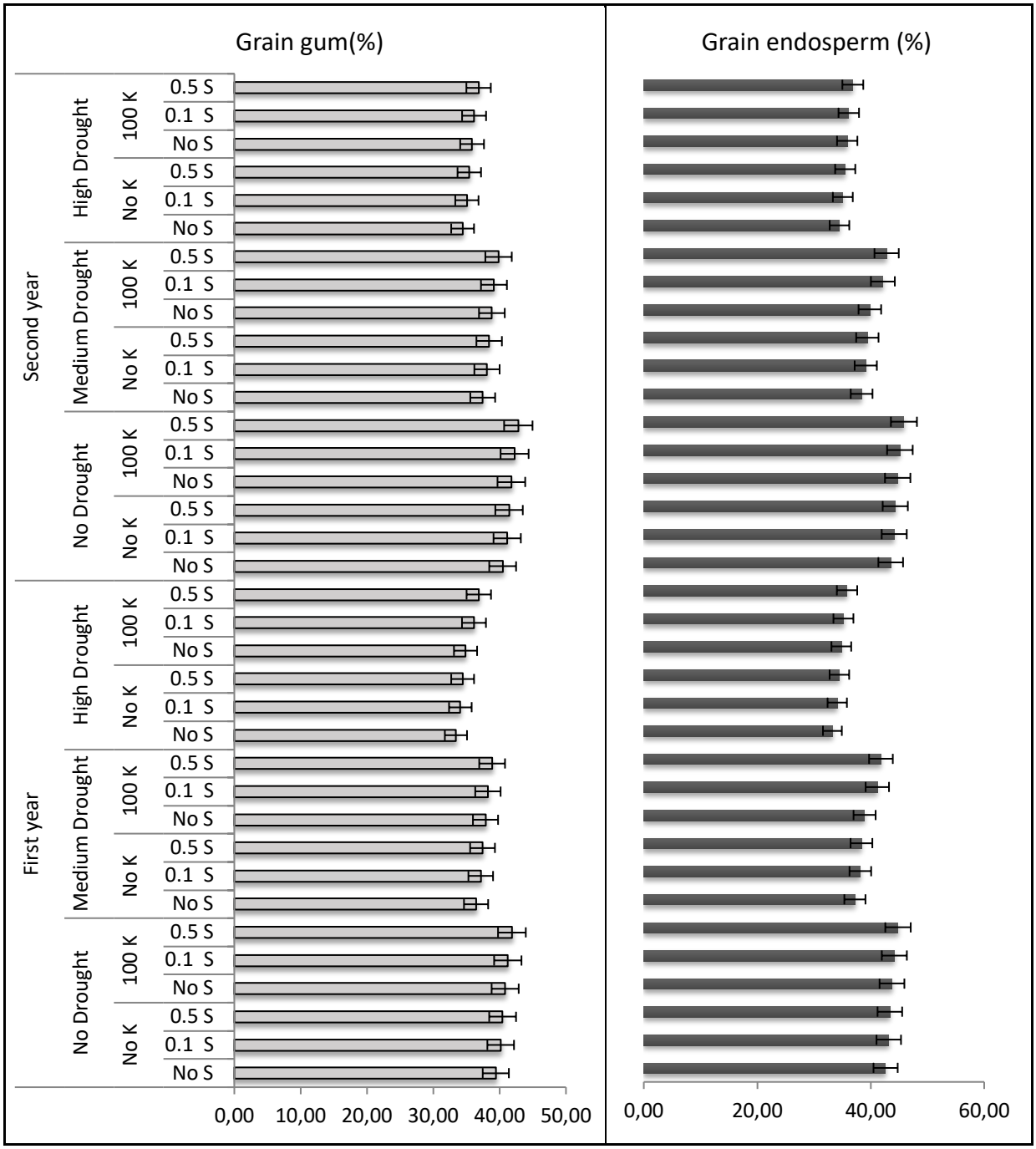

Figure 3. Average comparison of the three effects of drought stress, salicylic acid and potassium treatment on guar in two years on grain factors.

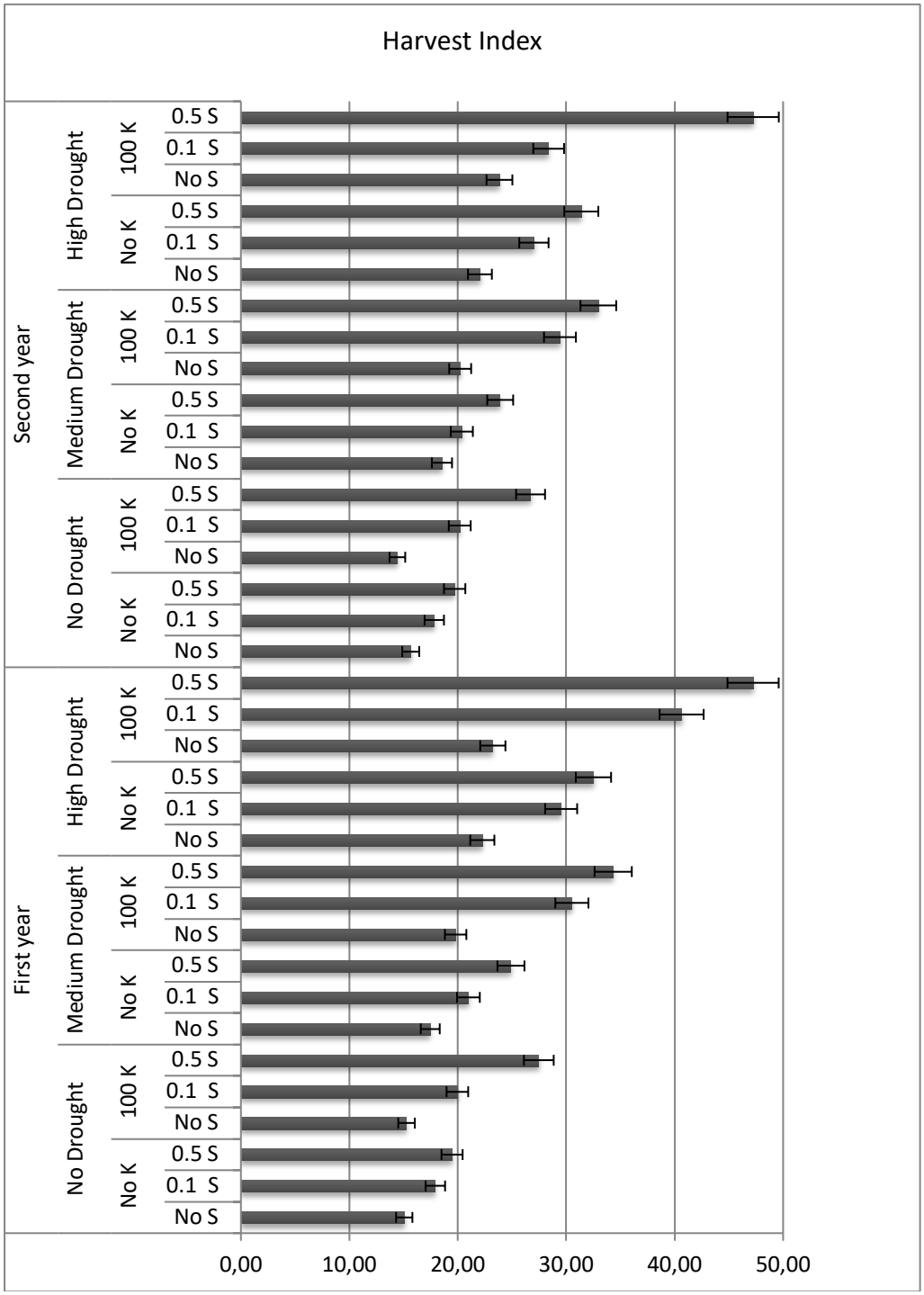

Figure 4. Average comparison of the three effects of drought stress, salicylic acid and potassium treatment on guar in two years on harvest index.

In order to more accurately assess the relationships between the characteristics of drought stress treatments, a major component analysis was conducted (Figure 5). As the graph shows, the first and second components represented about 75% and 22.1%, respectively. Approximately every association between the traits was affected by drought stress and fertilization. Furthermore, GY can be more attributed to the moderate drought stress application of 100 K and 0.5 SA, while Ando, BY, and Gum were associated with no drought, the application of 100 K, and 0.5 SA. Moreover, K leaf, HI, and Pro were integrally occupied with a high correlation with high drought stress, application of 100 K and 0.5 and 0.1 SA.

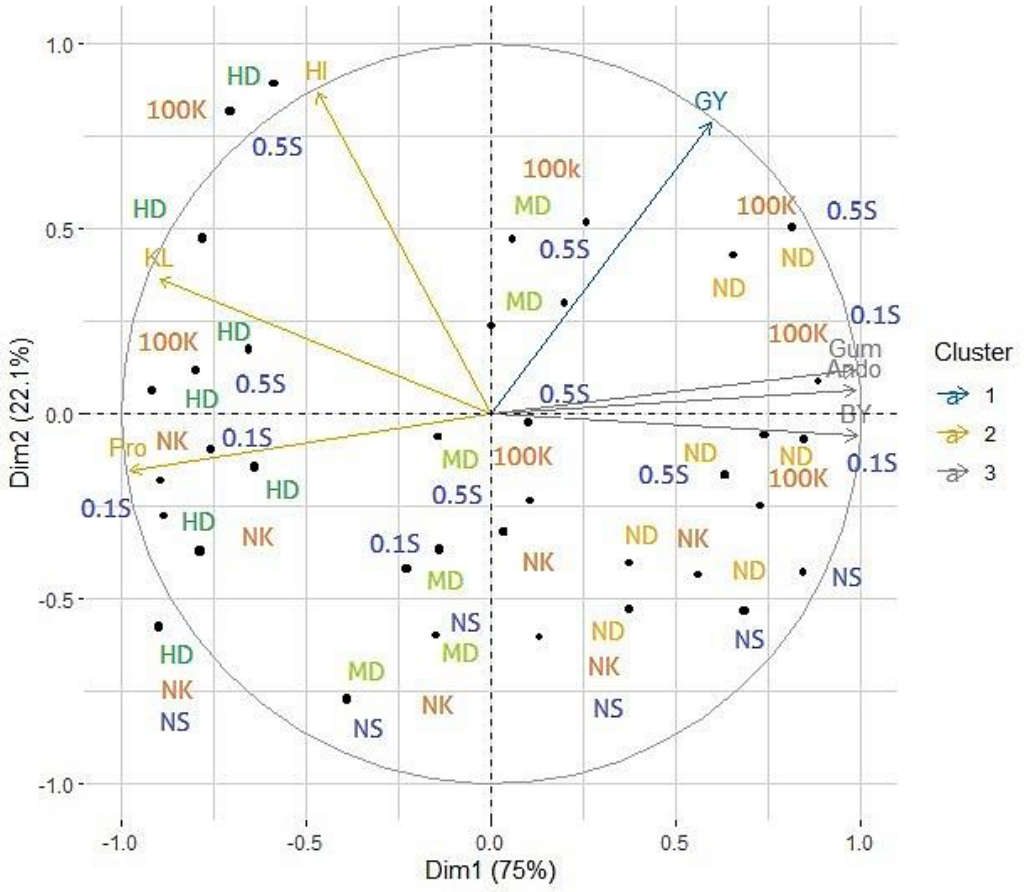

Figure 5. Principal component analysis for showing association among measured traits of C.tetragonoloba. KL: Leaf potassium (mg /g), HI: Harvest index, BY: Biological yield, Gum: Percentage of seed gum, Pro: Percentage of grain protein, Ando: Seed endosperm percentage. ND: without drought stress, MD: moderate drought stress, HD: high drought stress, NS: No salicylic acid, 0.1 S: 0.1 mM salicylic acid, 0.5S: 0.5 mM salicylic acid, NK: non- potassium, 100K: application of 100 (kg/ha) potassium.

4. Discussion

In this study, the combined effect of foliar application of salicylic acid and varying levels of potassium fertilization on Guar productivity and nutrients was assessed. The application of salicylic acid by irrigation or spraying has made it possible to improve the mechanisms of tolerance to abiotic stress in plants subjected to environmental stress (Khan et al.,2015; Gondor et al.,2016). Hoang et al., (2019) reported that drought tolerance is a major challenge in breeding rice in unfavorable environments that stopping irrigation in each growth period reduces the vegetative and reproductive growth of the plant.

Our result showed that a high level of K slightly increased grain protein, leaf potassium, grain and biological yield, harvest index, and grain endosperm and gum under drought stress conditions . About each combination of traits was affected by drought stress and fertilization. The current results were similar to those reported by Neseim et al. (2014) and Abdel-Motagally and Attia (2007). The highest cereal protein and foliar potassium content were found in the high drought stress due to the heavy application of potassium and salicylic acid over the last two years. Salicylic acid is also essential for plant growth, physiological performance, and crop productivity under abiotic stress conditions (War et al., 2011). The highest grain protein and foliar potassium content were observed in the high drought stress due to a high application of potassium and salicylic acid in two years. Potassium is an influential macronutrient that plays a key role in improving plants' water conditions, stomatal movements, enzyme

activity, osmoregulation, and membrane stability which may help the plants to tolerate the adverse effect of drought stress (Ahmad et al., 2014; Erel et al., 2015). Raza et al. (2013) found that the application of K improved the absorption of K, N, P, and Ca into wheat during drought conditions.

Consumption of potassium and salicylic acid at all levels of stress caused by dryness and even under non-critical conditions had a positive effect on guar yield and yield components. As drought stress increased, the effect of potassium and salicylic acid on increased guar gum resistance to drought stress was very apparent. The reason for this can be attributed to the effect of salicylic acid in reducing sodium uptake and increasing potassium uptake, as well as increasing the activity of antioxidant enzymes and increasing drought tolerance in guar gum.

Conclusion

In conclusion, Guar is resistant to high drought stress and had a significant increment in yield under the application of potassium and salicylic acid. However, with increasing drought stress up to 80%, a significant decrease in biological yield was observed. In general, the application of potassium and salicylic acid at all levels of drought stress and even non-stress conditions had a positive effect on yield and plant nutrients. The reason for this can be attributed to the effect of salicylic acid in reducing sodium uptake and increasing potassium uptake, as well as increasing the activity of antioxidant enzymes and increasing drought tolerance in guar gum.

Conflict of interest

The authors declare that they have no conflict of interest.

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Research Article

Estimating the Soil Water Retention Curve Using Different Empirical Models and A Piecewise Regression Method

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Water retention curve,
Soil moisture,

Abstract: In this study, the aim was to compare experimental and empirical methods used for estimation the soil water retention under different soil conditions. Soil samples were chosen to represent examples of heavy, medium and light soil structures. Water retention curves were obtained in the laboratory using the standard method. The van Genuchten (1980) (vG), and the Brooks and Corey (1964) (BC) methods were used empirically. Model parameters were determined by artificial neural networks and Solver optimization methods. In addition, soil water retention SWR curves were obtained by using a piecewise regression (PR) method. As a result of the study, determination coefficient R^2 values from 0.8946 to 0.9879 were obtained for the vG model, while the Solver method gave better results. R^2 values from 0.8914 to 0.9267 were obtained for the BC method and finally from 0.9598 to 0.9717 for the PR method. No clear differences were observed for different soil structures. Finally, the use of PR has been suggested for water retention curves where breakpoints are to be included, and it is also easy to use. In addition, the vG and BC models gave reasonable results for different soil groups. It is understood that the Rosetta method provided with the HYDRUS software program can be used in the case of limited data to determine model parameters. However, the Solver method provided more reliable results and was easy to use with both models.

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1. Introduction

Water is one of the most basic elements in terms of agricultural production. The soil–plant–water relationship needs to be well understood in order to increase product quality and productivity in sustainable agriculture. Soil moisture is the primary source of this relationship. Soil moisture is accepted as one of the most important factors for irrigation planning, fertilizer applications, transport of solutes and pollutants, and for drainage and soil mechanics (Topp et al., 2008; Jaiswal et al., 2020; Er et al., 2020).

Moisture in the soil is expressed as the water held by the pores between the soil grains. Water in the soil is classified as leaking or retained water. The water which is retained in the soil at less than

1/3 of atmosphere pressure, is drained out by force to the lower levels. (Okuroglu and Yaganoglu 2015; Demir et al., 2019). Soil water is divided into capillary water and hygroscopic water. Capillary water is defined as water held, in the soil at approximately 1/3 to 31 atmosphere pressures (Novák and Hlaváčiková, 2019). Hygroscopic water is water held with forces greater than about 31 atmospheres (Zimmermann et al., 1967; Arthur et al., 2021).

The soil water content, which characterizes the state of water in the soil and its availability for plants, is described by soil water constants. Saturation capacity, field capacity, wilting point, oven-drying, and available water are expressed by the soil water constants used in applications (Savage et al.,1996; Santra et al., 2018). The soil water content ranges represented by soil moisture constants for different structured soils are given in Table 1.

Table 1. The average volumetric soil water contents of soil moisture constants (Viliam and Hana, 2019)

Soil Moisture Parameters	**Soil Water Content (cm^3-cm^3)**
Oven Drying	0.01 – 0.20
Wilting Point	0.02 – 0.30
Field Capacity	0.10 – 0.40
Saturation Point	0.25 – 0.60

Soil water retention curves describe the relationship between the volumetric amount of water in the soil and the soil water potential (Castellini et al., 2018). This curve is characteristic of different soil types and is also called the soil moisture characteristic. Water retention curves are directly related to soil texture, structure, pore properties, and organic matter content (Hudson, 1994; Vogelmann et al., 2013, Göçük and Demir, 2021). Soil water retention curves are part of the basic soil hydrophysical properties. The water holding capacity of the soil is expressed as the amount of moisture retained between the field capacity and the wilting point. The water holding capacity is low in light textured soils and higher in heavy textured soils.This is high in low, heavy textured soils in light textured soils (Saxton et al., 1986; Sebastian et al., 2017).

The water retention curve is found by obtaining volumetric soil moisture amounts at different moisture tensions on the soil sample. The extraction of water retention curves of soils is significant when calculating the amount of water to be delivered to the soil and the required irrigation interval in agricultural areas (Fredlund and Rahardjo, 1993; Bolotov et al., 2019). Soil water retention curves can be created or estimated by using a tensiometer, filter paper, a pressure plate, TDR, a dew point hygrometer (WP4C), via a gravimetric method, regression and mathematical model-based calculations, and different software programs (Munsuz, 1982; Yongwei et al., 2021).

The most popular method of estimating soil retention at specific water potentials, which are relatively difficult to determine, is to estimate it by regression analysis using easily measurable soil properties.. The estimation of water retention curves with regression equations based on soil properties has emerged due to alternative costly and time consuming procedures in laboratory settings (Mavroulidou et al., 2013).

Jaiswal et al., (2020) performed the extraction and evaluation of water retention curves by pedotransfer in Indian soil. The soil moisture content, the bılk density, texture, and amount of organic carbon of the soil samples taken from different regions were determined using the pressure table method, the soil moisture content, the bulk density, the amount of texture and organic carbon, by applying pedotransfer function-based models. Similarly, Sysuev et al., (2013) conducted studies on pedotransfer functions and the prediction of water retention curves.

Water retention curves are often expressed in equations using mathematical models. These include van Genuchten (1980), and Brooks and Corey (1964) equations. In the study conducted by Büyüktas and Hakgoren (2005), using the Brooks and Corey and van Genuchten approaches that are widely used in determining the characteristic curves of soil water in Aksu Unit soils of the West Mediterranean Agricultural Research Institute, The functional relationships between the water content and the soil water pressure were obtained by with an MS EXCEL program, and then the Brooks and Corey (Θr, β, λ) and van Genuchten (Θr, α, n) shape parameters were determined. The shape parameters of these approaches were obtained, and their relationships to each other were examined. Chen et al.

(2016), Benson et al. (2014), and Chi (2014) found many studies on the estimation of van Genuchten (Ɵr, α, n) parameters.

In this study, the moisture content of soil samples in sandy, loamy and clayey texture classes was determined at different pressures by the pressure plate method. Based on the determined moisture levels, the aim was to evaluate the best model by making comparisons between Rosetta (2003), van Genuchten MS EXCEL solver, Brooks-Corey MS EXCEL solver, and regression analysis. Thus, the usability of the selected empirical methods in different soil texture conditions will be demonstrated.

2. Material and Methods

2.1. Soil properties

Different textured soils used in the study were taken from agricultural lands on the Bingöl Plain. Disturbed and undisturbed soil samples were taken from fields. Texture analysis included the proportional distribution of the sand, clay, and silt fractions of the soil water determined by using the Bouyoucos hydrometer method (Gee and Bauder, 1986), the specific gravity using the pycnometer method (Blake and Hartge, 1986), and the bulk density using the cylinder method for the undisturbed soil samples (Demiralay, 2011). The total porosity was determined by a formula using the specific density and bulk density (USSL, 1954). Hydraulic conductivity values were determined using the disturbed soil samples according to the constant water level method with a laboratory permeameter (Demiralay, 2011). The physical and hydraulic characteristics of the soil used in the study were given in Table 2. The texture class of S2 and S3 soils is the same, but however, it was used in the experiment because the clay content was quite different compared to the other two classes.

Table 2. The properties of the soil used in the study

Case	Sand (%)	Silt (%)	Clay (%)	Textural Class	Bulk density (g cm^{-3})	Porosity (%)	Hydraulic Conductivity (cm h^{-1})
S1	32	30	38	CL	1.22	51.00	0.23
S2	36	40	24	L	1.49	45.22	0.61
S3	46	40	14	L	1.42	46.21	1.63

2.2. Determination of the Soil Water Retention Curve Using the Pressure Plate Method

Many methods are used to determine soil water potential (such as the pressure plate apparatus, thermocouple psychrometry, heat dissipation sensors, and dew point potentiometer (Campbell and Gee, 1986). Among these methods, the most widely used is the pressure plate method. Pressure plates are very common empirical devices that are applied to evaluate the soil water retention curve (Richards, 1948, 1965; Klute, 1986). The method relies on the application of air pressure to the soil sample and the removal of water from the porous media. The soil sample saturated with water is placed in a pressurized container with a semi-permeable ceramic plate. The pressure range applied to the soil samples varies depending on the soil type and the technical parameters of the apparatus used. Generally, air pressures are in the range of 33 kPa (Hw-330 cm) to 1500 kPa (Hw-15000 cm) (Richards, 1953; Tinsley, 1967). It can calculate the soil water content with a value between 2 and 4.5 on the pF curve (Toll, 2012; Kim et al., 2016).

2.3. Soil water retention curve models

2.3.1. The van Genuchten model

The van Genuchten (vG) function is often used to describe the soil water retention curve (SWRC) of unsaturated soils and has the following form:

$$\theta_h = \theta_r \frac{\theta_s - \theta_r}{(1 + (\alpha h)^n)^m} \quad (1)$$

where Θh is the effective soil water content as a function of pressure head, and Θs is the saturated soil water content that was assumed to be equal to soil porosity (P) obtained at a laboratory

$$P = (1 - \frac{\gamma_s}{\gamma_t}) \quad (2)$$

γ_s is the soil particle density obtained by the pycnometer method at a laboratory
γ_t is the soil bulk density obtained with the weight of dry soil per unit of volume at a laboratory

Θr is the residual water content defined here as the water content for which the gradient (dΘ/dh) is zero. Residual water content measurements are not always made routinely, in which case it is usually determined with estimating techniques.

h is the soil suction (cm) α, n and m are the empirical shape parameters.
Many researchers assume that m = 1 and it has been successfully used in many studies to describe the soil water retention data. However, it can be assumed to be (m = 1−1/n) with the integrated results. α, n and Θr estimated by using the rosetta model

The Rosetta model

The Rosetta model can estimate the vG parameters Θr, n, and α. This model has five options for input data: a) soil texture class, b) sand, silt, and clay percentages, c) sand, silt, and clay percentages and bulk density, d) sand, silt, and clay percentages, bulk density, and field capacity (33 kPa) water content and e) sand, silt and clay percentages, bulk density, field capacity and wilting point (1500 kpa) water content. This study used option e) with six inputs to estimate the vG model parameters. These estimates were generated by combining the artificial neural networks with the bootstrap method (Schaap et al., 1999). For this purpose, RETC (van Genuchten et al., 1991) computer code was used to implement the Rosetta model.

The solver optimization method

Solver is a function in EXCEL that can calculate the maximum or minimum value of one cell by changing other cells. This function was used to estimate Θr, n, and α by the minimization of the sum of squared deviations from the measured water content values (Anlauf, 2014).

2.3.2. Brooks and Corey (1964) model

Brooks and Corey (1964) suggested an equation for SWRC as follows:

$$\frac{\theta-\theta_r}{\theta_s-\theta_r} = (\alpha h)^{-n} \quad (3)$$

where α is the inverse of the air-entry value (1/cm) and n is the curve-fitting parameter. The saturated soil water content Θs is assumed to be equal to the soil porosity, and the α and n parameters were estimated by the Solver optimization method.

2.3.3. Piecewise regression model

Regression analysis was conducted to determine the relationship between the soil suction and water content. Due to the complex shape of the soil water retention curve, regression analysis could not produce satisfactory results. Therefore, the data was divided into two pressure groups: 1 to 2000 kpa and 2000 to 15000 kpa, depending on the shape of the curve. Linear, logarithmic, polynomial, exponential, and power curve fitting approximations were applied, and the best approximation equation with the highest coefficient of determination (R^2) was selected according to Toms and Lesperance, (2003).

2.4. Statistical analysis

The accuracy of the results of the models used to derive the soil water retention curve was evaluated using the coefficient of determination (R^2) and the root-mean-square error (RMSE) between the measured and predicted values expressed as follows:

$$R^2 = \frac{\sum_1^N (y_i - \hat{y}_i)^2}{\sum_1^N (y_i - \bar{y}_i)^2} \quad (4)$$

$$RMSE = \sqrt{\frac{\sum_1^N (y_i - \hat{y}_i)^2}{N}} \quad (5)$$

where γ_i represents the measured value, $\hat{y}_i$ is the predicted value, $\bar{y}_i$ is the average of the measured value γ, and N is the total number of observations. Analyses were performed using SPSS software (SPSS 2013).

3. Results

3.1. Water retention curves obtained under laboratory conditions

Soil moisture values kept at different negative pressure values (0.1, 10, 33, 50, 100, 300, 500, 700, 900, 1200, and 1500 kPa) for each soil group were determined by the pressure plate method to obtain the water retention curves (Figure 1.). It was determined that the water retention values changed depending on the constitution class of the soil. In other words, the moisture content of soils under the same pressure followed the progression S1> S2> S3. The highest moisture values were obtained in the S1 group and the lowest in the S3 group. Moisture values staying above the wilting point (1500 kPa) value were not considered in this study because they are insignificant for agricultural irrigation.

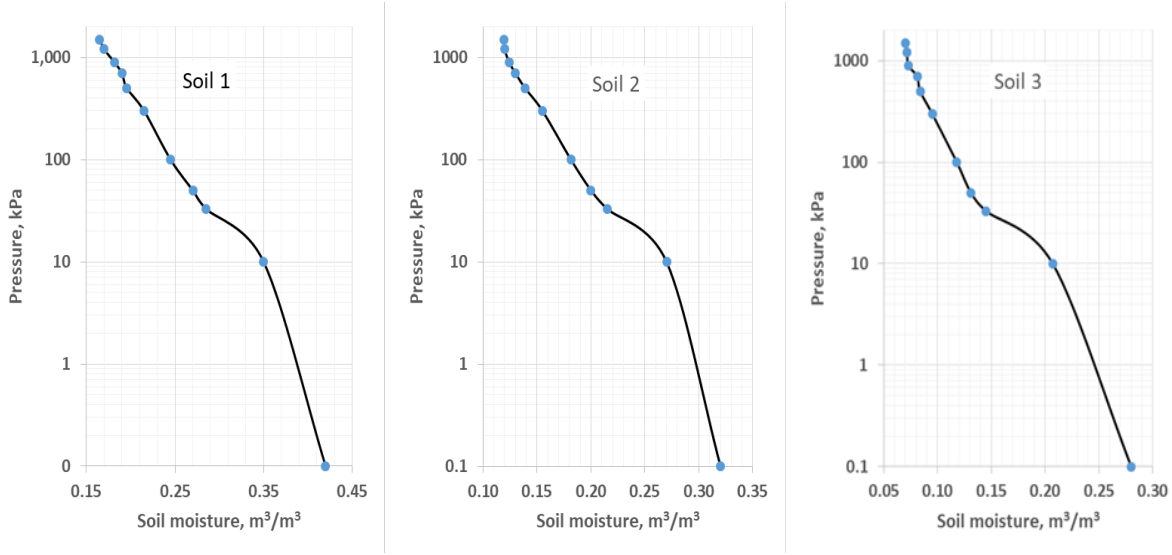

Figure1. Water retention curves determined by the pressure plate method.

3.2. Results Obtained By The Models

The results determined by the van Genuchten (1980) and Brooks and Corey (1964) model parameters are shown in Table 3. The van Genuchten parameters α, n, m, and Θs (measured) and Θr for three different soil types were determined using the Rosetta and Solver methods. In the Brooks and Corey model, α, ʎ, Θs (measured), and Θr parameters were determined by the Solver method.

Table 3. The van Genuchten (1980) and the Brooks and Corey (1964) model parameters

The van Genuchten parameters						
Soil group	**Estimating method**	**Model parameters**				
		α	**n**	**m**	**θ_s**	**θ_r**
S1	Rosetta	0.0255	1.3137	0.2388	0.4853	0.0740
	Solver	0.0001	0.2198	2.2671	0.5100*	0.0000**
S2	Rosetta	0.0295	1.3405	0.2540	0.3818	0.0525
	Solver	0.0001	0.18383	2.62696	0.4522*	0.0000**
S3	Rosetta	0.0464	1.4351	0.3032	0.3757	0.0334
	Solver	0.0002	0.1728	3.3067	0.4621*	0.0000**
Brooks and Corey model parameters						
Soil group	**Estimating method**	**Model parameters**				
		α	**ʎ**	**θ_s**	**θ_r**	
Soil 1	Solver	43.4334	0.0901	0.5100*	0.000**	
Soil 2	Solver	167.4351	0.0978	0.4522*	0.000**	
Soil 3	Solver	280.5307	0.1347	0.4621*	0.000**	

* measured value.
** assumed value.

Regression Model Results

Considering the breaking points in the water retention curves, the regression equation for pressures from 0.1 to 100 kPa and from 100 kPa to 1500 kPa were obtained by dividing them into two parts. The best fit was obtained with an exponential function, and the regression parameters are given in Table 4, while the curves are given in Figure 2.

Table 4. Regression models for estimating water retention curves

Soil group	**model parameters($y = a \cdot x^b$)**					
	Part 1(<100kpa)			**Part 2(>100kpa)**		
	a	**b**	**R^2**	**a**	**b**	**R^2**
S1	0.3681	0.0740	0.8901	0.4840	0.1470	0.9894
S2	0.2800	0.0780	0.8636	0.3903	0.1660	0.9885
S3	0.2243	0.1230	0.8945	0.2939	0.1990	0.9865

In order to compare the performances of water retention curves obtained by using experimental and empirical equations, the root mean square error (RMSE) and determination coefficient (R2) were compared. The results related to these are given in Table 5 and Figure 3.

Table 5. Statistical comparision of models

Soil group	**Statistical criteria**	**vanGenuchten *Rosetta***	**van Genuchten*Solver***	**Brooks and Corey *Solver***	**Regression model**
S1	RMSE	0.0908	0.0086	0.0231	0.0132
	R^2	0.8946	0.9879	0.9125	0.9717
S2	RMSE	0.0279	0.0106	0.0211	0.0121
	R^2	0.9780	0.9726	0.8914	0.9650
S3	RMSE	0.0829	0.0098	0.0174	0.0129
	R^2	0.8978	0.9764	0.9267	0.9598

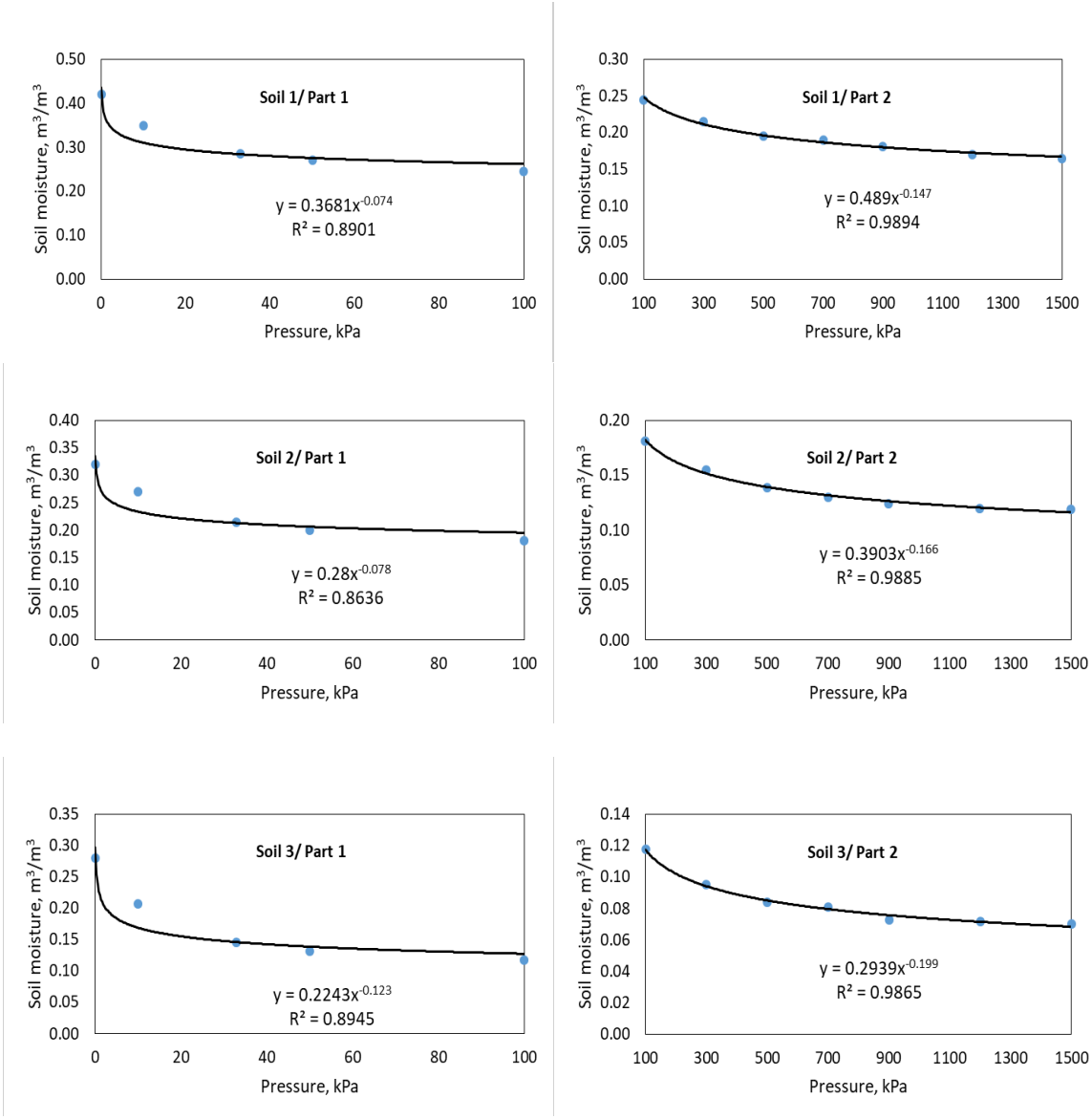

Figure 2. Regression equations.

The van Genuchten Solver ($R^2 = 0.9878$) method gave the best results for different negative pressure ranges within the methods for S1 soil. The Regression model ($R^2 = 0.9716$), Brooks Excel Solver ($R^2 = 0.9125$), and van Genuchten Rosetta ($R^2 = 0.8946$) followed this model.

For S2 soil, the van Genuchten Rosetta ($R^2 = 0.9780$) method gave the best results for different negative pressure ranges within the methods. The van Genuchten Solver ($R^2 = 0.9726$), Regression model ($R^2 = 0.9650$), and Brooks Excel Solver ($R^2 = 0.8914$) followed this model (Table 5).

It was obtained the best result in the van Genuchten Solver ($R^2 = 0.9764$) for different negative pressure ranges in S3 soil. The Regression model ($R^2 = 0.9598$), Brooks Excel Solver ($R2 = 0.9267$), and van Genuchten Rosetta ($R2 = 0.8978$) followed this model.

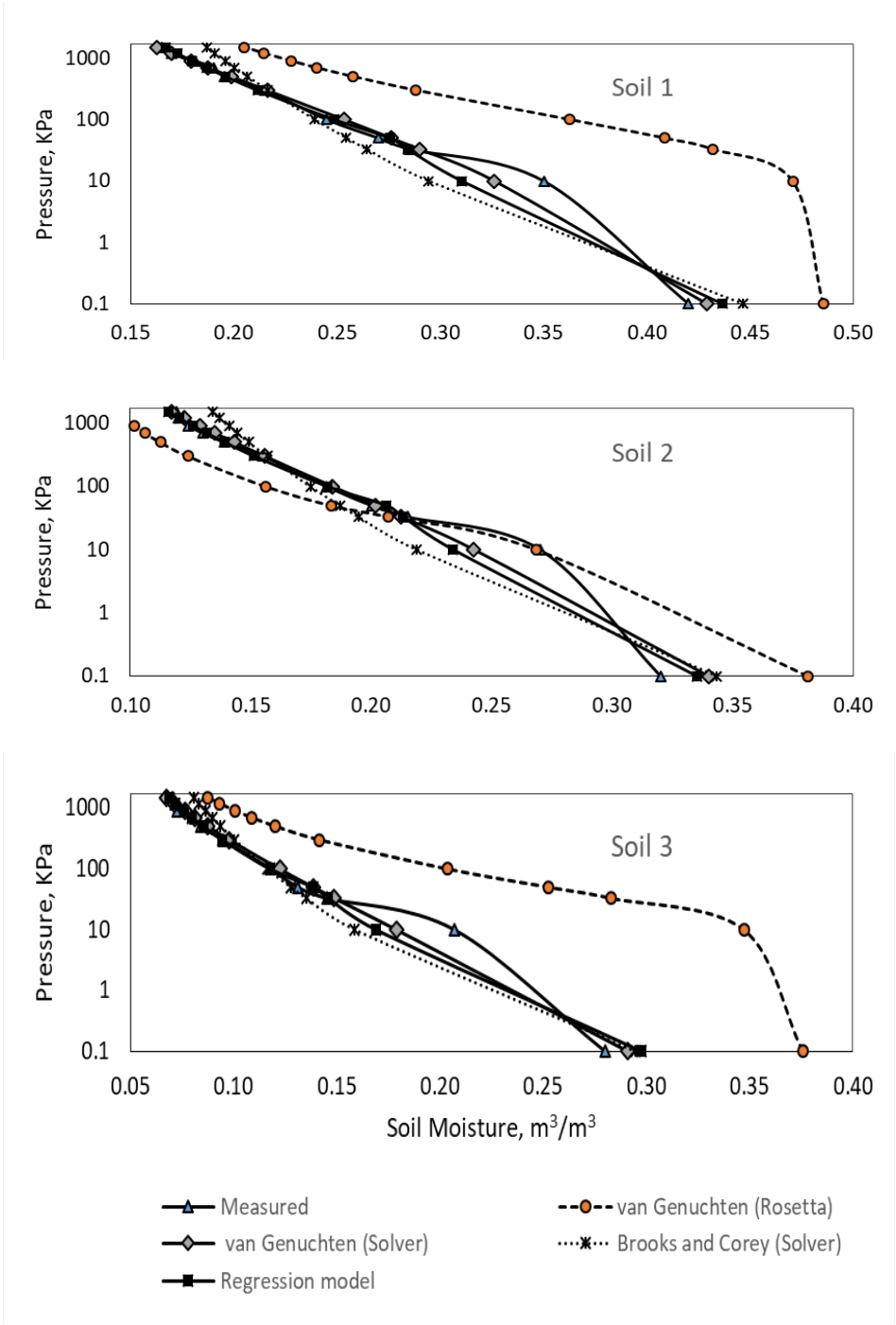

Figure 3. Soil water retention curves for the models.

Discussion

Pan et al. (2019) obtained R^2 values from 0.958 to 0.997 for van Genuchten and the Brooks and Corey models. The researchers stated that both models gave a higher variation and error in the 10 to 50 kPa range compared to the higher pressures. This situation showed the significance of the Piecewise regression application considered in our study. The R2 values in the Piecewise regression model study were from 0.9598 to 0.9717, with RMSE values varying from 0.0121 to 0.0132. These values showed that the model generally gave stable and realistic results. It has advantages such as being easier to obtain, with the equations being simpler and easier to use. Barker et al. (2019), stated that more reasonable

results were obtained with the Piecewise regression method for the relationship curves with fragile shapes.

In the study, when the two different approaches used in determining van Genuchten model parameters were compared, it was seen that the Solver optimization method gave better results. Similarly, Babangida et al. (2014) used the model parameters of the Solver method for optimization. The method is easily usable in EXCEL and is its biggest advantage. On the other hand, Rosetta gave R2 values from 0.8946 to 0.9780 with the model parameters obtained by using the basic properties of the soil (texture, field capacity, and wilting point). The method makes estimates with fewer easily available inputs. One of the weaknesses of these predictions is that they do not take into account the structure and mineralogy of the soil and instead assume that soils of similar texture have similar soil hydraulic properties. (Domínguez-Niño et al., 2020).

Conclusion

Soil water retention curves are of great importance in irrigation planning and in agricultural productivity, and protection of land and water resources. Many researchers have modeled the water retention curves by measuring the moisture values maintained at certain pressures using the pressure plate method under laboratory conditions. In this study, it was determined that it provided reasonable results for different soil groups as a result of an evaluation with the van Genuchten, and Brook and Corey models. It is understood that the Rosetta method provided with the HYDRUS package program can be used with limited data in determining the model parameters. The Solver method provided more reliable results and easy operation for both models. However, the fact that the water retention curves contain shape breaking points has highlighted the use of Piecewise regression. As a result, as far as the applicability of the model studies is concerned, it is understood that it depends on the number of experimentally acquired inputs and the easy availability of these inputs.

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Research Article

Determination of *In Vitro* True Digestibility and Relative Feed Values of Alternative Roughage Sources

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In vitro digestibility,
Oak tree leaves

Abstract: The aim of this study was to compare chemical composition, relative feed values, and in vitro true digestibility of the giant fennel (*Ferula communicus* L. -F), helis (*Prangos ferulacea* L. -H), gum- tragacanth (*Astragalus microcephalus WILD*. -G) and leaves oak tree (*Quercus robur* L. -O), a naturally growing plant on the high- altitude plateaus of the Eastern Anatolia, with those of alfalfa. In vitro true digestibilities (IVTD) of roughages were determined with the Ankom Daisy^{II} incubator. In terms of crude protein (CP) content, the lowest value was determined in H herb with 7.35 %, and the highest value was determined in alfalfa hay (A) with 19.28% ($p<0.05$). G hay had higher acid detergent fiber (ADF) and neutral detergent fiber (NDF) content and O leaves had higher ether extract (EE) and condensed tannin (CT) content. While the lowest IVDMD were found in G hay (42.91%) and O leaves (56.22%) with the highest cell wall structural components and CT content, the highest digestibility value was determined for F (70.47%) and A (71.60%) ($p<0.05$). Considering the analyzed parameters, it can be said that F hay is more suitable for ruminant feeding than other roughages.

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1. Introduction

Livestock animals make critical and valued contributions to society throughout the world and play a key role in agriculture. The main role of ruminants in the ecosystem is the ability to break down the structural carbohydrates in plants and metabolize them to make meat and milk that can be consumed by humans or other animals in the food chain. Roughage is an important main component of ruminants' feeds. Roughage is necessary to keep the maintenance of rumen health as it should. Considering the existence of animals in our country, there is a large deficit in roughage production in terms of both quantity and quality.

According to official data of TUIK (2021a;b), Türkiye has 18 million 26 thousand bovine animal unit livestock existence, and 71.3 million tons of quality roughage is needed to feed the animal existence. However, the total quality roughage production in Türkiye is 67.2 million, 11.7 million from the meadow and grassland lands, and 55.5 million from forage crops. Accordingly, the roughage gap in the country is 4.1 million tons. Ruminant producers are forced to look at alternative sources of roughage to see their animals due to a shortage of roughage.

Ferula communis L. (giant fennel-locally called çakşır or kerkol) and *Prangos ferulacea* L. (locally called helis) is a naturally growing plants in the high- altitude plateaus of the Eastern Anatolian region. This plant produces seeds every other year, but it is not cultivated yet. It is a perennial plant and is most densely diversified in Iran-Turan phytogeographic region (Duran et al., 2005; Hakan et al., 2009; Çelikezen et al., 2019). It is claimed to be effective on oustrus and ovulation of sheep and goats (Keskin et al., 2004; Önal et al., 2004). It is reported that this effect is caused by a phytoestrogenic substance called ferutin (Appendino et al., 2001). Helis is used as hay and winter fodder for ruminant animals in Turkiye, Iran, Central Asia, North India, and the Caucasus (Razavi, 2012). Because it grows on the steep slopes of the mountains, it is mostly evaluated by sheep. Caksir, helis, gum-tragacanth, and oak tree leaves, which grow in the high plateaus of the Eastern Anatolia Region, are harvested in June-July, dried, and used as a source of roughage in winter. Azarfard (2008) suggested that alfalfa hay can be replaced with *P. ferulacea* in the finishing rations of lambs at 35% to 60% levels, while 100% levels increased the fat in the carcass and tail of lambs.

Astragalus species have anti-inflammatory (Kim et al., 2013), immunostimulatory (Qin et al., 2012), antioxidant (Kim and Yang, 2005), and antiviral activities (Sanpha et al., 2013). Due to these properties, it has been reported that Astragalus species are used in the roughage ration, and their by-products are used as additives to the feed of farm animals (Qiao et al., 2018). *Quercus robur* L. is the most common oak species in the forest areas of the Eastern Anatolia Region, where winters are colder and longer, rainfall is heavy, and temperature differences are high. *Quercus robur* L. and *Quercus brandii* L. are common in Hakkari, Bitlis, Muş, Bingöl, Elazığ and Malatya (Günal, 2013). In these regions, oak leaves are an important source of roughage for small ruminants, especially goats. It has been reported that the leaves of trees and shrubs have superior mineral and protein composition compared to some hay and can be used to meet the protein and mineral needs of ruminants which can make good use of low-quality roughage (Kongmanila, 2012).

In this study was compared with traditionally used alfalfa hay of relative feed values and *in vitro* true digestibility of giant fennel, helis, gum-tragacanth, and oak tree leaves, which are widely grown in the high-altitude plateaus of the Eastern Anatolia Region and used by the local people for small ruminants feeding.

2. Material and Methods

2.1. Ethical statement

This study was approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Approval no: 2019/07).

2.2. Plant samples

In the experiment *F. communis* L., *P. ferulacea* L., *A. microcephalus WILD*. and *Q. robur* L. leaves, which are grown in the meadow-pasture and forest areas of Bitlis Province Hizan District and are widely used by the people of the region for feeding small ruminants, were used as feed materials in the experiment. In addition, alfalfa hay (*Medicago sativa* L.), which is traditionally used in ruminant feeding, was compared with other roughages used.

The forages were harvested and dried from the meadow-pasture and forest areas of the villages of Hizan district of Bitlis province in July (giant fennel, helis, and gum-tragacanth samples) and August (oak tree leaves). Plant sampling was done from 3 villages representing Bitlis Province Hizan District and 3 samples from each village. Villages were taken as replicates. All plant samples in the same village were combined. The giant fennel, helis, and gum-tragacanth samples were taken from all parts of the plant, while leaf samples were taken from the oak tree. Plant samples dried in laboratory conditions were ground on a mill and sieved in 1 mm diameter sieve to make the samples ready for chemical analysis.

2.3. Chemical analysis

Dry matter (DM), crude protein (CP) and ash of all the samples were determined according to the standard methods of AOAC (1998). Ether extract (EE) analysis was carried out as described by AOCS Am 5-04 (Komarek et al., 2004). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods of Van Soest et al. (1991) using an ANKOM 220 Fiber

Analyzer (ANKOM Technology Corporation, NY, USA). Condensed tannin (CT) was determined by butanol-HCl-iron method as described by Bate-Smith (1975). All chemical analyses were carried out in quadruplicate. The CT (%) of samples was calculated using equations as follows:

CT (%) = Absorbance (550 nm×156.5×dilution factor) / Dry weight (%)

2.4. *In vitro* true digestibilities

In vitro true digestibility (IVTD) was determined in the Ankom Daisy incubator by using the filter bag technique according to Van Soest *et al*. (1991) and Ankom technology (2002) procedures. In the Ankom Daisy incubator, rumen fluid was used as the inoculum source. The rumen content was obtained post-mortem from the rumens of two Simental cattle aged 22 months old that were slaughtered in a commercial abattoir in Van. Animals were fed twice daily with a diet containing grass, hay, and straw (60%) and concentrates (40%). After filtering the rumen fluid through two layers of cheesecloth under CO_2 into a thermos at 39 °C, approximately 100 g of solid rumen content was added and delivered to the laboratory within 20-25 minutes. The Daisy incubator instrument contains 4 cylinder jars. 400 ml of rumen fluid as inoculum and 1600 ml of buffer solution (solution B and A, mixing ratio 1/5 and mixing pH 6.98) and 24 filter bags were placed in each cylinder jars . The jars were closed immediately after bubbling with CO_2 and allowed to incubate for 48 hours.

The IVTD (%) of samples was calculated using equations as follows:

$$IVTD\ (\%) = 100 - ((W3 - (W1 \times C1)) \times 100)/W2 \quad (1)$$

W1: Weight of filter bag, W2: Weight of sample, W3: Final weight after NDF analysis,
C1: The bag without a sample was also prepared for correction

2.5. Determination of relative feed value and quality *in vitro* true digestibilities

The relative feed value (RFV), which is used as an important tool in forage quality evaluation and marketing, was calculated according to the following equations (Rohweder et al., 1978).

Dry matter intake (DMI, live weight %) =120/(NDF%) (2)
Dry matter digestibility (DMD, %) = 88.9 – (0.779× ADF%)
RFV (%) = (DMI × DMD)/1.29

According to the Quality Standard assigned by The Hay Marketing Task Force of the American Forage and Grassland Council, the RFV was evaluated as roughages based on reject <75 (5), 75-86 poor (4), 87-102 fair (3), 103-124 good (2), 125-151 premium (1) and prime >151.

2.6. Statistical analysis

The data obtained from the experiments were analyzed one-way ANOVA completely randomized design (SAS, 2014). The differences between roughages chemical composition, forage quality, and IVDMD were tested by using Duncan's multiple range test.

3. Results

The chemical composition, the cell wall structural elements of roughages, and condensed tannins are presented in Table 1. Accordingly, H and A hay had the lowest DM content than the other roughages, while the other roughages had no significant differences from each other. The highest ash level was found in H ($p<0.05$), followed by A>F>G=O. The CP content of the roughages ranged from 7.35% in the H hay to 19.28% in the A. The A hay, on the other hand, had higher CP content when compared to its other roughages ($p<0.05$). The EE content of roughages ranged from 5.37 to 0.65%, the highest being in O leaves. The NDF content of the roughages ranged from 34.27 to 64.64%, the highest NDF content was found in G with 64.64%, and this value was followed by O with 53.70%. The ADF

content of the roughages ranged from 26.39 to 54.31%, the highest being in the G hay and the lowest in the A hay ($p < 0.05$).

The condensed tannin concentration of the roughages was significantly different ($p<0.001$). The CT concentration ranged between 0.94 to 6.97 DM%. Among the roughages A hay contained significantly ($p<0.05$) the lowest CT, while O leaves had the highest concentration of CT.

Table 1. Chemical compositions of selected roughages (DM %)

Roughage	DM	Ash	CP	EE	NDF	ADF	CT
F	93.52±0.74^{a}	7.49±0.21^{c}	9.05±0.59^{b}	2.68±0.22^{b}	34.27±1.08^{e}	31.55±1.03^{c}	2.08±0.12^{c}
H	90.92±0.13^{b}	12.28±0.55^{a}	7.35±0.14^{c}	2.74±0.04^{b}	43.16±0.89^{c}	36.37±0.77^{b}	1.85±0.06^{c}
G	93.70±0.15^{a}	6.20±0.15^{d}	8.24±0.48^{bc}	0.65±0.07^{c}	64.64±0.65^{a}	54.51±0.65^{a}	3.12±0.14^{b}
O	93.42±0.30^{a}	6.04±0.30^{d}	9.00±0.51^{b}	5.37±0.27^{a}	53.70±0.91^{b}	35.68±0.79^{b}	6.97±0.28^{a}
A	90.44±0.21^{b}	9.01±0.52^{b}	19.28±0.09^{a}	2.32±0.09^{b}	37.72±0.92^{d}	26.39±059^{d}	0.94±0.03^{d}
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^{a,b,c} Values within a row with different superscripts differ significantly at $p<0.05$.

F: *Ferula communicus* L.- caksir, H: *Prangos ferulacea* L. -helis, G: *Astragalus microcephalus WILD*. - gum-tragacanth and O: *Quercus robur* L.- leaves oak tree, A: *Medicago sativa* L.-alfalfa.

The IVDMD, DMD, DMI, RFV, and RFV quality classes of roughages are presented in Table 2. A hay and F hay outperformed H, O leaves, and G hay with respect to IVDMD, DMD, DMI, and RFV ($p<0.001$). The IVDMD of roughage feeds ranged from 42.81 to 71.60%, being highest for A hay and lowest for G hay ($p<0.05$). The RFV adopted in marketing and pricing of roughage in many developed countries was found to be 67.06 on average for G hay in this study. According to RFV results, G hay was classified in "5-reject" quality class.

Table 2. Forage quality and IVDMD values of different roughages

Roughage	DMD,%	DMI, BW%	RFV	RFV Quality	IVDMD,%
F	64.32±1.69^{ab}	3.53±0.24^{a}	154.58±5.88^{a}	Prime	70.47±0.38^{a}
H	60.57±1.11^{b}	2.79±0.11^{b}	131.09±7.56^{b}	Premium (1)	64.74±0.39^{b}
G	46.43±1.13^{c}	1.86±0.06^{c}	67.06±3.78^{c}	Reject (5)	42.81±1.05^{d}
O	61.10±0.98^{b}	2.24±0.05^{c}	105.98±3.40^{b}	Good (2)	56.22±0.68^{c}
A	67.96±0.50^{a}	3.02±0.08^{b}	159.35±5.40^{a}	Prime	71.60±0.77^{a}
P value	<0.001	<0.001	<0.001	<0.001	<0.001

^{a,b,c} Values within a row with different superscripts differ significantly at $p<0.05$.

F: *Ferula communicus* L.- caksir, H: *Prangos ferulacea* L. -helis, G: *Astragalus microcephalus WILD*. - gum-tragacanth and O: *Quercus robur* L.- leaves oak tree, A: *Medicago sativa* L.-alfalfa; DMD: Dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed value, BW: Body weight, IVDMD: *In vitro* dry matter digestibility.

4. Discussion

In this study, it was determined that the dry matter contents of F hay, G hay, and O leaves are higher than A and H hay. As it is known, trees and shrubs have higher DM and fiber concentrations and lower CP concentrations in summer than herbs (Demir and Keskin, 2016; Dökülgen and Temel, 2020). In this study, the CP content of hays ranged from 7.35 to 19.28%. In this study, the CP values found for H hay and G hay were similar to the ones found for *P. ferulacea* L. (3.92-10.1%) and *A. gummifer* L. (6.38-9.66%) (Yurtseven, 2011). The CP content of 19.28% determined in A hay seems to be well above the daily protein requirement of ruminants (NRC, 1996). In addition, the CP content of the ration should not fall below 7% for the ammonia nitrogen level required in the rumen in order for the microbial activity in the rumen to continue in its normal course and for the continuity of the microbial activity (Norton, 1994; Cappellozza, 2013). In this study, it is seen that the roughages contain 7% and more CP. The H hay had the lowest (7.35%) CP ratio among the other roughages used in the study. In ruminant rations, the protein value of the rations can be balanced according to the yield level of ruminants by mixing a little F, H, G, and O leaves together with A hay.

While G hay and O leaves had the lowest (0.65%) and the highest (5.37%) EE content, respectively, F, H, and A hay were found to be similar in terms of EE content. It has been reported that

the CF content of shrubs and tree leaves varies according to the season, and is generally low in spring and summer and higher in autumn and winter (Alatürk et al., 2014). In a study, the EE content of Kermes oak leaves in spring and summer was 6.03% and 4.53%, respectively. It has been reported that this rate increases to 8.06% and 8.10% in the summer and autumn months (Alatürk et al., 2014).

The quality of the forage depends on the amount and ratio of fiber in it. This is because fiber is more difficult to digest than the non-fiber components of forage. Also, the rate at which fiber is digested slows as plants mature. While ADF is an indicator of the digestibility of the plant, NDF is a good predictor of ruminant dry matter consumption and gastrointestinal fullness (Van Soest, 1982). The NRC recommendation for NDF in a diet is 30%, and the primary source of a minimum of 21% NDF is roughage. As a general rule, feedstuff which has 11-13% CP are capable of supplying adequate protein for maintenance and growth, while feedstuffs with low NDF (20-25%) are more digestible than those with more than 35% NDF (Norton, 1994). The CP (12.2% and 10.2%), CF (3.4% and 4.7%), and ADF (24.4% and 23.2%) contents reported by Shawrang et al. (2013) for *P. ferulacea* and *F. orientalis* are in the ranges reported for H hay and F hay in the current study.

In an evaluation to be made in terms of condensed tannin as secondary components that prevent the use of nutrient matters in forage, oak tree leaves and gum-tragacanth were the most striking. We found the highest condensed tannin concentration (6.97%) in oak tree leaves. This was followed by gum-tragacanth with 3.12% and caksir with 2.08%. Ataşoğlu et al. (2010) found that condensed tannin content in Kermes oak varies between 5.83% and 13.8% in dry matter, Sevim and Sarı (2014) found that condensed tannin content in kermes oak leaves was 9.61%, Kamalak et al. (2015) 9.22%, Imik (1997) 8.02% and Alatürk et al. (2014) reported that it varies between 17.09 g/kg DM and 19.26 g/kg DM. Examining the phytochemical and biological properties of 4 different gum-tragacanth species commonly used in folk medicine, Jaradat et al. (2017) stated that the total tannin content varies between 12.78 mg TA/g and 22.54 mg TA/g, and its content varies according to gum-tragacanth species. Studying the potential nutritional value of gum-tragacanth species at different maturation periods, Çacan et al. (2017) reported that the condensed tannin content is between 0.47% and 0.78%, and the said values vary according to the gum-tragacanth species and harvest periods. It has been reported that the total tannin content in roots and fruits varies between 3.76% and 5.70% in different *Ferula sp*., and gallic tannin is not found in the mentioned parts (Baytop, 1967). When the condensed tannin content is examined, it is thought that if oak leaves are used alone as a source of forage, feed consumption will decrease (Kamalak, 2007). In addition to these, 6% in the rations of sheep; it has been reported that goats can tolerate the presence of 8-10% tannin in their rations (İmik and Şeker, 1999). However, since goats have a higher tolerance (8-10%) against condensed tannin than sheep, it is estimated that oak leaves with higher condensed tannin content (6.97%) will be more suitable for use in goat rations.

A and F hay mark the highest IVDMD, DMD, and RFV values, while G hay showed the lowest values. The amount of dry matter consumption of the animals is related to the NDF content of the feeds, and the feeds with high NDF content have a lower DMI value (Özcan and Kılıç, 2018). From this point of view, it can be said that the F hay can be consumed more willingly by animals. Jančík et al. (2017) suggested that the *in vitro* digestibility value of alfalfa hay by Daisy II is 79.6%. This value is similar to the value found for alfalfa hay in this study, while Ekinci et al. (2018) are higher than the value of the IVDMD value (62.53 %) found for alfalfa hay. The mean values obtained for IVDMD in the K hay (72.43%) are higher than that obtained by Shawrang et al. (2013) for effective DM degradability (56.0%) of *Ferula orientalis* by nylon bag technique. It is possible to see the differences between the changes in the plant composition during the harvest stage and the methods used to determine the digestibility value.

The H hay marked the high IVDMD, DMD, DMI, and RFV values, while O leaves and G hay showed the lowest values. The reason behind this finding was that G hay and O leaves is poor in terms of carbohydrate and protein contents that can be used by rumen microorganisms while being rich in cell wall structural elements and condensed tannin. Indeed, especially the increase in ADF content adversely affects digestibility. Aldemir et al. (2015) and Coşkun et al. (2004) suggested that the in vitro dry matter digestibility of *Prangos ferulacea* hay is 79.15 and 80.60%, respectively. These values are higher than the values found H hay in this study, while Shawrang et al. (2013) are similar to the effective dry matter degradability value found for *Prangos ferulacea*. Yurtseven (2011) suggested that DMD (55.83% - 66.77%), DMI (2.13- 2.67% BW), and RFV (112.57-115.98) values for helis hay are adversely affected by delayed harvest time.

In the present study, IVDMD, DMI, DMD and RFV values of O leaves were determined to be 56.22%, 2.24%BW, 61.10%, and 105.98, respectively. According to Dökülgen and Temel (2020), the DMD of Kermes oak leaves in the autumn and spring were 57.5% and 69.00%, respectively. This was due to the increase in leaves and shoots in the spring and the lower NDF and ADF contents compared to the autumn months. They also stated that RFV values in autumn (125.09) are lower than in spring months (173.66). Parlak et al. (2011) declared that dry matter digestibility of Kermes oak was the highest in spring (70%) and was between 43.6 to 51.4% in other mounts of the year.

The lowest IVDMD value of 42.81% was determined in the G hay. As RFV falls below 100, feed quality decreases (Richardson, 2020). As seen in Table 1, the highest ADF (54.51%) and NDF values (64.64%) are in G hay. The increase in NDF and ADF causes a decrease in RFV of feeds, and an increase in intracellular components causes an increase in RFV. The value of DMD calculated from the ADF value (46.43%) and the level of DMI calculated using the NDF value (1.86%) was the lowest in G herb. Therefore, it is seen that the values calculated in terms of RFV are in parallel with the *in vitro* digestion values. Yurtseven (2011) determined that the gum-tragacanth plant contains higher ADF and NDF, probably due to its thorny structure, and that the DMD value determined by the gas production technique is lower (37.39%-42.84%) compared to alfalfa hay and helis.

5. Conclusion

As a result, considering the problems in roughage in Türkiye, the use of alternative feed sources such as tree leaves is of particular importance. Considering their nutrient content, it is not possible to use caksir, helis, gum-tragacanth, and oak tree leaves alone as an alternative roughage source to alfalfa hay. It can be recommended to use caksir, helis, gum-tragacanth plant, and oak leaves together with other roughage in the roughage ration, depending on the quality of the roughage. Additional studies, however, are needed to elucidate the longer associative effects of roughage source on rumen fermentation and digestibility in small ruminants.

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Research Article

Effect of Supplementation of *Nigella Sativa* Oil on Nutrient Digestibility, Some Blood Metabolites and Rumen Parameters in Karadi Lambs

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Blood and Rumen parameters, Digestibility, *Nigella sativa* oil

Abstract: To examine the effect of supplementation of varying levels of the oil of *Nigella sativa* in Karadi lambs rations on nutrients digestibility, blood metabolites, and some rumen parameters, 18 Karadi lambs were allocated into three groups, and the first group was fed a basal diet as control whereas, the second (T2) and the third (T3) groups fed the basal diet being supplemented with 0.15 and 0.30% of DM *Nigella sativa* oil (NSO) respectively. All animals were fed individually on 1.5 kg/lamb/day. Results showed that dry matter (DM), crude protein (CP), organic matter (OM), and crude fiber (CF) digestibility was not affected (P>0.05) by NSO supplementation. Also, supplementing NSO had no significant effect on serum total protein (TP), albumin (Alb), globulin (Glb), triglycerides (TG), cholesterol (Chol), high density lipoprotein (HDL), and very low-density lipoprotein (VLDL) concentrations. There was an increasing trend (P=0.07) in LDL concentration of lambs fed on T2 and T3 as compared to control. Neither treatment nor interaction between time and treatment had an effect on rumen fluid pH. A significant decrease (P=0.008) was noted in rumen fluid pH value with the advances of time post feeding. The ammonia-nitrogen concentration in rumen fluid was generally lower upon oil supplementation, and it was significantly (P=0.03) decreased in the T2 group at 4 hours following morning feeding. It can be concluded that supplementing with 0.15 and 0.3% /DM of NSO showed a reduction in rumen ammonia-nitrogen while it had no effects on nutrient digestibility and blood metabolites in Karadi lambs.

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1. Introduction

Nowadays, feeding natural additives is appraised as a crucial principle in healthy nutrition. The prohibition of using antibiotics as feed additives in animal nutrition because of their residues in animal products and the elevated level of consumers' awareness on the occurring hazards on health caused more research on safe and natural additives (Khamisabadi et al., 2016) due to improvement caused on animals'

health when used as additives in feeds. Several findings (Calsamiglia et al., 2007; Benchaar et al., 2008; Hart et al., 2008) suggested that adding plants or plant extracts containing many bioactive compounds to ruminant's diets might have a positive effect on ruminal fermentative status, thus supporting the degradation processes and mitigating ruminal methane (CH_4) formation (Calsamiglia et al., 2007; Benchaar et al., 2008; Hart et al., 2008). Essential oils (EO) are known as secondary plant metabolites that can be steam volatilized or extracted using organic solvents (Calsamiglia et al., 2007). Essential oils act as antimicrobial compounds against microbes, counting bacteria, protozoa, and fungi which could result in improved digestibility (Greathead. 2003). *Nigella sativa* (NS) is an annual herb belonging to *Ranunculacae* buttercup family and is globally regarded as an important medicinal plant because of its advantageous actions. It is considered as antibiotics' natural alternative for improving the health of animals and raising the quantitative and qualitative features of animal's products (Longato et al., 2015). It is demonstrated by El-Naggar et al., 2018 that adding oil of NS at 20g kg^1 DM to the diet of ruminants led to improve weight gain, feed conversion, digestibility of nutrients, and nitrogen balance. Similarly, Cherif et al., (2018) found that the addition of *Nigella Sativa* seeds to the concentrate (12g kg^1) significantly improved average daily gain and affected rumen fermentation by rising ammonia nitrogen (NH_3-N) concentration, reducing protozoa count. It is reported that biochemical and hematological measurements are critical indicators during diagnostics (Kaneko et al., 2008 and Nicoll et al., 2004). Furthermore, evaluating the state of metabolism in order to predict and prevent the occurrence of many diseases in critical periods circumstances (Celi et al., 2008). In this regard, Idris et al., (2014) found that the addition of 47g of NSO seeds/kg DM caused a significant rise in Cho, LDL, HDL levels, and live body weight. Since no sufficient information was found available through literature about the effects of NSO on nutrient digestibility, blood metabolites, and rumen parameters in Karadi ewes, therefore, this work pointed to investigate the impacts of dietary addition of NSO on nutrient digestibility, some parameters of rumen and blood in Karadi lambs.

2. Material and Methods

The experiment was performed following the procedure of the Institutional Committee on Animal Use Ethics (Approval No. AEC 06052021). This experiment was conducted at the farm of the Animal Production Department, College of Agricultural Engineering Science, University of Duhok. All procedures were approved by the ethics committee of the College of Agricultural Engineering Sciences, University of Duhok. Eighteen Karadi lambs, with an average body weight of 32.43±0.39 kg, were used and fed individually on three different diets at a rate of 1.5 kg/lamb/day. The concentrate diets consisted of 40.0 % ground barley, 20.0 % ground yellow corn, 15.0% soybean meal, 10.0 % wheat bran, 11.0% wheat straw, 1.5% vitamins and minerals mixture, 1.7 % limestone and 0.8 % salts. The diet composition is shown in Table 1. Three levels (0, 1.5, and 3g kg^1) of oil from NS (as extracted using a hydraulic press system by coaled pressing) were added to concentrate and considered as the three experimental groups; T1, T2, and T3, respectively. N. sativa oil was manually supplemented to the basal diet for the experimental groups. The lambs were individually kept in pens (1×2m) for 14 days as an adaptation period and then transferred to metabolic cages for 7 days as a samples collection period. Fresh drinking water was freely available during the entire experiment. Ruminal fluid was obtained on the 7^{th} day of collection period at 0 and 4 hrs. after morning meal via esophageal tube and suction strainer, and collected into bottles then filtered through a double thickness of cotton gauze and pH was recorded immediately using portable pH meter. Ammonia nitrogen was measured according to MAFF (1986) and kept under -18° C in plastic bottles for further analysis. The daily feed intake and fecal output were recorded, and fecal samples were collected on day 7 of the collection period in metabolism crates. The nutrient digestibility was determined as described by the method of McDonald et al., (2010). The blood samples were collected by draining through Jugular venipuncture into plastic tubes. The samples were refrigerated overnight and then centrifuged at 4500g for 10 minutes. The serum was separated and frozen at -18˚C for subsequent analysis.

2.1 Statistical analysis

The experimental results were analyzed by factorial one-way ANOVA by applying the package of Genstat Statistical Software (Genstat 20^{th} edition, VSN International Ltd, U.K.). All analyzed data

sets were normally distributed. The datasets were analyzed to compare between the three supplemental levels (0, 1.5, and 3g kg^{1}) of NSO experimental groups (T1, T2, and T3) for nutrient digestibility coefficients (%), blood metabolites (mg dl^{1}), NH_3-N (mg/dl) in rumen fluid, and ruminal fluid pH. Repeated measure ANOVA analyses of NH_3-N concentration and pH of rumen fluid datasets were also analyzed to compare between groups of treatment (T1, T2, and T3) during different times (hours) and the treatment x time interaction. Tukey test was used to compare different groups for all parameters. Differences were revealed as significant at $P<0.05$, and trends were revealed when P was between <0.1 and >0.05.

3. Results and Discussion

Table 1. Showed the composition of rations used in experiment

Nutrient%	DM	Crud Protein (CP)	Ether Extract (EE)	Ash	Crude Fiber (CF)	Nitrogen Free Extract (NFE) [1]	Metabolizable energy[2] (MJ Kg^{1} DM) (ME)
T1	93.96	13.01	4.25	8.05	19.77	48.88	11.80
T2	93.75	13.70	4.41	8.12	14.90	52.62	12.21
T3	93.85	13.38	4.21	8.03	16.77	51.46	12.02

Chemical analysis was carried out (on the basis of dry matter) at the nutrition lab. Animal Production Department.
[1]NFE%= 100- (Moisture + Ash + EE + CP + CF contents).
[2]ME was calculated according to MAFF,(1975) ME= (CP*0. 2+EE*0. 31+CF*0.05+NFE*0.14).

3.1. Nutrient digestibility

The effect of dietary oil supplementation of NSO on nutrient digestibility in Karadi lambs is shown in Table 2. Results of the present study revealed that nutrients digestibility of DM, OM, CP, and CF were not affected by NSO supplementation. Such results are in agreement with the findings of Khateri et al., (2017), who noted that the apparent digestibility of DM, CP, OM, and NDF was not influenced by the mixture of essential oil supplementation. Similarly, Khattab et al., (2011) concluded that adding black seed oil did not significantly affect nutrients digestibility in dairy buffaloes. Also, Metwally et al., (2015) found that in cows, the addition of EO caused no significant change to the digestibility of DM, CP, CF, and OM. However, Benchaar et al., (2006) revealed no change in apparent digestibility of DM, NDF, and CP in dairy cows consuming a diet supplemented with EO at 2 g/d. In contrast in sheep, El-Naggar et al., 2018 found that nutrients digestibility and rations nutritive value as supplemented with varying levels of NSO resulted a significant increase ($P<0.05$) in the DM, CP, OM and NFE digestibility. Results of nutrients digestibility in dairy goats fed the diet supplemented with 7.5 g *Nigella sativa*/head are also showing a significant ($P\leq0.05$) escalate in DM, EE digestibility (El-Basiony et al., 2015). Moreover, Klevenhusen et al., (2015) found that 50 mg supplementation of NSO tended to elevate DM and OM disappearance when compared with control as studied *in vitro*. Essential oils as bioactive compounds are complex plant metabolites with variable composition, so they differently affect the rumen microbial growth and activity depending on the dose rate of bioactive compounds and the chemical composition of the diet (Klevenhusen et al., 2012). Therefore, the difference in digestibility coefficients from the present experiment and that of the other studies may be attributed to the variation in essential oil composition as a bioactive compound and also due to the animal species and diet composition.

Table 2. Effect of feeding experimental diets on nutrients digestibility of Karadi lambs

Item	T1	T2	T3	SED	P-value
Nutrient digestibility %					
Dry Matter DM	65.45	67.65	75.00	6.88	0.36
Organic Matter OM	69.04	71.30	78.02	6.50	0.38
Crude Protein CP	62.53	64.79	72.53	6.80	0.32
Crude Fiber CF	64.05	55.81	70.49	8.43	0.25

T1: fed the basal diet. T2: fed the basal diet supplemented with 0.15% of DM *Nigella sativa* oil. T3: fed the basal diet supplemented with 0.30% of DM *Nigella sativa* oil.

3.2. Blood metabolites

The impacts of supplementing the *Nigella sativa* oil on serum blood metabolites are presented in Table (3). Results revealed that supplementing NSO did not affect the TP, Glb, and Alb concentrations in the serum of Karadi lambs. The result is in accordance with the findings shown by Khattab et al., (2011) who stated no effect (P≥0.05) of the NSO on plasma TP, Alb, and Glo concentrations in buffalo calves. Similarly, Abdullah and Farghaly, (2019) in lamb; and El-Hawy et al., (2018) in Barki ewes, observed that feeding *Nigella sativa* meal had no significant impact on concentrations of total TP, Glb, and Alb. These results were in contrast with observations of El-Saadany et al., (2008) who reported that *Nigella sativa* seeds supplementation significantly increased (P<0.05) TP and Glo concentration.

Table 3. Effect of feeding experimental diets on serum metabolites in Karadi lambs.

Parameters	T1	T2	T3	SED	P-value
Total Protein mg dl^{1}	6.39	6.07	6.36	0.48	0.76
Albumin mg dl^{1}	2.83	2.71	2.83	0.13	0.62
Globulin mg dl^{1}	3.56	3.53	3.54	0.42	0.86
Triglyceride mg dl^{1}	18.60	12.00	14.67	4.05	0.29
Cholesterol mg dl^{1}	45.00	46.33	41.00	6.26	0.68
High density lipoprotein HDL mg dl^{1}	24.67	22.50	19.33	3.98	0.42
Low density lipoprotein LDL mg dl^{1}	15.53^{a}	21.43^{b}	18.73^{ab}	0.42	0.07
Very low-density lipoprotein VLDL mg dl^{1}	4.80	2.40	2.93	1.18	0.13

^{a,b}: the difference between the values with different letters in the same raw is significant (P<0.05).
T1: fed the basal diet. T2: fed the basal diet supplemented with 0.15% of DM Nigella sativa oil. T3: fed the basal diet supplemented with 0.30% of DM Nigella sativa oil.

In the present study, NSO supplementation had no effect on serum TG concentration. This result resembled with those obtained by Khattab et al., (2011), who found that buffalo calves fed supplemented ration with NSO had no significant contrast (P>0.05) in TG concentrations. Similarly, Abdullah and Farghaly, (2019) reported that there were no significant deviations (P>0.05) in TG concentrations of lambs due to *Nigella sativa* meal diet. Also, El-Basiony et al., (2015) noted no significant effects on TG concentration when NS was added to the rations of lactating goats. In contrast, Idris et al. (2014) found that the mixing of 47g kg^{1} of NSO in the diets of sheep resulted in a significant (P<0.05) rise in serum triglycerides concentration. In this investigation, serum Chol concentration was not got affected by feeding of NSO. Similarly, Otaru et al., (2011) found that supplementation of palm oil until 16% in a concentrate had no effect on serum Chol of Red Sokoto goats. Also, Khattab et al., (2011) observed no significant effect of black seed oil supplementation on blood plasma Chol concentration of pregnant buffaloes. Unlike our findings, Habeeb and El-Tarabany, (2012) reported that supplementing the diet of growing Zaraibi goats with NS significantly decreased serum total Chol. Saleh, (2005), found that lactating ewes supplemented with NS seeds decreased (P<0.05) total plasma Chol as compared with ewes on a basal diet. This decline in the level of serum Chol could be related to low levels of thymoquinone and monounsaturated fatty acids when hepatocytes synthesize Chol (Padhye et al., 2008).

Serum HDL level was numerically decreased in groups fed NSO as compared to the control animals. This result is in concord with that revealed by (El-Essawy et al., 2019a and 2019b), who noted that plasma levels of HDL were not influenced by the addition of EO to the diet in Barki lambs and ewes; however, El-Hawy et al., (2018) noticed that feeding ration containing *Nigella sativa* meal in Barki ewes led to a significant lessen in HDL level comparing to control. There was a trend (P=0.07) in LDL concentration to be higher in lambs fed on T2 (21.43 mg dl^{1}) compared to the control. In sheep, Idris et al., (2014) showed that NSO, increased significantly (P<0.05) the HDL level and stated that this could be due to the biohydrogenation of the unsaturated fatty acids in the rumen together with a possible effect of the rumen atmosphere to the thymoquinone, which is the active ingredient of NS.

3.3. Rumen fluid

The rumen fluid pH values and ammonia-nitrogen concentrations in Karadi lambs fed black seed oil before the morning feeding and at 4 hrs. post morning feeding are given in Table 4. Neither treatment nor interaction between time and treatment had an effect on rumen fluid pH. A significant decrease (P=0.008) was noticed in rumen fluid pH value at post feeding compared with pre-feeding.

Table 4. Effect of feeding experimental diets on rumen fluid parameters in Karadi lambs

Parameter	Time	T1	T2	T3	SED	P value	Repeated Measures Analysis					
							SED			P value		
							Tr.	Time	Time*Tr	Tr.	Time	Time*Tr
pH												
Before feeding		6.72	6.72	6.72	0.02	0.97	0.02	0.01	0.03	0.97	0.008	0.97
4hrs post feeding		6.67	6.67	6.66	0.04	0.97						
NH_3-N (mg/dl)							5.10	3.89	6.98	0.03	0.95	0.97
Before feeding		37.4	25.1	25.7	7.55	0.22						
4hrs post feeding		38.2a	23.6b	25.8ab	6.36	0.08						

[a,b]: the difference between the values with different letters in the same raw is significant (P<0.1 and >0.05).
T1: fed the basal diet. T2: fed the basal diet supplemented with 0.15% of DM *Nigella sativa* oil. T3: fed the basal diet supplemented with 0.30% of DM *Nigella sativa* oil.

Time post feeding and interaction between time and treatment had no effect on ammonia-nitrogen concentration in rumen fluid, but there was a significant effect of treatment (P=0.03) as it was reduced to 23.6 mg dl^1 in the lambs fed a diet supplemented with 0.15% NSO as compared to those fed control diet (38.2 mg dl^1) (Figure1).

The results are in line with that of Klevenhusen et al., (2015), who found during an in vitro study that supplementing the rumen fluid with either 50 or 500 mg/l resulted in no change in rumen fluid pH, but it caused a significant reduction in NH_3-N. Also, it was reported by Mclntosh et al., (2003) that feeding essential oils caused a reduction in the deamination process in the rumen as a result of the inhibitory effect of EO on most cultures of ruminal bacteria. The same authors showed that *Clostridium sticklansii* and *Prevotella anaerobius,* which are considered hyper-ammonia producing bacterial species, remained sensitive to the inclusion of EO in the diet. In the current study, the decline in NH_3-N concentration may be associated to the suppressing impact of NSO on ammonia-producing bacteria, which might lead to a reduction in the rate of deamination in the rumen.

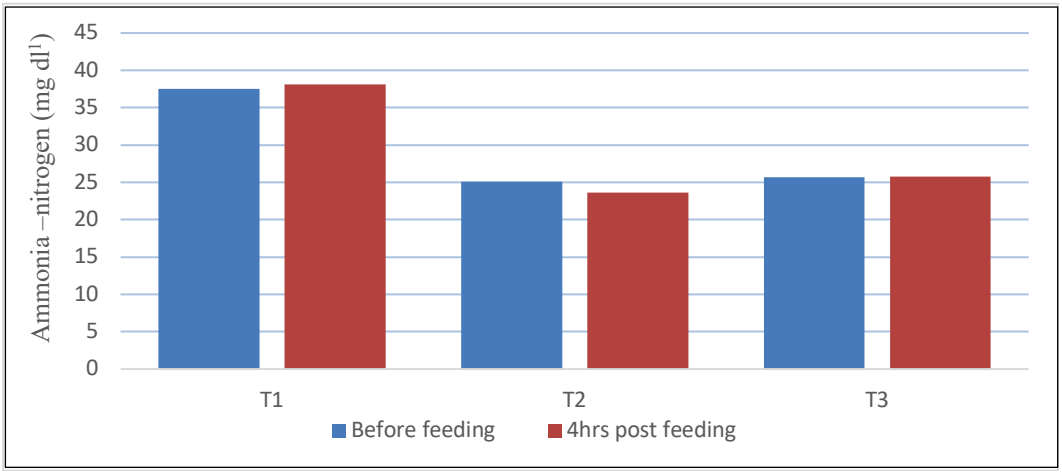

Figure1. Effect of feeding experimental diets on rumen NH_3-N in Karadi lambs.

Conclusion

With the exception of serum HDL concentration, supplementing with 0.15 and 0.30% /DM of NSO had no effects on nutrient digestibility or blood metabolites. *Nigella sativa* oil supplementation caused a reduction in rumen ammonia-nitrogen in Karadi lambs. Further research is required to study NSO supplementation on rumen function at wider time intervals.

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Research Article

First Insight into Genetic Variation and Population Structure of The Emerging *Citrus chlorotic dwarf-associated virus* (CCDaV, genus *Citlodavirus*)

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Abstract: *Citrus* spp. is widely planted in tropical and subtropical regions, including in Turkey and other Mediterranean countries. Due to its widespread vector and climate change, Citrus chlorotic dwarf-associated virus (CCDaV), a member of the newly formed genus *Citlodavirus*, is one of the emerging viruses that can be a serious constraint to *Citrus* crops production in the coming years. Therefore, *in-silico* analysis on all available isolates in NCBI GenBank was performed to provide the first insight into the genetic population and evolution of CCDaV, which may contribute to its control. CCDaV phylogroups based on full genome, complete movement protein, and complete coat protein sequences were found to be not associated with isolate origins or host species, and all isolates also shared a high genetic identity among them. However, neutrality tests indicated that the current populations are expanding, driven by new mutations. Low Fixation index (F_{ST}) values (0.00000-0.36207) confirmed no genetic separation among different ORFs of isolates from three countries. The constructed TimeTree suggested that CCDaV emergence was very recent compared to the other three members of the genus *Citlodavirus*. Therefore, the obtained results of this study could also expand our knowledge on other even more obscure citladovirus and even other plant DNA viruses, which are still less studied than RNA viruses.

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1. Introduction

Citrus chlorotic dwarf-associated virus (CCDaV, genus *Citlodavirus*, family *Geminiviridae*) is the causal agent of the destructive 'citrus chlorotic dwarf disease' (CCCD) affecting *Citrus* spp. and its hybrids such as lemon (*C. limon*), pomelo (*C. maxima*), orange (*C. sinensis*), mandarin orange (*C.*

reticulata), bitter orange (*C.* × *aurantium*), grapefruit (*C.* × *paradisi*), and tangelo (*C.* × *tangelo*) (Zhou et al., 2017; Karanfil and Korkmaz, 2019). CCDaV genome is a monopartite DNA of 3640 nucleotides in a full-length genome sequence, organized into five open reading frames (ORFs): ORF V2 (encodes V2-like protein), ORF V1 (coat protein - CP), and ORF V3 (movement protein - MP) are comprised in the virion-sense strand whilst ORF C1 (C1:C2-like protein) and ORF C2 (RepA-like) are in the complementary-sense strand (Loconsole et al., 2012). Phylogenetic analysis based on a limited number of full genome sequences suggested a correlation between phylogroups and geographical origins as isolates from Turkey were clustered in a group distinct from those from China (Zhou et al., 2017; Karanfil and Korkmaz, 2019).

CCDaV transmission occurs by grafting and its putative vector, the bayberry whitefly (*Parabemisia myricae*), but does not happen mechanically (Korkmaz et al., 1995). Despite the virus has only been reported in Turkey (Loconsole et al., 2012), China (Guo et al., 2015), and Thailand (Yang et al., 2020), the presence of *P. myricae* in many citrus-growing countries around the world has raised epidemiological concerns that CCDaV might spill into other regions, especially those surrounding Turkey (Loconsole et al., 2012). Thus, CCDaV is regarded as one of 11 emerging viruses that currently pose serious threats to citrus crops in the Mediterranean region (Catara et al., 2021).

Recombination and reassortment are the most frequently found evolutionary forces in populations of both RNA and DNA plant viruses, thus significantly affecting their genetic variation (Chare and Holmes, 2006; Gibbs et al., 2008). While population genetics of many emerging plant RNA viruses have been discussed in recent papers (Randa-Zelyüt and Ertunç, 2021; Tokhmechi et al., 2021; Morca et al., 2022), the genetic variability and evolutionary mechanisms of populations of DNA viruses were remained less studied (Sanz et al., 1999; Ng et al., 2011). *Citrus* spp., themselves, as commercially important fruits in Turkey, are constantly improved through breeding (Kurtuluş et al., 2021). However, our grasp on the molecular profiles of CCDaV is still at a very early stage and needs to be further advanced as the data gained in genetic diversity, and population studies could contribute to the understanding of the molecular evolution of viruses and in the management of the viral disease through the development of either more specific or universal detection methods as well as determination of resistant gene(s) in the plant (Sokhandan-Bashir and Melcher, 2012; Stobbe and Roossinck, 2016; Santosa and Ertunç, 2021). Therefore, results of the first population analysis on CCDaV were presented in this study to learn more about some evolution aspects of the virus, which might also provide vital information for the even more obscure other three *Citlodavirus* species.

2. Material and Methods

2.1. Multiple sequence alignment and phylogenetic analysis

In-silico analysis using different bioinformatic software was carried out within this study. Twenty-three CCDaV isolates from Turkey, China, and Thailand with full genome sequences were retrieved from NCBI (National Center for Biotechnology Information) GenBank on February 7, 2022, and aligned using ClustalW (1.6) performed in MEGA X software v.10.2.4 (Kumar et al., 2018), then trimmed to extract their complete genome and five gene regions (V2, coat protein (CP), movement protein (MP), C1:C2-like, and RepA-like) sequences. Complete CP sequences of the other 19 global isolates were then aligned with the constructed CP alignment to create a dataset of 42 isolates. The pairwise nucleotide (nt) and amino acid (aa) sequence's identity matrix of isolates at the complete genome, CP, and MP levels were generated using Sequence Demarcation Tool (SDT) v1.2 software (Muhire et al., 2014).

The complete genome and CP alignments were subjected to recombination analysis using RDP, GENECONV, Chimaera, MaxChi, Bootscan, Siscan, and 3Seq algorithms implemented in the Recombinant Detection Program (RDP v.4.56) software with default parameters (Martin et al., 2015). Phylogenetic anomalies identified by less than five methods and with a Bonferroni-corrected *P*-value of < 0.05 were ignored (Martin et al., 2015).

The best DNA models to study the evolutionary relationship of isolates at the complete genome, CP, and MP levels were determined using MEGA X. Phylogenetic trees for the three regions comparisons were constructed using Maximum-Likelihood (ML) statistical method based on the Kimura

2-parameter model (Kimura, 1980) with uniform rates as implemented in MEGA X software, and branches were supported by bootstrap method with 1000 replications.

2.2. Current CCDaV population structure

The complete picture of variation among different CCDAV populations in each of the complete and five genome regions (V2, coat protein (CP), movement protein (MP), C1:C2-like, and RepA-like) was estimated according to four parameters: the number of haplotypes (H), haplotype diversity (Hd), average pairwise nt diversity (π), and transcriptional constrain (ω=dN/dS) suited in DnaSP software v.6.12.03 (Rozas et al., 2017). Any tested genome region having a dN/dS ratio > 1, = 1, or < 1 was considered to have been under negative (purifying), neutral, or positive (diversifying) constrain, respectively (Rozas et al., 2017). The results of three suites of neutrality constrain tests: Tajima's D (Tajima, 1989), Fu and Li's D* and F* (Fu and Li, 1993), as well as the determination of FST KS*, Z*, and Snn metrics (Hudson et al., 1992; Hudson, 2000), were presented using DnaSP v.6.12.03 to allow the quantitative estimation of genetic variation and gene flow between CCDaV populations for each of the datasets. Infrequent gene flow and expanding genetic divergence among CCDAV populations were determined by having FST (fixation index) value > 0.33 (Rozas et al. 2017).

2.3. Molecular dating analysis

The divergence time of four *Citlodavirus* species, including CCDaV, camellia chlorotic dwarf-associated virus (CaCDaV), passion fruit chlorotic mottle virus (PCMoV), and paper mulberry leaf curl virus 2 (PMLCV-2) with two *Mulcrilevirus* species: mulberry mosaic dwarf associated virus (MMDaV) and paper mulberry leaf curl virus 1 (PMLCV-1) as outgroup sequences were estimated based on the age evaluation of internal nodes (Kumar et al., 2018). TimeTree was reconstructed using the fast-dating RelTime-ML computational method under the Tamura-Nei parameter model (Tamura and Nei, 1993) implemented in MEGA X software, with default calibration of most recent common ancestors (MRCA) (Mello, 2018).

3. Results

3.1. Multiple sequence alignment and phylogenetic analysis

The phylogenetic trees showed that there was no clear consensus on the isolates clustering in comparisons based on complete genome, CP, and MP regions (Figure 1). Although the three trees showed different topologies, no significant recombinant signal indicative of recombinant isolates was detected by RDP software.

Pairwise identity analysis indicated that all analyzed CCDaV isolates shared high identities in both their nt and aa sequences, but they had a greater percentage of nt than aa identities in all compared regions; as an example, sequences of CCDaV isolates were 97.3-99.9% aa and 98.8-99.9% nt identical at the complete genome level (Table 1). Since CP was the most conserved region at both nt and aa levels, and more likely to be targeted in future identification studies of CCDaV, group naming was then done based on the result of the CP region comparison in which isolates were clustered into two distinct major groups (1 and 2) (Figure 1).

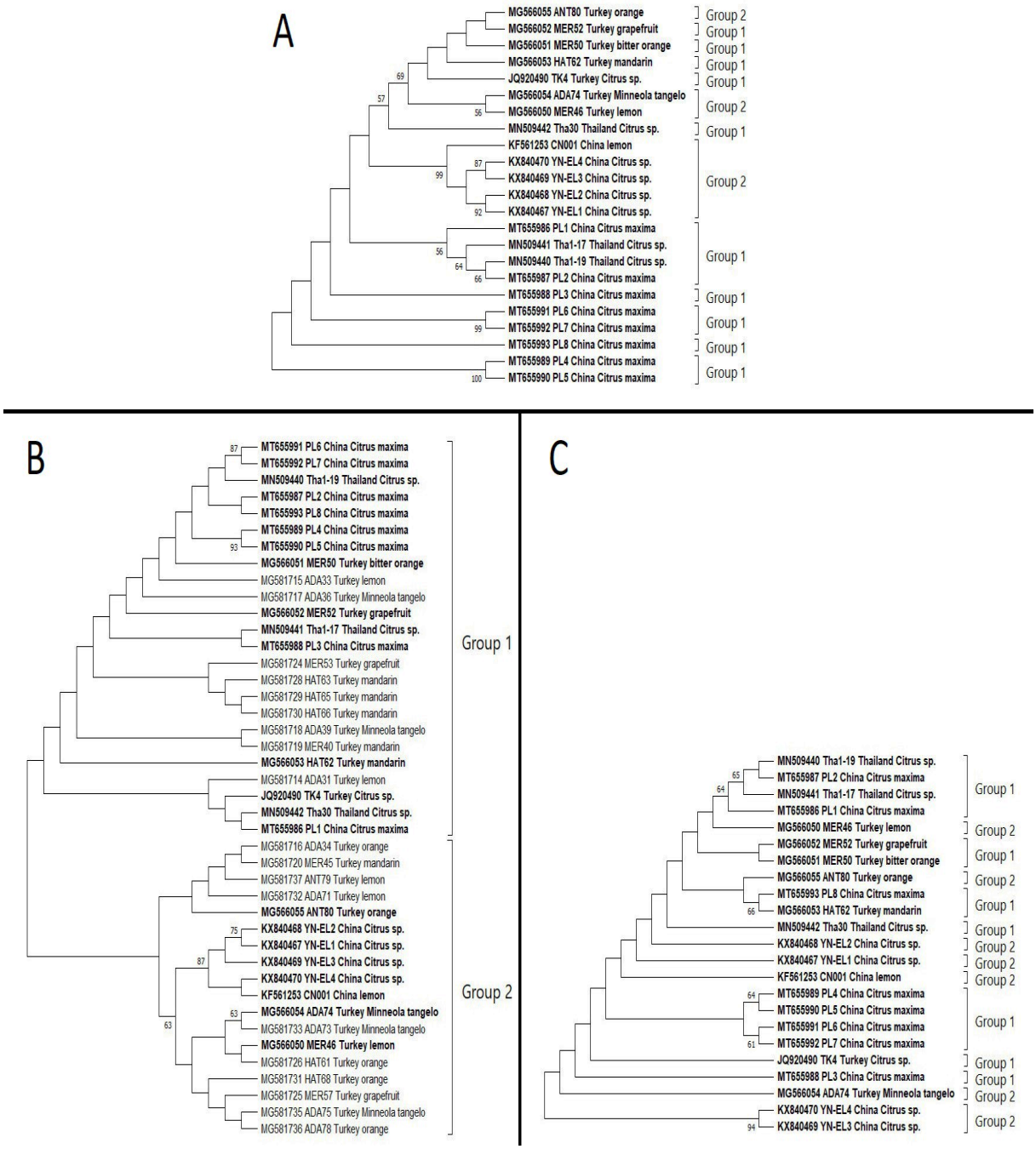

Figure 1. Maximum-Likelihood phylogenetic analysis of the nucleotide sequences of three different CCDaV genomic regions using Kimura 2-parameter model (K2) with Uniform Rates among Sites by MEGA X software, branches were supported by 1000 bootstrap replicates (only >50% values were shown). A. Phylogenetic analysis of the complete genome of 23 isolates, B. complete CP of 42 isolates, C. complete MP of 23 isolates. Group naming was based on the results of the complete CP analysis. Names of isolates that have complete genome sequences were printed in bold.

Table 1. Identity percentage of nucleotide and amino acid sequences of different CCDaV genomic regions and phylogroups

Comparison	Identity (%)	
	Nucleotide	Amino acid
Complete genome	98.8-99.9	97.3-99.9
V2	98.8-100	97.8-100
CP all	98.8-100	98.4-100
CP group 1	99.2-100	98.4-100
CP group 2	99.2-100	98.8-100
CP group 1 x group 2	98.8-100	98.8-100
MP	98.7-100	96.4-100
C1:C2-like	98.3-100	95.3-100
RepA-like	98.6-100	96.1-100

3.2. Population structure

Genetic variation and polymorphism on the full genome and five genes of CCDaV populations from three countries were determined by four genetic diversity parameters. At the full genome level, all three countries and world populations obtained the maximum Hd value of 1.000. The nucleotide diversity of all analyzed populations in different genome regions was observed to be low values (π between 0.00106 - 0.00741), with China population obtaining the highest π values in comparisons based on V2, CP, C1:C2-like, and RepA-like genes (Table 2). In general, CP and MP genes experienced more vigorous negative constraints than other genes. The analysis also found that the RepA-like region and several countries' populations of various gene regions are undergoing diversification pressure, as shown by their dN/dS ratio of > 1 (Table 2).

Table 2. Results of analysis of genetic diversity and polymorphism of partial five ORFs of CCDaV from different countries

ORF (Protein)	nt position (no of aa)	Group/geography	H	Hd	π	dN/dS
Full genome	1-3642 (1103)	All (n=23)	23	1.000	0.00582	0.7378
		Turkey (n=7)	7	1.000	0.00327	0.6118
		China (n=13)	13	1.000	0.00642	0.8645
		Thailand (n=3)	3	1.000	0.00439	1.1084
ORF1 (V2 protein)	194-613 (139)	All (n=23)	11	0.838	0.00427	0.5566
		Turkey (n=7)	5	0.857	0.00340	nd
		China (n=13)	6	0.859	0.00464	0.3433
		Thailand (n=3)	2	0.667	0.00317	0.2640
ORF2 (Coat protein)	417-1181 (254)	All (n=42)	29	0.959	0.00458	0.1257
		Turkey (n=26)	16	0.902	0.00258	0.0584
		China (n=13)	11	0.974	0.00741	0.0986
		Thailand (n=3)	3	1.000	0.00523	0.2983
		Group 1 (n= 24)	17	0.938	0.00387	0.1957
		Group 2 (n= 18)	13	0.961	0.00432	0.0876
ORF3 (Movement protein)	1211-2131 (306)	All (n=23)	17	0.953	0.00412	0.3353
		Turkey (n=7)	7	1.000	0.00434	1.6831
		China (n=13)	9	0.936	0.00384	0.2369
		Thailand (n=3)	3	1.000	0.00362	0.0702
ORF4 (C1:C2-like protein)	2300-2707 (135)	All (n=23)	14	0.929	0.00680	0.7074
		Turkey (n=7)	6	0.952	0.00630	1.1009
		China (n=13)	7	0.846	0.00647	0.5623
		Thailand (n=3)	6	0.952	0.00630	0.9429
ORF5 (RepA-like protein)	2611-3420 (269)	All (n=23)	15	0.945	0.00513	1.1572
		Turkey (n=7)	3	0.524	0.00106	0.5759
		China (n=13)	9	0.949	0.00630	0.8831
		Thailand (n=3)	3	1.000	0.00329	nd

N, number of isolates; Hd, haplotype diversity; π, nucleotide diversity; ω, dN/dS; nd, not determined.

The result of molecular variation patterns analysis suggested that Tajima's *D*, Fu and Li's *D** and *F** evaluated significantly and non-significantly negative values for CCDaV from three countries as well as worldwide populations in the full genome and all five genes, except for Turkey and world populations in C1:C2-like gene which were assigned positive values by Fu and Li's *F** test (Table 3).

Low K_S*, *Z**, and *Snn* values and the low F_{ST} values of < 0.33 were distributed among three countries in the full-length genome sequence and five gene comparisons, except Turkey vs. Thailand in RepA-like gene comparison (F_{ST} = 0.36207). Likewise, Group 1 vs. Group 2 in CP gene comparison revealed a low F_{ST} value (0.19991) (Table 4). Therefore, it can be suggested that the currently characterized global CCDaV isolates share low genetic variation.

Table 3. Results obtained from demography test statistics on five ORFs of CCDaV from different countries

ORF (Protein)	Group/geography	Tajima's *D*	Fu and Li's *D**	Fu and Li's *F**
Full genome	All (n=23)	-1.94372*	-2.38068 ns	-2.63291*
	Turkey (n=7)	-1.66129*	-1.74916*	-1.91293*
	China (n=13)	-1.04083 ns	-0.72544 ns	-0.92816 ns
	Thailand (n=3)	nd	nd	nd
ORF1 (V2 protein)	All (n=23)	-1.86429*	-1.91374 ns	-2.21294 ns
	Turkey (n=7)	-1.48614 ns	-1.56696 ns	-1.68344 ns
	China (n=13)	-0.51618 ns	0.24334 ns	0.05126 ns
	Thailand (n=3)	nd	nd	nd
ORF2 (Coat protein)	All (n=42)	-2.27745**	-3.65182**	-3.76783**
	Turkey (n=26)	-2.23397**	-3.71053**	-3.81047**
	China (n=13)	-0.51656 ns	-0.22630 ns	-0.34689 ns
	Thailand (n=3)	nd	nd	nd
	Group 1 (n= 24)	-2,90983*	-3,12995*	-2,14468*
	Group 2 (n= 18)	-1,40286 ns	-1,58014 ns	-1,27100 ns
ORF3 (Movement protein)	All (n=23)	-2.25491**	-2.89175*	-3.15463**
	Turkey (n=7)	-1.65112*	-1.74976*	-1.89968*
	China (n=13)	-1.65473 ns	-1.39773 ns	-1.67472 ns
	Thailand (n=3)	nd	nd	nd
ORF4 (C1:C2-like protein)	All (n=23)	-1.79576 ns	-2.63241*	2.77779*
	Turkey (n=7)	-1.59446 ns	-1.68667*	1.82427 ns
	China (n=13)	-0.72083 ns	-0.61664 ns	-0.73434 ns
	Thailand (n=3)	nd	nd	nd
ORF5 (RepA-like protein)	All (n=23)	-1.62927 ns	-1.37921 ns	-1.70176 ns
	Turkey (n=7)	-1.35841 ns	-1.42725 ns	-1.52246 ns
	China (n=13)	-0.89097 ns	-0.44861 ns	-0.64717 ns
	Thailand (n=3)	nd	nd	nd

Statistical significance: *, P < 0.05; **, 0.10 > P > 0.05; ns, not significant; nd, not determined.

Table 4. Genetic difference, gene flow and migration rate results of five ORFs of CCDaV from different countries

Genomic region	Comparisons	K_s* (*P* value)	*Z** (*P* value)	*Snn* (*P* value)	F_{st}
Full genome	Turkey(n=7)/Thailand(n=3)	2.58027 (0.0100*)	2.50957 (0.0310*)	0.83333 (0.0560 ns)	0.18384
	Turkey(n=7)/China(n=13)	2.89176 (0.0010**)	3.93619 (0.0010**)	0.97500 (0.0000***)	0.21155
	Thailand(n=3)/China(n=13)	3.03646 (0.2700 ns)	3.77678 (0.2480 ns)	0.75000 (0.3600 ns)	0.09064
ORF1 (V2 protein)	Turkey(n=7)/Thailand(n=3)	0.79623 (0.6720 ns)	2.98372 (0.5770 ns	0.48000 (0.9180 ns)	0.00000
	Turkey(n=7)/China(n=13)	1.76667 (0.0680 ns)	4.27803 (0.1580 ns)	0.64286 (0.1030 ns)	0.09608
	Thailand(n=3)/China(n=13)	0.93537 (0.4500 ns)	3.88041 (0.4520 ns)	0.71667 (0.2650 ns)	0.07246
ORF2 (Coat protein)	Turkey(n=2)/Thailand(n=3)	1.01896 (0.0530 ns)	4.99596 (0.0700 ns)	0.89655 (0.1310 ns)	0.02900
	Turkey(n=26)/China(n=13)	1.24881 (0.0000***)	5.40646 (0.0000***)	0.91209 (0.0000***)	0.12500
	Thailand(n=3)/China(n=13)	1.77963 (0.5680 ns)	3.83233 (0.5040 ns)	0.69167 (0.4340 ns)	0.07143
	Group 1(n= 24)/ Group 2 (n= 18)	3.10888 (0.0000***)	5.65866 (0.0000***)	0.79932 (0.0000***)	0.19991
ORF3 (Movement protein)	Turkey(n=7)/Thailand(n=3)	1.53634 (0.3470 ns)	2.87517 (0.3570 ns)	0.46667 (0.7260 ns)	0.15385
	Turkey(n=7)/China(n=13)	1.40241 (0.1840 ns)	4.26661 (0.1740 ns)	0.61500 (0.1250 ns)	0.01437
	Thailand(n=3)/China(n=13)	1.34516 (0.3120 ns)	3.81914 (0.3530 ns)	0.62813 (0.6720 ns)	0.11258
ORF4 (C1:C2-like protein)	Turkey(n=7)/Thailand(n=3)	1.17124 (0.1750 ns)	2.75185 (0.1450 ns)	0.73333 (0.0470*)	0.05172
	Turkey(n=7)/China(n=13)	1.16112 (0.0980 ns)	4.20756 (0.0920 ns)	0.64667 (0.0880 ns)	0.08782
	Thailand(n=3)/China(n=13)	1.16748 (0.0980 ns)	3.76973 (0.0920 ns)	0.75000 (0.3060 ns)	0.10776
ORF5 (RepA-like protein)	Turkey(n=7)/Thailand(n=3)	0.62559 (0.0090**)	2.52964 (00090**)	0.76190 (0.0090**)	0.36207
	Turkey(n=7)/China(n=13)	1.26787 (0.0000***)	3.93528 (0.0000***)	0.96875 (0.0000***)	0.32713
	Thailand(n=3)/China(n=13)	1.59277 (0.4300 ns)	3.79660 (0.2440 ns)	0.78750 (0.1340 ns)	0.09281

PM test: Probability obtained by the permutation test with 1000 replicates); ns, not significant; **, 0.001<P<0.01; ***, P<0.001.

3.3. Divergence time analysis

The phylogenetic Timetree showed that four *Citlodavirus* species can be clustered into three groups (Figure 2). CCDaV has a closer genetic relationship with camellia chlorotic dwarf-associated virus (CaCDaV) than passion fruit chlorotic mottle virus (PCMoV) and paper mulberry leaf curl virus 2 (PMLCV-2), in accordance with Zhang et al. (2018) finding. The molecular clock estimation between CCDaV and CaCDaV was 0.43 Mya (0.5 - 1.00 in Timescale).

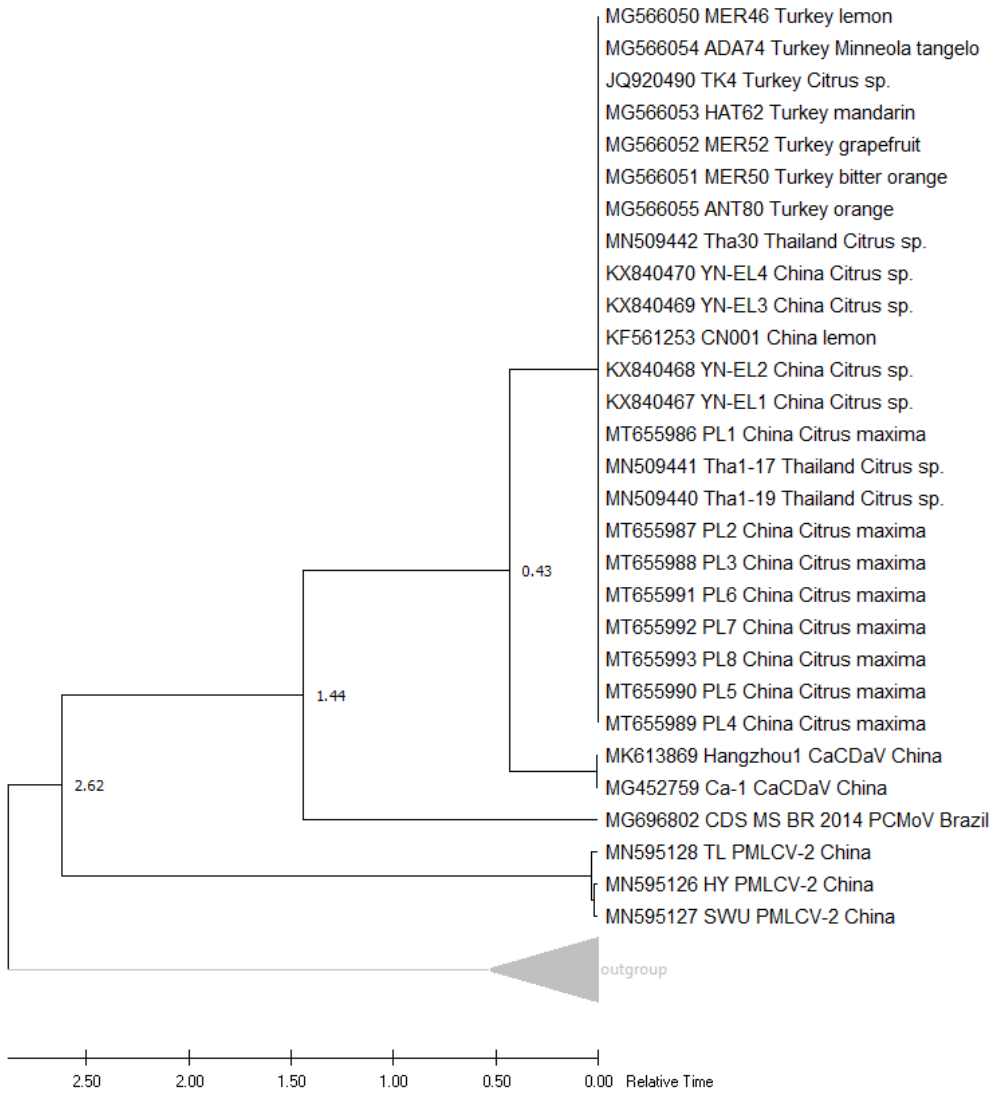

Figure 2. Divergence time estimation of four *Citlodavirus* species: citrus chlorotic dwarf associated virus (CCDaV), camellia chlorotic dwarf-associated virus (CaCDaV), passion fruit chlorotic mottle virus (PCMoV), and paper mulberry leaf curl virus 2 (PMLCV-2) by RelTime-ML in MEGA X. Two *Mulcrilevirus* species: *mulberry mosaic dwarf associated virus* (MMDaV, 4 isolates) and *paper mulberry leaf curl virus* 1 (PMLCV-1, 2 isolates) were used as out-group.

4. Discussion

The study on the population structure of CCDaV presented in this paper did not only enrich our understanding of the evolution of plant DNA viruses but also gave early insight into members of *Citlodavirus*, a newly established genus within the family *Geminiviridae* (Roumagnac et al., 2021). Even though CCDaV has been better studied than other citlodaviruses, the research on the virus is still in need of further advancement than the phylogenetic stage.

Phylogenetic trees constructed using complete genome, complete CP, and complete MP alignments were shown to have different topographies. However, no solid recombinant signals were detected by RDP analysis. These results suggested that the evolution of different genome regions did not occur simultaneously. It also provides evidence that reassortment might have a greater contribution than recombination in the direction of CCDaV evolution. Analysis using MEGA X software on the three observed regions indicated that there is no clear association between phylogroups with host or origin, contrasting a previous report by Karanfil and Korkmaz (2019), which clustered Turkish and Chinese isolates into two distinct groups.

CCDaV isolates from three different countries compared here shared high nucleotide (nt) and amino acid (aa) homology, in line with previous reports (Zhou et al., 2017; Karanfil and Korkmaz, 2019). The main transmission mode of the virus via grafting could be contributed to the high identity of isolates from around the world, as infected plants were probably grafted from the same infected source(s). The percentage identities on all studied genomic regions were higher at nt than aa level, suggesting most nt changes produced nonsynonymous aa substitutions.

DnaSP software estimated that different genome regions were under negative selection pressures, except ORF 5, which was supposedly under positive pressure. The negative constraints on the full genome, ORF1 and ORF4, were less than ORF 2 and ORF3, which are experiencing strong negative pressures (Table 2). These data further suggest that the evolution of different genome regions of CCDaV did not occur at the same rate and direction.

Three neutrality tests assigned negative numbers to the observed populations, indicating the recent expansion of CCDaV populations through new mutations. Vector transmission may have a greater influence on the shaping of future CCDaV evolution as the virus populations were shown to survive bottleneck selections which could be caused by graft transmission from the same source(s) (Roossinck and Ali, 2017). The F_{ST} values among different populations in comparisons of genomic regions were all < 0.33, except in Turkey vs Thailand at ORF5 (0.36), demonstrating once again that CCDaV populations are highly similar and almost no genetic isolation among currently known isolates from different countries.

CCDaV shared a common basal node with CaCDaV in the phylogenetic TimeTree of *Citlodavirus,* which indicated that CCDaV is related closer to CaCDaV than to PCMoV and PMLCV-2. These could add data to the ongoing discussion on citlodaviruses evolution as previously Fontenele et al. (2018) showed that CCDaV is phylogenetically closer to PCMoV while Qiu et al. (2020) determined that CCDaV is closer to PMLCV-2. The results of gene flow and time tree analyses of this current study also showed that many more isolates from other countries are needed to resolve the origin and ancestor of CCDaV.

Conclusion

The global population of CCDaV currently only consists of isolates from three countries: China, Thailand, and Turkey, which showed high genomic identity among each other. The constructed TimeTree also suggested that CCDaV separation as a distinct species was recent in the context of the evolution course of the genus *Citlodavirus*. However, different CCDaV populations were all estimated to be expanding, likely by the means of low-frequency polymorphism. This report presented an early insight, and more isolates from different regions are called in the future to further understand the evolution of the emerging CCDaV.

Acknowledgments

The authors declare no conflicts of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

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Research Article

First Report of the Endophytic Bacteria Associated with *Phormidium* sp.

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Abstract: Recent molecular studies on endophytic bacterial diversity have revealed a large richness of species. Associations between endobiotic bacterial-algae interactions have been studied for more than 40 years but were, up to now, never molecularly analyzed within the filamentous Cyanobacteria *Phormidium*. Therefore, the endophytic bacteria associated with fresh microalgae *Phormidium*, a group of ubiquitous photosynthetic organisms that play an important role in aquatic ecosystems, has been investigated. To study this partnership, *Phormidium* sp. was cultured in BG-11 medium using optimal conditions, and after the incubation period, cell biomass was obtained. Total genomic DNA from biomass was extracted and used for endophytic bacteria determination by using the 16S rRNA gene. Sequencing results revealed that a total of seven endophytic bacteria living within the cytoplasm of the host *Phormidium* sp. have been identified, including six bacteria belonging to three genera, namely *Sphingomonas*, *Sphingopyxis,* and *Stenotrophobacter* and while one bacteria remained unidentified due to low sequence homology in the GenBank database. The results highlighted the importance of endophytic bacteria associated with *Phormidium* sp. for the first time by using sequence-based identification.

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1. Introduction

Endophytic microbial communities, which reside in symbiotic associations inside the cell, particularly bacteria and fungi, are known that live in algae or plants without inducing disease in the development and growth of various host organisms (Yaish et al., 2015; Gouda et al., 2016). Among endophytic microorganisms, especially bacteria, have an ingenuity for living in internal alga or plant tissues and performing beneficial impacts to host growth by having a symbiotic association and having co-evolved an intimate ecological relationship that helps hosts adapt to biotic and abiotic stress (Nouh et al., 2021). Similarly, the surface of algae organisms like often represents a highly active association between hosts and microbes. In some cases, microorganisms play significant roles in hosts. In other cases, endophytic bacteria help supply the defense chemical and metabolism in algae with vitamins, heat, salinity, drought, fatty acids, pathogens, infections, and pollutants (Wahl et al., 2012; Singh and Reddy, 2014; Flewelling et al., 2015; Manomi et al., 2015; Karthick and Mohanraju, 2018; Mandelare et al., 2018; Ismail et al., 2020).

For over 40 years, the relationships between microalgae and bacteria have been widely studied by culturing in the laboratory. In recent studies, it is seen that there are methods using bacterial gene sequence analysis obtained from DNA isolated from host cells without culturing bacteria. (Reiter et al., 2002; Miyamoto et al., 2004). Over the past two decades, three revolutionary techniques, the development of the polymerase chain reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE), and the establishment of a classification system for bacteria based on the phylogeny of 16SrRNA, have changed our understanding of the microbial world (Rappé and Giovannoni, 2003; Muyzer et al., 2004).

Phormidium sp. (phylum cyanobacteria) is a genus of blue-green microalgae that is single-cell, filamentous, unbranched, and about 3 to 4 μm in diameter. Used extensively in biotechnology processes due to its endurance and simple nutritional requirements, a few species of this genus live in extreme environments like contaminated areas, hot springs, and desert lands (Guiry and Guiry, 2016).

The major objective of the present study was to explore the potential of endophytic bacterial communities within *Phormidium* microalgae by using the modified molecular method. *Sphingomonas* sp., *Sphingopyxis* sp., and *Stenotrophobacter* sp. as endophytic bacteria associated with *Phormidium* sp. were the first time identified. The outcome of this study will open a framework for controlling which of the endophytic bacterial members likely maintain an endosymbiotic relationship with the algae host.

2. Material and Methods

2.1. Algal material and culture condition

Phormidium sp. (BDCC 002) used in this study was obtained from Manisa Celal Bayar University Culture Collection of Biology Department in Manisa, Turkey. The algal biomass is grown in 250 mL Erlenmeyer flasks containing 100 mL of BG-11 medium. The aqueous biomass is incubated in a light incubator at 26 °C, 36 μmol m^{-2} sec^{-1} irradiances, 110 rpm magnetic stirring, and 2000-3000 lux fluorescent light on 16:8 h photoperiod. The medium was fixed to pH 5-7 using 1 M NaOH or 1 M HCI. *Phormidium* cells were harvested after the 12th and 15th days of incubation (Stanier et al., 1971). After the completion of incubation, the algal biomass was harvested from the media by centrifugation (6000 rpm, 5 min, 25 °C) and then subjected to total genomic DNA isolations.

2.2. Total genomic DNA extraction

The collected culture biomass was washed with distilled water and centrifuged to remove the remnants of the medium. The culture biomass was collected and then washed with distilled water and centrifuged for the removal of the medium. Afterward, the filamentous thalli were cut into 1–2 cm pieces and washed with double distilled water 2 times between the time interval of 5 min. The sample in test tubes was initially surface sterilized with 0.5% (w/v) EDTA solution for 5 min, followed by 70% (v/v) ethanol for 5 min, and later washed with PCR grade water for 1.5 min. This procedure was repeated at least 4-5 times. Sections about 1 mm thick were cut with a sterile lancet, and biomass was placed in a new sterilized 2 mL microtube and centrifugated for 5 min at 15000 rpm. Total genomic DNA from this sample was extracted using the WizardR Genomic DNA Purification Kit (Promega, A1120) by following per under the conditions specified by the Promega instructions. The total DNA samples were checked by 1% (w/v) agarose gel electrophoresis, by adding 10 μL DNA dye (Invitrogen, SYBR, Safe DNA Gel Stain) and with a 1 kb DNA marker (Geneaid DL006) using TAE buffer.

2.3. The 16S rRNA gene amplification

The 16S rRNA gene was amplified by PCR reaction using two universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3') (Lane, 1991). A reaction mixture optimized for PCR was done in a total volume of 50 μL, and each reaction contained 5 μL of 10X DreamTaq PCR buffer (with $MgCI_2$), 2 μL of 100% DMSO, 1 μL of 10 mM dNTPs, 0.75 μL of each 20 μM primer, 0.4 μL of DreamTaq DNA polymerase (ThermoScientific, EP0702), 39.6 μL of PCR grade water and 0.5 μL of template DNA (approximately 50 ng).

PCR amplification was carried out with a thermal cycler (Applied Biosystems Veriti Thermal Cycler, USA) and conditions were as follows: 4 min at 95 °C, 36 cycles of 30 sec at 95 °C, 30 sec at 49 °C, and 1 min at 72 °C, followed by 10 min final extension at 72 °C, then cooling at 4 °C. PCR products

were electrophoresed in 0.8% agarose gels containing SYBR (Invitrogen) using TBE buffer. Afterward, the PCR products were examined in a UV transilluminator (gLite gel scanner), and those that formed a single pure bank were separated for sequence analysis and stored at -20 °C. The PCR products were commercially sequenced by the GATC biotech company in Germany.

2.4. Phylogenetic analysis

The raw sequence data were edited with the BioEdit Sequence Alignment Editor program (V, 7.2.5.) (Hall, 1999) for the counterpart of degenerative bases, and the forward and reverse reading sequences were combined using the same program. The edited sequences were then blasted to compare with the data in the gene bank to identify possible bacteria. Using CLUSTALW, the 16S rRNA gene sequence was aligned with multiple sequences from the GenBank database. Phylogenetic trees were created with representative sequences using algorithms with a bootstrap test (1000 replicates) (Felsenstein, 1985; Saitou and Nei, 1987; Tamura et al., 2004) in the MEGA X software (V, 10.0.4) (Kumar et al., 2018).

3. Results and Discussion

Molecular characterization of endophytic bacteria is the first step in differentiating them at the strain level. 16S rRNA gene sequencing allowed accurate identification of endophytes from various host species. In the current research, the identification of endophytic bacteria from *Phormidium* sp. was performed using 16S rRNA gene sequencing. The phylogenetic tree was constructed based on the obtained sequences along with the closely related taxa from GenBank (NCBI).

All of the endophytic bacteria were classified to the genus level because of the low sequence coefficient of similarity between 83-96% of that the bacterial species collected with GenBank.

According to the bioinformatics analysis, seven endophytic bacteria were identified from *Phormidium* sp. (BDCC 002) by using a sequence-based method into three genera: *Sphingomonas* sp. (3), *Sphingopyxis* sp. (2), and *Stenotrophobacter* sp. (1) and an unidentified bacteria (1) (Figure 1).

On the other hand, five endophytic bacteria (three of *Sphingomonas* sp. and two of *Sphingopyxis* sp.) and one bacteria (*Stenotrophobacter* sp.) belonged to the largest group of bacteria- phylum Proteobacteria and Acidobacteria, respectively.

The sequences of seven endophytic bacteria were deposited at the GenBank with accession numbers (MW759557–MW759563). Previous research has shown that these bacteria are isolated and identified from different hosts (Zhang et al., 2014; Battu et al., 2017; Wang et al., 2020; Cheng et al., 2021). However, these genera were not found in the *Phormodium* sp. before.

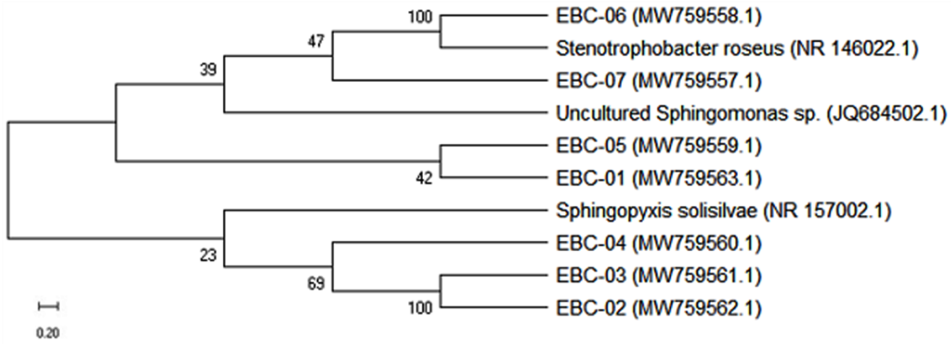

Figure 1. Neighbour-joining phylogenetic tree based on analysis of the 16S rRNA gene sequences showing the phylogenetic relationships between endophytes and representatives. Bootstrap values calculated for 1000 replications are indicated. GenBank accession numbers are given in parentheses. Only bootstrap values >50% are shown. Bar, 0.20 substitutions per nucleotide position.

The genus *Sphingomonas* is a Gram-negative, rod-shaped, chemoheterotrophic, strictly aerobic bacteria that typically produce colonies with yellow pigment and belongs to the class Alphaproteobacteria. Phylogenetically, *Sphingomonas* is well delineated from other genera, *Sphingobium*, *Sphingopyxis,* and *Novosphingobium* of the sphingomonads group, family

Sphingomonadaceae of Proteobacteria. This microorganism was defined by Yabuuchi et al. (2002) as generally non-pathogenic to humans (Glaeser & Kampfer, 2014). *Sphingomonas* sp. was previously isolated and identified in different plants such as *Sedum alfredii* (Bao et al., 2014), *Solanum lycopersicum* (Halo et al., 2015), *Solanum pimpinellifolium* (Khan et al., 2017), *Allium tuberosum* (Feng et al., 2017), *Glycine max* L. (Bilal et al., 2018) and *Oryza sativa* (Cheng et al., 2021). According to these researches, *Sphingomonas* sp. can be considered to be the dominant genus and may be represented in large numbers in the host plant. Table 1 shows the occurrence of related 3 endophytic bacteria in some plants. They appear more of *Sphingomonas* type than the other endophytic bacteria.

Table 1. Examples of reported bacterial endophytes and organisms harboring them

Endophytes	Host organisms	Reference
Sphingomonas SaMR12	*Sedum alfredii*	Bao et al., 2014
Sphingomonas sp. C40	*Oryza sativa*	Cheng et al., 2021
Sphingomonas sp. LK11	*Solanum pimpinellifolium*	Khan et al., 2017
Sphingomonas sp. LK11	*Solanum lycopersicum*	Halo et al., 2015
Sphingomonas sp. LK11	*Glycine max* L.	Bilal et al., 2018
Sphingomonas strain HJY	*Allium tuberosum*	Feng et al., 2017
Uncultured *Sphingomonas* sp. EBC-01	*Phormidium* sp.	In this study
Uncultured *Sphingomonas* sp. EBC-04	*Phormidium* sp.	In this study
Uncultured *Sphingomonas* sp. EBC-07	*Phormidium* sp.	In this study
Sphingopyxis granuli	Rice cultivar RP Bio-226	Battu et al., 2017
Sphingopyxis sp.	Potato tubers	Liu et al., 2011
Uncultured *Sphingopyxis* sp. EBC-02	*Phormidium* sp.	In this study
Uncultured *Sphingopyxis* sp. EBC-05	*Phormidium* sp.	In this study
Stenotrophobacter sp.	Korean Pine Forests	Wang et al., 2020
Uncultured *Stenotrophobacter* sp.EBC-06	*Phormidium* sp.	In this study
uncultured bacterium EBC-03	*Phormidium* sp.	In this study

On the other hand, the genus *Sphingopyxis* belongs to the class Alphaproteobacteria and the family Sphingomonadaceae. *Sphingopyxis* cells are aerobic Gram-negative, chemoheterotrophic bacteria that typically produce rod colonies with yellow pigment (Peter Kampfer et al., 2002). The genus *Sphingopyxis* was first proposed by Takeuchi et al. (2001). Relatively less studied strains of *Sphingopyxis* are mostly found in environments such as anaerobic sludge cover, seawater, wastewater treatment plant, hydrocarbon contaminated soil, and hexachlorocyclohexane contaminated soil (Jindal et al., 2013; Glaeser and Kampfer, 2014; Verma et al., 2015).

Sphingopyxis was isolated and identified by Battu et al. (2017) on rice culture and by Liu et al. (2011) on potato tubers. Battu et al. (2017) reported the use of high throughput plant genomic data to identify *Sphingopyxis granuli* endophytic bacteria colonizing rice plants by using novel next-generation sequencing-based computational methods.

Stenotrophobacter is a rod feeding on a few substrates. *Stenotrophobacter* belongs to the class Acidobacteria and the family Blastocatellaceae. *Stenotrophobacter* Gram-negative, nonmotile, short rods. Based on the 16S rRNA gene sequence analysis, *Stenotrophobacter* occurs in various habitats such as freshwater and marine water, microbial mats, hot springs, human body, as well as additional uncultured representatives in bulk soils, rhizosphere of wild and crop plants (Pascual et al., 2017). *Stenotrophobacter* sp. was identified as an endophytic species only in Korean pine forests by Wang et al. (2020).

It has been stated in different studies that the identified endophytic bacteria contain chlorophyll a, and it is predicted that these bacteria can affect development and growth positively by nitrogen fixation and production of some growth factors such as indole-3-acetic acid, HCN production, or synthesis of 1-ACC-deaminase to the host *Phormidium.* From the existing literature, it is known that these bacteria might also have the potential to adapt the host to extreme conditions such as desert soils, thermal springs, and polluted environments. The genus *Sphingomonas* and *Sphingopyxis* are commonly isolated from freshwater and marine habitats, soils, plant rhizosphere, or activated sludge. Some are antagonistic against plant pathogens and can produce exopolysaccharides (sphingans), which are gelling

agents that are used for pharmaceutical, food, or industrial applications including bioremediation of wastewater or contaminated dumping sites (Glaeser and Kampfer, 2014).

It should be noted that attempts to the isolation of these bacteria, depending on the growth media in both solid and liquid growth media such as R2A, Luria–Bertani agar, Tryptic Soy Agar, and conditions could not be cultured. It is thought that the reason for this is that endosymbiotic bacteria cannot be cultured in individually artificial growth conditions and media without a host.

This study, which provides the first information on the identity and phylogenetic diversity of bacterial communities within *Phormidium*, although they cannot be cultured in different synthetic media, shows that *Phormidium* harbors endophytic bacteria that are not very complex but taxonomically diverse, including members of *Sphingomonas, Sphingopyxis,* and *Stenotrophobacter*.

Conclusion

Endophytic bacteria associated with *Phormidium* sp. (BDCC 002) algae have been identified for the first time. The molecular approach revealed three bacteria, *Sphingomonas* sp., *Sphingopyxis* sp., and *Stenotrophobacter* sp. belonging to endophytic. As a future perspective will be to focus on the biological function and the potential biotechnological relation between endophytic bacteria and host *Phormidium* microalgae, such as the production of phytohormones and siderophores, as well as nitrogen fixation. The results highlight the importance that *Phormidium* is closely associated with well-defined endophytic bacterial communities.

Data Availability

The nucleotide sequences obtained in this study have been deposited in the GenBank database with accession numbers: MW759557–MW759563.

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Research Article

Determination of Separation Performance in CFD-DEM Simulation Using Straw Particles in A Standard Cyclone

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Abstract: Although flow in biological materials sometimes behaves like a continuous one, it cannot be simulated with continuity-based modeling when it comes to discontinuous flow behavior. The Discrete Element Method (DEM) in combination with Computational Fluid Dynamics (CFD) is a computational method for modeling particles in fluid flow by tracking their motion. DEM is widely used in the field of engineering, and its use in the agricultural field is increasing. This study analyzes the CFD-DEM relationship of biological material in aerodynamic systems and reviews current applications. In the article, the definition of aerodynamic systems as a basic principle, particle-fluid and particle-particle interaction forces in the system, modeling of particle motions, CFD-DEM coupling method, and analysis applications of agricultural aerodynamic systems are examined. In this study, simulation experiments were carried out at 100 g/s and 200 g/s straw feeding values at each value of 18-15-12-10-8-6-4 m/s air and straw inlet velocities. The flow near the cyclone walls caused the straw particles to be directed towards the lower exit end of the cyclone. At feed densities of 100 g/s and 200 g/s, the least particle output was obtained at a rate of 18 m/s. The highest cyclone output efficiency was obtained at feed densities of 100 g/s and 200 g/s at a velocity of 12 m/s. The compatibility of the trial simulation results with the literature showed that the CFD-DEM application is an important approach to study the behavior of particulate matter in fluids.

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1. Introduction

The cyclone separator in the agricultural processing industry is considered one of the simplest and least expensive pieces of equipment for gas particulate matter separation. But cyclones have a complex flow pattern. Traditionally, the cyclone has been widely used in various industrial applications due to its high efficiency, simple design, relatively low cost of maintenance, flexible structure, and low power consumption (Wang et al., 2002; Marinuc and Rus, 2011;). One of the widely preferred areas is its use in separation processes (Avcı and Erel, 2003).

Cyclones are designed tangentially or axially according to the inlet type. Generally, tangential inlets are more commonly used for separation in cyclones. The fluid entering the cyclone gains a rotational motion with the effect of inertial forces. This rotational motion causes the fluid to be affected by the vortex motion. A relative movement in the radial direction occurs under the effect of different inertia forces on phases with different densities in the fluid. Part of the dense phase is removed from the flow area by the effect of centrifugal force (Şendoğan, 2012).

The cyclone's input section, body length, discharge pipe diameter, fluid flow, and physical qualities of the material employed all have an impact on cyclone efficiency. For these parameters to work in harmony with each other, their values must be determined optimally. More experimental studies should be done to determine these values (Şendoğan, 2012).

There are two most important points in cyclone design and selection. These are efficiency and pressure loss. The most important factors affecting the efficiency of the cyclone are the rate of entry of the particles into the cyclone and the amount of particle feed.

Inlet velocity affects both efficiency and pressure drop. As a result, the velocity must be improved. Cyclone performance can be enhanced under non-specific conditions by employing high input velocities, but performance can be diminished by increasing cyclone diameter (Faulkner and Shaw, 2006). Especially in the optimization of cyclone-style separators where the design parameters are dependent on each other, the use of programs that work according to computational fluid dynamics models facilitates the design and optimization. The flow parameters of high eddy and turbulent fluids may be easily calculated using computational fluid dynamics (CFD) models (Chu et al., 2011). As a result, crucial information for forecasting the aerodynamic behavior of the cyclonic-style separation process may be gained. Recently, high-order turbulence models using the unstable Reynolds mean Navier-Stokes (RANS) formulation, such as the Reynolds Stress Model (RSM), have shown adequate numerical estimate capabilities (Jakirlic et al., 2002; Gronald and Derksen, 2011). Cortes and Gil (2007) conduct a thorough examination of numerical CFD turbulence models for cyclone separators, concluding that proper resolution of flow characteristics is required for a successful simulation. The inclusion of particle matter in the cyclone's air flow necessitates extra modeling computations. As a result, the best numerical approach is Cundall and Strack (1979)'s Discrete Element Method (DEM), which uses the Lagrangian formulation technique to monitor each particle matter in the system independently. One can accurately predict the particulate flow behavior by considering the fluid-particle contact fundamentals of particle-particle, particle-boundary, and Newton's laws of motion (Peng et al., 2020; Zhu et al., 2008; Chu et al., 2011) and can analyze all physical phenomena. A schematic flowchart is shown in Figure 1 (Peng et al., 2020; Zhou et al., 2020) to provide an overview of the DEM algorithm.

Today, agricultural products (wheat, barley, etc.) are generally harvested with a combine harvester, and the remaining straw is either made into bales or chaff is made with a straw shredder machine (thresher machine). In small lands where the combine does not enter, the straw and seed parts of agricultural products are separated by threshing machines with or without storage. In addition, the harvesting and threshing processes of legumes such as chickpeas and beans are also carried out with threshing machines, with or without storage. The straw coming out of these threshing machines needs to be loaded, transported, and stored. The bagging process is widely used in the transportation or storage of hay. The bagging process is costly in terms of labor and causes a lot of time loss.

This study aims to optimize the design of the cyclone (separator) part of the cyclone regular straw bagging system, for which a patent application has been made for the straw bagging process by using CFD and DEM methods. In this context, flow analysis and particulate flow (air-straw) analyses of the cyclone, designed according to the literature, were carried out at different air inlet velocities with CFD and DEM based programs. Time-dependent separation and cyclone yield analysis of straw at different air velocities and feeding conditions in the cyclone were performed with CFD ANSYS Fluent and ROCKY DEM software. In this context, simulation experiments were carried out at 100 g/s and 200 g/s straw feeding values at each value of 18-15-12-10-8-6-4 m/s air and straw inlet velocities.

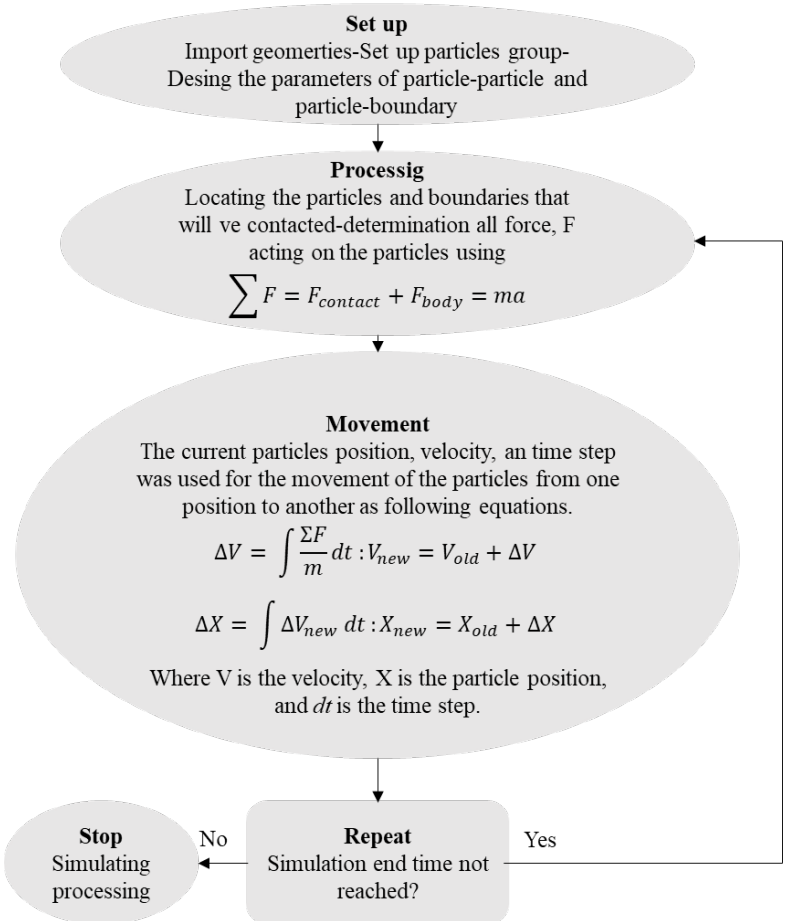

Figure 1. Flowchart of the discrete element method.

2. Material and Method

The fluid phase motion is estimated using the Navier-Stokes equations in the continuous flow model, which divides the system area into tiny cells (Drew, 1983; Fluent, 2009; Neuwirth et al., 2013; Fonte et al., 2015; He et al., 2018). Fluid control in the cyclone is estimated in the CFD simulation using Equation (1-2) to determine continuity/conservation of mass and conservation of momentum, respectively. A finite volume approach was used to solve these equations using the CFD ANSYS-Release-19 software within Selçuk University.

$$\frac{\partial(\rho_f)}{d_t} + \nabla(\rho_f v_f) = 0 \tag{1}$$

$$\frac{\partial(\rho_f v_f)}{d_t} + \nabla(\rho_f v_f v_f) = -\nabla p + \nabla\tau_f + \rho_f g + \nabla(-\rho_f \overline{u_i' u_j'}) \tag{2}$$

Where ρ_f is the density of the fluid, t is the real-time, ∂t is indeed the difference in operators at period, v_f is the mean flow rate, p is the pressure, and τ_f is the viscous stress tensor of the fluid, g represents gravity, $u_i' u_j'$ represents the mean variable fluid velocity, and $\rho_f u_i' u_j'$ represents the Reynolds stress nonlinear component due to turbulence.

The Reynolds stress model (RSM) was adopted for the current investigation because of the severe eddies and anisotropic turbulence within the cyclone (Gimbun et al., 2005; Xiang and Lee, 2005; Wan et al., 2008; Gronald and Derksen, 2011; El-Emam et al., 2021).

The primary strategy to follow the particle phase trajectory in the area under examination is to use the Lagrangian method in DEM software (El-Emam et al., 2021). The motion of particulate matter is split into two categories: translational motion, which is defined by Newton's second law (Equation 3), and rotational motion, which is defined by Euler's law (Equation 4).

$$m_i \frac{du_i}{dt} = \sum_{j=1}^{n_i^c} F_{ij}^C + F_i^f + F_i^g \tag{3}$$

$$I_i \frac{d\omega_i}{dt} = \sum_{j=1}^{n_i^c} M_{ij} \tag{4}$$

Where m_i is the particle mass and u_i and ω_i are the particle i's translation and rotation velocities, respectively. The contact force and torque exerted on particle I by particle j or the wall, respectively, are F_{ij}^c and M_{ij}. F_i^f fluid-particle interaction forces, F_i^g gravitational force I_i is the second moment of particle i and t is time.

The Hysteretic Linear Spring Model (Walton and Braun, 1986) is utilized in this work to reduce the overhead of extensive simulation times. Because the velocities and charge rates of surrounding particles are ignored, the energy loss is unaffected by other connections. This model can also be due to repeated compressible materials incorporating non-adhesive particle flow. (Freireich et al., 2009; Fonte et al., 2015; Almeida et al., 2016). The DEM approach produced and employed the following Equation (5-8) to mathematically describe this model (Walton and Braun, 1986; Rocky, 2018).

$$F_i^{n,T} = \begin{cases} \min(F_i^{n,(T-\Delta T)} + K_u^n \Delta S^n, K_l^n S^{n,T}), \text{if} \Delta S^n \geq 0 \\ \max(F_i^{n,(T-\Delta T)} + K_u^n \Delta S^n, 0.001\, K_l^n S^{n,T}), \text{if} \Delta S^n < 0 \end{cases} \tag{5}$$

$$\Delta S^n = S^{n,T} - S^{n,T-\Delta T} \tag{6}$$

$$\frac{1}{K_l^n} = \begin{cases} \frac{1}{E_i Z} + \frac{1}{E_j Z} & \text{Particle-Particle} \\ \frac{1}{E_i Z} + \frac{1}{E_w Z} & \text{Particle-Wall} \end{cases} \tag{7}$$

$$K_u^n = \frac{K_u^n}{\varepsilon^2} \tag{8}$$

Where $F_i^{n,T}$ and $F_i^{n,(T-\Delta T)}$ are the actual contact forces at time T and time T−ΔT, respectively, and ΔT is the time step. ΔS^n stands for normal overlapping variation, whereas $S^{n,T}$ and $S^{n,T-\Delta T}$ stand for normal overlap in the present and past, respectively. The unloading and loading contact stiffnesses are K_u^n and K_l^n, respectively, and the wall or boundary is w. ε is the restoration ratio, and Z is the particle size. E_i and E_w are the particle and boundary Young's moduli, respectively.

The Linear Spring Coulomb Limit Model (El-Emam et al., 2021) was used in this research to determine the total tangential contact force of the particle shear force during tangential contact (Equation 9).

$$F_i^{t,T} = min(F_i^{t,(T-\Delta T)} + K_l^n \Delta S^t, \mu F_i^{n,T}) \quad (9)$$

$F_i^{t,T}$ and $F_i^{t,(T-\Delta T)}$ are the tangentially contacting forces at the current and previous times, respectively, St is the change in tangential overlap throughout the time step, and is the friction coefficient indicating contact slip.

This numerical study used a one-way link between CFD and DEM, as well as an appropriate friction correlation model, to anticipate the actual behavior of the particulate matter flow in the cyclone separator (Peng et al., 2020; El-Emam et al., 2021).

The Ganser drag model (Ganser, 1993; Fonte et al., 2015; Almeida et al., 2016) has been successfully used since it is based on a single particle drag model. A discrete phase flow is a diluted flow whereby each particle is dealt with separately (El-Emam et al., 2021). Another strong reason to employ this model is that the particle matter in the simulation is non-spherical, having changing forms, densities, characteristics, alignment, and concentration. Therefore, the Ganser drag model can be accurately concluded using the particle relative Reynolds number and the two shape factors in the governing equations, as shown in (Equation. 10-13).

$$\frac{C_d}{K_2} = \frac{24}{R_{ep}K_1K_2}[1+0.1118(R_{ep}K_1K_2)^{0.6567}] + \frac{0.4305}{1+\frac{3305}{R_{ep}K_1K_2}} \quad (10)$$

$$K_1 = \left(\frac{d_p}{3d_v} + \frac{2}{3}\emptyset^{-0.5}\right)^{-1} - 2.25\frac{d_v}{D} \quad (11)$$

$$K_2 = 10^{1.8148(-\log_{10}\emptyset)^{0.5743}} \quad (12)$$

$$R_{ep} = \frac{\rho_f|v_f - u_i|d_p}{\mu_f} \quad (1)$$

Where the coefficient of friction is Cd, the Reynolds number is R_{ep}, and the shape factors are Stokes and Newton, respectively. d_p is the diameter of a spherical particle whose reflected area is equal to the reflected area of the real particle. The particle's sphericity is ∅, the diameter of a solid sphere with the same volume as the real particle is d_v, the fluid viscosity is μ_f, and the particle diameter is D.

Numerical simulations were run in CFD ANSYS-Release-19 on a machine with an Intel(R) 2.10 GHz CPU and 16GB of RAM for this work, as well as in ROCKY DEM software, which was provided with a 6-month trial version. Here, the unidirectional coupling of the CFD-DEM is used since the movement of particles is only affected by the airflow (dilute flow), and the particle velocity at the inlet of the cyclone is considered to be the same as that of the surrounding air (Elghobashi, 1994; Elsayed and Lacor, 2014; El-Emam et al., 2021). It is also recommended for the simulation of large particles in one-way coupling and unlimited homogeneous flows with different particle densities (Elsayed and Lacor, 2011; Fonte et al., 2015; Almeida et al., 2016). The general view of the cyclone used in this study is given in Figure 2, and its dimensions are given in Table 1 The cut-off size of this cyclone was not determined in this study because the basic working principle of the cyclone in this study is that it will be used for packing or bagging the straw by separating the air from it.

Table 1. Dimensions of thedesign componentse

Companent	Geometry	Dimensions (mm)
Cyclone diameter	Dc	320
Cylinder length	Lc	420
Cone length	Zc	950
Vortex diameter	Dv	120
Vortex length	Lv	130
Straw outlet duct	Do	100
Straw and air intake duct	Di	100

First, three-dimensional unstructured networks (Slacket al., 2000; Chu et al., 2011) were arranged on the designed cyclone (Fig. 2). The goal here is to resolution-independent is network resolution independent. It has been found that the solution has changed with the network improvements made in the cyclone. This indicates that a network-independent solution has yet to be found. As a result, network enhancements were made, and the process was repeated until the solution remained unchanged as the network changed. The y+ value was assumed to be between 30 and 100 for these operations (El-Emam et al., 2021). The cyclone mesh network has a total of 98.255 cells. The CFD solution was then transferred to DEM to execute the CFD-DEM merger simulation after network independence was established.

In CFD simulations, the liquid film flow (air) conditions were used (El-Emam et al., 2021). The air characteristics in the cyclone were modified to be turbulent, with a temperature of 25°C, a density of 1,225 kg/m^3, and kinematic viscosity of 1.7894 106 kgm/s. To show the impacts of airflow, the RSM turbulence model is combined with typical wall functions. For each calculation model, the y+ value is between 30-100. This shows that the nodes closer to the wall are in the log-log layer and not in the laminar substrate (El-Emam et al., 2021). The terminal velocity for wheat straw varies between 4.80-6 m/s (Khoshtaghaza and Mehdizadeh, 2006). In this study, straw and air intake velocities were applied as 18-15-12-10-8-6-4 m s^{-1} inside the cyclone. Each velocity application has been tested at 100 g/s and 200 g/s straw inlet feed densities. Thus, a total of 200 g and 400 g straws have been entered at the cyclone input for each velocity value application. The DEM simulation values for the straw are given in Table 2. A straw particle of the same size was used to get a faster and more accurate result of the simulation time.

Table 2. DEM simulation values of straw

Property	Unit	Straw
Particle Shape	-	Straight fiber
Dimensions	mm	5 (Equivelant diameter) x 20
Density	kg/m^3	200 (O'dogherty et al., 1995; Annoussamy et al., 2000)
Number of particles	-	18000
Coefficient of friction (straw-straw)	-	0.3 (Sitkei, 1987)
Coefficient of friction (straw-steel)	-	0.8 (Liu et al., 2018)
Coefficient of restitution		0.2 (Li et al., 2018)
Rolling resistance		0.3
Poisson's ratio		0.4 (Li et al., 2012; Liu et al., 2018)
Shear modulus of straw	GPa	0.001 (Liu et al., 2018)

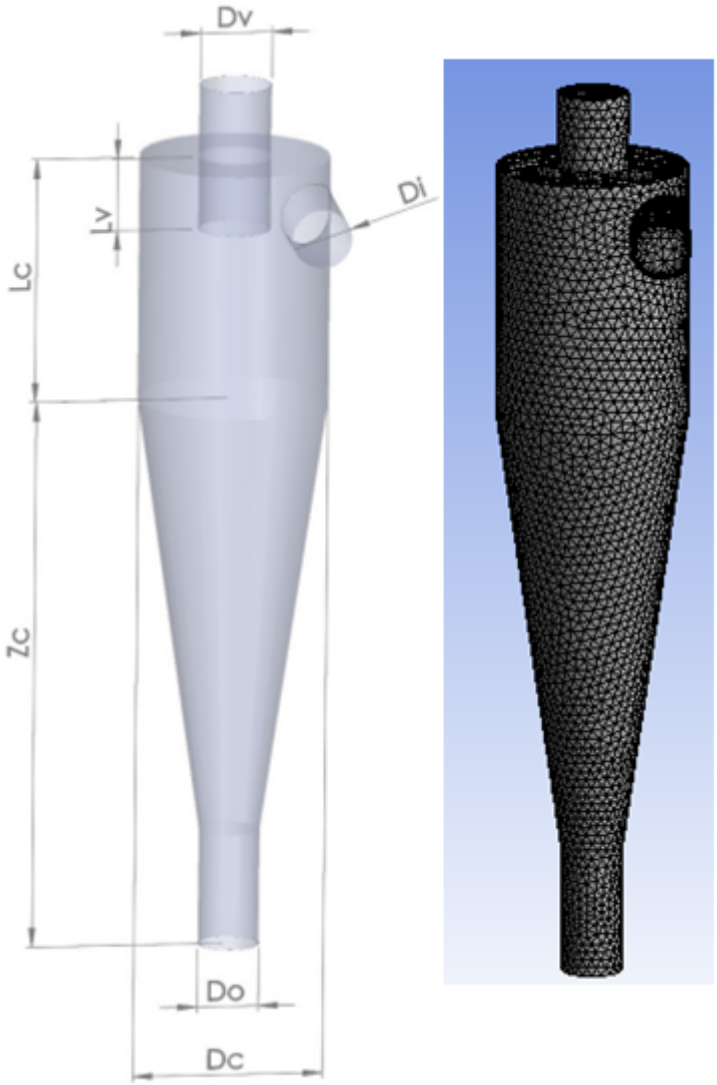

Figure 2. Cyclone model and mesh network.

One-way CFD-DEM coupling simulation was performed for each velocity value. A total of 14 different simulations were run, including air velocities and different feed densities. For each operating case, particulate matter was given proportionately to the air velocity at the inlet of the cyclone. The total simulation time was selected as 6 seconds before starting the simulations. The airflow was operated until the conclusion of the simulation period, while the cyclone was fed constantly till the 2nd second of the simulation time. Cube was made using the Processes component of Rocky's Particle program. With this Cube feature, the inlet and outlet mass values of the cyclone from both the inlet channel (Di) and the outlet channel (Do) of the straw to the straw are graphically and numerically taken with 0.1 second intervals from the first moment of the straw starting the flow (0th second) to the 6th second. Calculations under these conditions took approximately 40 to 60 hours, depending on each velocity value.

The output efficiency (s) was obtained in this study to evaluate the cyclone efficiency. The output efficiency (Equation 14) was computed by comparing the number of straws at the cyclone intake (feed) to the number of straws at the outflow (product) (Vose, 1978; Farran and Macmillan, 1979; Stepanenko, 2017; El-Emam et al., 2021).

$$\eta_s = \frac{\text{The amount of straw entering the cyclone}}{\text{The amount of straw coming out of the cyclone}} \text{x}100 \qquad (14)$$

3. Results and Discussion

Due to severe turbulence and violent vortex, cyclone separators have a complicated velocity profile at high air intake velocities. The findings of the CFD simulations looked at in this study were compared to varied velocities and velocity variations along the cyclone borders. To acquire an exact result of extraction efficiency and to depict the rotational properties of the airflow, a precise estimate of velocity variation levels is necessary. The Rankine vortex type is defined by the velocity profile that forms the typical flow shape (Peng et al., 2002; Cortes and Gil, 2007; Kozołub et al., 2017). The flow within reverse flow cyclone separators must follow this flow arrangement (Figure 3). On the outside, there is a free vortex, and in the middle, there is a forced vortex. The hay is forced out by the flow towards the cyclone barriers. The fluid flow direction in the core part of the cone section is inverted upwards, directed from the vortex finder to the cyclone exit. Figure 3 depicts the velocity profiles for the cyclone's various air input velocities.

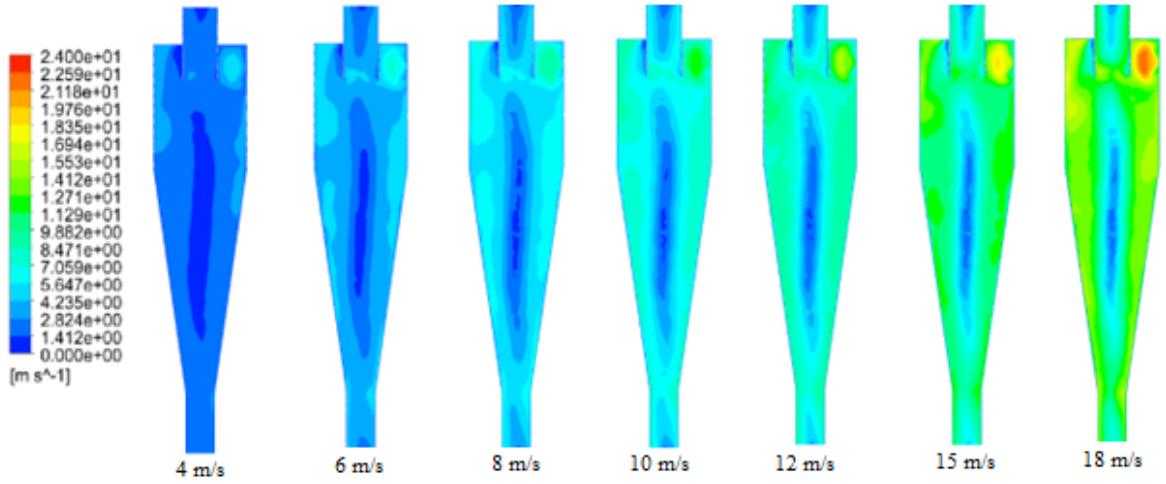

Figure 3. Velocity profiles in the cyclone of different inlet velocities.

In all of the different velocity combinations, the sidewalls of the cyclone had higher velocities than the center. The pressure distribution depending on the velocity profiles within the cyclone is shown in Figure 4. Positive high-pressure is seen at the entrance of the cyclone and on the side walls (cylinder (Lc) walls). This might imply that the wind in this location will have a greater influence on the straw particons than in others. While the pressure decreases in the middle of each cyclone, the pressure becomes negative at the vortex outlet (Dv). The streamline reverses its path towards the center of the cyclone near the end of the negative pressure zone, and air exits the cyclone through the vortex outlet. This situation is seen in Figure 4. The pressure on the sidewalls of the cyclone also increased due to the increase in air entry velocity into the cyclone. The flow near the cyclone walls is intended to push heavy particles towards the lower outlet end of the cyclone.

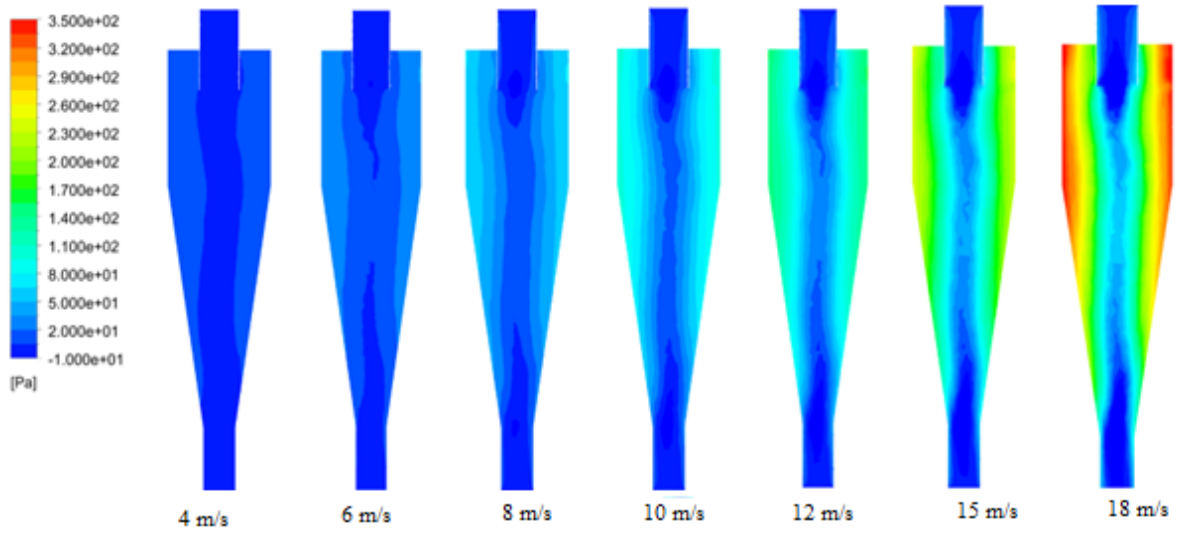

Figure. 4. Pressure distributions in the cyclone of different inlet velocities.

It is known that the majority of flow particles accumulate on the cyclone intake wall shortly after entering and then drop in strips (Wang et al., 2006; Li et al., 2009). As seen in Figures 5-6 and 7, particle behavior can be accurately captured by the current CFD-DEM model. It is seen that the flow reaches a steady state after 2 seconds, which is consistent with the literature (Chu et al., 2011).

The separation efficiency found in the cyclone is reflected in the spiral flow pattern of the straw. Particle flow usually enters the perimeter from the cyclone intake, and spirals are created around the barrel portion towards the top of the cyclone, as images of the simulation result from different time steps plainly demonstrate. They flow tangentially down to the conical section of the cyclone, generating an outer vortex. The higher air velocity in the outer vortex of the barrel section causes the heavier particles (straws) to be separated from the flow stream and gathered towards the cyclone wall due to increased centrifugal force. When the flow reaches the conical part, the air velocity drops drastically and creates an internal vortex. During this process, the straw fall from the end of the conical section as the airflow escapes the vortex finder. The flow of straw in the cyclone at rates of 4 m/s, 10 m/s, and 18 m/s is given as an example in Figure 5-6 and 7, respectively. It is seen that the straw moves from the cyclone walls to the straw outlet channel with the effect of the pressure on the cyclone wall. Similar relationships were also seen in other velocity trials and feeding values.

The time-dependent changes in the amount of straw particles coming out of the cyclone were examined at each velocity value. The output amounts of the straw particles were observed at a rate of at least 18 m/s at both 100 g/s (Figure 8) and 200 g/s (Figure 9) feed densities. The high velocity value prolonged the residence time of the straw in the cyclone and caused it to leave the cyclone later. With the increase in the cyclone inlet velocity, the straw output decreased at different simulation times due to the increase in the centrifugal force acting on the straw and the increase in the number of eddy rotations (Karpov and Saburov, 1998; Fıçıcı, 2006). This was particularly evident at the cyclone input velocities of 15 m/s and 18 m/s. The decrease in time-related product output at high air and hay input velocities was caused by the increase in the length of time the straw remained in the cyclone. Researchers have reported that gas velocity, particle inlet density, and particle diameter have increased particle residence time (Dibb and Silva, 1997; Corrêa et al., 2004; Corrêa et al., 2004; Farias et al., 2013). Corrêa et al. (2004a) study foundthe that if cyclone air flow rate increases from 8.6 kg/s to 9.25 kg/s, the residence time of the particle in the cyclone increases from 0.67 s to 1.27 s.

When we examine the Figures, it can be seen that many straws reach a steady state at the base of thclone in the 2.1 the second of the simulation period. However, it is seen that the straw flow at the highest velocity of 18 m/s is more unstable than the straw flow at the lower velocity values. We can explain the reason for this as the turbulent flow that occurs in the cyclone at high velocity.

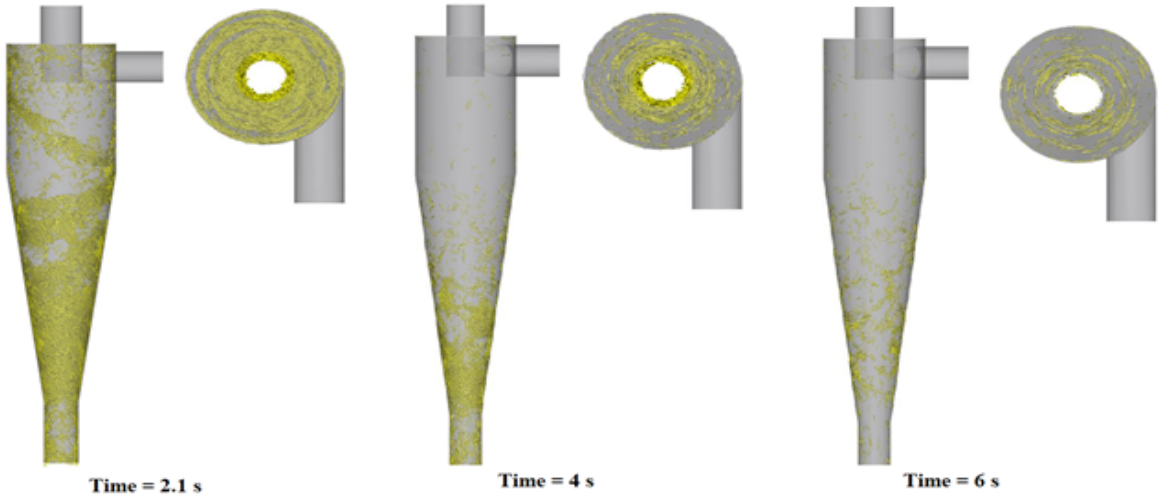

Figure 5. Time-dependent movement of straw in the cyclone at an inlet velocity of 4 m/s.

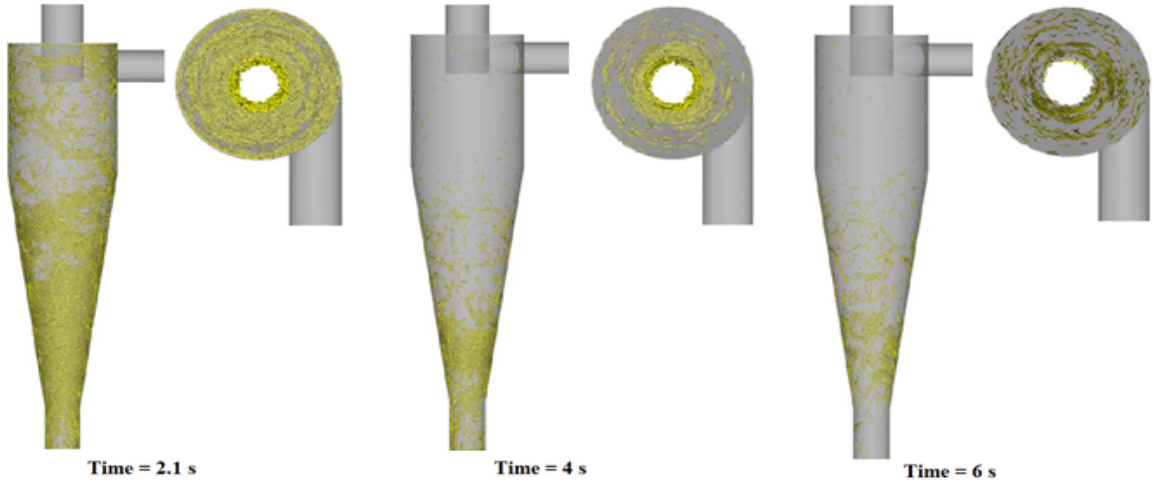

Figure 6. Time-dependent movement of straw in the cyclone at an inlet velocity of 10 m/s.

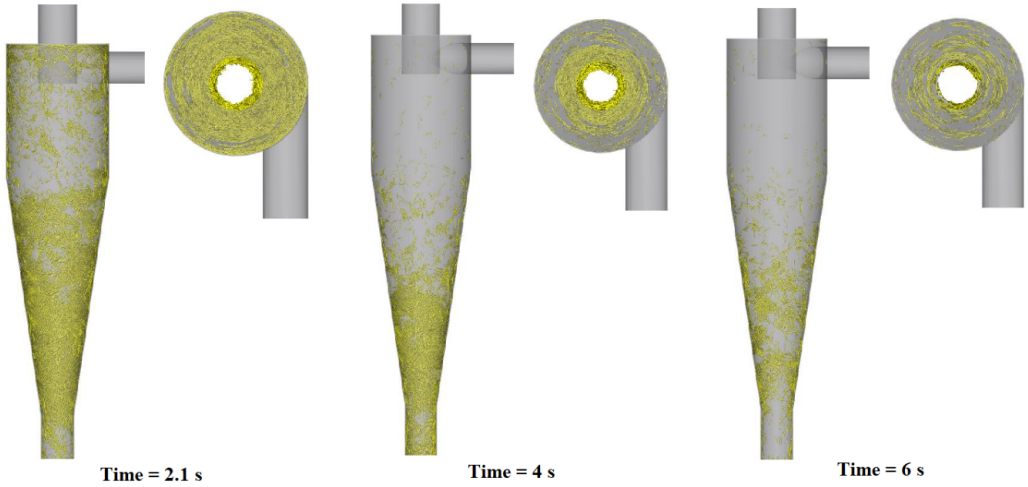

Figure 7. Time-dependent movement of straw in the cyclone at an inlet velocity of 18 m/s.

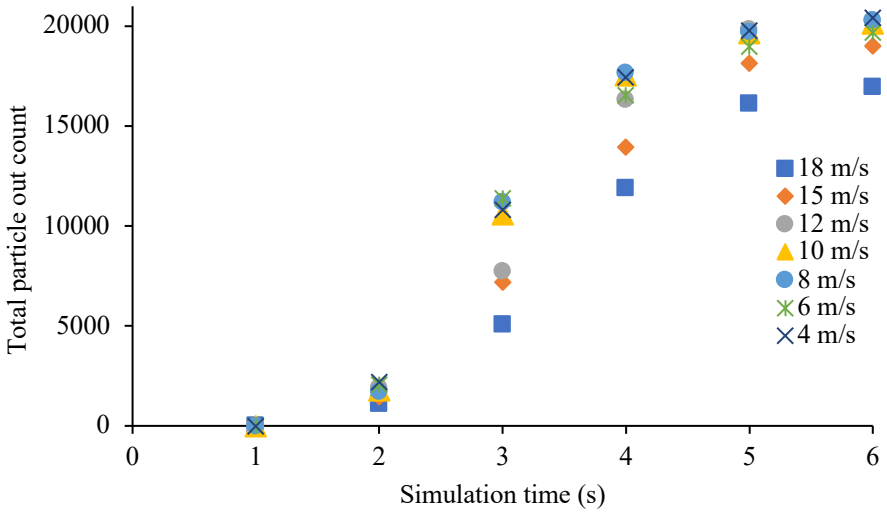

Figure 8. Total particle output amount of different velocities (at 100 g/s feed density).

The output efficiency at the end of the 6th second of the simulation was investigated at different air and straw inlet velocities and different straw inlet dinties into the cyclone (Figure 9). Output efficiency of both 100 g/s and 200 g/s straw inlet densities decreased at high inlet rates of 15 m/s and 18 m/. The highest output efficiency at 100 g/s and 200 g/s inlet densities were calculated as 93.8% and 96.4% at 12 m/s air inlet velocity, respectively. At low flow rates, the centrifugal force is weak, making regular flows of particles difficult. As a result, the output efficiency may be lower. At higher flow rates, however, the centrifugal force is strong, and the turbulent kinetic energy increases. This also affects the stability of the cyclone inflow field. This effect will intensify the back-mixing of particles and cause a reduction in output efficiency. In this study, there was a decrease in cyclone output efficiency at very low velocity and very high velocity. Ma et al. (2015) reported that in their three different cyclone designs and different flow rates (30-40-50-60-70 m^3/h), they achieved the highest output efficiency of 50 m^3/h flow rate. They reported reduced output efficiency at very low and very high flow rates in each cyclone model.

The increased feed density in the cyclone at the same air inlet rates has increased the hay output efficiency. Lim et al. (2003) reported that increased particle inlet density increases cyclone yield. Huang et al. (2018) explained in their study results that increasing particle mass loading improves separation efficiency. The separation efficiency is proportional to the residence time of the straw particle in the cyclone.

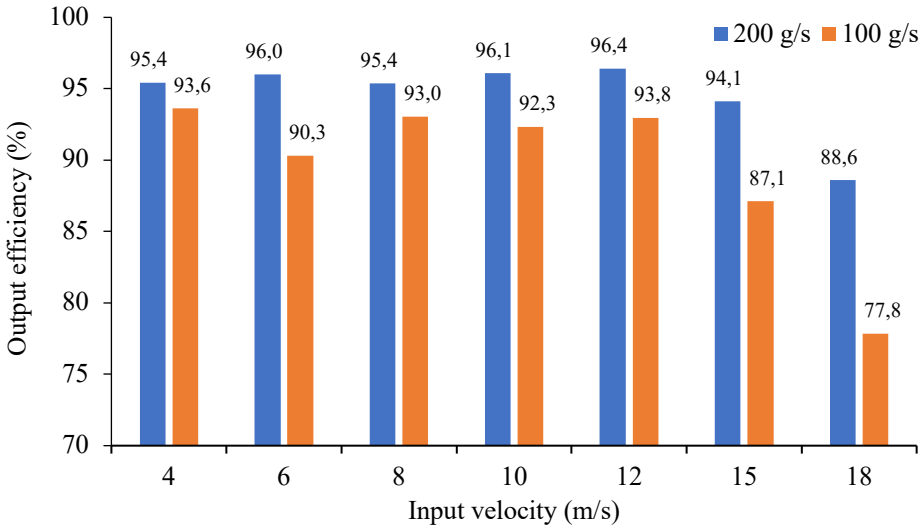

Figure 9. Cyclone output efficiency at different inlet velocities and feed densities.

4. Conclusion

The amount of straw particles exiting the cyclone was analyzed in a time-dependent manner in this study using CFD and DEM methods in a designed cyclone.

Reynolds stress turbulence model was used since the cyclone has a complex turbulent flow type as its flow type. During the simulation, the drag model caused the straws to form trajectories towards the bottom of the cyclone. According to the simulation results, there was an increase in pressure on the side walls of the cyclone due to the increase in the air inlet velocity to the cyclone. The flow near the cyclone walls caused the straw particles to be directed towards the lower exit end of the cyclone. At feed densities of 100 g/s and 200 g/s, the least particle output was obtained at a rate of 18 m/s. The high velocity value extended the residence time of the straw in the cyclone and delayed the separation of the straw from the cyclone. The highest cyclone output efficiency was obtained at feed densities of 100 g/s and 200 g/s at a velocity of 12 m/s. In addition, the increase in feeding density at constant air inlet velocity entering the cyclone increased the straw output efficiency. The simulation analysis results of the straw flow in the cyclone were found to be compatible with the literature. Thus, straw bagging or packaging will also guide in terms of improving the designs by using CFD and DEM simulation methods before cyclone designs. This research was the first attempt to model the airflow of straw particles using a cyclone separator.

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Research Article

Evaluation of the Relationship Between Infiltration Rate and Some Soil Properties under Different Land-Use

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Land conditions,
Morphology,
Factor analysis

Abstract: Soil infiltration rate (IR) is an important parameter and a good indicator of soil quality and fertility. The most influential factors for all conditions where the best performance in infiltration surveys is achieved are soil properties and land-use type. Therefore, a detailed understanding of infiltration is required for different land-use complexes. In this study, the effects of soil properties on IR in soils under different land-uses (pasture, fallow, and orchard) were investigated. Soil samples were taken from 30 points determined by GPS from 3 land-uses within the border of the Çubuk district of Ankara Province, Turkey. IR (with Minidisc infiltrometer, MDI), bulk density, and penetration resistance were measured in undisturbed soil samples. Saturated hydraulic conductivity (K_s) and sorptivity were obtained from infiltration measurements. Soil parametric analyses and morphological descriptions were made in disturbed soil samples. In order to digitize the morphological properties, the coding system was created with the help of soil identification cards. The average IR value was found to be the highest in the orchard and the lowest in pasture samples. Correlation analysis, one-way ANOVA, and factor analyses were used to evaluate the relationships between soil variables and IR. IR showed the highest correlation with sorptivity (0.72), sand (0.69), and K_s (0.86) in the pasture, fallow, and orchard, respectively. IR in different land-uses was loaded on the same factors with different soil variables. Due to different land management practices, such additional measurements need to be made to accurately assess the potential impact of land-use and management changes on agricultural activities.

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1. Introduction

Infiltration is one of the main processes controlling water flow from surface to groundwater and is a complex dynamic process dependent on many factors (Jakab et al., 2019). Knowledge about the infiltration process, which has a fundamental role in agriculture and water research, is essential to be able to evaluate research results well (Pedretti et al., 2012). Therefore infiltration process continues to attract attention from researchers (Vand et al., 2018). Infiltration is, by definition, the initial stage of

water flow into a relatively dry soil profile in which gravity plays only a minor role (Philip, 1957) (Equation 1). Infiltration can be measured in many ways as cumulative infiltration and IR. Cumulative infiltration is defined as the total of water flowing from the soil surface into the profile throughout a certain time (Chu, 1978).

$$I = St^{0.5} + At \quad (1)$$

Where I: cumulative infiltration (cm s^{-1}), S: sorptivity (cm $s^{-1/2}$), t: time (min), for one-dimensional vertical infiltration, A is proportional to the K_s of the soil.

The IR of soil depends on various factors such as initial conditions, structure, and mechanical behavior of soils (Angelaki et al., 2013). Another factor that has remarkable effects on infiltration due to the dynamics of soil properties is land-use (Biro et al., 2013).

In different land use, soil tillage tools, methods, and technological processes can affect soil's physical, chemical, and biological properties differently (Yankov and Drumeva, 2021). Due to the loss of land that can be used for agricultural purposes, different land uses, landuse planning (Şatır and Berberoğlu, 2021) and their effects have gained importance today. One of the soil processes affected by land use is soil infiltration capacity. It has been noted in many previous studies that soil infiltration capacity is controlled by the land-use type caused a significant change in the physical properties of the soil, and thus affected soil IR (Horel et al., 2015; Sun et al., 2018; Dionizio and Heil, 2019; Dionizio and Costa, 2019). However, in previous studies, general conclusions about land-use change effects on infiltration capacity could not be fully drawn due to the complexity of the plant and soil system. Although the land-use pattern is considered as one of the main factors affecting infiltration, the differences in the infiltration capacity of the soil are not very clear (Sun et al., 2018). Therefore, it is more important to reach the necessary information about soil management after the land is transformed into different land-use. Adequate knowledge of a soil's IR is essential for reliable prediction and control of soil and water-related environmental hazards (Patle et al., 2019). The aim of this study is to evaluate the relationships between IR of soils under different land-use (pasture, fallow, and orchard) and some physical, chemical, and morphological soil properties.

2. Material and Methods

2.1. Materials and soil sampling

This study was carried out in soils under 3 different land-uses in the Çubuk district in Ankara Province, Turkey (Figure 1). Pasture has less calcareous, high organic matter, neutral pH, unsalted, and generally clayey soils. Fallow soils are slightly alkaline and calcareous, with medium organic matter, unsalted and clayey. Orchard soils are slightly calcareous and alkaline, generally with weak organic matter, unsalted and clayey (Sarıdemir, 2010). For sampling, a total of 30 sample points were determined by GPS (Global Positioning System), 10 randomly from each land-use (Figure 1). Undisturbed soil samples were taken with a sampling cylinder (100 cm^3) after the topsoil was cleaned for infiltration measurements and bulk density. Disturbed soil samples were taken from the same points at a depth of 0-10 cm for basic soil analyses.

2.2. Methods

Infiltration measurements were made at a suction ratio of 2 cm (Decagon Devices, Inc. 2014). MDI is a useful device like a classical tension infiltrometer for predicting hydrodynamic properties of soils (Alagna et al. 2016). The soil surface isn't disturbed when using MDI (White and Perroux 1987). MDI prevents the water flow through the macropores because a negative potential was applied during measurement (Minasny and George 1999). Before measurements, ground vegetation was trimmed and surface litter carefully removed, and a very small amount of fine-grained sand was used to fully contact the infiltrometer with the soil. For the calculation of infiltration values, the simple method commonly used in dry soils suggested by Zhang (1997) was used (Equations 2 and 3).

$$I = C_1 t + C_2 \sqrt{t} \tag{2}$$

$$k = \frac{C_1}{A} \tag{3}$$

Where C1 (m min^{-1}, relates to k) and C2 (m min$^{-1/2}$, corresponds to the soil sorptivity value) are the parameters. k is hydraulic conductivity (K_s) and C1 is the slope of the cumulative infiltration curve versus the square root of time. A is a value that relates van Genuchten parameters to the suction velocity and radius of the infiltrometer disc for a given soil type. Ks values were calculated by Equation 3. C1 was obtained from infiltration graphs. For A, values were taken corresponding to 4.5 cm disc diameter and -2 cm suction value (6.36 for silty clay and 4.30 for clay classes) (Decagon Devices Inc. 2014).

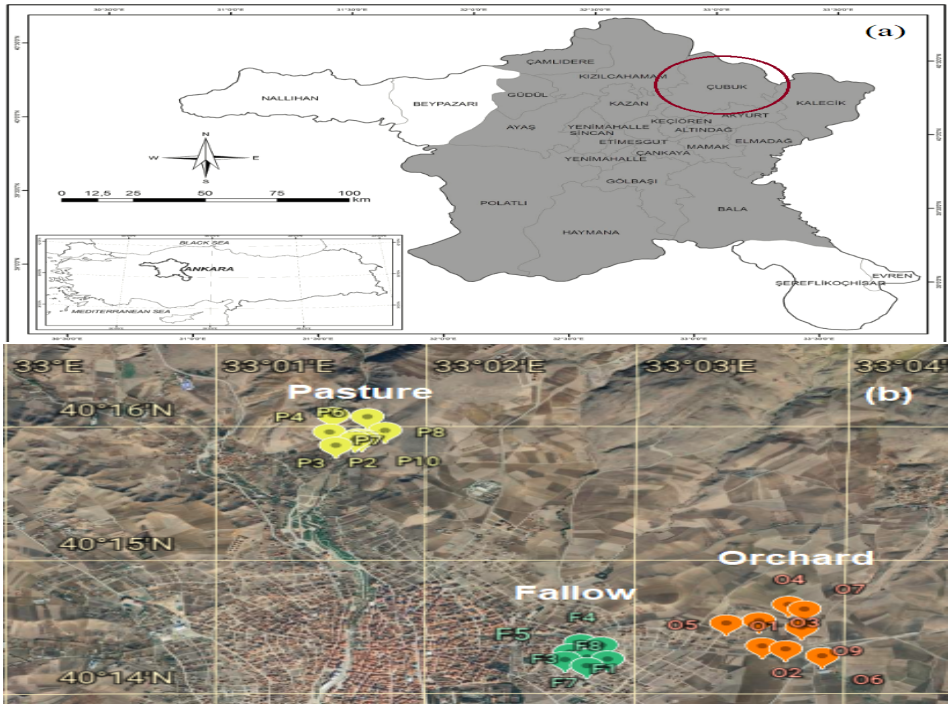

Figure 1. (a) Study areas (Orkun et al., 2014) and (b) soil sampling points (P: Pasture, F: Fallow, O: Orchard) (The map was downloaded from Google earth, sampling points and coordinates were edited).

The cumulative infiltration (I) was plotted as a function of the square root of time according to the Philip (1957) equation. The sorptivity values were obtained from the slope of the regression equations of these graphs for each sample (Baranian Kabir et al., 2020). Soil bulk density (Black and Hartge, 1986), soil texture (Gee and Bauder, 1986), field capacity (FC) and wilting point (Klute and Dirksen, 1986), aggregate stability index (Kemper and Rosenau, 1986), pH, organic matter and $CaCO_3$ content (Page et al., 1982), and electrical conductivity (Rhoades, 1982) were measured. Soil description charts were used for digitizing the morphological properties such as soil structure, pores, color, consistency, stickiness, plasticity, roots (Schoeneberger et al., 2012), and coefficient of linear extensibility (COLE) (Schafer and Singer, 1976) (Karahan and Erşahin, 2017). Correlation analysis was performed to evaluate the relationships between IR and soil properties. In order to create more meaningful and independent factors by reducing the number of variables, factor analysis (principal components) (SPSS Inc., 2015) was used. Factors with an eigenvalue of ≥1 were selected according to the factor analysis line graph of soil variables, and the critical factor load was taken as 0.5 for soil variables (Kalaycı, 2010). For reducing the number of variables loaded on more than 1 factor, varimax rotation was applied in the analysis.

3. Results

Descriptive statistics for some soil samples were given in Table 1. IR, K_s, soil structure type, and root properties were included in very variable classes in all land-use (Mulla and Mc Bratney, 2001). The highest average infiltration value is in an orchard, and the smallest is in pasture soil samples. IR classes are in very low class in all land-use (Kohnke, 1968). Infiltration values have positive kurtosis in all applications, but it showed high kurtosis (5.6) in fallow soils (Webster, 2001).

Table 1. Descriptive statistics of some soil variables for each land-uses

Soil variables	Max.	Min.	AM	SD	CV%	Skewness	Kurtosis
Pasture							
Infiltration rate (cm s^{-1})	0.011	0.001	0.005	0.003	66.780	1.0882	0.200
Clay (%)	61.200	46.200	55.275	5.138	9.300	-0.910	-0.475
K_s (cm s^{-1})	0.003	$3x10^{-5}$	$43x10^{-5}$	$91x10^{-5}$	213.900	2.963	8.932
Bulk density (gr cm^{-3})	1.298	1.025	1.118	0.082	7.300	1.108	1.551
Organic matter (%)	10.013	4.627	6.825	1.477	21.600	0.968	1.783
EC (dS m^{-1})	0,476	0,290	0,347	0,054	15,50	1,582	3,392
pH	7.435	6.555	7.079	0.299	4.200	-0.903	-0.319
PR (KPa)	783,333	446,667	560,667	110,630	19,70	0,937	0,230
Structure type	5.000	4.000	4.400	2.348	53.400	0.609	-3.33
Pore size	4.000	2.000	2.700	0.675	25.000	0.434	-0283
Stickiness	2.900	2.500	2.790	0.145	5.200	-1.156	0.201
Root quantity	2.000	1.000	1.000	0.966	60.400	0.111	-0.623
Fallow							
Infiltration rate (cm s^{-1})	0.045	0.004	0.014	0.012	90.440	2.2461	5.648
Clay (%)	64.150	60.400	62.100	1.092	1.800	0.429	-0.002
K_s (cm s^{-1})	$85x10^{-5}$	$5 x10^{-5}$	$44 x10^{-5}$	$26 x10^{-5}$	59.091	-0.024	-0.989
Bulk density (gr cm^{-3})	1.313	1.142	1.235	0.059	4.700	-0.398	-1.310
Organic matter (%)	2.435	1.773	2.157	0.202	9.400	-0.636	-0.005
EC (dS m^{-1})	0.214	0.168	0.191	0.014	7.100	0.101	0.041
pH	8.050	7.505	7.731	0.161	2.100	0.487	0.558
PR (KPa)	377.500	220.000	289.250	53.826	18.600	0.459	-1.224
Structure type	5.000	4.000	4.800	2.547	53.100	-2.236	5.000
Pore quantity	3.000	3.000	3.000	0.000	0.000	0.000	0.000
Stickiness	2.600	2.300	2.410	0.120	5.000	0.738	-0.878
Root quantity	3.000	1.000	1.400	0.843	60.200	1.779	1.406
Orchard							
Infiltration rate (cm s^{-1})	0.073	0.006	0.032	0.0192	59.430	1.028	1.299
Clay (%)	66.200	60.700	64.525	1.882	2.900	-0.981	0.271
K_s (cm s^{-1})	0.001	$0,5x10^{-5}$	$72.7x10^{-5}$	$34.1 x10^{-5}$	46.900	-0.979	1.021
Bulk density (gr cm^{-3})	1.199	1.007	1.107	0.056	5.000	-0.197	0.021
Organic matter (%)	2.842	1.406	1.920	0.381	19.900	1.579	3.946
EC (dS m^{-1})	0.209	0.167	0.191	0.015	7.90	-0.481	-1.515
pH	7.915	7.810	7.850	0.035	0.400	0.620	-0.488
PR (KPa)	212.500	112.500	172.250	32.112	18.60	-0.733	0.047
Structure type	5.000	4.000	4.667	2.547	54.600	-0.968	-1.875
Stickiness	2.800	2.600	2.720	0.120	4.400	-0.407	-1.734
Root quantity	1.000	0.000	0.100	0.316	316.200	3.162	10.000

IR: Infiltration rate, K_s: Saturated hydraulic conductivity, EC: Electrical conductivity, PR: Penetration resistance, pH: Soil reaction.

3.1. Infiltration rates of soil samples

IR graphs were created using cumulative infiltration values versus time (Zhang, 1997) (Figure 2). One-way analysis of variance was performed for the significance of the differences between the average IR and average sorptivity values in land-use (Table 2). The method indicated the soil IR and sorptivity properties among the land-use were statistically significant at 0.05 level ($p \leq 0.05$).

Table 2. One-way analysis of variance of average IR and sorptivity for land-use

Land-use	Average infiltration rate	Average sorptivity
Pasture	$0.0045±0,00096^a$	$0.035±0,0025^a$
Fallow	$0.0136±0,0039^b$	$0.127±0,0073^b$
Orchard	$0.0323±0,0061^c$	$0.096±0,012^c$

Means indicated with different letters in the same column are different at the level of 0.05.

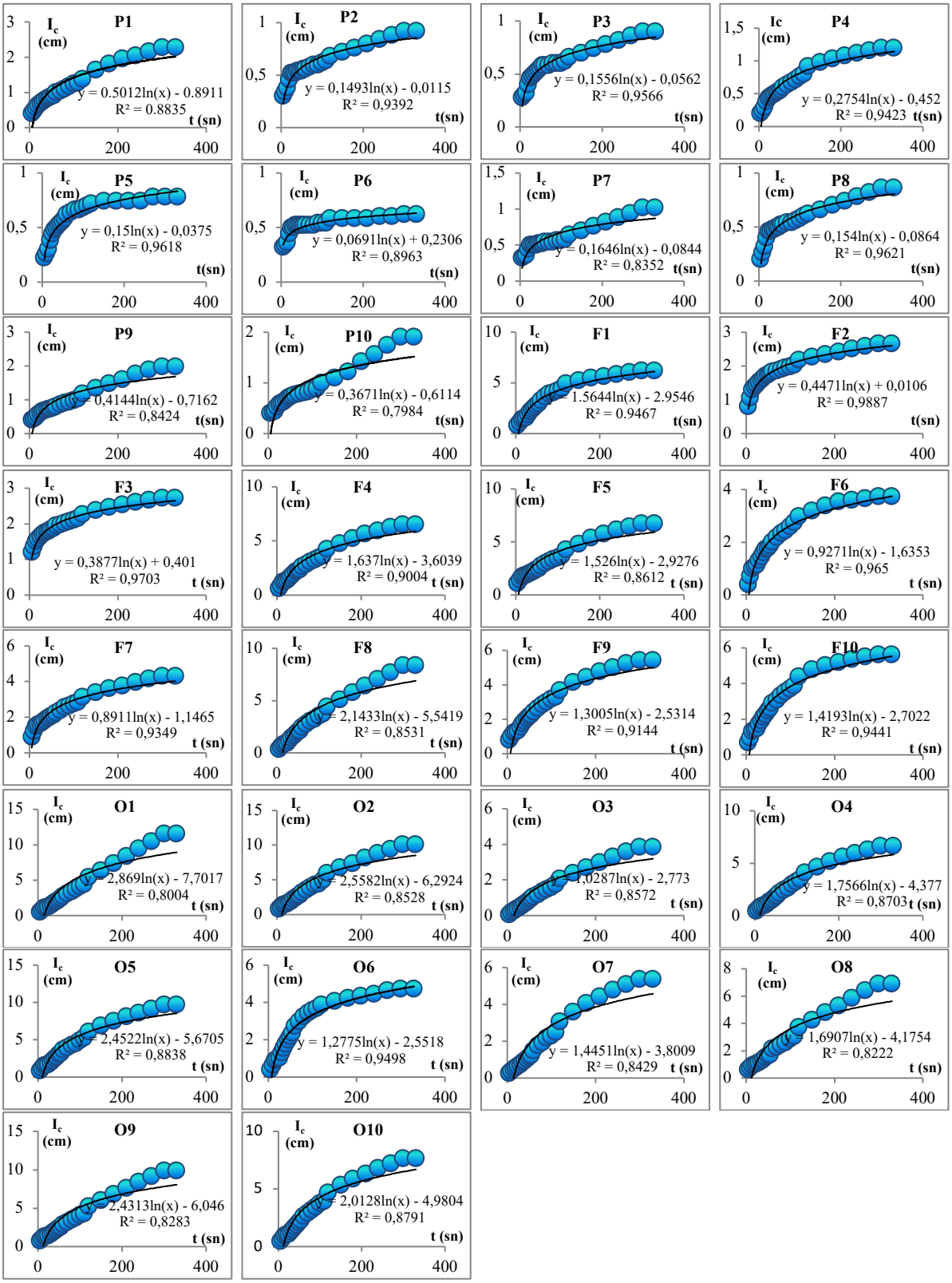

Figure 2. IR graphs of pasture (P), fallow (F) and orchard (O) samples.

3.2 Factor analysis of soil variables

Seven factors for pasture (the highest value is structure class and type, and the lowest value is wilting point), 8 factors for fallow (the highest value is structure size, the lowest is COLE), and 8 factors for the orchard (the highest value is color, the lowest is EC) were selected (Tables 3, 4, 5).

Table 3. Factor analysis of soil parametric and morphological variables for pasture soils

PASTURE	Factors				Factors			
Soil variables	**1**	**2**	**3**	**Soil variables**	**4**	**5**	**6**	**7**
Structure class	0.989			**pH**	0.814			
Structure size	0.989			**ASI (%)**	0.778			
EC (dS m^{-1})	-0.822			**$CaCO_3$ (%)**	0.696			
Root size	0.746			**Silt (%)**	0.657			
Sand (%)		-0.943		**Soil moisture (%)**	-0.651			
Stickiness		0.846		**IR (cm s^{-1})**	0.618			
Clay (%)		0.735		**Plasticity**	0.603			
COLE		0.658		**PR (KPa)**		0.930		
Bulk density (gcm^{-1})			0.932	**Organic matter (%)**		0.807		
K_S (cm s^{-1})			0.893	**Field capacity (%)**		0.626		
Pore size			0.850	**Wilting point (%)**		0.559		
Sorptivity (cm $s^{-1/2}$)			0.599	**Structure type**			0.926	
				Color			0.782	
				Consistency			0.719	
				Root quantity				0.899
Variation, %	20.880	15.100	14.470		14.130	12.150	11.090	7.230
Total variation, %								95.07

EC: Electrical conductivity, COLE: Coefficient of linear extensibility, K_s: Saturated hydraulic conductivity, pH: Soil reaction, IR: Infiltration rate, PR: Penetration resistance, ASI: Agregatte stability index, $CaCO_3$: Calcium carbonate.

Table 4. Factor analysis of soil parametric and morphological variables for fallow soils

FALLOW	Factor				Factor				
Soil variables	**1**	**2**	**3**	**Soil variables**	**4**	**5**	**6**	**7**	**8**
Structure size	0.929			**Stickiness**	0.901				
Color	0.811			**Pore quantity**	0.892				
$CaCO_3$ (%)	0.776			**Pore size**	-0.615				
Wilting point (%)	0.761			**COLE**	0.543				
EC (dS m^{-1})	-0.748			**PR(KPa)**		-0.911			
Structure class	0.729			**FC (%)**		0.787			
Structure type	-0.581			**IR (cm s^{-1})**			0.961		
Clay (%)		-0.954		**K_s (cm s^{-1})**			0.671		
Sand (%)		0.894		**Plasticity**				0.949	
Sorptivity (cm $s^{-1/2}$)		-0.842		**OM (%)**				-0.641	
Soil moisture (%)		0.609		**Consistency**				0.583	
pH			-0.898	**ASI (%)**					-0.920
Bulk density (cm g^{-1})			0.806						
Silt (%)			-0.632						
Variation, %	19.34	13.54	13.00		11.75	11.49	10.65	9.54	8.170
Total variation, %									97.51

$CaCO_3$: Calcium carbonate, EC: Electrical conductivity, pH: Soil reaction, COLE: Coefficient of linear extensibility, PR: Penetration resistance, FC: Fcapacity, IR: Infiltration rate, K_s: Saturated hydraulic conductivity, OM; Organic matter, ASI: Agregatte stability index.

Table 5. Factor analysis of soil parametric and morphological variables for orchard soils

ORCHARD	Factors				Factors				
Soil variables	**1**	**2**	**3**	**Soil variables**	**4**	**5**	**6**	**7**	**8**
Color	0.982			**Root quantity**	0.971				
Wilting point (%)	0.962			**Consistency**	0.971				
Field capacity (%)	0.932			**Structure class**	0.753				
$CaCO_3$ (%)	-0.804			**Structure type**		-0.951			
Soil moisture (%)	0.794			**Pore quantity**		-0.605			
Clay (%)	0.711			**OM (%)**			0.960		
IR (cm s^{-1})		0.947		**Silt (%)**				0.791	
K_s (cm s^{-1})		0.840		**pH**				0.706	
Sorptivity (cm $s^{-1/2}$)		-0.744		**Pore size**				0.657	
Sand (%)		0.670		**ASI (%)**					0.762
EC (dS m^{-1})		0.554		**Structure size**					-0.679
COLE			-0.840						
Bulk density (gr cm^{-3})			0.838						
Stickiness			0.824						
PR (KPa)			0.614						
Plasticity			0.594						
Variation, %	21.800	16.890	13.470		11.520	10.570	8.790	8.220	7.470
Total variation, %									98.750

$CaCO_3$: Calcium carbonate, Ks: Saturated hydraulic conductivity, EC: Electrical conductivity, COLE: Coefficient of linear extensibility, PR: Penetration resistance, IR: Infiltration rate, OM; Organic matter, pH: Soil reaction, ASI: Agregatte stability index

Table 6. The number of factors and definitions of the study area soil variables

PASTURE		FALLOW		ORCHARD	
FN	**Factor definition**	**FN**	**Factor definition**	**FN**	**Factor definition**
1	Morphology and EC	**1**	Morphology and chemistry	**1**	Soil physics
2	Texture and morphology	**2**	Texture and soil water	**2**	Soil water and EC
3	Soil water	**3**	Bulk density and pH	**3**	Bulk density and soil mechanics
4	Parametric ve plasticity	**4**	Morphology	**4**	Root and structure
5	Soil water and OM	**5**	Resistance and saturation	**5**	Structure and pore
6	Color and consistency	**6**	Conductivity	**6**	Organic matter
7	Pores	**7**	Consistency and organic matter	**7**	Pore and pH
		8	Stability	**8**	Stability and structure

FN: Factor number, OM; Organic matter, EC: Electrical conductivity.

According to the results of the factor analysis, the total 27 soil variables defined 95.07% of the total variability in the pasture, 97.51% in fallow, and 98.75% in orchards. In the pasture, the variables soil structure, EC, and root size were loaded on factor 1, and this factor explained 20.8% of the variance of the data set. Therefore, Factor 1 was named 'Morphology and EC'. In fallow, the variables structure size, color, $CaCO_3$, WP, EC, structure class and type were loaded on factor 1, and this factor explained 19.34% of the variance of the data set. Therefore, factor 1 was named ' Morphology and chemistry '. In the orchard, the variables color, WP, FC, $CaCO_3$, soil moisture, and clay were loaded on factor 1, and this factor explained 20.8% of the variance of the data set. Therefore, factor 1 was named ' Soil physics ' (Table 6). Similarly, factors were named according to the dominant soil characteristics loaded on each factor in pasture, fallow, and orchard (Table 3-6).

Soil variables loaded on the first factors show that morphological features are more dominant. It is seen that soil structural properties such as structure and pore, mechanical properties such as COLE, and root properties are positively related to infiltration in pasture land soil properties. However, EC, sand content and soil moisture content were negatively correlated with infiltration rate (Table 3). There were many previous studies that found soil infiltration was affected by soil properties (Tejedor et al., 2013; Sajjadi et al., 2016; Patle et al., 2019; Saputra et al., 2021). Saputra et al. (2021) reported that different land use shows variations in processes like infiltration due to they have different vegetation covers. Each type of vegetation has a different root system and therefore has different amounts of soil organic matter. These are important factors that affect the infiltration rate due to improvements in soil physical properties such as structure and soil porosity.

4. Discussion

It was noted that the soil infiltration process was generally affected by vegetation and soil characteristics (Leung et al., 2015). Therefore, many studies were performed on soil infiltration capacity using different soil variables such as water content, organic matter, and porosity under different land-use (Huang et al., 2017; Wu et al., 2016). In this study, we evaluated the relations between infiltration rate and some soil properties under 3 different land-use (pasture, fallow, and orchard) using One-way analysis of variance and factor analysis. The results showed that, contrary to expectations, the infiltration rate value was found to be the lowest in the pasture. For example, Fischer et al. (2015) stated that in the pasture, infiltration capacity increased due to water flow through macropores. However, our study showed the opposite result. Pasture samples have higher organic matter and penetration resistance and lower average IR, pore size, and clay and sand content than fallow and orchard samples (Table 1). Although the lowest clay and the highest organic matter content, the lowest average IR value was found in pasture samples. However, although clay content in the pasture is lower than in fallow and orchard samples, stickiness and plasticity were measured higher. Generally, these properties represent the clay fraction in soil, and their values increase as the clay ratio (Hardjowigeno 2016). Despite the low clay content, the effect of higher values of stickiness and plasticity may have resulted in a low measurement of IR in the pasture. This difference can be attributed to the different effects of clay content, stickiness, and plasticity, as stated in Karahan and Erşahin (2017). They reported that although soil stickiness and plasticity are tidily correlated with soil clay content, they may affect macropore flow in a different way to clay content, depending on the amount of 2:1-type active clays. This result also supports the effect of soil morphological features on water flow. Ferreira et al. (2015) studied hydrological properties including infiltration capacity in different land-use in central Portugal. They noted that infiltration

capacity increased with sand content in both surfacesoil (r = 0.228) and subsurfacesoil (r = 0.201) soil, but decreased with clay fractions (r = -0.140). Therefore, we also attributed the lowest IR finding in the pasture to soil compaction. We can say that soil compaction suppresses the low moisture (5.81, 8.04 %, 8.33 % in pasture, fallow, and orchard, respectively) and high organic matter content, therefore causing low IR. Zhao et al. (2013) found the soil infiltration rate for five grasslands greater than that of cropland, and they attributed the reduction in IR of cropland to the effect of soil compaction. According to our results, the lower IR in pasture than in fallow and orchard could be attributed to the effect of soil compaction caused by more vehicle and human traffic and grazing. Similarly, as reported in Sun et al. (2018) and Alaoui et al. (2011), Radke and Berry (1993) noted that soil compaction might decrease the infiltration rate of soils by affecting soil structure. Saputra et al. (2021) associated the clogging of the soil pores t with human activity above the soil surface.

On the other hand, besides the low clay and sand content, therefore the number of macropores is also low in the pasture. These macropores that are few may have been destructed by soil compaction. This might be because the pasture area is used as a promenade and for overgrazing purposes. It has been reported in previous studies that land use due to soil compaction has an effect on infiltration capacity. Haghnazari et al. (2015) studied the infiltration rates of agricultural soils and reported that the movement of heavy machines and excessive grazing reduced the infiltration rate. Soil compaction causes a decrease in soil macropores and an increase in soil dry bulk weight and penetration resistance, and thus has the effect of reducing the rate of water infiltration. Alaoui (2015) investigated the hydrological parameters of four representative grassland soils on the Swiss plateau and found that the interaction between bulk density and macroporosity could facilitate water infiltration. This interaction is related to the soil sand content. The average sand content value in the pasture soils was found to be lower (10.62) than the fallow (19.6) and orchard (17.2). Contrary to Alaoui (2015), low sand content may also have resulted in low IR value in the pasture. Colloff et al. (2010) expected that the infiltration rate would increase when the vegetation cover produces macropores by altering soil structure in the pasture. This result is in line with ours that the infiltration rate decreases due to the destruction of macropores by soil compaction. In this study, it confirms that the highest bulk density and penetration resistance values are measured in the pasture confirms this result (Table 1). Benevenute et al. (2020) evaluated PR as an indicator of soil compaction and noted that animal trampling increased soil compaction and soil degradation in pastures. As a matter of fact, measuring the average penetration resistance (PR) value in the pasture area (560 kPa) is about twice as much as in fallow (289 kPa) and orchard (172 kPa) can be considered an indicator of compaction. Keller and Dexter (2012) and Bayat et al. (2017) emphasized the impact of animal trampling, especially after rainy weather, and indicated that increasing the plasticity and susceptibility to compaction can significantly increase soil PR in wet soils.

In addition, Wu et al. (2016) reported that root systems abundant in grasslands improved the infiltration capacity of the soils. Huang et al. (2017) also emphasized the importance of roots in the infiltration process in relation to belowground biomass. However, the results of these studies are not in line with the result of the lowest IR, although the total plant roots are higher in the pasture. Because the fact that there are mostly small diameter roots (Table 1) that have the effect of increasing the water flow in the soil may be due to overgrazing in the studied pasture area, moreover, roots in pasture may have clogged the pores in soil due to the compaction effect, and thus they may have decreased the soil infiltration rate. Cui et al. (2019) reported that there is a relative effect of roots that is not fully understood with different diameters in the infiltration stages. Considering that small roots can also block the pores, this may explain the lower infiltration rate in the weak roots pasture samples compared to the other land-use. In fallow, sand content, bulk density, root quantity, and size were found to be the highest compared to other land-use soil samples (Table 1). The fact that the fallow area has higher IR values due to having a higher sand content than the pasture is consistent with the result of Mazaheri and Mahmoodabadi (2012) and Mousavi (2015). Santra et al. (2021) measured soil infiltration of 15 sites in Jaisalmer, India, and reported that higher sand content results in higher steady-state IR in contrast to the clay content. Higher IR measurement in the fallow compared to pasture samples was attributed to higher sand content and bulk density values and lower clay content. In addition, the lowest organic matter content was measured in fallow samples.

In the orchard, clay and water content and pore size were found to be the highest, and bulk density, organic matter, PR were found to be the lowest compared to other land-use (Table 1). According to these measurements, only the high clay content and the low bulk density complement each other.

Contrary to expectation, although low organic matter and PR, and high clay content were measured, the IR value was found to be the highest in orchard samples in all land-use. The contrasts determined in relation to IR and soil properties in the pasture were also seen in the orchard. Therefore, different factors, such as plant roots and soil living organisms that lead to higher IR in the orchard, were evaluated. Saputra et al. (2021) reported that a low bulk density might indicate more development of roots and water in the soil. Dwiratna and Suryadi (2017) found that high total porosity is inversely proportional to its bulk density. Fischer et al. (2014) noted that roots increase the organic matter content of soil and help to form soil pores, therefore changing the earthworms' burrowing activity and biomass and affecting the infiltration capacity of soils. However, since the trees in the orchard (including the trees of apples, pears, plums, cherries, sour cherries, apricots, and peaches) have deeper roots and there is no graze cover among these trees, roots were not found in soil samples. However, we can say that tree roots contribute to the water flow in the lower part of the soil. Ow and Ghosh (2017) noted that tree roots could also increase IR by facilitating water flow in subsoils where the soil is more compacted. In addition, we can say that IR increases due to the gaps created by the activities of soil creatures that live in orchards. Observed earthworms in the orchard have contributed in this way to the increase in infiltration. These findings are consistent with studies (Fischer et al., 2014; van Schaik et al., 2014) which reported that burrowing activity and biomass of earthworms effects IR. In addition, Zadeh (2015) reported that soil organisms could lead to loosening in the soil with their activities, thus facilitating infiltration.

In pasture soils, soil water content was found to be negatively associated with infiltration rate (Table 3). According to Alaoui (2015), the lower initial soil water content may increase the infiltration. The negative correlation between soil moisture content and IR in pasture soils seems to be in agreement with this study. In addition, previous studies reported that there were significant negative correlations between soil infiltration and saturated and initial moisture contents (Neumann ve Cardon, 2012; Lun ve Liang, 2014). In fallow soils, negative relations were found between IR and clay content and penetration resistance (Table 4). In general, increased clay content reduces the formation of macropores (Karahan and Erşahin, 2017), which results in the IR decreasing. In orchard soils, negative relations were found between IR and COLE and pore quantity (Table 5). COLE and PQ are properties that are positively related to the clay content. The total amount of pores is higher in clay soils and small pores slow down the infiltration rate. Therefore, the water holding capacity of clay soils can be very high, but their water transmission capacity is quite low. Since PQ will increase with the amount of clay, it is negatively related to IR. Patle et al. (2018) estimated the infiltration rate using texture fractions, and they reported that an increase in clay would decrease IR significantly.

Conclusion

In this study, IR of soils under different land-use (pasture, fallow, and orchard) within the boundaries of the Çubuk district of Ankara, Türkiye were compared. Correlation analysis was performed for the IR of the variables belonging to the soil samples and the direction and degree of their relations with each other. It was seen that in the pasture, sorptivity had an effect (r=0.72) on IR while K_s was seen to be effective in fallow (r=0.69) and orchard (r=0.86) soils. It was found that the average IR and average sorptivity values in all land-uses were different at the 5% significance level according to the ANOVA test results. In pasture samples, although the organic matter content is 3 times more (6.8%) than fallow (2.2%) and orchard (1.9%) and mean clay content is the lowest, the IR value was found to be the lowest. Similarly, in the orchard, the clay content and bulk density were found to the lower according to pasture and fallow, but the highest IR was measured in the orchard. These are contradictory results contrary to expectations. Therefore, these obtained results show that there can be more dominant factors than clay or organic matter content in pasture and orchard that affect the infiltration rate of soils. These results were attributed to soil compaction for the pasture. It can be said the reason for soil compaction is the use of the pasture area for grazing and recreation. The measured maximum PR value in the pasture area confirms this soil compaction.

Soil compaction due to machine, animal, and human traffics destroys macropores which are the soil's structural properties, and the possibility of roots clogging the pores that provide water transmission can be said to be the reason for low IR in the pasture. Since there are trees with deeper and larger roots such as apple, pear, plum, cherry, sour cherry, apricot, and peach in the orchard and there is no weed cover among these trees, roots were not found in soil samples. In addition, due to the soil surface being

cleaned during the sampling, it can be said that the litter consisting of tree leaves doesn't contribute to the organic matter content; therefore, it can be said that organic matter was measured as low. Therefore, we can attribute that it increases the IR due to the gaps created by the activities of soil creatures that live in orchards. Contrary to expectations, high IR in the orchard and low IR measurement in pasture show that land use rather than dominant soil characteristics may be more effective in the relationships between soil properties and infiltration rate. Obtained results in the study will provide useful information to researchers in modeling soil water relations and to farmers in making important application decisions. In addition, we used soil morphological variables as a different factor that affects IR, as well as using soil physical and chemical variables. The loading of morphological variables with high values on the factors shows that soil morphologic variables such as stickiness, plasticity, structure, pores, and roots are effective on IR. Therefore, especially an increase in studies that investigate the relationships between soil infiltration and structural properties under different field conditions will be beneficial in terms of obtaining more accurate results.

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Research Article

Effect of Potassium Sulphate Fertilizer Doses on Sugarcane Growth Yield and Quality Grown in Sudan

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Potassium sulphate

Abstract: Sugarcane is one of the most important crops in Sudan which played a leading role in the local and foreign trade. Research has been conducted in two sites in Sugarcane Research Center Farm in Guneid-Sudan from 5/7/2019 to 5/9/2020. The study aimed to evaluate the effect of potassium sulphate fertilizer doses on the growth, yield, and quality of sugarcane. The variety tested was Co 6806. The treatments consist of five doses of potassium sulphate (SOP) fertilizer which contains (50% K_2O and 18% S); K1: 0.0, K2: 40, K3: 80, K4: 120, and K5: 160 kg SOP kg ha^{-1} arranged in a randomized complete block design (RCBD) replicated three times. The results obtained showed that there was a significant effect on plant growth due to the application of different levels of potassium sulphate fertilizer in the two sites. Statistically, the treatment K3: (80 SOP kg ha^{-1}) significantly recorded the highest cane and sugar yield values compared to the other potassium sulphate levels and the control in the two different experimental sites.

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1. Introduction

Sugarcane (*Saccharum officinarum* L.) is a perennial grass belonging to the genus Saccharum, family Poaceae, and tribe Andropogoneae, according to Kolo et al. (2005). Approximately 79% of the world's sugar is produced from sugar cane, while sugar beet represents about 21% of global sugar production. All sugarcane schemes in Sudan lie in the central clay plain soils, which soils have a few limitations. Fertilization with the major elements of nitrogen, phosphorous and potassium is a necessary agricultural practice, and the cultivated plants need greatly. It also has direct effects on both vegetative growth and fruit growth and clearly affects the quantity and quality of fruits (Toprak, et al. 2021). The main characteristics of the central clay plain soils are high clay content, alkaline pH, low organic matter,

and poor in both N and P nutrients. Dawelbeit (2010) recorded a deficiency of potassium in some parts of the central clay plain soils in Sudan. Sugarcane, as a long-duration, labor-intensive crop, removes a significantly greater amount of plant nutrients from the soil. Jagtap et al. (2006) reported that sugarcane production of 100 tons per hectare removes 207 kg N, 30 kg P_2O_5, and 233 kg K_2O from the soil. Hence, it is essential to replenish the depleted soil with plant nutrients at the desired levels. Potassium (K) is commonly called potash, which is defined as K_2O. Potassium is the most absorbed nutrient by the sugarcane crop, and it plays various metabolic functions in plants, including photosynthesis, protein synthesis, the activation of several enzymes, and the functioning of the stomata (Hawkesford et al., 2012). The main sources of potassium were muriate of potash (MOP) and sulfate of potash (SOP). In tropical soils, usually with scarce K availability, fertilization with K nutrient should induce positive responses in sugarcane since K is the nutrient most extracted by agriculture (Korndorfer and Oliveira, 2005). Low levels of available K in the soil contribute to reduced sugarcane longevity. Therefore, it is considered an important element in restoring the productivity of sugarcane. There is a general understanding that the central clay plain soils in which all sugarcane schemes lie are rich in K and that there is no need to add potassium to the soil based on a few research studies. El-Tilib et al. (2004) reported that fertilization of cane fields in Sudan was geared towards using nitrogen and phosphorus to a small extent. Kwong and Pasricha (2002) reported that potassium is a relatively expensive nutrient, and deficiencies can often be corrected with a moderate rate of application, which can result in excellent economic returns. Many factors are responsible for the decline in sugarcane yield. Among these factors is the unbalanced nutrition of sugarcane with NPK fertilization. Therefore, the main objective of this study was to evaluate the effect of different levels of potassium sulfate fertilizer on the growth of sugarcane plants.

2. Material and Methods

From 5/7/2019 to 5/9/2020, the research was conducted at two sites in the Sugarcane Research Center Farm in the Guneid area (33o 19″ E, 14o 47″ N). The soil of the experimental site was classified as the Suleimi soil series, which is clayey Smectitic alluvium and clayey vertisol with moderate chemical fertility. The variety tested was Co 6806. Potassium sulphate (SOP) fertilizer, which contains (50% K_2O and 18% S), was distributed evenly to the experimental units using the broadcasting application method. The experimental design was a Randomized Complete Block Design (RCBD). The treatments included five levels of potassium sulphate fertilizer K1:0.0, K2:40, K3: 80, K4: 120, and K5: 160 kg ha^{-1} replicated three times. The time of application is 150 days after planting. The observation of growth parameters included:

1) Potassium concentration (mg/kg) from leaf sheath samples taken at 60, 90, 120, 270 and 360 days after planting. The samples were weighed and then oven dried and finely powered in a Willey Mill to pass through a 2 mm sieve, and potassium concentration were determined using the wet digestion method in which dilute acid (HN03: HCI04) was used with the help of a flame photometer (Tandon, 1998),

2) Plant height (cm), measured from soil surface to a first leaf joint,

3) Stalk diameters were measured from the middle of the sample stalk,

4) The number of millable stalks per hectare,

5) Cane yield and quality parameters (the sugar cane juice quality parameters, which include sucrose percent (pol %), purity percent of cane juice, and sugar yield tons per hectare (tons/ha), were determined from juice analyzed according to ICUMSA (2007) methods of analysis)

Analysis of variance (ANOVA) was used to compare the different treatments, and the least significant difference (LSD) at the 5% level of significance was used to separate the means. (USDA, NRCS. March 2007, USA).

3. Results

3.1. Effect of potassium sulphate fertilizer levels on plant cane growth and yield parameters

Potassium concentration from the plant cane leaf sheath was determined at 60, 90, 120, 270, and 420 days after planting. The experimental results data in Figure 1 showed that potassium concentration in the plant cane leaf sheath starts with high K concentration values at 60 days of age and then decreases

at 90 and 120 days after planting depending on the soil solution before the addition of different potassium sulphate fertilizer levels, which were added to the soil at 150 days from the planting date. Most of the potassium is absorbed in the canopy completion period or tillering, as confirmed to that by Medina et al. (2013), who found a higher concentration of potassium at the beginning of plant development and, over time, reaching a lower concentration in the adult plant at harvesting. After the addition of K_2SO_4 fertilizer levels at the exact time, K- concentration began to increase and then decrease with the increase of the crop age till it reached its lower K- concentration values at harvesting.

Regarding plant cane growth parameters, the experimental results from Table 1 showed that there was no significant difference in means between treatments due to the application of different potassium sulphate levels in all growth parameters; (plant height, number of tillers/$m^{2,}$ and number of internodes) in the two different experimental sites of the plant cane experiment.

Results in Table 2 showed that there was a significant difference between different potassium sulphate fertilizer levels in all cane yield parameters except for cane diameter. The treatment K3 recorded the highest cane length values (220.9 and 190.4 cm) in the two different experimental sites, respectively. The treatment K2 recorded cane length values of 195.7 and 185.9 cm in the two different experimental sites, respectively. The treatment K4 recorded cane length values of 187.0 and 177.9 cm in the two different experimental sites, respectively. The treatment K5 recorded cane length values of 192.6 and 170.5 cm in the two different experimental sites, respectively. The treatment K1: (the control) - recorded the lowest cane length values (185.2 and 183.1 cm) in the two different experimental sites, respectively. Experimental results data in Table 2 showed that there was a significant difference between different potassium sulphate levels in the number of millable stalks per hectare in the two different experimental sites. The treatment K3 recorded the highest number of millable stalks values (129.0 and 87.9), the treatment K2 recorded a number of millable stalks values of 109.0 and 84.5, the treatment K4 recorded a number of millable stalks values of 110.0 and 81.6), the treatment K5 recorded a number of millable stalks values of 115.0 and 82.4 in the two different experimental sites, respectively. The treatment K1 (the control) - recorded a number of millable stalks values of 114.0 and 78.4 in the two different experimental sites, respectively. Experimental results data in Table 1 also showed that there was a significant difference between different potassium sulphate levels on the cane yield in the two different experimental sites. The treatment K3 recorded the highest cane yield values of 95.2 and 99.5 tons of cane/ha, the treatment K2 recorded cane yield values of 76.6 and 84.7 tons of cane/ha, the treatment K4 recorded cane yield values of 80.4 and 89.4 tons of cane /ha, the treatment K5 recorded cane yield values of 84.1 and 89.5 tons of cane/ha in the two different experimental sites, respectively. In the two different experimental sites, the treatment K1 (the control) recorded cane yield values of 86.6 and 85.9 tons of cane/ha. Kadarwati (2020) concluded that 180 kg/ha of potassium in the form of K_2O increased sugarcane stalk diameter, weight, and yield. Ahmad et al., (2013) recommended 90 kg ha^{-1} to get a heavier stalk weight. The experimental results obtained from this study confirmed those of Kolln et al. (2013), who observed that increases in soil potassium content increased sugarcane productivity in Brazil. In addition, El-tilib et al. (2004) found that potassium application had a significant effect on plant density, stalk diameter, cane, and sugar yield. These results agree with Jafarnejadi (2013), Khan et al. (2005), and Kadarwati (2020), who found that optimum and balanced use of potassium fertilizers in different forms the improved cane yield and quality of different cultivars gave maximum economic benefit to the farmers.

3.2. Effect of potassium sulphate fertilizer levels on plant cane quality parameters

Experimental results data in Table 3 showed that there was a significant difference between different potassium sulphate levels on plant cane quality parameters, which include purity percent cane juice and sugar yield tons per hectare. In the two different experimental sites, there was no significant difference between different potassium sulfate levels on pol% and fiber% cane. Experimental data showed that there was a significant difference between different potassium sulphate levels in purity percentage of cane. In the two different experimental sites, the treatment K3 had the highest purity percent cane values (90.0 and 89.6%). In the two different experimental sites, treatments K4 and K5 recorded purity percent cane values (89.4 and 89.5%), while treatment K2 recorded purity percent cane values (87.7 and 88.4%). The treatment K1 (the control) recorded the lowest purity percent cane values of 84.6 and 86.6 in the two different experimental sites, respectively. Experimental results data in Table

3. showed that there was a significant difference between different potassium sulphate levels on sugar yield in the two different experimental sites. The treatment K3 recorded the highest sugar yield values (10.2 and 9.5 tons of sugar ha^{-1}) in the two different experimental sites, respectively. The treatment K4 recorded sugar yield values (9.6 and 9.4 tons of sugar ha^{-1}). The treatment K5 recorded sugar yield values (9.0 and 8.7 tons of sugar/ha), and the treatment K2 recorded sugar yield values (8.7 and 8.8 tons sugar ha^{-1}) in the two different experimental sites, respectively. The treatment K1 (the control)-recorded the lowest sugar yield values (8.4-and 8.3-tons of sugar ha^{-1}) in the two different experimental sites, respectively. According to the experimental results, potassium sulfate fertilizer application was effective in sugar formation and increased the percentage of sugar in the plant cane crop. The results obtained for cane quality characteristics are like those reported by Jafarnejadi (2013), who reported that applying potassium sulfate fertilizer at the appropriate dosage may improve cane quality and sugar yield. Also, the results of Phonde et al. (2005) confirmed that on crop quality, adequate potassium supply ensured a higher sugar yield. The results are also in agreement with that of Ng & Kwong (2002), who reported that the application of potassium sulfate fertilizer was the probable reason for the increase in sugar yield.

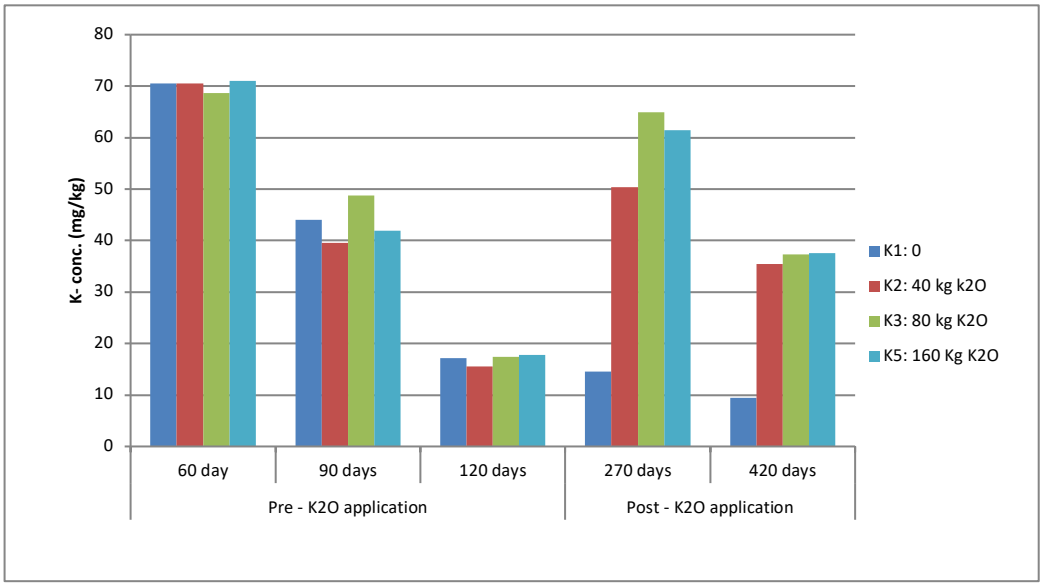

Figure 1. Potassium concentration (mg/kg) in plant cane leaf sheath.

Table 1. Effect of different potassium sulphate fertilizer levels* on plant cane growth parameters

Expt. Site	Treatments	Plant height (cm)			No of tillers/m^2			No of internodes		
		3 months age	6 months age	9 months age	3 months age	6 months age	9 months age	3 months Age	6 months Age	9 months Age
Site 1	K_1	44.0 a	66.8 a	172.8 a	10.5a	12.8a	15.5a	0.0 a	3.3a	11.0a
	K_2	39.0 a	67.5 a	175.3 a	10.3a	12.3a	14.8 a	0.0 a	3.0a	12.5a
	K_3	39.3 a	69.3 a	175.8 a	12.0 a	13.5 a	15.5 a	0.0a	3.5a	12.0a
	K_4	37.3 a	63.0 a	175.8 a	11.3 a	13.0 a	15.5 a	0.0a	2.8a	12.8a
	K_5	35.0 a	66.0 a	168.3 a	11.8a	13.5a	15.8 a	0.0a	3.0a	11.0a
	Mean	38.9	66.5	173.6	11.2	13.0	15.4	0.0	3.1	12.2
	CV%	17.00	13.4	2.84	12.1	10.20	4.78	0.0	4.3	10.6
	LSD (0.05)	10.2	13.7	7.6	2.1	2.0	1.1	0.0	1.4	2.0
Site 2	K1	42.3a	71.8a	179.3a	9.5a	13.0a	15.5a	0.0a	3.8a	9.8a
	K2	42.8a	73.3a	184.0a	9.3a	12.0a	15.5a	0.0a	3.5a	9.8a
	K3	47.5a	74.8a	185.3a	10.3a	12.8a	15.3 a	0.0a	3.8a	10.0a
	K4	43.0a	68.5a	184.3a	9.8a	12.3a	15.3a	0.0a	3.5a	10.3a
	K5	38.0a	71.5a	180.0a	9.0a	12.0a	15.3a	0.0a	3.5a	10.5a
	Mean	42.7	72.0	182.0	9.6	12.4	15.3	0.0	3.6	10.7
	CV%	12.3	12.7	2.6	11.4	7.5	5.3	0.0	2.8	11.3
	LSD (0.05)	8.1	14.1	7.2	1.7	1.4	1.2	0.0	1.2	1.9

*Five levels of potassium sulphate fertilizer K1:0.0, K2:40, K3: 80, K4: 120 and K5: 160 kg ha^{-1}.

Table 2. Effect of different potassium sulphate fertilizer levels* on plant cane yield parameters

Treatments	Cane length (cm)		Cane diameter (cm)		Number of millable stalks\hectares		Cane yield (Tons per hectare)	
	Site1	Site2	Site1	Site2	Site1	Site2	Site1	Site2
K_1	185.2^{b}	183.1^{ab}	2.0^{a}	2.0^{a}	114.0^{b}	78.4^{b}	86.6^{ab}	85.9^{b}
K_2	195.7^{b}	185.9^{ab}	2.1^{a}	2.0^{a}	109.0^{b}	84.5^{ab}	76.6^{ab}	88.7^{ab}
K_3	220.9^{a}	190.4^{a}	2.1^{a}	2.1^{a}	129.0^{a}	87.9^{a}	95.2^{a}	99.5^{a}
K_4	187.0^{b}	177.9^{bc}	2.0^{a}	2.1^{a}	110.0^{b}	81.6^{ab}	80.4^{bc}	89.4^{ab}
K_5	192.6^{b}	170.5^{c}	2.0^{a}	2.0^{a}	115.0^{b}	82.4^{ab}	84.1^{bc}	89.5^{ab}
Mean	184.4	181.6	2.1	2.0	115.0	83.0	84.0	92.6
CV%	8.5	6.3	3.3	5.1	8.4	12.1	8.5	12.8
LSD(P<0.05)	18.7	12.2	0.2	0.2	12	9.3	8.8	12.6

*Five levels of potassium sulphate fertilizer K1:0.0, K2:40, K3: 80, K4: 120 and K5: 160 kg ha^{-1}.

Table 3. Effect of different potassium sulphate levels* on plant cane quality

Treatments	Pol % cane Juice		Purity % cane Juice		Fiber % cane		Sugar yield Tons per hectare	
	Site1	Site2	Site1	Site2	Site1	Site2	Site1	Site2
K_1	12.8^{a}	11.6^{a}	84.6^{d}	86.7^{b}	18.6^{a}	18.5^{a}	8.4^{c}	8.3^{b}
K_2	12.6^{a}	11.8^{a}	87.7^{cd}	88.4^{a}	18.4^{a}	18.5^{a}	8.7^{c}	8.8^{ab}
K_3	12.7^{a}	12.1^{a}	90.0^{a}	89.6^{a}	18.9^{a}	18.6^{a}	10.2^{a}	9.5^{a}
K_4	12.8^{a}	11.7^{a}	89.4^{ab}	89.5^{a}	18.1^{a}	18.3^{a}	9.6^{b}	9.4^{a}
K_5	12.7^{a}	11.7^{a}	88.9^{bc}	88.4^{ab}	18.4^{a}	18.2^{a}	9.0^{bc}	8.7^{ab}
Mean	12.7	11.7	88.7	88.6	18.4	18.4	9.2	8.9
CV%	3.9	3.4	1.6	2.0	4.2	4.6	10.8	12.4
LSD (P< 0.05)	0.6	0.8	0.5	1.8	0.8	0.9	1.1	1.2

*Five levels of potassium sulphate fertilizer K1:0.0, K2:40, K3: 80, K4: 120 and K5: 160 kg ha^{-1}.

Conclusion

For the cultivation of sugarcane in Guneid-Sudan, application of different levels of potassium sulphate fertilizer resulted in a significant effect on plant growth, and the treatment K3 (80 SOP kg ha-1) recorded the highest cane and sugarcane value in terms of yield and yield quality.

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Research Article

Comparison of Changing Cultivation Pattern on Morphological and Biochemical Characteristics of Forage of Two Types of Crop Legumes in The Tropical Climate of Southern Kerman Province

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Abstract: Reduction of the quantity and quality of forage is one of the main restrictions on the productivity of livestock systems. Tropical legumes are the most important crops to improve livestock feeds and, thus, for providing livestock products for human consumption in arid regions. In order to investigate the shift of cultivation date of two legumes from summer to spring in arid weather conditions, a factorial experiment in a randomized complete block design with three replication was conducted at the Agricultural Research Institute of south Kerman, Iran, during two cropping seasons. Treatment was planting in three and two tropical legumes (Tapary bean and cowpea). The results showed that changing planting dates led to a significant effect on seed yield and forage quality of two legumes in the region. All agronomic traits for cowpea increased compared to Tepary bean due to differences in their genetic backgrounds. The two legumes were not different in terms of nitrogen, crude protein, and ash. On all three planting dates, the hemicellulose-free cell wall of cowpea was higher than Tepary. In contrast, neutral detergent fiber for Tepary was observed more than cowpea. The highest dry matter index was recorded for cowpea. Whereas the highest dry matter digestibility, the net energy of lactation, and metabolizable energy were related for Tepary. According to the different physiological and phenological responses of the two legumes, it is necessary to examine the selection of suitable planting dates for improving the quantitative and qualitative yield of forage.

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1. Introduction

Human nutrition is the most important challenge of the future. FAO estimated that the population will increase to nine billion by 2050. Hence, to satisfy the demand for population growth,

food production will have to increase by 60% by 2050 (FAO, 2010). On the other hand, there are growing concerns about the impact of climate change on agricultural production, especially livestock. Climate change results in increased global warming, which changes rainfall patterns in different regions. So that, in some areas, there will be floods, and in others, there will be droughts (Cevik, 2021). Consequently, general strategies for facing climate change are adaption to environmental conditions, such as shifting cropping patterns (Schultze-kraft et al., 2018). Demand for livestock products is expected to increase significantly in the future, especially in the south, east, and Southeast Asia, and with smaller distribution in sub-Saharan Africa because of the increased global standard of living (Robinson and Pozzi, 2011). Hence, livestock products play an important role in human nutrition (Mottet et al., 2017). Livestock production in tropical areas, especially when based on pasture use, will lead to an irreversible impact on the environment (Schultze-Kraft et al., 2018). A proper option to improve rangeland productivity, reduce production costs, and sustainability is to introduce legumes that aid in diversifying the forage system and reduce the risk of pests and diseases and rangeland destruction (Lista et al., 2019).

Forage-based livestock production plays a crucial role in the affordable supply of nutrient-rich foods for humans (Baath et al., 2020). Reducing the quantity and quality of livestock feed, especially in arid regions, increased the cost of meat and dairy production (Paul et al., 2020). On the other hand, rising temperatures due to climate change lead to a decrease in the nutritional value of forage and emissions of methane from ruminants (Lee et al., 2017). Tropical legumes are considered because of their benefits, including a positive effect on ecosystem conservation and sustainable livestock production in tropical regions (Nouri et al., 2020. a). Among the benefits of these plants is nitrogen fixation (15 to 158 kg $N.ha^{-1}$ per year) (Thomas, 1995), high nutrition value, deep root system (improving soil mineral cycle and soil compaction, increasing water productivity), extensive genetic diversity (approximately 20000 species) and the existence of secondary metabolites. Therefore, forage legumes have a high potential to address environmental concerns and food security (Schultze-kraft et al., 2018).

Livestock is an important national resource in Iran (Kazemzadeh et al., 2008). Whereas shortage of forage is one of the main problems for livestock in Iran (Rad et al., 2020). On the other hand, climate change led to drought in Iran. So, water reserves in many parts of this country are exposed to serious threats due to inefficient exploitation, and the continuation of this trend led to irreversible economic and environmental consequences in the region (Nouri et al., 2020. b). In order to achieve goals of sustainable development of agricultural products, proper design of planting patterns is essential to achieve maximum productivity and increase income. So, crop production could benefit from changing plantation patterns and crop rotation (Zabel et al., 2014).

The current study was carried out by using long-term data of Meteorology and drawing an Ambrothermic diagram of the Jiroft region. The objective of this paper is to evaluate the effect of changing growth seasons from summer to spring on the agronomic, biochemical, and nutritional characteristics of two tropical legumes, with the aim of designing a new plantation model appropriate to the policy and goals of each region.

2. Material and Methods

A factorial experiment was carried out randomized complete block design with three replications in Jiroft, Iran, during the 2018 and 2019 crop seasons. This region has a longitude of 56° 45' to 58° 31' E and latitude of 28° 10' to 29° 20' N and is located at 630 meters above sea level. Treatments were planting in three different dates (PD1= Jan-30, PD2= Feb-8, and PD3= Feb-18) and two legumes (Tepary bean (*Phaseolus acutifolis*) and cowpea (*Vigna unguiculata*)). The meteorological data of the region during the years of the experiment are presented in Table 1. Before planting, the physiological zero of the two legumes was determined, the Ambrothermic diagram was drawn using long-term meteorological data of region, and proper planting date was conducted with Ambrothermic diagram and cumulative of growth-day-degree (GDD) for two legumes.

Table 1. Monthly temperature and precipitation during the growing season in 2018-2019

2018	Temperature (ºC)Min.	Max.	Humidity (%)Min.	Max.	Total precipitation (mm)	Total sunny hours
January	6.8	23	26	74	2.4	212.1
February	12.5	27.5	23	82	10.2	228.3
March	16.1	34.1	17	65	2.3	259.3
April	20.4	37.5	14	45	1.3	269.5
May	26.1	44.2	12	43	0	283.8
June	27.2	45.6	9	45	0	329.1
Sum.	109.1	211.9	101	354	16.2	1582.1
Average	18.1	35.3	16.8	59	2.7	263.6
2019	**Temperature Min. (ºC)**	**Max.**	**Humidity (%)Min.**	**Max.**	**Total precipitation (mm)**	**Total sunny hours**
January	7	20.9	33	85	72.1	190.5
February	9.1	22.9	31	87	49.7	235.8
March	15.3	28.5	32	85	52	173.3
April	18.2	36.3	15	65	5.6	248.5
May	22.8	42.8	9	49	1.6	312.1
June	26.8	45.7	12	60	0	301.4
Sum.	99.2	197.1	132	431	181	1461.6
Average	16.5	32.8	22	71.8	30.1	243.6

After deep tillage and disk leveler, seeds were planted on the ridge. The length of each ridge in every plot was 6 m, and the distance between ridges was 50 cm. Based on soil results (Table 2), triple super phosphate fertilizer was applied at the rate of 150 $kg.ha^{-1}$, a quarter of nitrogen as a starter at the planting time, and zinc and manganese sulfate fertilizers were distributed in plots at the rate of 50 $kg\ ha^{-1}$ at the planting date.

Table 2. Physical and chemical properties of the soil

Depth of sampling (cm)	A.V.K (ppm)	A.V.P (ppm)	N (%)	EC ($ds.m^{-1}$)	pH	Soil texture
0-25	78.5	20.2	0.039	0.46	7.8	Loam-sandy
25-50	131.2	12.2	0.012	0.47	7.5	Loam-sandy

A.V.K: Available potassium, A.V.P: Available phosphorus, N: Nitrogen.

Morphological and grain-related traits such as plant height, branch number, pod number, pod length, number of grains per pod, 1000-grain weight, grain yield, straw yield, and harvest index were studied after harvesting.

Plant's samples were cut in the field and instantly transferred to the laboratory were dried in an oven at 75˚C for 24h. The dried samples were then grounded and passed through a 2-mm sieve, and biochemical traits were then assessed. The studied traits included nitrogen, crude protein (CP), ash (Hollman et al., 2013), cell wall-hemicellulose free (ADF), and neutral detergent fiber (NDF) (Asp et al., 1992). Then, traits related to livestock nutrition such as dry matter intake (DMI), digestible dry matter (DDM), the net energy of lactation (NE_L), metabolizable energy (ME), and relative feed value (RFV) were obtained using the following formulas (Lithourgidis et al., 2006).

$$DMI=120/\ \%NDF\ dry\ matter\ basis \quad (1)$$

$$DDM= 88.9 – (0.779\ *\%ADF\ dry\ matter\ basis) \quad (2)$$

$$NE_L=[1.044-(0.0119*\%ADF)]*2.205 \quad (3)$$

$$ME\ (Mj.kg^{-1}) = 0.17\ \%DDM – 2 \quad (4)$$

$$RFV= \%DDM*\%DMI*0.775 \quad (5)$$

All parameters were analyzed using the analysis of variance (ANOVA). Several comparisons have been performed on partial data sets by applying Duncan's test at the probability level of $p<0.05$. All statistical analyses were carried out in SAS software (9.3).

3. Results

3.1. Agronomic traits

The analysis of the variance of agronomical traits (Table 3) indicated that the experimental years have a significant effect on all agronomical traits, except grain yield and harvest index, due to significant differences in rainfall in 2019 compared to 2018 (Table 1). The analysis of variance (Table 3) indicated that there were significant differences between planting dates in terms of pod length, number of grains per pod, and grain yield. Since the two legumes were morphologically different from each other, all their agronomic traits showed significant differences. There was a significant interaction between the planting dates and legumes for branch number and 1000-grain weight.

Figures 1 and 2 represent mean comparison for morphological and yield-related traits regarding panting date and two legumes. The highest pod length was observed on PD1and the lowest was on Feb-30. The highest number of grains per pod was recorded on PD1and the lowest was on PD2. However, no difference between planting dates on PD2 and two planting dates was observed for number of grains per pod. The planting dates of PD1and PD2 had the highest and lowest grain yield, respectively. The Grain yield showed no difference on PD3and two planting dates.

Based on Table 6, Cowpea obtained the highest branch number on PD3. Although the Tepary bean showed the lowest number branch on PD2, its difference with PD3 was not significant. Tepary bean obtained the highest number branch on PD1. The highest pod number per plant was observed on the planting date of Tepary bean on PD1, while its lowest pod number, no difference was detectable among the February planting dates. Planting of cowpea on PD1 and PD3 was a higher pod number than on PD2. The highest 1000-grain weight of cowpea was related to planting on PD2, whereas planting on PD1 and PD3 decreased its 1000-grain weight. Conversely, the highest 1000-grain weight of Tepary bean was obtained on PD1, and the late planting date led to a decrease 1000-grain weight of this legume.

Table 3. Analysis of variance of morphological traits and yield of legume plants as affected in different planting date

S.O.V	d.f	Mean square								
		P.H	Branch No.	Pod No.	P.L	grain No.	T.G.W	grain.Y	Straw. Y	HI
Year	1	900^{**}	30.2^{**}	87^{**}	70.8^{**}	36^{**}	4053.4^{**}	2^{ns}	15^{*}	0.00001^{ns}
r	2	31.5^{ns}	3.6^{ns}	0.08^{ns}	0.006^{ns}	0.4^{ns}	68.8^{ns}	0.7^{ns}	1.6^{ns}	26.2^{ns}
r (Year)	2	0.5	0.08	0.1	0.001	0.0001	13	0.005	0.009	0.0004^{ns}
P.D	2	3.5^{ns}	0.7^{ns}	82.3^{**}	5.6^{**}	2.1^{*}	233.3^{ns}	3^{**}	8.4^{ns}	31.5^{ns}
P.D (year)	2	0.08^{ns}	0.0001^{ns}	0.4^{ns}	0.09^{ns}	0.0002^{ns}	2.5^{ns}	0.01^{ns}	0.05^{ns}	0.00003^{ns}
Leg	2	277.7^{*}	12.2^{*}	113.7^{**}	269.5^{**}	28.4^{**}	8487.5^{**}	55.8^{**}	78.8^{**}	948.4^{**}
Leg (year)	1	1.7^{ns}	0.2^{ns}	2.7^{ns}	3.8^{**}	0.0002^{ns}	711^{**}	0.3^{ns}	0.4^{ns}	0.0007^{ns}
P.d×Leg	2	13.3^{ns}	7^{**}	59^{**}	0.09^{ns}	0.2^{ns}	350^{**}	1.2^{ns}	4.8^{ns}	10^{ns}
year×P.d×Leg	2	0.8^{ns}	0.0001^{ns}	0.7^{ns}	0.0008^{ns}	0.0001^{ns}	1^{ns}	0.007^{ns}	0.03^{ns}	0.00005^{ns}

ns = non-significant difference*and**: Significant at 5% and 1% probability level, respectively (r: replication, P.d: planting date, Leg: legumes, P.H: plant height, branch No.: branch number, pod No.: pod number, P.L: pod length, grain. No.:grain number per pod, T.G.W: Thousand grain weight, G.Y: grain yield,. Y: straw yield, HI: harvest index).

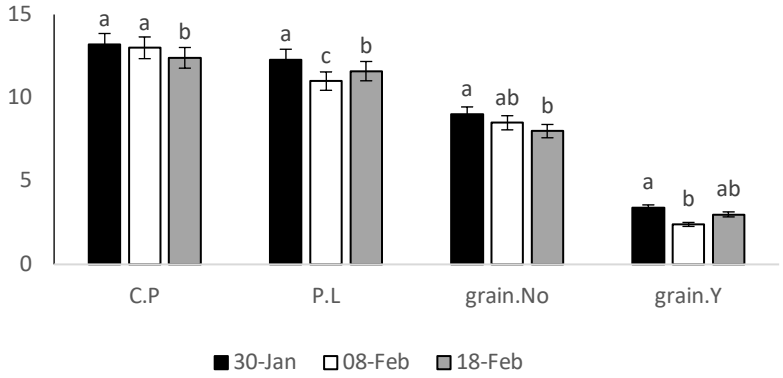

Figure 1. Mean comparison of planting date studied traits.

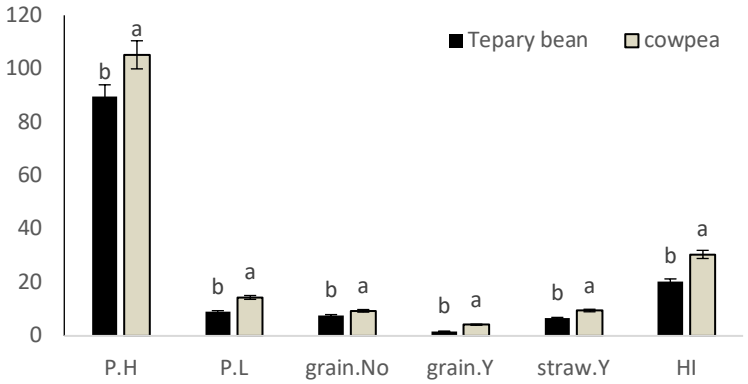

Figure 2. Mean comparison of legumes for studied traits.

3.2. Biochemical characteristics

Based on the analysis of variance, because of superior weather conditions in 2019 compared to 2018, all traits related to the foraging quality of legumes in the second year significantly increased (p<0.01) compared to the first year. The effect of planting date, except crude protein, on other traits was not statistically different. The interaction between treatments was significant for all traits except crude protein and ash (Table 4).

A mean comparison of some biochemical traits related to the effect of year on the quality in two legumes is presented in Table 7. The amount of nitrogen, crude protein, ash, ADF, and NDF of legumes increased slightly in the second year compared to the first year. According to the results (Table 6), the interaction between planting date and legume showed that the amount of nitrogen in cowpea, and Tepary bean under different dates was slightly different from each other. So, planting on PD3 decreased the nitrogen content of the two legumes. In all three planting dates, ADF of the Tepary bean was lower than cowpea. The lowest amount of ADF in Tepary bean was observed in planting on PD1, and late planting led to increasing ADF of this legume. In contrast, ADF levels of cowpea were slightly different under different planting dates. NDF content in Tepary bean was higher than cowpea on all three planting dates. The highest amount of NDF in two legumes was recorded on PD1, and the late planting was caused by their NDF.

Table 4. Analysis of variance of biochemical traits of legume plants as affected in different

S.O.V	d.f	N	C.P	Ash	ADF	NDF	DMI	DDM	NE_L	ME	RFV
year	1	0.03^{**}	2.8^{**}	39.2^{**}	101.6^{**}	14.8^{**}	0.3^{**}	61.3^{**}	0.09^{**}	1.7^{**}	1487.3^{**}
r	2	0.2^{**}	10.7^{**}	41.8^{**}	402^{**}	58.7^{**}	1.2^{**}	245.2^{**}	0.2^{**}	7^{**}	5862.4^{**}
r(year)	2	0.0002	0.005	7.6	0.2	0.01	0.003	0.1	0.0008	0.003	2.8
P.d	2	0.03^{**}	2^{**}	0.4^{ns}	46.2^{**}	1043.7^{**}	23.2^{**}	28.4^{**}	0.03^{**}	0.8^{**}	7376.8^{**}
P.d(year)	2	0.008^{*}	0.1^{ns}	1.6^{ns}	3.7^{ns}	2.2^{ns}	0.001^{ns}	2.2^{ns}	0.0001^{ns}	0.05^{ns}	4.8^{ns}
Leg	2	0.006^{ns}	0.1^{ns}	0.1^{ns}	246^{**}	338^{**}	15.8^{**}	148.8^{**}	0.1^{**}	4.4^{**}	22.2^{**}
Leg(year)	1	0.0002^{ns}	0.08^{ns}	0.5^{ns}	3.4^{ns}	0.04^{ns}	0.02^{*}	2^{ns}	0.001^{ns}	0.07^{ns}	102.6^{**}
P.d×Leg	2	0.01^{*}	0.2^{ns}	0.01^{ns}	90^{**}	47.3^{**}	5^{**}	51.6^{**}	0.07^{**}	1.5^{**}	2384.5^{**}
year×P.d×Leg	2	0.0002^{ns}	0.01^{ns}	2.2^{ns}	0.7^{ns}	0.1^{ns}	0.002^{ns}	0.4^{ns}	0.001^{ns}	0.01^{ns}	4.5^{ns}
CV (%)	-	2.3	2.4	0.9	2	4.7	1.3	5	24	9	4.2

ns = non-significant difference*and** significant at 5% and 1% probability level, respectively (r: replication, P.d: planting date, Leg: legumes, N: nitrogen percentage, C.P: crude protein, ADF: Cell wall-hemicellulose free, NDF: Neutral Detergent fiber, DMI: Dry Matter Index, DDM: Digestible Dry Matter, NEL: Net Energy of Lactation, ME: Metabolisable Energy, RFV: Relative feed value).

3.3. Quality characteristics of products

According to analysis variance (Table 5), experimental years and interaction between treatments showed significant effects on all traits related to nutrition livestock. Considering the inverse ratio of dry matter intake, digestible dry matter, the net energy of lactation, metabolizable energy, and relative feed value with ADF, these traits increased in 2018 compared to 2019 (Table 7). Based on Table 6, in two legumes, the amount of DMI increased with late planting. The highest DMI was obtained in the planting of cowpea on Feb. 18, while its lowest content was recorded for Tepary bean on PD1. Planting of Tepary bean on PD1 showed the highest mean value for digestible dry matter. Cowpea showed maximum DDM on Jan-8. However, the digestible dry matter of cowpea showed little difference among the three planting dates.

Based on a meaningful comparison of interaction between treatments (Table 6), the planting date of Tepary bean on PD1 had the highest net energy of lactation, while late planting reduced NE_L for this legume. Conversely, with Tepary bean, the planting date in PD1 had the lowest NE_L for cowpea and its planting in February led to NE_L. Among compared two legumes, the Tepary bean obtained the highest mean value for metabolizable energy but late in planting decreased its ME. Planting of cowpea and Tepary bean on PD3 had the highest relative feed value.

Table 5. Analysis of variance of biochemical traits of legume plants as affected in different planting date

S.O.V	d.f	N	C.P	Ash	ADF	NDF	DMI	DDM	NE_L	ME	RFV
year	1	0.03^{**}	2.8^{**}	39.2^{**}	101.6^{**}	14.8^{**}	0.3^{**}	61.3^{**}	0.09^{**}	1.7^{**}	1487.3^{**}
r	2	0.2^{**}	10.7^{**}	41.8^{**}	402^{**}	58.7^{**}	1.2^{**}	245.2^{**}	0.2^{**}	7^{**}	5862.4^{**}
r(year)	2	0.0002	0.005	7.6	0.2	0.01	0.003	0.1	0.0008	0.003	2.8
P.d	2	0.03^{**}	2^{**}	0.4^{ns}	46.2^{**}	1043.7^{**}	23.2^{**}	28.4^{**}	0.03^{**}	0.8^{**}	7376.8^{**}
P.d(year)	2	0.008^{*}	0.1^{ns}	1.6^{ns}	3.7^{ns}	2.2^{ns}	0.001^{ns}	2.2^{ns}	0.0001^{ns}	0.05^{ns}	4.8^{ns}
Leg	2	0.006^{ns}	0.1^{ns}	0.1^{ns}	246^{**}	338^{**}	15.8^{**}	148.8^{**}	0.1^{**}	4.4^{**}	22.2^{**}
Leg(year)	1	0.0002^{ns}	0.08^{ns}	0.5^{ns}	3.4^{ns}	0.04^{ns}	0.02^{*}	2^{ns}	0.001^{ns}	0.07^{ns}	102.6^{**}
P.d×Leg	2	0.01^{*}	0.2^{ns}	0.01^{ns}	90^{**}	47.3^{**}	5^{**}	51.6^{**}	0.07^{**}	1.5^{**}	2384.5^{**}
year×P.d×Leg	2	0.0002^{ns}	0.01^{ns}	2.2^{ns}	0.7^{ns}	0.1^{ns}	0.002^{ns}	0.4^{ns}	0.001^{ns}	0.01^{ns}	4.5^{ns}
CV (%)	-	2.3	2.4	0.9	2	4.7	1.3	5	24	9	4.2

ns = non-significant difference*and** significant at 5% and 1% probability level, respectively (r: replication, P.d: planting date, Leg: legumes, N: nitrogen percentage, C.P: crude protein, ADF: Cell wall-hemicellulose free, NDF: Neutral Detergent fiber, DMI: Dry Matter Index, DDM: Digestible Dry Matter, NEL: Net Energy of Lactation, ME: Metabolisable Energy, RFV: Relative feed value).

Table 6. Mean comparison of interaction of legumes and planting date for studied traits

Characters	30-Jan		8-Feb		18-Feb	
	T	C	T	C	T	C
No. branch	7±0.6	6.6±0.4	5.8±0.4	7.3±0.6	5.8±0.4	8.3±1
No. pod	21.8±2	14.5±0.6	15.3±1	10.6±0.7	15.6±1.2	17±1.2
T.G.W (gr)	65±3.8	158±7.7	60.3±2.7	169.6±9.2	61.8±3	150.6±6.7
N (%)	2.05±0.07	2.15±0.07	2.1±0.05	2.08±0.06	2±0.03	2±0.03
ADF (%)	71.7±2.7	82.5±3.2	81±2.1	81±2.2	77.3±1.4	82.06±1.5
NDF (%)	40.5±1.5	37.4±1.4	26±0.6	21.2±0.5	27.4±0.5	16.8±0.3
DMI (%)	3±0.1	3.2±0.1	4.6±0.1	5.6±0.1	4.3±0.08	7±0.1
DDM (%)	33±2	24.6±2.5	25.8±1.6	25.7±1.7	28.6±1	25±1.2
NE_L ($Mcal.kg^{-1}$)	0.43+0.06	0.13±0.08	0.18±0.06	0.18±0.06	0.25±0.04	0.15±0.04
ME ($Mj.kg^{-1}$)	3.6±0.3	2±0.4	2.4±0.2	2.3±0.3	2.8±0.1	2.2±0.2
RFV (%)	77.2±7.8	62.8±8.8	93.8±8.4	113.8±10.8	97.6±5.8	139±3.5

Mean values ±ES (T: Tepary bean, C: cowpea, No. branch: number of branches, No. pod: number of pod, T.G.W: Thousand grain weight, N: nitrogen percentage, ADF: ADF: Cell wall-hemicellulose free, NDF: Neutral Detergent fiber, DMI: Dry Matter Index, DDM: Digestible Dry Matter, NEL: Net Energy of Lactation, ME: Metabolisable Energy, RFV: Relative feed value).

3.4. Correlation results

Evaluation of relationships related to measured traits was represented in Table 8. There were differences in values of the correlations between measured traits, but in some cases, the sign of the correlation was also changed. The correlation of crude protein with nitrogen was positive. Ash content showed positive correlations with nitrogen and crude protein. Correlations of NDF with ADF and DDM with nitrogen, crude protein, and ash were negative. Similarly, the correlation of digestible dry matter with nitrogen, crude protein, and ash was negative and its correlations with DMI were positive. The ME showed negative correlations with nitrogen and ADF, while, its correlation with plant height was positive. Also, the number branchesand pods length showed a positive correlation with RFV. NE_L showed negative correlations with nitrogen, crude protein and ash, and positive correlations with DMI and DDM. Plant height had positive correlations with the number branchesand number pods. 1000-grain weight showed positive relationships with plant height and number pod. Correlation of grain yield with number branch was also positive. In addition, the harvest index showed a correlation positive with the number branches and grain yield.

Table 7. Mean comparison of year for studied traits

Year	P.H (cm)	Branch No.	Pod No.	P.L (mm)	Grain No.	T.G.W (gr)	G.Y. (ton ha^{-1})	N (%)	C.P (%)	Ash (%)	ADF (%)	NDF (%)	DMI (%)	DDM (%)	NE_L (Mcal kg^{-1})	ME (Mj kg^{-1})	RFV (%)
2018	62.6^b	6^b	14.2^b	10.2^b	7.5^b	100.3^b	2.7^b	2.03^b	12.6^b	96.2^b	77.6^b	27.5^b	4.7^a	28.4^a	0.2^a	2.8^a	103.8a
2019	72.6^a	7.7^a	17.3^a	13.0^a	9.5^a	121.5^a	3.2^a	2.09^a	13.1^a	98.3^a	81.0^a	28.8^a	4.5^b	25.8^b	0.1^b	2.3^b	91^b

Table 8. Correlation between studied traits

	N	C.P	Ash	ADF	NDF	DMI	DDM	NE_L	ME	RFV	P.H	Branch No.	Pod No.	P.L	No. seed	T.G.W	Grain. Y	Straw. Y	HI
N	1																		
C.P	0.8^{**}	1																	
Ash	0.9^{**}	0.9^{**}	1																
ADF	0.5^{ns}	0.2^{ns}	0.2^{ns}	1															
NDF	-0.5^{ns}	-0.2^{ns}	-0.1^{ns}	-0.9^{**}	1														
DMI	-0.9^{**}	-0.9^{**}	-0.9^{**}	-0.2^{ns}	0.1^{ns}	1													
DDM	-0.6^{*}	-0.8^{**}	-0.5^{**}	0.1^{ns}	0.05^{ns}	0.8^{**}	1												
NE_L	-0.9^{**}	-0.9^{**}	-0.9^{**}	-0.2^{ns}	0.1^{ns}	0.9^{**}	0.8^{**}	1											
ME	-0.8^{**}	-0.5^{ns}	-0.5^{ns}	-0.8^{**}	0.9^{**}	0.5^{ns}	0.3^{ns}	0.5^{ns}	1										
RFV	0.2^{ns}	0.4^{ns}	0.2^{ns}	-0.1^{ns}	0.02^{ns}	-0.2^{ns}	-0.2^{ns}	-0.2^{ns}	-0.1^{ns}	1									
P.H	0.1^{ns}	0.4^{ns}	0.3^{ns}	-0.2^{ns}	0.2^{ns}	-0.3^{ns}	-0.4^{ns}	-0.3^{ns}	0.01^{ns}	0.9^{**}	1								
Branch No.	0.3^{ns}	0.5^{ns}	0.3^{ns}	0.1^{ns}	-0.2^{ns}	-0.3^{ns}	-0.3^{ns}	-0.3^{ns}	-0.3^{ns}	0.8^{**}	0.7^{*}	1							
Pod No.	0.0008^{ns}	0.2^{ns}	0.2^{ns}	-0.09^{ns}	0.1^{ns}	-0.2^{ns}	-0.4^{ns}	-0.2^{ns}	0.006^{ns}	0.4^{ns}	0.6^{*}	0.4^{ns}	1						
P.L	0.5^{ns}	0.6^{ns}	0.4^{ns}	0.2^{ns}	-0.3^{ns}	-0.4^{ns}	-0.2^{ns}	-0.4^{ns}	-0.5^{ns}	0.8^{**}	0.7^{*}	0.9^{**}	0.3^{ns}	1					
Grain No.	0.4^{ns}	0.4^{ns}	0.3^{ns}	0.03^{ns}	-0.1^{ns}	-0.3^{ns}	-0.1^{ns}	-0.3^{ns}	-0.3^{ns}	0.8^{**}	0.6^{*}	0.6^{*}	0.06^{ns}	0.7^{*}	1				
T.G.W	0.2^{ns}	0.3^{ns}	0.3^{ns}	0.09^{ns}	-0.07^{ns}	-0.3^{ns}	-0.4^{ns}	-0.4^{ns}	-0.2^{ns}	0.5^{ns}	0.7^{*}	0.5^{ns}	0.9^{**}	0.5^{ns}	0.3^{ns}	1			
grain Y.	0.04^{ns}	0.1^{ns}	0.1^{ns}	-0.07^{ns}	0.07^{ns}	-0.1^{ns}	0.005^{ns}	-0.1^{ns}	-0.02^{ns}	0.5^{ns}	0.5^{ns}	0.6^{*}	0.4^{ns}	0.5^{ns}	0.5^{ns}	0.5^{ns}	1		
Straw Y.	0.3^{ns}	0.3^{ns}	0.3^{ns}	0.2^{ns}	-0.2^{ns}	-0.3^{ns}	-0.5^{ns}	-0.3^{ns}	-0.4^{ns}	0.07^{ns}	0.2^{ns}	-0.01^{ns}	0.4^{ns}	0.1^{ns}	-0.07^{ns}	0.5^{ns}	-0.3^{ns}	1	
HI	0.04^{ns}	0.1^{ns}	0.1^{ns}	-0.2^{ns}	0.2^{ns}	-0.1^{ns}	0.1^{ns}	-0.1^{ns}	0.2^{ns}	0.6^{ns}	0.4^{ns}	0.7^{*}	0.1^{ns}	0.5^{ns}	0.5^{ns}	0.1^{ns}	0.6^{*}	-0.5^{ns}	1

ns = non-significant difference*and** significant at 5% and 1% probability level, respectively Mean with same letter(s) in not significantly different using Duncan,s multiple range tests ($p \leq 0.05$) (P.H: plant height, branch No.: number of branches, pod No.: number of pod, P.L: pod length, grain No.: number of grain per pod, T.G.W: Thousand grain weight, grain.Y: grain yield, N: nitrogen percentage, C.P: crude protein, ADF: Cell wall-hemicellulose free, NDF: Neutral Detergent fiber, DMI: Dry Matter Index, DDM: Digestible Dry Matter, NEL: Net Energy of Lactation, ME: Metabolisable Energy, RFV: Relative feed value).

4. Discussion

Cowpea is one of the legumes which widely distributed throughout the tropics regions (Ezeaku et al., 2015). Therefore, identifying the most proper planting pattern in tropical regions is necessary to obtain its maximum yield per unit area (Madani et al., 2010). Tepary bean is a drought-tolerant crop that has been neglected. Hence, the planting of Tepary should be considered, and this legume has the potential to provide greater resilience to cope with climate change (Molosiwa and Kagokong 2018). Afshar Manesh (1998) study in Jiroft, the highest yield-related traits of cowpea and Tepary bean under summer planting date showed 2.9 and 1.2 tons per hectare, respectively. Whereas, in the current study, grain yield of cowpea and Tepary were obtained 4.2 and 1.7 tons per hectare, respectively. Consequently, shifting planting dates from summer to spring in the Jroft region significantly affected the yield-related traits of the two legumes. The difference in yield between the two seasons could be attributed to the amount of rainfall and increased reproductive period in spring compared to summer, which this finding is in agreement with Ezeaku et al. (2015). Among the three planting dates studied, the highest grain yield of legumes was related to early planting (January), and Late planting (February) led to a decrease in their yield. The study of planting dates (December, January, and February) on the Tepary bean by Molosiwa and Kagokong (2018) in South Africa showed that the highest yield component was obtained in January.

Since the highest forage yield of cowpea and Tepary bean in summer planting of the region reported 2.4 and 1.8 tons per hectare, respectively (Madani et al., 2010). Thus, the present study showed a significant effect of changing planting patterns on the forage yield of two legumes. In addition, because of more rainfall in 2019 than in 2018, yield and yield components were observed to be higher in the second year compared to the first year. Therefore, due to the role of good soil moisture in the production of grain beans, the proper planting date is the wet season (Porch et al., 2013). Other studies by Canavar and Kaynak (2008) in Turkey and Ezeaku et al. (2015) in Nigeria on cowpea showed that early planting is higher yielding than late planting. Thus, further research on the best sowing dates for legumes, especially in wet seasons, is suggested in tropical regions.

Based on the results of the interaction of treatments showed that two legumes had a different response to branch and pod numbers under all three planting dates. This case could be due to the physiological and phenological responses of different plant varieties. So, there is a significant difference among varieties in terms of the number of pods under different planting dates (Sadeghipour and Aghaei, 2012). This difference might be because of the different activities of plant meristems. Therefore, varieties with more meristematic activity of along the stem produce more pods. On the other hand, the activity of meristems is related to temperature, and response of meristems to temperature is very enormously between species (Ali et al., 2009). According to the fact that improvement of grain yield is linked with these traits, varieties of plants that have more branches and pods per plant could produce more yield. Thus, selecting of proper planting in dates for different plants is an important factor in increasing traits that affect grain yield. Similarly, our results showed that planting in January increased pod length and the number of grains per pods of two legumes and led to increased grain yield. These results confirmed by Mussavi et al. (2005) reported that late in planting reduced of vegetative growth period and production of vegetative organs; as a result, assimilation decreased, early flowering, and reduced yield and yield components. Consequently, an early planting date might increase the survival of upper plant organs such as branches and pods (Santalla et al., 1993). On the other hand, the interaction between legume and planting date had no difference for the length of pod and number of grains per pod, and these traits are influenced by genetics (Bahrami, 2006). Kiyanbakht et al. (2015) reported a significant effect of genotype on the number of grains per pod of bean plants. Also, the interaction between planting date and legumes showed that late planting decreased 1000-grain weight for Tepary bean, but it was increased for cowpea; this could be the correlation of 1000-grain weight with pod number. In general, late planting led to a decreased growth period and early maturation; therefore, 1000-grain weight which is determined at the end of the growing season, is reduced (Afshar Manesh, 1998).

Cowpea and Tepary bean are rich in proteins that are the most important legumes in terms of protein after soybean (Madani et al., 2010). Early planting dates had the highest crude protein for two legumes. According to the results, two legumes showed no significant difference in nitrogen and crude protein under different planting dates; on the other hand, early planting dates increased protein content in the two legumes. Since forage plant yield and protein content are important traits, early planting could

increase quantity and quality in both legumes than late planting. In the study, quality-related traits reported that an increase in protein content is due to high absorption of minerals by roots, and increased vegetative growth under early planting leads to more nitrogen supply for plants (Sood et al., 1994; Yilmaz,et al., 2020).

Fiber content is one of the main components in digestion forage by ruminants and is widely used in measuring the quality of forage. Hence, two important chemical compounds, including neutral detergent fiber (NDF) and cell wall-hemicellulose free (ADF) are evaluated (Eskandari, 2017). Different planting dates for cowpea were no significant difference in terms of ADF, while late planting significantly decreased its NDF. Since, forage with less ADF and NDF has higher quality than forage with more ADF and NDF (Bahreininejad, 2019), for cowpea studied, planting dates increased forage quality due to no significant difference in protein and ADF and decreased NDF.

Digestive ability is one of the most important traits to determine forage quality. So, increasing fibers leads to reduce forage consumption by livestock (Ahmadi et al., 2016). In fact, highly fibered forage crops remain in the rumen for more time due to their slower rate of digestion and decreasing dry matter intake (Ronga et al., 2020). In the current study, dry matter intake in cowpea was more than in Tepary bean, which is because of the negative correlation of DMI with NDF. The Planting date in January for Tepary bean increased DMI which is due to decreased ADF on this date. Similar to DMI, the difference among all three planting dates was not significant for ADF content of cowpea; therefore, its DDM showed no significant difference. These results are confirmed by the study of Yolcu, et al. (2009). Dry matter digestibility is the portion of dry matter in a feed that is digested by livestock at a specified level of intake (Undersander et al., 1993). In addition to reducing ADF, unsurprisingly, an increase of nitrogen availability led to a greater feed of forage (No'am and Sinclair., 1995). Based on the mean comparison, the highest net energy of lactation was related to the planting date in January of the Tepary bean, and its amount decreased with late planting. Whereas the net energy of lactation of cowpea was no different under all three planting dates. According to the present study, Jahanzad et al. (2013) reported that an increase in NE_L is attributed to improving access to nutrients, especially nitrogen, and reduce in ADF.

Relative feed value is an index for forage ranking based on estimates of digestibility and consumption potential, which is derived from DMI and DDM and indicates the energy and consumption of forage (Lithourgidis et al., 2006). According to the results, in two legumes, late planting led to increasing inRFV, which it's to increasing in cowpea is higher than in Tepary bean. Its case is related to the increase of DMI and DDM in two legumes under late planting. Valentine and Horrocks (1999) reported that forage has RFV between125-151 is considered to be good in terms of livestock feed. Based on the standard forage quality table, planting of cowpea on PD3 produced forage with a good quality degree. Metabolizable energy is the amount of energy per kilogram of dry forage, and high forage digestibility could increase ME (Abdullah et al., 2010). The results of interaction between planting date and legumes indicated that ME for Tepary bean was higher on January compared to February, while late planting decreased ME. Different planting dates showed no difference for ME in cowpea; hence, ME depends on the genotype of plants (Holchek et al., 2004). Metabolizable energy for Tepary bean increased in January planting because of an increase in its digestible dry matter and decreased ADF.

Conclusion

In the current study, changing the planting pattern of cowpea and Tepary bean in the Jiroft region led to a significant increase in grain and forage yield in two legumes. Two legumes obtained the most yields in January, and late planting decreased their yield. Different planting dates had not different in the nitrogen of the two legumes; thus, early planting compared to late planting was suitable in terms of quantity and quality of forage. Mean comparison of two legumes also showed that because of no significant difference in planting dates on protein, ADF, DDM, and NE_L for cowpea and its increased DDM and RFV, cowpea was proper forage quality than Tepary bean. In general, due to different physiological and phenological responses of two legumes to different planting dates, their agronomic, biochemical, and nutritional traits were different; thus, further investigation to determine proper planting dates for varieties of crop plants was necessary to increase quantitative and qualitative forage.

Conflict of interest

The authors declare that they have no conflict of interest.

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