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Phone	Fax	e-mail
+90 (432) 225 10 56; 225 10 24	+90 (432) 225 11 04	yyujagrsci@gmail.com

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Yuzuncu Yil University
Journal of Agricultural Sciences
Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi
https://dergipark.org.tr/en/pub/yyutbd

CONTENTS

Yuzuncu Yil University
Journal of Agricultural Sciences
Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi
https://dergipark.org.tr/en/pub/yyutbd

Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

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Research Article

New Record in Summer Squash and Infestation of Branched Broomrape (*Phelipanche ramosa* (L.) Pomel) in Vegetable Areas in Van/Türkiye

Reyyan YERGİN ÖZKAN*[1], Işık TEPE[2], Enes FİDAN[3]

[1,2,3]Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Plant Protection, Van, Turkey.

[1]https://orcid.org/0000-0003-2319-404X, [2]https://orcid.org/0000-0002-9156-9467, [3]https://orcid.org/0000-0002-4567-2375

*Corresponding author e-mail: reyyanyergin@yyu.edu.tr

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Phelipanche ramosa,
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Abstract: The increasing vegetable production in Van/Türkiye province and its districts, where the study was carried out. The most significant of these issues is the parasitic and highly invasive broomrapes. A survey was conducted in August-October 2019 in order to determine the extent of the broomrape problem in the areas of vegetables. In this study, the type of broomrape found in vegetable areas was identified first, then the infestation rates and the average number of shoots in each plant were determined. The results concluded that the branched broomrape [*Phelipanche ramosa* (L.) Pomel] was found as a single species in all areas. It was also detected that branched broomrape was infested with tomato, eggplant, cucumber, and summer squash; with related densities of 4.2, 0.4, 0.26, and 0.78 %, on average, respectively. No infestation was encountered in pepper. As a result of the study, this infestation detected in summer squash is the first record in terms of the host series of branched broomrape. Although less infestation was seen in other areas, it is predicted that the branched broomrape could be spread rapidly to non-infested areas over time due to its character.

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1. Introduction

The broomrape genera (*Orobanche* and *Phelipanche*) in the Orobanchaceae family include more than 170 holoparasitic herbaceous plant species (Joel, 2009). Broomrapes cause yield losses as parasitic plants in many crops (Joel, 2007; Thorogood et al., 2009). Broomrapes whose native range is the Mediterranean regions, have nowadays invaded more than 73 million hectares of agricultural lands in many states of the USA, as well as in the Middle East, Southern and Eastern Europe, Türkiye and North Africa (Abang et al., 2007; Üstüner et al., 2020; Üstüner and Aksoy Orel, 2021). Despite intensive control measures, it is observed that there are new records and invasions in the world every day (Rubiales et al., 2011).

It was stated that the branched broomrape had a large host range as a holoparasite in Solanaceae (tomato, eggplant, pepper, potato, and tobacco), Brassicaceae (mustard and rapeseed), Cannabaceae (hemp), Fabaceae (chickpea, clover, peanut, broad bean, lentil, and pea), Apiaceae (carrot, celery, fennel, and parsnip) and Asteraceae (lettuce, sunflower and a few ornamental species) families (Parker, 2013).

It is understood from some studies that the broomrapes are also a problem in the members of the Cucurbitaceae family. *Orobanche crenata* and *Phelipanche aegyptiaca* were reported to cause damage to Cucurbitaceae members. Although *O. aegyptiaca* and *O. ramosa* show great similarity in terms of the host sequence they parasitize, it was reported that *O. aegyptiaca* had more problems than *O. ramosa* in members of the Cucurbitaceae family (Eizenberg et al., 2002).

In Türkiye, the broomrapes are common in all regions (Aksoy et al., 2011). Although there are more than 100 species in the world and 37 species in Türkiye (Gilli, 1982); only four species [*Phelipanche aegyptiaca* (Pers.) Pomel, *P. ramosa* L., *Orobanche crenata* Forsk*., O. cernua* Loefl., and *O. cumana* Wallr.] cause economic damage (Orel-Aksoy and Uygur, 2003; Bülbül and Uygur, 2009; Ekiz, 1970). According to Aksoy et al., (2009) among the broomrape species, *O. ramosa* L. and *O. aegyptiaca* Pers. prefer tomato, eggplant, and potato from Solanaceae, as well as cultivars such as lentils and broad beans from legumes; *O. crenata* Forsk. is seen in many legumes, especially in broad beans and lentils, and *O. cumana* in sunflowers (Üstüner et al., 2020).

Since broomrapes are parasitic weeds, their control is difficult, chemical control is limited, and it is an invasive plant for Türkiye (Nemli et al., 2010). With the increase in vegetable production in the region many plant protection problems have arisen. In the questionnaire conducted by Bingölbali (2019), it was stated that broomrapes were among the species that growers complained about the most. The aim of this study was to determine the problems of the growers in the region and to contribute to the literature, the species, densities, and infestation of broomrape were determined in the vegetable areas in Van province.

2. Material and Methods

This study was carried out in fields where vegetables (cucumber, tomato, pepper, eggplant, and summer squash) were grown in Van province between August and October 2019. The research was carried out in 6 of the 13 districts of Van province, where intensive vegetable cultivation is done. These six districts evaluated cover a total area of 95.6 hectares. Family lands, which are less than one decare and don't grow vegetables commercially, were excluded from the study. According to TURKSTAT (2018) data, it was understood that cucumber, tomato, pepper, eggplant, and summer squash were grown in a total of 1.2 thousand hectares area in Van province. The surveyed areas constituted approximately 8% of this area (Table 1).

Table 1. Surveyed districts and areas

Districts	Surveyed area (ha)
İpekyolu-Edremit	2.4
Gevaş	46.4
Erciş	14.8
Tuşba	28.0
Gürpınar	4.0
Total	95.6

Observations were made once in every three rows, as the cultivated plants in which the research was carried out were planted in rows. The species of broomrape found in vegetable areas were identified first, then the infestation rates (%) and the average number of shoots in each plant were determined during the surveys. In the areas where the infestation is seen, the root zone of each crop plant was opened and closely examined to determine whether or not the broomrapes hold onto the plant. In order to eliminate the influence of edge effects, the first and last two rows of the sampled area were not included during observation.

In calculating the number of infested vegetables in the field, first, the total number of plants was found by multiplying the number of vegetables in the row by number of rows in the field. Then, the number of infested plants in a row of every three rows was counted. The number of infested plants in the field was determined and the infestation rates were the contamination rates were calculated with the equation developed by us (Eq. 1) proportioning these values with the number of plants in the field. In

order to determine the average number of broomrapes per plant, the number of broomrape branches in each three infested plants was counted and the averages were calculated.

$$\text{Infestation rate (\%)} = (\text{Number of infested plants/Total number of plants}) \times 100 \quad (1)$$

The infestation rates were calculated for each district in general and specifically. The general infestation was calculated on the basis of all planting areas, and the special infestation was calculated based on only infested areas. These calculations were made separately for tomato, eggplant, pepper, summer squash, and cucumber.

3. Results and Discussion

The identification of broomrape species found in tomato, eggplant, pepper, cucumber, and summer squash growing areas in Van province was made according to Gilli (1982) and it was determined that branched broomrape [*Phelipanche ramosa* (L.) Pomel; *Syn: Orobanche ramosa* L.] was found in all vegetable areas. Parker (2012 and 2013) stated that *P. ramosa* was a host in Solanaceae (tomato, eggplant and tobacco as well as pepper and potato), Brassicaceae (rapeseed and mustard), Cannabaceae (hemp), Fabaceae (chickpeas, alfalfa, peanuts, broad beans, lentils, and peas), Apiaceae (carrot, celery, fennel, and parsnip) and Asteraceae (lettuce, sunflower and a few ornamental species) families. It was also found that this type of broomrape had been a host in wild species in Chenopodiaceae, Amaranthaceae, Malvaceae, Rosaceae, and many other families. Although it was reported to be a host in onions, it was stated that broomrape was not seen in other monocotyledonous plants. In another study carried out, it was determined that *Phelipanche ramosa* was the most significant pest of tomato in Iran (Minbashi Moeini, 2004). In studies conducted in Türkiye, *P. ramosa* was found to be most harmful among the hosts of tobacco (Ekiz, 1970; Uludağ and Nemli, 2009), tomato (Aksoy et al., 2001; Uludağ and Nemli, 2009), sunflower (Ekiz, 1970), lentil (Aksoy and Uygur, 2003), and eggplant (Demirkan, 1992).

Only table varieties are grown in tomato fields in Van. In these areas, surveys were carried out in 30 fields in total. The infestation was detected in 13 of the fields and no infestation was detected in İpekyolu, Edremit, and Gürpınar districts. The general and specific infestation rates were detected as 4.3% and 6.0%, respectively. The average number of broomrape shoots in tomatoes throughout the province was found to be 29.2 branches. This high number can be explained by the fact that the species in question gives a large number of tillering and has a number of shoots. It was determined that the general infestation rate in the Gevaş district, which has the highest tomato growing area, was 5.3%, and the infested fields were very close to this rate at 5.4%. The average number of broomrape shoots per plant was determined as 31.5 branches. It was determined that five of the 11 fields surveyed in Erciş were infested. The general infestation rate was found as 4.9%, and the rate in the infested fields is 7.3% (Table 2). Aksoy (2003), stated that *O. ramosa* caused 24.8% of product loss in tomato fields in Türkiye. According to Aksoy and Uygur (2003), Orobanche aegyptiaca and O.ramosa shoots were detected at a rate of 3.3% per m-2 in tomato fields. In a study conducted by Ruşen and Yazlık (2009) on tomato fields in the Marmara Region, they stated that they had never encountered *P. ramosa* in greenhouses; but detected it in 58%, 14%, and 50% in Bursa, Kocaeli, and Sakarya provinces, respectively. In the survey carried out in the tomato fields in Samsun province, it was determined that the density of *P. ramosa* was 22.3% and the number of shoots was 1.1 branches (Işık and Kaya 2009). In another study conducted by Özaslan and Kendal (2014) in tomato planting areas in Lice/Diyarbakır, it was determined that *P. ramosa* was among the species with the highest density with 3.7 plant m^{-2}. Bülbül et al., (2009) found that 27.7% of the greenhouses and 80% of the fields were infested with *Orobanche aegyptiaca* and *O.ramosa* in the Eastern Mediterranean region and that these species had an average shoot number of 0.4 branches per tomato root in greenhouses. Compared to other studies conducted in Türkiye, it was seen that branched broomrape infestation was higher in tomatoes in Van province.

There is limited cultivation land for eggplant and it is grown in the districts of Tuşba, Gürpınar, and Gevaş. Eggplant studies were conducted in seven districts and evidence of infection was found in three fields in the Gevaş district. Thus, infestations were found in three of the five fields, with general and specific infestations at 0.4% and 0.6%, respectively. These infestation rates also represent the Van province. Even though the infestation is limited to a particular district, Table 2 indicates that there are

an average of 11.6 broomrapes per plant. The eggplant is on the host list of *P. ramosa* (Musselman, 1987). According to reports, *O. aegyptiaca* reduces eggplant yields in India by 30–35% (Prasad et al., 2009; Singh et al., 2017). According to a study by Akhter and Khan (2020), *P. ramosa* densities in eggplant areas ranged from 15 to 35 percent.

Table 2. Survey values in districts of Van

Vegetables	Districts	Total number of fields	General infestation rate (%)	Total number of infested fields	Special infestation rate (%)	Average number of broomrape branches
Tomato	İpekyolu-Edremit	3	0	0	0	0
	Gevaş	9	5.30	7	5.42	31.57
	Erciş	11	4.94	5	7.37	25.75
	Tuşba	6	0.45	1	10.41	15
	Gürpınar	1	0	0	0	0
	Van	30	4.27	13	6.04	29.27
Eggplant	İpekyolu Edremit	No planting	No planting	No planting	No planting	No planting
	Gevaş	5	0.48	3	0.6	11.6
	Erciş	No planting	No planting	No planting	No planting	No planting
	Tuşba	1	0	0	0	0
	Gürpınar	1	0	0	0	0
	Van	7	0.43	3	0.6	11.6
Pepper	İpekyolu Edremit	1	0	0	0	0
	Gevaş	6	0	0	0	0
	Erciş	2	0	0	0	0
	Tuşba	2	0	0	0	0
	Gürpınar	1	0	0	0	0
	Van	12	0	0	0	0
Cucumber	İpekyolu Edremit	1	0	0	0	0
	Gevaş	6	0.26	2	0.35	10
	Erciş	No planting	No planting	No planting	No planting	No planting
	Tuşba	1	0	0	0	0
	Gürpınar	1	0	0	0	0
	Van	9	1.68	2	0.35	10
Summer squash	İpekyolu Edremit	No planting	No planting	No planting	No planting	No planting
	Gevaş	4	1.07	1	3.69	37
	Erciş	1	0	0	0	0
	Tuşba	1	0	0	0	0
	Gürpınar	1	0	0	0	0
	Van	6	0.78	1	3.69	0.52

Pepper is grown in all districts where surveys are carried out in Van province. The infestation was not detected in any field in the region where both pointed and bell pepper varieties are grown. Although *P. ramosa* was observed in pepper cultivation areas in Van province, when the root zone was examined in detail it was understood that the attachment was not in pepper plants but in different types of weeds in the field (Table 2). Qasem and Foy (2007), tested pepper as a trap plant in the greenhouse to determine the hosts of *O. ramosa*. Although it is not on the host list of broomrape, they reported that they had obtained moderate infestation (11–30 shoots/pot) in pepper. They explained this situation as exudates in the soil slightly increasing seed germination in pepper. Hershenhorn et al. (1996) also noted that pepper was parasitized by *O. aegyptiaca*. However, no record of pepper has been found in the references in Türkiye.

Similar to eggplant, cucumbers are only grown in a small portion of Van province. Infestation of branched broomrape in cucumber was found only in the Gevaş district. In the whole survey region, observations were made in nine fields, although only two of those areas had an infestation. The general and particular infection rates in this district were found to be 0.26% and 0.35%, respectively. Although the infestation rates are low, the average number of branched broomrape shoots in the plant was determined as 10 (Table 2).

The growing area of summer squash is limited, as is the case with cucumbers and peppers. The survey was carried out in nine fields, four of which were only in Gevaş. Although the general infestation rate is low, the specific infestation rate originating from a single district was determined as 3.6%. The average number of branched broomrape shoots in summer squash was found to be 37, and this value is seen as the highest average among all survey areas (Fig. 1, Table 2).

Figure 1. Branched broomrape infestation in summer squash.

It was stated that *P. aegyptiaca* species in the Cucurbitaceae family caused more parasitism than *P. ramosa* (Eizenberg et al., 2002). In addition, Musselman and Parker (1982) stated that the Cucurbitaceae family was also among the hosts of *O. crenata. Cucurbita moschata, Cucumis melo var. flexuosus,* and *Cucumis sativus* species were among the lowest infestation rates (≤ 10 shoots/pot), and *Cucurbita maxima* was among the species with moderate infestation rates, which were used as trap plants in a greenhouse study carried out by Qasem and Foy (2007) to determine the hosts of *O. ramosa* used. Labrada and Perez (1988) stated that beans, sorghum, corn, and cucumber can be used as trap plants for the germination of *O. ramosa* seeds. In the literature review, no record of *P. ramosa* infestation was found in summer squash (*Cucurbita pepo*) both in the world and in Türkiye; therefore, the results obtained from this study are considered to be the first record of summer squash.

4. Conclusion

According to the results obtained, it was determined that the branched broomrape [*Phelipanche ramosa* (L.) Pomel] was a problem in the tomato, eggplant, pepper, cucumber, and summer squash cultivation areas in Van province. The infestation was found to be high throughout Van province, and it was determined that this ratio was 4.2%, 0.4%, 0.26%, and 0.78% in tomato, eggplant, cucumber, and summer squash, respectively. No infestation was found in the pepper. The fact that no weed management method is applied and the same crops are grown in these areas every year without alternation has caused the density to increase. The infestation detected in summer squash in this study is the first record in terms of the host series of branched broomrape. The highest rate of infestation was determined in the district of Tuşba with 10% among the districts of Tuşba, İpekyolu, Edremit, Gevaş, Gürpınar, and Erciş, where the study was carried out. Although this situation may seem like a local infestation, the mentioned areas are the places where vegetable farming is done most intensively. It is anticipated that branched broomrape will spread rapidly to non-infested areas due to its invasive character in the following years.

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Research Article

Exploration and Investigation of Antifungal Activity of Plant Leaf Extracts on Growth of *Scletorium rolfsii* Sacc.

Moralita CHATRI*[1], Linda ADVINDA[2], Fitria OKTIANA[3], Puja MALONA[4], Silvi LATHIFAH[5] Dwi Hilda PUTRI[6]

[1,2,3,4,5,6]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Padang, 25131

[1]https://orcid.org/0000-0002-2833-1763, [2]https://orcid.org/0000-0002-5425-7173, [3]https://orcid.org/0009-0002-2232-9256
[4]https://orcid.org/0009-0004-0901-6380, [5]https://orcid.org/0009-0004-3187-033X, [6]https://orcid.org/0000-0002-4070-8869

*Corresponding author e-mail: moralitachatri@gmail.com

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Leaf extract,
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Abstract: Botanical fungicides are fungicides derived from plants that produce chemical compounds that potentially inhibit microbial growth. These fungicides are safe because to its not harmful to humans and the environment. In the present study, the plant materials used often compete with plant materials used for food and medicine such as galangal rhizomes and betel leaves. Therefore, it is necessary to explore materials derived from plants that have not been widely utilized. So the research was conducted to determine the effect of leaf extracts from several plants on the growth of *Sclerotium rolfsii* Sacc. the fungus that causes wilt disease in plants and determines the level of antifungal activity. This research was conducted using a completely randomized design (CRD). The leaf extracts used were from the plants *Muntingia calabura*, *Terminalia cattapa, Syzygium oleina, Morinda citrifolia, Dimocarpus longan,* and *Artocarpus altilis* with concentrations of 10%, 20%, 30%, 40%, and 0% as control. The data obtained were analyzed using variance analysis (ANOVA) with Duncan's New Multiple Range Test (DNMRT). The results showed that all treatments used could inhibit the growth of *S. rolfsii* Sacc because they were significantly different from the control. It was determined that antifungal activity in leaf extracts of *M. calabura*, *T. cattapa*, *S. oleina*, and *D. longan* was very strong, and also *A. altilis* had a strong antifungal activity, while *M. citifolia* had a moderate antifungal activity.

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1. Introduction

The *Sclerotium rolfsii* Sacc. is a soil-borne fungus that causes stem rot disease, consequently, the plants wilt and die (Sektiono et al., 2019). It also causes the dumping of disease and is easily recognized by the presence of white mycelium on the infected plant parts and in subsequent attacks will form sclerotia. Globally, this disease affects plants at all stages of growth, including seeds, and mature plants (Agrios, 2005).

The stem rot disease begins with the yellowing at the base and is accompanied by the emergence of mycelium. On the underside of the leaves and near the plant, sclerotia were found and the leaves close to the soil surface will experience chlorosis and change into brown, thereby causing plants to wither,

rot, and die (Kator et al., 2015; Martinius et al., 2019). This fungus has a wide host range, which makes it difficult to control (Semangun, 2004), and can survive in the soil in the form of sclerotia for several years (Punja, 1985). According to Agrios (2005), sclerotia can last for 2-3 years depending on the availability of organic matter.

S. rolfsii Sacc. attack on plants begins by infecting the roots or stems of plants close to the soil surface. Furthermore, it will infect the roots or stems, causing nutrient and water transportation to be blocked (Xie and Vallad, 2016). Early symptoms begin with yellowing of the stem base, accompanied by the emergence of mycelium. Sclerotia are found on the underside of leaves and near the plant. Leaves that are close to the soil surface will experience chlorosis and turn brownish. Blockage of nutrient and water transportation causes plants to wilt, rot, and eventually die (Martinius et al., 2019). *S. rolfsi* can produce oxalic acid (Monazzah, et al., 2018) which is directly toxic to plant tissues (Kyoung, 2008).

Control of plant diseases is generally achieved through the use of pesticides. Pesticides are chemicals used in agriculture to protect plants by destroying unwanted organisms. Such as insecticides, rodenticides, herbicides, fungicides, and others. However, these chemicals can cause a large number of negative impacts on health and the environment because they can cause toxic effects or poisoning due to excessive exposure to chemicals or doses (Alewu and Nosiri, 2011; Stamati, et al., 2016). According to Mesnage and Seralini (2018), the human population exposed to pesticides is currently in increasing numbers due to continuous use.

Pesticides used to control plant diseases caused by fungi are fungicides. Due to the negative impact caused by fungicides, it is necessary to find alternative sources of new pesticides, where the use of these pesticides is expected to be efficient, safe, and selective against target pests and pathogens (Shukla, et al., 2012). One of them is biological control such as the use of botanical fungicides. Botanical fungicides are fungicides obtained from plant organ extracts, such as from tubers, roots, stems, or leaves (Husein and El-Anssary, 2018). Mazid et al., (2011), explained that plants can produce chemical compounds or secondary metabolites that can protect themselves from pathogen attacks because these compounds are antibiotics, antifungal, and antiviral. These secondary metabolite compounds have a broad spectrum such as terpenes, phenols, flavonoids, tannins, alkaloids, saponins, and others. Each plant chemical derivative can be utilized based on differences in the content of its biological properties (Dubey, et al., 2008; Shukla et al., 2012). Secondary metabolite compounds can be utilized as botanical fungicides. Botanical fungicides have advantages, including being environmentally friendly, easily degraded, and abundant local resources so that they are easily available (Dalimunthe and Rachmawan, 2017).

Some plants have been utilized as botanical fungicides, such as extracts from garlic, betel and cloves (Prasetyorini, 2020), lemongrass (Martinius et al., 2019), basil (Nugroho et al., 2019) and some seaweed plants (Mabrouki, 2020; Inci et al., 2021; Ozakin et al., 2021) have been discovered to inhibit the growth of the fungus *S. rolfsii* Sacc. However, the plants used as botanical fungicides are plant organs that are widely used as raw materials for medicines and food flavorings. So that if used as a botanical fungicide for plant disease control, it will certainly compete in the supply of raw materials; as a result, the price becomes expensive and will make it difficult for farmers. The existence of different interests is one of the challenges in biological control. Based on this, it is necessary to explore plant organs such as leaves, which are not widely used by humans and have the potential to be applied as botanical fungicides. So a study was conducted on several plant leaf extracts to see their effect on inhibiting the growth of *S. rolfsii* Sacc. and its antifungal activity.

2. Material and Methods

2.1. Plant material

The choice of plant leaves is a plant that grows many leaves and the leaves are not widely used by people like for food or medicine. There are six plants, namely *Muntingia calabura (kersen), Terminalia cattapa (ketaping), Syzygium oleina* (Redbud), *Morinda citrifolia* (noni), *Dimocarpus longan* (longan), and *Artocarpus altilis* (breadfruit).

2.2. Methods

This study was carried out in January-April 2022 at the Research Laboratory at the Department of Biology, Faculty of Mathematics and Natural Sciences, Padang State University. The experiment was conducted using a Completely Randomized Design (CRD). CRD is one where the treatments are assigned completely at random so that each experimental unit has the same chance of receiving any one treatment. Each of these plant extracts was distinguished by concentrations of 10%, 20%, 30%, and 40%. The choice of concentration is based on previous research, in which a concentration of 20% of *Hyptis suaveolens* leaf extract was able to inhibit the growth of S. *rolsii* Sacc. Then, in this study, the concentration was reduced to 10% and increased to 30% and 40% (Chatri et al., 2019). The negative control was the treatment without leaf extract and the positive control was the treatment with chemical fungicides (Antracol 70 WP), intending to see if the leaf extract gives the same effect as chemical fungicides. The positive control concentration used was the lowest concentration of leaf extract (10%).

2.3. Preparation media incubation

Approximately 7.8 g PDA media was put into a 250 mL Erlenmeyer and dissolved to 200 mL of distilled water, then heated using a hot plate until boiling and homogeneous. After homogeneous, it was left until the temperature of the solution decreased, and then the Erlenmeyer was closed with a cotton plug, aluminum foil, and plastic wrap. Then the PDA media was sterilized in an autoclave at 121°C at a pressure of 15 psi for 15 minutes. After that, the PDA media was poured into petri dishes and allowed to solidify.

2.4. Preparation of leaf extract

Fresh leaves of the six plants were rinsed with distilled water, then finely chopped, and then dried, after that, the leaves were pulverized using a blender, then put into an opaque bottle of 300 grams, and soaked with 96% ethanol. The container was tightly closed and placed in a place protected from the light and left for 5x24 hours, then filtered using filter paper. The leaf extract solution obtained was purified by the evaporation process using a vacuum rotary evaporator to obtain a thick extract (Renisheya et al., 2012). Furthermore, the pure extract obtained was diluted according to the treatment, namely 10%, 20%, 30% and 40%. For a concentration of 10%, 1 gram of leaf extract was taken and then added with distilled water to a volume of 10 mL, and so on.

2.5. Leaf extract essay

Leaf extract testing was carried out by taking 2 mL of extract from each treatment and then adding it to 8 mL of PDA in a test tube, homogenizing it using a vortex, then it was poured into a Petri dish, after which it was allowed to freeze perfectly. For the control, 10 mL of PDA medium that was not added with leaf extract was used. *S. rolfsii* Sacc. that has been grown (3 days of age), inoculated on PDA medium that has been added with leaf extract according to the treatment. The size of the fungal colony taken was approximately 0.5 cm x 0.5 cm (length x width) taken using a scalpel, then placed in the center of a petri dish that contained a mixture of medium with leaf extract, and then incubated at room temperature.

2.6. Measurement of *S. rolfsii* growth

Fungal growth was done by measuring the diameter of S. *rolfsii* Sacc. (cm) on day 2 to day 5 after incubation. The data analyzed was the data on day 5. Measurement of the diameter of fungal colonies is done by making a horizontal and vertical line on the surface of the Petri dish. The cutting point of both lines was right in the center of the fungal colony that grew as shown in Figure 8.

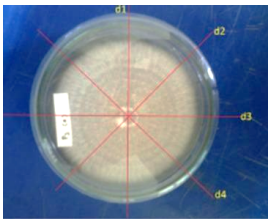

Figure 1. Diameter measurement of fungal colonies (d = diameter).

2.7. Assessment antifungal activity

To determine the antifungal activity of the leaf extract, the percentage of inhibition of *S. rolfsii* Sacc. growth was calculated by the formula (Ouoba, et al., 2018):

$$P = \frac{D1 - D2}{D1} \times 100\% \quad (1)$$

Description:
P = Percentage of inhibition
D1 = Average diameter of fungus in negative control (cm)
D2 = Average diameter of fungus in each treatment (cm)

Based on the percentage of growth inhibition of *S. rolfsii* Sacc. the criteria for antifungal activity were determined as shown in Table 1.

Table 1. Antifungal activity (Mori, et al., 1997)

Inhibition Percentage	Activity Level
P≥75	Very strong
75≤P<50	Strong
50≤P<25	Moderate
25≤P<0	Weak
0	Inactive

2.8. Data analysis

Colony diameter data were examined using Analysis of Variance (ANOVA) and was continued with Duncan's New Multiple Range Test (DNMRT) at α=0.05. The antifungal activity data were analyzed descriptively.

3. Results and Discussion

3.1 Growth of *S. rolfsii* Sacc.

The results of the investigation on the effect of several leaf extracts on *S. rolfsii* Sacc. are shown in Table 2. According to Table 2, it was discovered that at the lowest concentration of 10%, several leaf extracts such as *M. calabura, D. longan, S. oleina,* and *A. altilis*. effectively inhibit the growth of *S. rolfsii* Sacc. The results of statistical analysis showed that the diameter of the *S. rolfsii* Sacc. colonies was significantly different from the control (-). This is because the compounds contained in the secondary metabolites of these plants can act as antifungals. Tiwari et al. (2009) stated that the ability of compounds contained in plants as antimicrobials depends on the concentration and chemical structure of the active components such as saponins, flavonoids, thiosulfinates, glucosinolates, phenols, and organic acids. However, the main components in plants that are active as antimicrobials are phenolic

compounds such as terpenes, aliphatic alcohols, aldehydes, ketones, and isoflavonoids. Leaf extracts of *M. calabura*, *D. longan*, *S. oleina*, and *A. altilis* contain saponins and flavonoid compounds. In this study, the leaf extracts of other plants also contained these two compounds, but in small concentrations. Therefore, it has not been able to inhibit the growth of *S. rolfsii* Sacc. According to Cushnie et al. (2005), saponins and flavonoid compounds can be found in fruit, seeds, stems, flowers, and leaves.

Table 2. Colony diameter (cm) of *S. rolfsii* Sacc. by treatment of several plant leaf extracts at different concentrations

Leaf Extract Concentration	***M. calabura***	***T. cattapa***	***D. longan***	***S. oleina***	***M. citrifolia***	***A. altilis***
control (-)	9.30 [a]	9.30 [a]	9.30 [a]	9.30 [a]	9.30 [a]	9.30 [a]
10	7.68 [b]	8.95 [a]	4.94 [b]	7.11 [b]	9.00 [a]	5.53 [b]
20	7.24 [b]	8.40 [a]	4.75 [b]	5.74 [c]	3.10 [b]	4.71 [c]
30	4.38 [c]	5.59 [b]	2.03 [c]	3.75 [d]	7.74 [c]	3.73 [c]
40	0.50 [d]	0.50 [c]	1.89 [c]	1.41 [e]	6.63 [d]	3.45 [c]
control (+)	--	--	--	--	--	--

Remarks: The number followed by the same letter is not significantly different in each treatment based on the Duncan test (α=0.05).

The diameters of *S. rolfsii* Sacc. colonies at a concentration of 40% (the highest concentration) are shown in Figure 1. It was discovered that the largest colonies of 6,63 cm were treated with *M. citrifolia* leaf extract and the smallest, namely 0.5 cm was treated with *M. calabura* and *T. cattapa*. Based on the analysis, all treatments with leaf extracts showed significant differences from the control. This proves that the leaf extracts of the tested plants can inhibit the growth of *S. rolfsii* Sacc. All leaf extracts have shown the ability to inhibit the growth of *S. rolfsii*. Even in the leaf extracts of *M.calabura* and *T.cattapa* with a concentration of 40%, the growth of *S.rolfsii* colonies did not exist at all until the end of observation (day 5), as can be seen in Figure 2-3. This inhibitory ability is due to the presence of active compounds contained in the leaves of these plants, such as alkaloids, phenols, flavonoids, saponins, tannins, steroids, and triterpenoids.

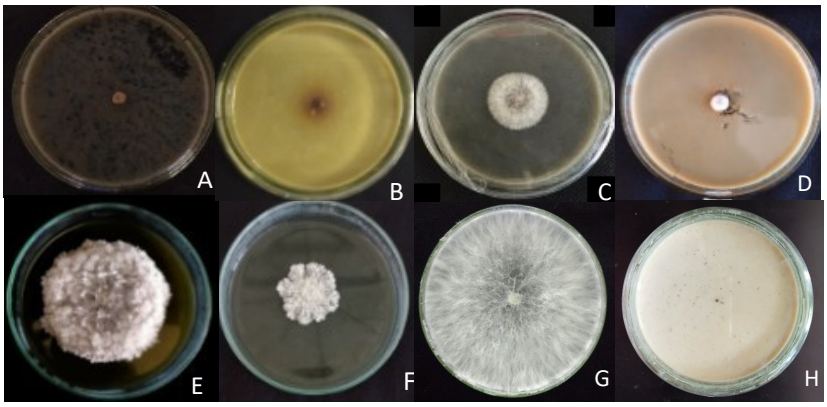

Figure 2. Diameter of *S. rolfsii* Sacc colonies at a concentration of 40% with the treatment of several plant leaf extracts. A. *M. calabura,* B. *T. cattapa,* C. *D. longan,* D. *S. oleina,* E. *M. citrifolia,*, F. *A. altilis,* G. control (-) and H. control (+).

Alkaloids are one of the secondary metabolites found in plants, which can be found in leaves, twigs, seeds, and bark. In general, alkaloids are often used in medicine. Alkaloids interfere with fungal growth by entering the cell wall and preventing DNA replication so that the formation of DNA and RNA will be disrupted (Aniszewski, 2007). Alkaloid compounds prevent replication of nucleic acid biosynthesis in fungi so that fungi cannot develop (Adegoke and Adebayo-tayo, 2009). Alkaloid compounds also bind strongly to ergosterol to form holes or channels, causing the cell membrane to leak

and lose some intra-cellular materials such as electrolytes (especially potassium) and small molecules. This results in permanent damage to the cell and cell death in fungi (Mycek et al., 2001; Setiabudy and Bahry, 2007).

The phenolic compounds in flavonoids can denature cell proteins and shrink cell walls, causing fungal lysis, and disrupting growth, and death (Cowan, 1999). The mechanism of flavonoids inhibits fungal growth by disrupting cell membrane permeability. This can change organic components and interfere with nutrient transport, thereby creating a toxic effect on fungi (Komala and Siwi, 2019). Saponins can also function as antifungals, leading to the leakage of proteins and enzymes from the cell (Rijayanti, 2014). This leakage occurs because the saponins damage the permeability of the cell membrane by lowering the surface tension of the fungal cell wall. Furthermore, saponins will diffuse through the cytoplasmic membrane which destabilizes the membrane and the cytoplasm exits the cell leading to cell death (Sudarmi, 2017).

The mechanism of action of tannins as antifungals inhibits the synthesis of chitin which is used for the formation of cell walls in fungi and damages cell membranes, therefore, disrupting fungal growth (Watson and Preedy, 2007). Tannins can also cause cells to lyse because they target cell wall polypeptides and inhibit formation (Sapara, 2016). The imperfect formation makes the cells unable to withstand osmotic or physical pressure, thereby causing death (Rijayanti, 2014). Furthermore, tannins also inactivate fungal cell adhesins and enzymes as well as interfere with protein transport within cells (Egra, 2019). Triterpenoids inhibit fungal growth through the cytoplasm or interfere with the growth and development of fungal spores (Lutfiyanti, 2012).). *T.cattapa* has been shown to inhibit the growth of the fungus *Pyricularia grisea* (Zuraidah and Wahyuni, 2019) because the plant contains flavonoid, alkaloid, steroid, saponin, and tannin compounds (Salimi et al., 2022).

Testing of leaf extracts as antifungal was rarely carried out. Testing of leaf extracts in inhibiting bacterial growth has been carried out, such as *M. calabura* against *Escherichia* coli (Handoko et al., 2019) and *Porphyromonas gingivalis* (Muflikhah et al., 2017). Ethanol extract from *T. catappa* leaves can inhibit the growth of *Aeromonas hydrophila* bacteria (Purba et al., 2020). *D.longan* can inhibit the growth of *Staphylococcus aureus*, *Salmonella typhii,* and *Vibro mimicus* bacteria (Ripa et al., 2010), The results of research from Haryati et al., (2016) show that 96% ethanol extract of *S.oleina* leaves has antibacterial activity against *Staphylococcus aureus* bacteria and *Escherichia coli* bacteria.

The growths of *S. rolfsii* Sacc. colonies treated with several leaf extracts with different concentrations were observed until the 5th day after incubation, as shown in Figure 2-7. The growth of *S. rolfsii* Sacc. colonies still showed an increase in colony diameter until the last day of observation, except for the treatment of *M. calabura, T. cattapa*, and *S. oleina* leaf extracts. The colony diameter still increased because not all components of the active compounds in the leaf extract inhibited the growth of the fungus. The growth of *S. rolfsii* Sacc. colony diameter occurred very quickly in the 0% treatment or without treatment with leaf extract. This shows that the active compounds contained in the leaf extract affect the growth of fungi.

Positive control tests using chemical fungicides showed that *S. rolfsii* could not grow even at low concentrations such as the leaf extract (10%). *S. rolfsii* Sacc. mycelium grown on PDA media mixed with chemical fungicides initially formed sclerotia, but then the sclerotia died. This shows that chemical fungicides have very high toxicity, so they can kill microorganisms. In the principle of plant disease control, plant pest organisms are not eradicated to extinction, but only controlled until they do not interfere with plants which results in reduced production. The extinction of an organism or microorganism will result in a reduction in the diversity of living things. According to Mesnage and Seralini (2018), the use of pesticides can kill insects or fungi and have adverse long-term effects on agricultural systems due to a lack of biodiversity. Then, in this case, it is better to use botanical fungicides for plant disease control.

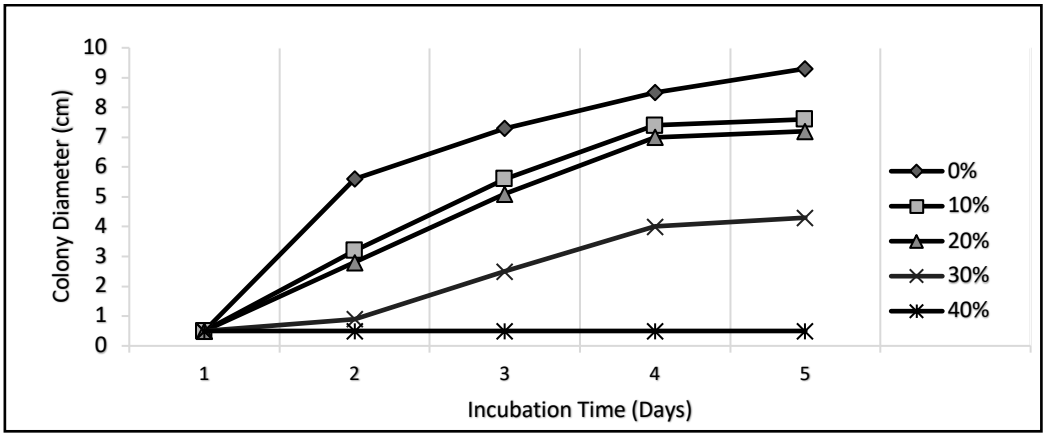

Figure 3. Growth of *S. rolfsii* Sacc with *M. carabola* leaf extract at different concentrations.

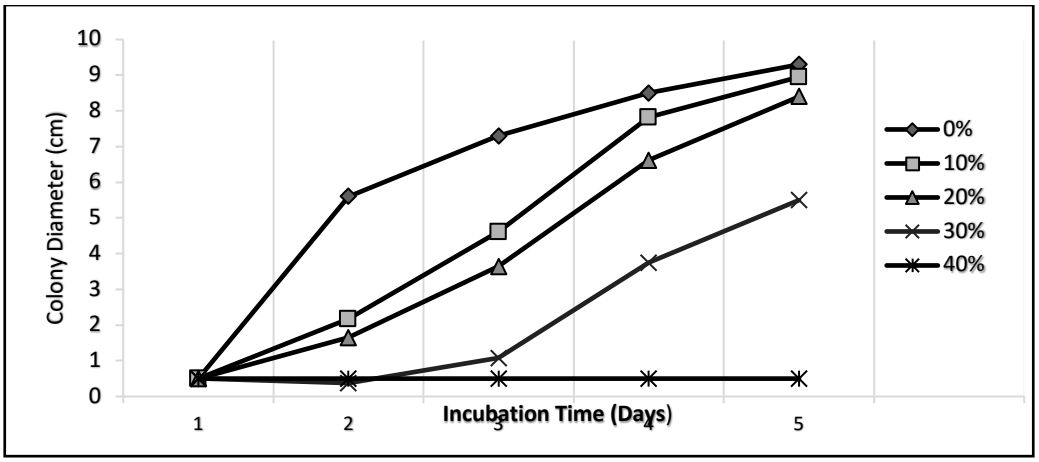

Figure 4. Growth of *S. rolfsii* Sacc with *T. cattapa* leaf extract at different concentrations.

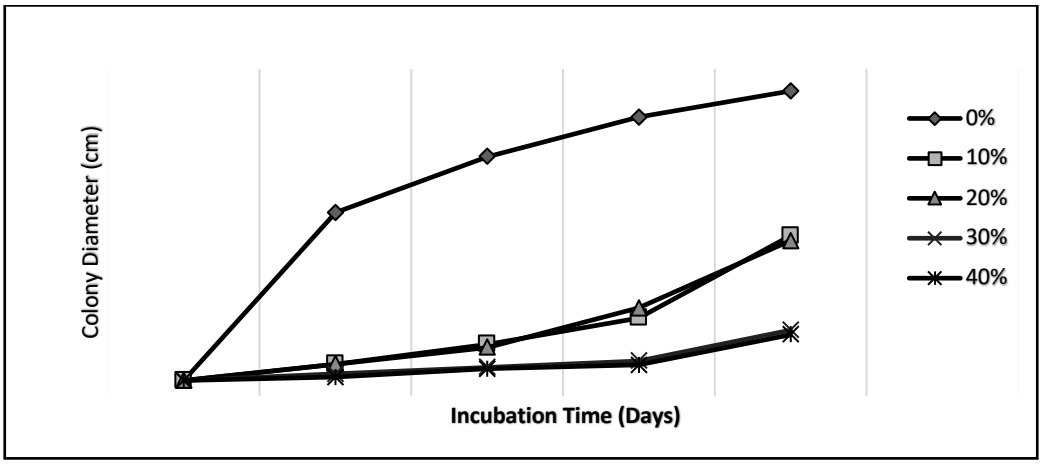

Figure 5. Growth of *S. rolfsii* Sacc with *D. longan* leaf extract at different concentrations.

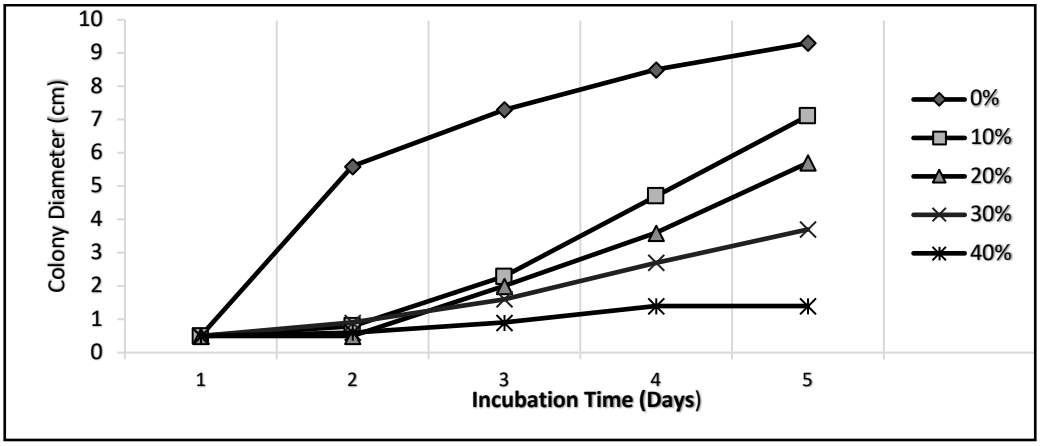

Figure 6. Growth of *S. rolfsii* Sacc with *S. oleina* leaf extract at different concentrations.

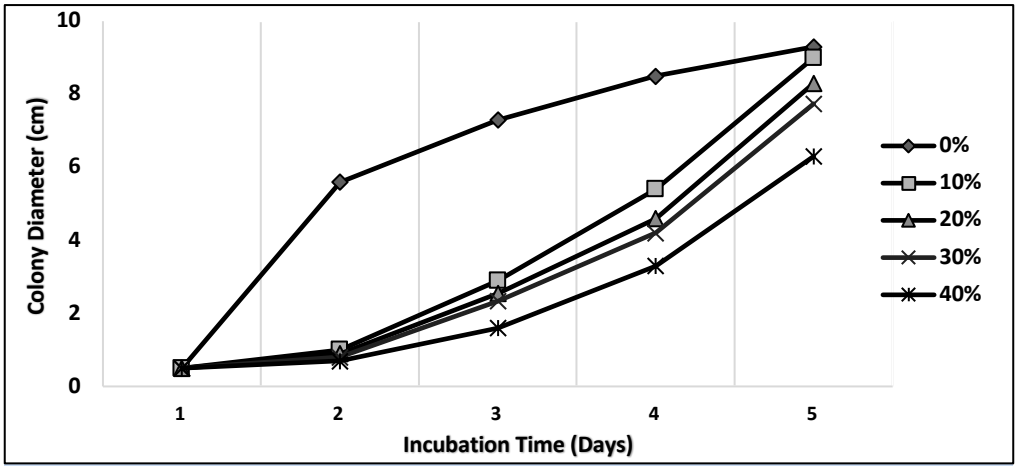

Figure 7. Growth of *S. rolfsii* Sacc with *M. citrifolia* leaf extract at different concentrations.

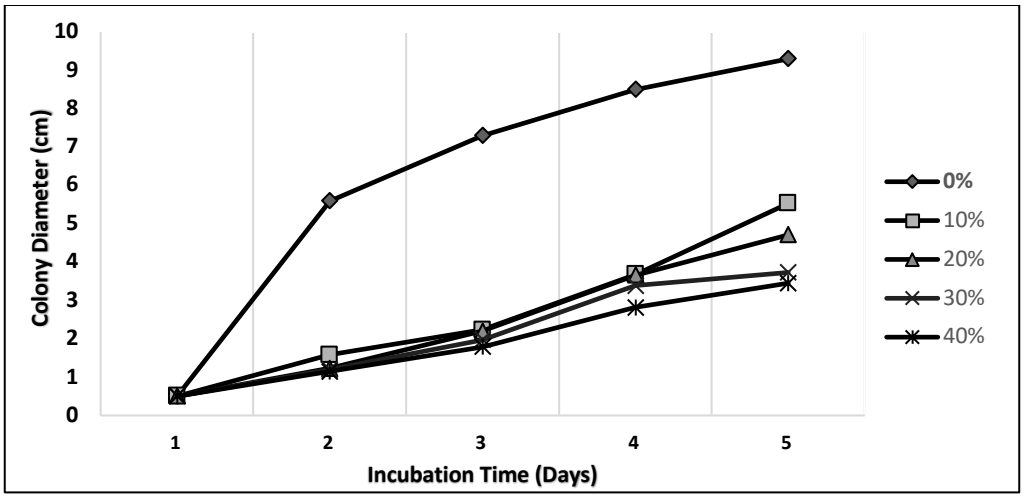

Figure 8. Growth of *S. rolfsii* Sacc with *A. altilis l*eaf extract at different concentrations.

3.2. Antifungal activity

The level of antifungal activity of leaf extracts can be known based on the percentage of growth inhibition (Table 3). It can be seen that some antifungal activities are the same and some are different in each plant extract treatment with different concentrations. At a concentration of 40%, treatment with *M. citrifolia* leaf extract showed a moderate level of antifungal activity (50%≤PP<25%), although the highest concentration and the results of statistical analysis have shown a significant difference with the

control on colony diameter. The treatment of *M. calabura*, *T. cattapa*, *D. longan,* and *S. oleina* leaf extracts at a concentration of 40% has a very strong antifungal activity because it is more than 75%. Treatment of *A. altilis* leaf extract with strong antifungal activity level. The occurrence of different levels of antifungal activity shows that the concentration of leaf extracts influences the growth of *S. rolfsii* Sacc. Because the difference in concentration will cause differences in the levels of active compound components or secondary metabolite compounds contained in plant leaf extracts. The same thing happened in the treatment of extracts of several Leguminosae plants, the difference in extract concentration gave a significant difference to the biomass of *S. rolfsii* Sacc mycelium (Sana et al., 2016). From the results of Chatri et al. (2022), the treatment of *Melastoma malabatrichum* L. leaf extract against *S. rolfsii* Sacc showed weak antifungal activity, even at 40% extract concentration. This indicates that the levels of antifungal substances in these plants are low. However, the treatment of *Hyptis suaveolens* L. leaf extract, at a concentration of 20% already showed a percentage of 100% growth inhibition with very strong antifungal activity (Chatri et al., 2019). This shows that the concentration of plant leaf extracts also affects anti-fungal activity. The higher the concentration of leaf extract used, the higher the content of active compounds that act as antifungal so that the antifungal activity will be greater. Conversely, the smaller the concentration of leaf extract, the less the content of active compounds that act as antifungals so antifungal activity will also be smaller. This is in line with Pelczar (1998) which states that increasing the concentration of an antimicrobial substance is proportional to its activity.

Table 3. Percentage growth inhibition of S. rofsii Sacc and antifungal level of leaf extracts of some plants

Plants	Leaf Extract Concentration (%)	Growth Inhibition Percentage	Antifungal Level
M. calabura	10	15.00	Weak
	20	15.20	Weak
	30	49.10	Moderate
	40	94.03	Very strong
T. cattapa	10	16.41	Weak
	20	27.80	Moderate
	30	61.70	Strong
	40	92.90	Very strong
D. longan	10	47.16	Moderate
	20	49.19	Moderate
	30	78.28	Very strong
	40	79.78	Very strong
S. oleina	10	22.80	Weak
	20	37.78	Moderate
	30	59.28	Strong
	40	84.69	Very strong
M. citrifolia	10	2.57	Weak
	20	10.93	Weak
	30	17.04	Weak
	40	32.15	Moderate
A. altilis	10	44.74	Moderate
	20	49.97	Moderate
	30	57.80	Strong
	40	60.69	Strong

Conclusion

According to the results of the research, all leaf extracts used can inhibit the growth of *S. rolfsii* Sacc. colonies. Because of the results of the statistical analysis, all treatments were significantly different from the control at the end of the observation. However, antifungal activity can be different even at the same concentration of different plant extracts. Antifungal activity in leaf extracts of *M. calabura, T. cattapa*, *S. oleina,* and *D. longan* is very strong, *A. altilis* has strong antifungal activity, whereas *M.*

citifolia has moderate antifungal activity. The results of this study are still on a laboratory scale (in vitro). To prove the real results, it is necessary to test it directly on plants (in vivo) with the right method. The results of the study can be used to control plant diseases at a lower price and do not cause negative effects, both on humans and the environment**,** as with chemical pesticides that have high toxicity**.**

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Research Article

Polyphenolic Profile and *in vitro* Antioxidant Activity of Three Algerian Date (*Phoenix dactylifera*) Varieties

Samir ZEROUAL[1], Randa GAOUAOUI[2], Amirouche DEGHIMA*[3]

[1,2,3] Laboratory of Genetics, Biotechnology and Valorization of Bio-resources, Faculty of Exact Sciences and Nature Sciences and the Life, University Mohamed Khider, Biskra, 07000, Algeria

[1]https://orcid.org/0000-0002-9066-7102, [2]https://orcid.org/0000-0003-4701-0081, [3]https://orcid.org/0000-0001-8046-1066

*Corresponding author e-mail: a.deghima@univ-biskra.dz

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Abstract: As known, dates are packed with antioxidants and bioactive compounds and provide various health benefits, as evidenced by their varying nitrite levels. Algerian dates, on the other hand, have not been thoroughly investigated for their bioactive compounds and overall antioxidant capacity. This research aims to tap into this potential by meticulously measuring total polyphenols, flavonoids, and condensed tannins in three popular Algerian varieties (*Phoenix dactylifera* L.) Ksiba, Hamraya, and Deglet Nour, and the determination of their antioxidant activity using (scavenging assays of 2,2-diphenyl-1-picryhydrazyl (DPPH) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, reducing power, and total antioxidant capacity. The analysis showed that the three varieties of date fruits are rich in total phenolics with an amount ranging between 71±51 and 7975±389 mg of gallic acid equivalent (GAE).100 g^{-1} of dry weight (DW), the flavonoid amount ranged from 31±3 to 767±4 mg of quercetin equivalent (QE) 100 g^{-1} DW and condensed tannins between 6± 2 and 653 ±64 mg of catechin equivalent (CE) 100 g^{-1} dry DW. The antiradical activity was quite promising and ranged between 0.5 and 24 µg AAE mg^{-1} extract for DPPH and between 2 and 113 µg AAE mg^{-1} extract for ABTS, while the reducing power and total antioxidant capacity values ranged from 16 to 154 µg ascorbic acid (AAE) mg^{-1} and 39 to 68 µg AAE mg^{-1} extract respectively. The results of this study show that Algerian date fruit can be regarded as a potential natural source of antioxidants, with ethyl acetate serving as the best extractant solvent, resulting in higher polyphenol content and antioxidant activities.

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1. Introduction

Date palm fruits (*Phoenix dactylifera* L.), commonly known as dates, hold significant importance in the Saharan regions as a crucial food source. Beyond its economic value, the date palm stands out for its rich nutritional content. Renowned for being a powerhouse of carbohydrates and antioxidants (Sawaya et al., 1983; Biglari et al., 2008), dates are extensively consumed worldwide and constitute a staple in the diets of numerous Arab countries (Hasnaoui et al., 2012). Moreover, beyond its culinary uses, the date fruit has long been employed in traditional medicine (Benchelah and Maka,

2008). It serves as a tonic for the muscular and nervous systems, recommended for conditions like asthenia, demineralization, tuberculosis, and anemia (Delille, 2007).

In Algeria, for instance, there are more than 13 million date palm trees and 940 varieties that have been recorded to date, with a total production of approximately 1.13 million tons annually (Harkat et al., 2022; Khirani et al., 2020). While extensive studies have scrutinized the nutritional and chemical composition of dates, including their carbohydrates, proteins, lipids, fibers, vitamins, and minerals, a significant gap exists in the exploration of the biological impact of phenolic components found in date palm fruits. Existing research is limited to specific varieties (Sawaya et al., 1982; Mohamed et al., 2014).

In, particular, it has been repeatedly reported that a diet based on date fruits, which are naturally enriched in plant polyphenols is effective against many diseases. In fact, date fruits, contain many classes of bioactive components, including phytochemicals such as flavonoids, carotenoids, polyphenols, phytoestrogens, and sterols. Therefore, there has been a surge of interest among researchers in the secondary metabolites of dates due to their potential health benefits, notably in guarding against cardiovascular diseases (Mansouri et al., 2005; Biglari et al., 2008). Phenolic compounds have been associated with various biological activities such as antibacterial, antioxidant, antiviral, anti-carcinogenic, anti-inflammatory, anti-allergic, and vasodilator effects. They also demonstrate inhibition against lipid peroxidation and platelet aggregation (Packer, 2001; Hurst, 2008).

Colorimetric procedures, such as the Folin-Ciocalteu assay, are widely used to assess phenolic content. They are quick, straightforward, and inexpensive, making them excellent for high-throughput screening and routine analysis. However, they lack selectivity and may be impacted by interfering chemicals in the date fruit extract. On the other side, chromatographic methods like HPLC offer excellent resolution and specificity, it enables the identification and measurement of individual phenolic chemicals. In this context, Colorimetric and chromatographic approaches provide complimentary advantages for determining polyphenol content in date fruits. As a result, combining both strategies can be beneficial. Colorimetry provides a quick and initial assessment of overall phenolic content, whereas HPLC provides extensive information about the types and amounts of particular phenolics found in the date fruit sample (Benouamane et al., 2022; Dominguez-lópez et al., 2023).

The current study aims to assess the quantity of phenolic compounds in three Algerian date varieties - Hamraya, Ksiba, and Deglet Nour - utilizing colorimetric and chromatographic analyses of diverse fruit extracts. Additionally, it seeks to estimate their antioxidant potential using various methods.

2. Material and Methods

2.1. Plant material and morphological characterization

The plant material used in this study consisted of three varieties of dates**,** Deglet Nour, Ksiba, and Hamraya growing in the Biskra region in Algeria. Deglet Nour variety was collected from the Tolga locality (GPS data: 34° 42′ 18″ N, 5° 23′ 01″ E), while the two other varieties Ksiba and Hamraya were collected from the Sidi Okba locality (GPS data: 34° 45′ 08″ N, 5° 53′ 25″ E) at full maturity and stored at 4 °C.

The morphological characterization of the whole date (IPIGRI, 2005) was carried out on a randomly sampled batch. The color was visually appreciated; the consistency was to the touch. The dimensions of the whole fruit and its kernel (length and width) were determined through a caliper, and the weight (pulp and core) was determined using a precision balance.

Elasticity is measured by inserting a date sample between the molar teeth, chewing, and calculating the force with initial chews. (sample pushback) (i.e. bite down evenly, evaluating the force required to compress at different degrees) (Singh et al., 2015).

2.2. Extracts preparation

The organic extracts were prepared following Diallo (2005) method. Initially, 200 grams of date paste were combined with a methanol-water blend (80/20: v/v) totaling 600 mL. Each mixture underwent an 18-hour agitation period and subsequent filtration through paper. The elimination of methanol was performed via a rotary evaporator operating at 45°C until complete evaporation.

The resultant crude extracts were reconstituted in 100 mL of water and sequentially subjected to extraction with four solvents of increasing polarities: Hexane, dichloromethane, ethyl acetate, and finally, butanol.

2.3. Extracts composition and characterization

The overall levels of polyphenols, flavonoids, and condensed tannins in three different Algerian date fruit extracts were measured. Total polyphenols were determined using the Folin-Ciocalteu method, as described by Waterman and Mole (1994) and Mansouri et al. (2005), and the results were expressed in gallic acid equivalents per 100 g of dry weight (DW). The flavonoid content was determined using methods described by Lamaison and Carnat (1991) and Bahorun (1997), with the results reported as quercetin equivalents per 100 g DW, and Condensed tannins were measured using Braca's (2002) method, and the results were presented as micrograms of ascorbic acid equivalents per milligram of extract (g AAE mg^{-1} extract).

High-Performance Liquid Chromatography (HPLC) was used to analyze different extracts to delve deeper into the polyphenolic composition of the three date fruit varieties. Organic extracts of date fruit were dissolved in methanol before HPLC analysis, which was carried out under the following conditions: C18 column (4.6 x 150 mm, 5 m), detector with a wavelength of 254 nm, mobile phase of acidified water/methanol, injection volume of 20 microliters, flow rate of 1 ml/min, and column temperature maintained at 25°C. Each sample's compound identification was based on detecting differences in retention times between the components determined and the standards.

2.4. Antioxidant activity

2.4.1. Antiradical activity

The antiradical activity of three different Algerian date extracts was measured against 2,2-diphenyl-1-picryhydrazyl (DPPH) as described by Braca (2002) and against 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) using the methods of Re et al. (1999). The activity was measured using an ascorbic acid standard curve in both tests, and the results were expressed as g ascorbic acid equivalents per mg of extract (g AAE mg^{-1} extract).

2.4.2. Reducing power

The study also examined the electron-donating mechanism as another aspect of antioxidant activity. The total antioxidant activity of the various extracts derived from the selected date fruits was determined using the method proposed by Prieto et al. (1999). Furthermore, their reducing power was assessed using the Bougandoura and Bendimerad (2012) methodology. These results were quantified and expressed as micrograms of ascorbic acid equivalents per milligram of extract (g AAE mg^{-1} extract).

2.5. Statistical analysis

Data were presented as the mean ± standard deviation (SD) (n=3). Normality distribution of the data was validated using the Shapiro test and the determination of significant differences among groups was made via two-way analysis of variance (ANOVA) and the Tukey test was selected as a post hoc ($p<0.05$) using GraphPad Prism 7.00 software (GraphPad Software Inc., San Diego, CA, USA). The Correlation tests were also performed using the same software to determine the relationship between phenolic compounds and antioxidant activity. The correlation was defined by Pearson’s correlation coefficient after validation of the normality distribution of the data using the Shapiro test.

3. Results and Discussion

3.1. Physical characteristics of fruits

Table 1 shows the physical characteristics of the studied varieties. Generally, Deglet Nour, Hamraya and Ksiba dates have an aromatic taste. When ripe, dates are brown, red, and somewhat dark brown, respectively, with a brown color of seeds and a semi-soft consistency, the peel is smooth and shiny (Figure 1).

Figure 1. Three Algerian date varieties; a) Deglet Nour; b) Ksiba and c) Hamraya.

Sensory properties are important in evaluations, which are typically conducted by expert panels, trained panels, and consumer panels. Ben Ismail et al. (2013) carried out a sensory profiling study on seven date fruit cultivars in Tunisia, revealing significant morphological and physicochemical diversity among the tested varieties. According to Ismail et al. (2001), understanding the physicochemical, mechanical, structural, textural, and sensory properties of dates is critical for their processing, storage stability, and consumer acceptance. Dates' preferred sensory quality attributes at the "tamar" stage of maturity were ranked as high for color and appearance, medium for fruit size, chewiness, solubility, and flesh thickness, and low for elasticity, mouthfeel, and shear force.

Table 1. Physical properties of Ksiba, Hamraya, and Deglet Nour varieties

Character of the fruit	Ksiba	Hamraya	DegletNour
Fruit shape	Egg-shaped	Egg-shaped	Egg-shaped
Color	Dark brown	Red	Brown
Consistency	Semi-soft	Semi-soft	Semi-soft
Plasticity	Tender	Tender	Tender
Texture	fibrous	fibrous	Smooth
Core shape	egg-shaped	egg-shaped	egg-shaped
Core color	Brown	Brown	Brown
Fruit quality	Common	Common	Common

3.2. Total polyphenols content (TPC)

Colorimetric analysis revealed significant differences in the total polyphenol content of the three date fruit varieties (Table 2). Notably, ethyl acetate proved the most effective extraction solvent for all three, showcasing the variety-specific influence on polyphenol extraction. Hamraya emerged as the richest in terms of polyphenol abundance, boasting a content ranging from 371 ± 20 mg GAE 100 g^{-1} DW (n-butanol extract) to a whopping 7975 ± 389 mg GAE 100 g^{-1} DW (ethyl acetate extract). This translates to a remarkable 95% confidence interval of -2220 to 8438, underscoring Hamraya's potential as a potent source of polyphenols. In contrast, Ksiba displayed the lowest polyphenol levels, with values ranging from 71 ± 51 mg GAE 100 g^{-1} DW (hexane extract) to 5200 ± 404 mg GAE 100 g^{-1} DW (ethyl acetate extract) and a 95% CI of -2129 to 5447. Deglet Nour GAE? Our findings align with Benmeddour et al. (2013) observations, who reported total polyphenol contents between 226 and 955 mg GAE 100 g^{-1} DW for ten Algerian date cultivars. This suggests similarities in polyphenol profiles within geographically close regions.

However, Bensaçi et al. (2021) reported lower values (154-278 mg GAE 100 g^{-1} DW) for four Algerian date varieties from Ouargla, possibly due to the influence of extraction solvent (hydromethanolic mixture) and/or regional variations. Interestingly, Kchaou et al. (2013) documented values between 199 and 576 mg GAE 100 g^{-1} on a fresh weight basis for some Tunisian cultivars, highlighting the impact of moisture content on reported values.

Table 2. Total polyphenol content of the extracts of the three date varieties (in milligram gallic acid equivalent (GAE) 100 g^{-1} dry weight date fruit)

Extract	DegletNour	Ksiba	Hamraya
Hexane	$1390\pm207^{1,2,a}$	$71\pm51^{1,b}$	$1725\pm339^{1,a}$
Dichloromethane	$1668\pm137^{2,b}$	$554\pm76^{1,2,c}$	$2365\pm212^{2,a}$
Ethyl acetate	$5972\pm434^{3,b}$	$5200\pm404^{3,c}$	$7975\pm389^{3,a}$
***n*-butanol**	$947\pm76^{1,4,a}$	$811\pm66^{2,b}$	$371\pm20^{4,bc}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

Various factors might be responsible for the observed differences such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage conditions, agricultural methods, process and stabilization conditions, climatic conditions, use of different analytical methods, and use of different phenolic acid standards (Besbes et al., 2004; Al-Farsi et al., 2007; Besbes et al., 2009; Ahmed et al., 2021; Benmehaia et al., 2022). Compared to popular fruits like cranberries (607 mg CE 100 g^{-1} DW), plums (551 mg CE 100 g^{-1} DW), and apricots (333 mg CE 100 g^{-1} DW) (Vinson et al., 2005), Algerian date varieties pack a remarkable punch of total polyphenols content.

3.3. Flavonoid content (TFC)

The flavonoid contents of the different extracts from the three varieties of dates are shown in Table 3.

Table 3. Average flavonoid content of the extracts of the three date varieties (in micrograms equivalent of quercetin (QE) 100 g^{-1} dry weight date fruit)

Extract	Deglet Nour	Ksiba	Hamraya
Hexane	$98\pm2^{1,a}$	$31\pm3^{1,b}$	$71\pm1^{1,c}$
Dichloromethane	$176\pm3^{2,a}$	$113\pm1^{2,b}$	$139\pm 2^{2,c}$
Ethyl acetate	$208\pm4^{3,a}$	$767\pm4^{3,b}$	$710\pm 21^{3,c}$
n-butanol	$117\pm6^{4,a}$	$162\pm2^{4,b}$	$49\pm4^{4,c}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

Continuing the trend observed for total polyphenols, ethyl acetate exhibited superior efficacy in extracting flavonoids from all three date fruit varieties (Table 3). Ksiba variety displayed the highest total flavonoid content, attaining a remarkable 767 ± 4 mg quercetin equivalents (QE) 100 g^{-1} dry weight (DW) within a 95% confidence interval (CI) of -268 to 804. Conversely, Deglet Nour exhibited the lowest content, averaging at 208 ± 4 mg QE 100 g^{-1} DW. Comparative analysis with existing literature reveals significant variations in flavonoid content among date cultivars. Bensaçi et al. (2021) reported considerably lower levels, ranging from 3 to 12 mg QE 100 g^{-1} DW (95% CI: 68.4 to 231) in four different Algerian cultivars. This observation aligns with the findings of Biglari et al. (2008) and Hasnaoui et al. (2012) who documented low total flavonoids in Moroccan and Iranian date cultivars. Our findings, however, resonate more closely with the work of Benmeddour et al. (2013), Kchaou et al. (2014), and Bouhlali et al. (2015). These studies reported total flavonoid content ranging from 15 to 300 mg QE 100 g^{-1}, 59 to 214 catechin equivalents (CE) 100 g^{-1} extract, and 69 to 209 mg rutin equivalents (RE) 100 g^{-1} DW in Algerian, Tunisian, and Moroccan date varieties, respectively.

3.4. Condensed tannins content (CTC)

The condensed tannin contents of the three varieties are presented in Table 4.

Table 4. Average condensed tannins content of the extract of the three date varieties (in micrograms equivalent of catechin (CE) 100 g^{-1} dry weight date fruit)

Extract	Deglet Nour	Ksiba	Hamraya
Hexane	$416\pm19^{1,a}$	$184\pm16^{1,b}$	$6\pm2^{1,c}$
Dichloromethane	$59\pm2^{2,a}$	$168\pm0^{1,b416}$	$150\pm11^{2,b}$
Ethyl acetate	$378\pm99^{1,a}$	$215\pm67^{1,b}$	$653\pm64^{3,c}$
n-butanol	$89\pm2^{2,a}$	$66\pm23^{2,a}$	$46\pm5^{1,a}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

For the Hamraya and Ksiba varieties, ethyl acetate was the best solvent in the extraction of condensed tannins, with the Hamraya variety presenting the highest CTC content among the three varieties (653±64 mg CE 100 g^{-1} DW; 95% CI: -262.1 to 689.6). However, for Deglet Nour the amounts of CTC extracted using hexane and ethyl acetate were statistically similar and higher than the remaining solvents. The tannin content in this study is significantly higher than that reported by Bouhlali et al. (2015) and Alahyane et al. (2018). Their research found a range of 57-92 mg CE 100 g^{-1} dry weight and 5-152 mg CE 100 g^{-1} dry weight, in some Moroccan cultivars. On the other hand, Benmeddour et al. (2013) reported similar results for the condensed tannin content in several Algerian date varieties (82 to 525 mg CE 100 g^{-1} DW). however, our CTC values, are lower than those reported by Sawaya et al. (1982). In their study of 25 Saudi Arabian cultivars, they observed significant variations in total tannin content, ranging from 600 to 2700 mg per 100 g of dry weight at the final stage of fruit maturity. Note that, tannic acid and procyanidin B2 were detected as hydrolyzable and condensed tannins, respectively by HPLC analysis (Dassamiour et al., 2022) and during storage of dates at room temperature, the relative quality of simple polyphenols and soluble tannins decreased. Flavones disappeared by giving brown oxidized compounds (Mohamed et al., 1985). Also, the level of these phenolic components was observed to be stable during storage at 4°C, as reported by Benmehaia et al. (2022).

3.5. HPLC analysis

Dichloromethane and ethyl acetate extracts of the three varieties were analyzed for their phenolic content using HPLC, and the results are shown in Table 5.

Table 5. Identified compounds by HPLC in Dichloromethane and Ethyl acetate extracts of the three date varieties

Phenolic compounds	Reten. Time [min]	Deglet Nour		Ksiba		Hamraya	
		Dichloromethane	Ethyl acetate	Dichloromethane	Ethyl acetate	Dichloromethane	Ethyl acetate
P.coumaric acid	25.21	-	+	-	-	+	-
3-hydroxy-4-cinnamic acid	28.28	+	-	-	-	+	+
cafeic acid	20.52	+	+	+	+	-	-
Ferulic acid	26.6	-	-	-	-	-	-
Gallic acid	6.5-7	-	-	+	-	-	-
M Anisic acid	33.03	+	+	-	-	+	+
Salicylic acid	30.74	-	-	-	-	-	-
Syringic acid	21.96	+	-	-	-	-	-
Trans-2.4-diméthoxycinnamic acid	39.28	+	-	-	-	-	-
Trans-cinnamic acid	25.17	-	-	-	+	-	-
Vanillic acid	22.7	-	-	-	-	-	-
Catechin	21.55	-	-	-	-	-	-
Epicatechin	22.50	-	-	-	-	+	-
Euleropein	32.36	-	-	-	-	-	-
Kaempferol	41.1	-	-	-	-	-	-
Myricetin	34-41	+	+	+	+	+	+
Quercetin	36.85	-	-	-	-	-	-
Berberine	24.52	-	-	-	-	-	-
Resorcinol	10.40	-	-	-	-	-	-
Rutin	30.68	-	-	-	-	-	-

Analysis of the phenolic compounds of dichloromethane and ethyl acetate in Ksiba, Hamraya, and Deglet Nour extracts by HPLC allowed the identification of the following phenolic acids: gallic acid, coumaric acid, manisic acid, caffeic acid, syringic acid, 3-hydroxy-4-cinnamic acid, Trans-cinnamic acid and trans-2,4-dimetoxic acid. The presence of two types of flavonoids was highlighted in the ECh and EAc extracts as myricetin and epicatechin compounds(Table 5). The table illustrates the diversity in polyphenolic compounds present in the different date varieties and their extracts. Some compounds are unique to specific varieties or extraction solvents, while others are common. Dichloromethane and ethyl acetate extracts vary in terms of the polyphenols they contain as the presence or absence of certain compounds can be attributed to the choice of solvent, highlighting the importance of solvent selection in polyphenol extraction. Each date variety exhibits a unique polyphenolic profile. For example, Hamraya contains quercetin and myricetin, which are not found in the other two varieties, Deglet Nour and Ksiba. While, some compounds, like 3-hydroxy-4-cinnamic acid and M Anisic acid, are common in both dichloromethane and ethyl acetate extracts of all three varieties. The presence of gallic acid and catechin, varies between varieties, indicating that the specific date variety influences the composition of polyphenols. Several compounds, including ferulic acid, salicylic acid, vanillic acid, kaempferol, berberine, resorcinol, and rutin, are absent in all the extracts, suggesting that they may not be prevalent in these date varieties. In conclusion, the HPLC results reveal that the three date varieties (Deglet Nour, Ksiba, and Hamraya) exhibit variations in their polyphenolic composition. The choice of extraction solvent (dichloromethane or ethyl acetate) also influences the presence of specific compounds. This information is valuable for understanding the diversity of bioactive compounds in different date varieties and can have implications for their potential health benefits and various applications in the food and pharmaceutical industries.

3.6. Antioxidant activity

The antioxidant effect of the date fruit extracts was measured using different methods including FRAP, total antioxidant TAC, DPPH, and ABTS free radical scavenging activity.

3.6.1. DPPH and ABTS radical scavenging capacities

The results of the radical scavenging ability (Table 6) revealed that the Ksiba and Hamraya had strong free radical scavenging ability, especially when ethyl acetate was used as solvent. The two varieties presented a similar DPPH scavenging capacity of 24±0.1 µg AAE.mg^{-1} extract (95% CI: -4.506 to 30.88). For Deglet Nour both dichloromethane and ethyl acetate gave the best DPPH scavenging capacity (17±0.04 µg AAE mg^{-1} extract; 95% CI: -0.32to 22.33), yet, it remains lower compared to the two other varieties. Hexane and *n*-butanol extracts of the three varieties exhibited low DPPH anti-radical activity.

Table 6. DPPH and ABTS Antioxidant capacities of three Algerian date cultivars

	DPPH (µg AAE mg^{-1} extract)				**ABTS (µg AAE mg^{-1} extract)**			
Variety/ Solvent	Hxn	DCL	EA	n-Btn	Hxn	DCL	EA	n-Btn
Deglet Nour	3±0.04^{1,c}	17±0.04^{2,a}	17±0.1^{2,a}	7±0.04^{2,b}	7±0.3^{1,d}	82±2^{1,b}	113±0.2^{1,a}	18±0.2^{1,c}
Ksiba	0.5±0.01^{2,d}	10±0.2^{3,b}	24±0.1^{1,a}	8±1^{1,c}	2±0.4^{2,d}	57±3^{3,b}	91±0.4^{2,a}	15±0.6^{2,c}
Hamraya	0.75±0.2^{2,d}	21±0.04^{1,b}	24±0.1^{1,a}	7±1^{2,c}	5±0.1^{1,c}	74±0.2^{2,b}	79±0.5^{3,a}	6±0.3^{3,c}

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

Our findings regarding DPPH radical scavenging activity (32-86%) for the three date varieties align with Benmeddour et al. (2013) who reported similar values in ten Algerian cultivars. This finding further corroborates the observations of Mansouri et al. (2005) and Hasan et al. (2010) highlighting the effective DPPH scavenging capabilities of date fruits. The ABTS method assesses an antioxidant's ability to neutralize the green-blue cationic radical ABTS+ (Antolovich et al., 2002). Interestingly, all three varieties exhibited stronger ABTS scavenging compared to DPPH, with ethyl acetate extracts demonstrating the highest activity. Deglet Nour emerged as the most efficient scavenger (113 ± 0.2 µg

AAE mg^{-1} extract), followed by Ksiba and Hamraya. Similar to DPPH, hexane, and n-butanol extracts exhibited weaker ABTS activity, ranging from 5 ± 0.1 µg AAE mg^{-1} for Hamraya's hexane extract to 18 ± 0.2 µg AAE mg^{-1} for Deglet Nour's n-butanol extract. Our ABTS findings resonate with Hasan et al. (2010) and Benmeddour (2013) who also reported potent ABTS scavenging activities in date fruits. The DPPH values were higher compared to Siddeeg et al. (2019) who documented lower activity (43-76%) in the ethanolic and methanolic flesh extracts of the Sukkari variety (Saudi Arabia/Iraq). Notably, Siddeeg et al. (2019) also confirmed strong ABTS activity in their date samples. The observed disparities in scavenging activity potentially stem from varietal differences, extraction solvents employed, and specific date fruit regions (Deghima et al., 2020). The abundant phenolic and flavonoid compounds in date fruits are recognized as potent hydrogen/electron donors, likely contributing to their effective radical scavenging capabilities.

3.6.2. Reducing power

The reducing power of the different solvents' extracts from the three varieties is presented in Table 7.

Table 7. The reducing power of the three varieties expressed as µg ascorbic acid equivalent (AAE) mg^{-1} of extract

Extract	Reducing power (µg AAE mg^{-1} extract)		
	Ksiba	Hamraya	Deglet-Nour
Hexan	21 ± 0.2^{4b}	18 ± 0.3^{3c}	30 ± 1^{3a}
Dichloromethan	29 ± 0.0^{3b}	60 ± 0.6^{1a}	19 ± 0.1^{4c}
Ethyl acetat	50 ± 0.2^{1c}	55 ± 0.4^{2b}	154 ± 1^{1a}
n-butanol	31 ± 0.4^{2c}	16 ± 0.5^{4b}	38 ± 0.3^{2a}

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

This method was developed to assess extracts' ability to reduce ferric iron ions (Fe^{3+}) found in ferrous iron complexes (Fe^{2+}). In fact, Fe^{3+} is involved in the formation of the hydroxyl radical (Benzie and Strain., 1996). The ethyl acetate extracts of studied varieties presented an important reducing power, with the highest value registered for the Deglet Nour variety (154±1 µg AAE mg^{-1} extract; 95% CI:-39.97 to 160.5) and the lowest registered for the Ksiba variety (50±0.2 µg AAE mg^{-1} extract; 95% CI:13.20 to 52.30). While for Hamraya variety the dichloromethane was the solvent with the best reducing power (60±0.6 µg AAE mg^{-1} extract; 95% CI:-0.1212 to 74.62). Benmeddour et al., (2013) reported higher values (272 to 1175 mg GAE 100 g^{-1} DW) for ten Algerian cultivars studied. Comparable results were reported by Zeroual et al. (2020), who observed that the best reducer was obtained by ethyl acetate extract with 118 µg EAA mg^{-1} extract from the Deglet Nour variety. The reducing power of a sample is determined by the electron transfer capacities of the reducers (antioxidants) in that sample. Because our extracts are high in electron-donating polyphenols, we may speculate that these compounds are responsible for our extracts' reducing power, which allows them to scavenge free radicals and function as chain-breaking antioxidants. They may also limit peroxide production and help in the regeneration of other damaged antioxidants (Deghima et al., 2020).

3.6.3. Total antioxidant capacity

The total antioxidant capacity of the different organic extracts from the three varieties is shown in Table 8.

The phosphomolybdate method quantifies the total antioxidant capacity (TAC) of the extracts, exploiting the reduction of Mo(VI) to Mo(V) in the presence of antioxidants and the subsequent formation of a green-colored phosphate-Mo(V) complex (Sahua and Laloo, 2011). Notably, the solvent employed significantly impacted the TAC values across varieties. Ksiba exhibited the highest TAC values (62 ± 0.1 and 61 ± 0.1 µg AAE mg^{-1} extract) for hexane and dichloromethane extracts, respectively. This may be attributed to the potential presence of lipophilic antioxidants in this variety (Deghima et al., 2020). Conversely, dichloromethane and ethyl acetate emerged as the superior solvents for Deglet Nour and Hamraya varieties. Overall, Deglet Nour achieved the highest TAC value (68 ± 3

µg AAE mg^{-1} extract, 95% CI: 37.99 to 69.01) associated with its dichloromethane extract. It is noteworthy that beyond polyphenols, other bioactive compounds such as carotenoids and α-tocopherol present in non-polar extracts (hexane and dichloromethane) might contribute to their overall antioxidant activity (Deghima et al., 2020). Our findings contrast with Ali Haimoud et al. (2016) who documented lower TAC values for dates, ranging from 43 to 90 µmol ascorbic acid g^{-1} extract, with Ali Ourached cultivar showcasing the highest recorded level.

Table 8. Total antioxidant capacity of the three varieties expressed as µg ascorbic acid equivalent (AAE) mg^{-1} of extract

Extract	Total antioxidant capacity (µg AAE mg^{-1} extract)		
	Ksiba	Hamraya	Deglet-Nour
Hexane	$62\pm0.1^{1,a}$	$41\pm0.5^{3,c}$	$49\pm0.6^{2,b}$
Dichloromethane	$61\pm0.1^{1,b}$	$63\pm3.8^{1,b}$	$68\pm3^{1,a}$
Ethyl acetate	$45\pm1.1^{2,c}$	$66\pm2.1^{1,a}$	$50\pm1.2^{2,b}$
n-butanol	$39\pm2.4^{3,b}$	$50\pm1.5^{2,a}$	$47\pm0.2^{2,a}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

3.7. Correlation study

A correlation study was performed to link the bioactive compounds to the observed activities and the results are presented in Table 9.

Table 9. Linear correlation coefficients between phenol content and antioxidant activity of extracts of *Phoenix dactylifera* L.

	TPC	TFC	CTC
DPPH	-0.3505	-0.38591	-0.12956
FRAP	0.74907	0.67693	0.67297
ABTS	0.93210	0.82561	0.64201
TAC	0.35289	-0.06963	0.25612

TPC: Phenolic compounds; TFC: Flavonoids; CTC: condensed tannins; FRAP: Reducing power; DPPH: Scavenger activity of the radical DPPH; ABTS: Scavenger activity of radical ABTS; TAC: Total antioxidant capacity.

Statistically significant positive correlations were observed between the FRAP and ABTS antioxidant capacities and the total phenolic content ($r = 0.75$ and $r = 0.93$, respectively). These findings corroborate those of Mansouri et al. (2005) and Biglari et al. (2008) who demonstrated a substantial contribution of phenolic compounds to the overall antioxidant potential of date fruits.

A similar trend emerged for the relationships between flavonoid content and condensed tannins with both FRAP and ABTS capacities ($r = 0.68$ and $r = 0.67$ for FRAP; $r = 0.83$ and $r = 0.64$ for ABTS). Interestingly, a weak negative correlation was observed between total antioxidant capacity and flavonoid content ($r = -0.06963$). Intriguingly, negative correlations were observed between total condensed tannins, phenolic compounds, and flavonoids concerning the DPPH antioxidant activity ($r = -0.13$, $r = -0.36$, and $r = -0.39$, respectively). These findings resonate with those of Ramchoun et al. (2017) and Alahyane et al. (2019) who reported similar negative correlations between these bioactive compounds and the DPPH scavenging capacity in their studies.

4. Conclusion

The study of the physicochemical and biochemical characteristics of three date varieties, namely: Hamraya, and Ksiba, harvested in the region of Sidi Okba, and Deglet Nour harvested in Tolga, revealed different characteristics from one cultivar to another. The morphological and organoleptic characteristics showed that the half-marrow dates have a dark color, a soft texture, and a fibrous appearance. The presence of considerable amounts of total phenolic compounds was revealed, especially in the more polar extracts, like ethyl acetate where the amount of polyphenols reached 7975 ±389 mg GAE/ mg extract for the Hamraya variety whereas the condensed tannin and flavonoid levels were

relatively lower with ethyl acetate being the best extractant solvent, except for Deglet Nour where hexane was the best extractant with an amount of 416±19 mg CE/ mg extract. High antioxidant activity was found in ethyl acetate extracts and dichloromethane compared to the hexane and n-butanol extracts which was positively correlated with phenolic content. Finally, the 3-hydroxy-4-cinnamic acid and M. Anisic acid, are common in both dichloromethane and ethyl acetate extracts of all three varieties.

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Research Article

The Influence of Slope Exposure, Profile Depth and Erosion Processes on Changes in the Content of Potassium, Phosphorus and Humus in Brown Soils of Mountain Pastures of Uzbekistan

Abdusamat AKHATOV[1], Viktoriya NURMATOVA*[2], Bahora USMONOVA[3]

[1,2]Research Institute of Environment and Environmental Protection Technologies under the Ministry of Ecology, Environmental Protection and Climate Change of the Republic of Uzbekistan, Bunyodkor Avenue, bldg 7a, 100043, Tashkent, Republic of Uzbekistan
[3]Jizzakh Polytechnic Institute, st. Halklar dustligi, 4130100, Jizzakh, Republic of Uzbekistan

[1]https://orcid.org/ 0000-0002-4895-2372, [2]https://orcid.org/0000-0001-9610-1727, [3]https://orcid.org/0000-0002-0174-4699

*Corresponding author e-mail: nurmatoffkennel@gmail.com

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Abstract: Humus, potassium, and phosphorus are key components of soil that play crucial roles in ecosystem productivity, plant growth, and development. They control a wide range of processes, including greenhouse gas fluxes, nutrient cycling, infiltration, and water retention. This article presents the results of evaluating humus, potassium (K), and phosphorus (P) content in the profile of brown soils in mountain pastures of Uzbekistan, as well as their distribution within these soils. The brown soils studied in the mountain pastures of Uzbekistan have a loamy granulometric composition, with the clay fraction not exceeding 20%. The carbonate content is low (2.5-9%), with the maximum amount found in the carbonate horizon. The soils exhibit weak leaching. The total humus content in the upper horizon varies from 1 to 6.6%. It was observed that the soils on the more moistened northern and western slopes contain more humus than those on the southern and eastern slopes, indicating a dependence of high humus content on slope exposition. For the first time, the article allocates phosphorus and potassium of near, labile, and potential reserves (as a percentage of the total content) to estimate the change in the nature of brown soils under economic use. It was found that the potential reserve of phosphorus and potassium (35.5-90%) prevails in soils. Further study of the features of humus, potassium, and phosphorus, their accumulation, and restoration in brown soils is essential for developing recommendations for the rational use, anti-erosion protection, and increased productivity of mountain pastures in Uzbekistan.

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1. Introduction

Humus, potassium, and phosphorus are critical soil components that profoundly impact ecosystem productivity, plant growth, and development. They regulate various processes, including greenhouse gas fluxes, nutrient cycling, infiltration, and water retention (Lehmann et al., 2020; Kassa et al., 2021). Their presence in soils effectively reflects both the dynamic and static changes in soil formation conditions, including those induced by anthropogenic activities like soil contamination. Agricultural land cultivation and its intensive use often result in the loss of soil fertility (Haddaway et

al., 2017; Chen et al., 2020; Franzluebbers, 2021). Therefore, reducing the rate of soil dehumidification while enhancing agricultural production efficiency is a critical challenge in land management, particularly in regions facing agricultural land scarcity due to unfavorable climatic conditions or topography.

In Uzbekistan, agriculture is a key sector of the economy, implementing a development strategy aimed at ensuring the country's food security. The country's territory is characterized by vast areas of infertile desert soils unsuitable for cultivation. The most favorable conditions for agriculture are found in river valleys, where farming on irrigated alluvial soils is traditional, and in the gentle sub-mountain zones and foothill plains, where low-productivity desert steppes on serozem are used as pastures and meadow-serozem soils as irrigated arable land (Kuziev et al., 2016). The Law on Pastures (2019) and the Agriculture Development Strategy of the Republic of Uzbekistan (2019) for 2020-2030 (both adopted in 2019) have spurred the more intensive use of mountainous areas in agriculture as pastures and hayfields. However, excessive grazing, leading to overgrazing, disrupts slope stability, promoting soil erosion, dehumidification, and a reduction in arable land area, restoration of which is challenging in mountainous terrain (Chen et al., 2019; Dou et al., 2022).

Despite numerous studies on humus, phosphorus, and potassium content and the factors influencing their dynamics in local soils (Akhatov et al., 2018; Kadirova et al., 2018; Normuratov et al., 2018; Raupova, 2018; Raupova and Abdullayev, 2018; Akhatov and Murodova, 2021; Askarov et al., 2021; Rakhmatov et al., 2021; Ruzmetov, 2021; Tashkuziev and Shadieva, 2021; Aliboeva et al., 2022; Turaev et al., 2022; Nabieva et al., 2023; Gafurova et al., 2024), the subject remains topical, especially given the current climate change trends in the region (Li et al., 2020). Of particular interest are the depletion of humus, potassium, and phosphorus reserves and the causes and factors of dehumidification of brown soils, which are widespread in the middle and low mountains and constitute the main part of the country's land fund. Therefore, this research aims to estimate the content of humus, potassium, and phosphorus, and their distribution in the profile of brown soils in mountain pastures in Uzbekistan.

2. Material and Methods

2.1. Areas and objects of research

The research was conducted in seven agricultural districts of the country between 2020 and 2023 (Table 1). Brown soils under mountain pastures (Gorbunov et al., 1975) were selected as the object of research. According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015), the studied soils belong to Cambisols and Kastanozems, with the most eroded variants belonging to Leptosols.

Table1. Geographic location of key soil profile cuts

№ section	Coordinates		Absolute height, m	Georeferencing of the section
	Latitude	Longitude		
74	40°57′41′′N	70°46′05′′E	1044	Kuraminsky ridge, western macro slope, near the settlement of Chodak, slope of western exposure, typical brown, calcareous, slightly eroded, medium humus, medium loamy soil on loess
54	39°52′21′′N	68°22′24′′E	924	Turkestan ridge, northern macro slope, near the settlement of Zomin, slope of western exposure, brown soil, leached, slightly eroded, medium humus, medium loamy on loess
40	39°30′38′′N	66°44′11′′E	885	Zarafshan ridge, western spur, northern macro slope, near the village of Sazagon, slope of eastern exposure, brown leached, slightly eroded, medium humus, medium loamy soil on loess loam
66	41°35′29′′N	70°07′17′′E	1382	Western Tien Shan, Koksu Range, southwestern slope, near the settlement of Burchmulla, slope of southern exposure, brown leached, medium eroded, medium humus, medium loamy soil on loess-like deluvium of granites

Table1. Geographic location of key soil profile cuts (continued)

№ section	Coordinates		Absolute height, m	Georeferencing of the section
	Latitude	Longitude		
28	40°25′47′′N	66°02′20′′E	839	Nuratau mountains (South-Nurata ridge), Aktau ridge, northern macro slope near the village of Chuya, slope of the northern exposure, brown leached, slightly eroded, medium humus, medium loamy soil on loess-like loam
13	39°12′54′′N	67°04′10′′E	1112	Zarafshan ridge, southwestern spur, western macro slope, near the village of Varganza, south-facing slope, south-facing slope, brown leached, medium eroded, medium humus, medium loamy soil on loess-like loam
1	37°42'24′′N	66°44′55′′E	824	Hissar ridge, southwestern spurs, Kugitangtau ridge, eastern macro slope, near the village of Pashkhurt, slope of southern exposure, slope of southern exposure brown leached, eroded, low humus, medium loamy soil on loess

Brown soils are widespread in the mountains of south-eastern and eastern Uzbekistan, where they occupy slopes of different steepness and exposition at altitudes from 800 to 1800 m. Soil-forming rocks are dealluvial and loess loams, carbonate rocks (Gorbunov et al., 1975). The climatic conditions are strictly continental, with an absolute maximum temperature of +45 ºC, and an absolute minimum of -30 ºC. Total solar radiation in the mountaisreaches up to 8350 MJ/m^2. In the foothills, precipitation ranges from 300-400 mm, increasing to 600-800 mm on the western and south-western slopes of mountain ranges. The highest amount of precipitation occurs in spring, reaching up to 600 mm, while the summer season experiences the lowest amount, with less than 100 mm. In the foothills (from 300-400 to 600-1000 m above sea level), stable snow cover does not form every winter. In the mountain zone (above 600-1000 m above sea level), snow cover begins at 800-1000 m and can reach a maximum thickness exceeding 1.5 m in some locations (Chub, 2007). The vegetation is represented by herb-bunchgrass steppes including pubescent wheatgrass (*Elytrigia aucheri* (Boiss.) Nevski), bulbous barley (*Hordeum bulbosum* L.), bulbous bluegrass (*Poa bulbosa* L.), bonfire (*Bromus* L.), prangos tall *(Prangos pabularia* Lindl.*)*, ferula (*Ferul*aspp.), cocksfoot (*Dactylis glomerata* L.), mortune oriental (*Eremopyrum orientale* L. Jaub. & Spach), bindweed pilose (*Convolvulus subhirsutus* Regel & Schmalh), cousinias hady (*Cousinia umbrosa* (Bunge) Kuntze) and others. On dry slopes, the growth of juniper-shrub forests with (*Juniperus turkestanica* Kom.), honeysuckle (*Lonicera* spp.), briar (*Rosa* spp.) is often observed (Gorbunov et al., 1975). In the whole country, desert pastures occupy 77%, piedmont pastures - 16%, mountain pastures - 4%, and high-mountain pastures - 3%. The natural foothill and mountain pastures of Uzbekistan occupy about 5 million ha, but their yields are low and amount to 3-7cwt ha^{-1}of air-dry mass, but in some areas and under favorable weather conditions this number can reach 12 cwt ha^{-1}.

In the Western Tien-Shan, brown soils typically form a distinct altitudinal belt. In the lower part of the belt, there is a subtype of slightly leached brown soils, while in the upper part, a subtype of typical brown soils is found. These soils are prone to erosion, and therefore, eroded types are often encountered to varying degrees. The typical profile of brown soils is characterized by a significant thickness, well-differentiated into humus-accumulative, metamorphic (median), and carbonate-illuvial horizons (Gorbunov et al., 1975).

The humus-accumulative horizon exhibits a gray or dark gray color with a brown tint, a loamy to medium loamy composition, a cloddy-flaser structure, and is saturated with roots (grassy sod). Carbonate types of soils effervesce at 10% hydrochloric acid from the surface. The median horizon is distinguished by its brown coloring, clayey granulometric composition, and nutty compound structure. The illuvial-carbonate horizon is compacted and easily identified by its whitish color, containing abundant new formations of secondary carbonates (loess with lime nodules, impregnation, pseudomycelium).

In strongly eroded brown soils, the profile is often broken to the carbonate horizon, with the upper part of the profile frequently absent. In moderately and slightly eroded soils, the upper part of the profile is fragmentary, and the differentiation into horizons is poorly expressed.

Seven profile cuts of brown soils with similar granulometric composition on slopes of different exposures were laid out for the studies.

2.1.1. Research methods

The objectives of the research included field studies of the morphological profiles of brown soils, soil sampling, and subsequent laboratory and analytical work. Field studies, sampling, and sample preparation followed generally accepted methods (Arinushkina, 1970; Rozanov, 1983). The degree of soil erodibility was evaluated according to Sobolev (1961). The total organic carbon and humus content were determined in the samples using the method of Tyurin (1937); inorganic carbon was determined using the method of Arinushkina (1970). Clay fractions were separated by centrifugation according to the method of Shaimukhometov and Voronina (1972). The carbonate content was determined by the acidimetric method (Scientific research Institute for Cotton Growing SoyuzNIKhI, 1963). The total potassium and phosphorus content was determined in one sample, with subsequent colorimetric determination using the method of Maltseva (Scientific research Institute for Cotton Growing SoyuzNIKhI, 1963). Mobile potassium and phosphorus (direct reserve) in soils and clay fractions were determined in a 1% $(NH_4)_2CO_2$ carbon-ammonium extract using the method of Machigin (Scientific research Institute for Cotton Growing SoyuzNIKhI, 1963).

The primary focus of the research was on a more detailed division into so-called potassium and phosphorus reserves and their calculation, carried out according to the method of calculating nutritional elements by Gorbunov (1978). In calculating reserves, the initial values were the overall content of potassium and phosphorus in the fraction less than 0.001 mm, in the agrochemical extract, and the quantity of the fraction less than 0.001 mm in soil, all expressed as a percentage. All calculations were made in milligrams per 100 g. The direct reserve (DR) represents the water-soluble, mobile form and is equal to the amount of potassium (phosphorus) in a 1% carbon-ammonium solution. The near reserve (NR) was defined as the potassium (phosphorus) in the clay fraction, considering a clay fraction of <0.001 mm (%). The potential reserve (PR) was obtained by subtracting the immediate and near reserve potassium (phosphorus) from the total reserve. All types of reserves were summed, and the percentage of each type of reserve was calculated from the sum (Gorbunov, 1978). Potassium and phosphorus reserves were calculated using the following equations:

$$NR = (P_{cl}\ x\ CF)\ /100 \quad (1)$$

where, NR is the near reserve of potassium (phosphorus), in mg 100 $g^{-1}$of soil;
P_{cl}- potassium content in the clay fraction, in mg 100 $g^{-1}$of soil
CF is the proportion of clay fraction, in %.

$$PR\ =\ TR\ -\ DR\ +\ NR \quad (2)$$

where, PR is the potential reserve of potassium (phosphorus), mg 100 g^{-1};
TR – total reserve of potassium (phosphorus), mg 100 g^{-1};
DR is the direct reserve of potassium (phosphorus), mg 100 g^{-1};
NR is the near reserve of potassium (phosphorus), mg 100 g^{-1}.

3. Results and Discussion

The total humus content in the upper horizon of the studied brown soils ranges from 1 to 6.6% (Table 2). The type of humus is defined as fulvate and humate-fulvate. The degree of erosion significantly affects the humus content of soils: Highly eroded soils show significant dehumidification due to almost destruction of the humus-accumulative horizon. Moderately eroded soils belong to the category of low-humus soils and contain, on average, 2-3% of humus, while slightly eroded brown soils have a higher humus content (4-5% of humus). The issue of preventing water erosion in the mountain regions of the country remains relevant. The vertical distribution of humus in the studied soils follows a regressive-accumulative pattern. The maximum amount of organic matter is concentrated in the upper

50-70 cm layer, with the humus content nearing 1% at a depth of about 1 m. The influence of exposure is noted in the distribution of humus: Soils on northern and western slopes accumulate a slightly higher amount than those on southern and eastern slopes.

Table 2. Content of humus, carbon and carbonates in mountain brown soils of Uzbekistan

Depth, (cm)	Humus, %	CO_2 carbonates, %	Soil C content, %			Content of the clay fraction, %	Humus Clay fraction, %	C_{org} Clay fraction, %
			C_{org}, %	C_{anorg}, %*	C_{total}, %			
Section 74								
0-7	6.58	3.82	3.82	1.04	4.86	3.0	16.93	9.82
7-26	2.79	1.62	1.62	0.44	2.06	8.3	6.81	3.95
26-75	2.38	1.38	1.38	0.38	1.76	8.6	6.81	3.95
Section 54.								
0-9	4.24	1.32	2.45	0.36	2.81	11.1	10.34	5.00
9-31	2.74	1.46	1.59	0.40	1.99	17.5	6.69	3.88
31-52	2.43	1.52	1.41	0.41	1.82	18.3	5.93	3.44
52-85	0.92	1.63	0.53	0.44	0.97	18.3	2.22	1.29
85-121	0.85	1.94	0.49	0.53	1.02	17.5	2.07	1.20
Section 40								
0-7	3.68	1.15	2.13	0.31	2.44	12.7	8.96	5.20
7-11	1.50	1.48	0.87	0.40	1.27	13.5	3.65	2.12
11-27	1.27	1.96	0.74	0.53	1.27	12.7	3.12	1.81
27-50	1.03	2.02	0.59	0.55	1.14	11.9	2.52	1.46
50-80	1.03	2.69	0.59	0.73	1.32	11.1	2.52	1.46
80-160	0.95	2.70	0.55	0.74	1.29	12.7	2.31	1.34
Section 66								
0-5	2.76	1.26	1.60	0.34	1,94	5.0	6.73	3.90
5-29	1.30	1.27	0.75	0.35	1,10	5.2	3.15	1.83
29-63	1.05	1.20	0.61	0.33	0,94	3.6	2.57	1.49
63-90	0.81	1.34	0.47	0.37	0,84	4.0	1.98	1.15
90-122	0.33	2.27	0.19	0.62	0.81	2.9	0.79	0.46
Section 28								
0-8	2.69	2.33	1.56	0.64	2.20	4.8	6.57	3.81
8-53	2.00	3.19	1.16	0.87	2.03	4.0	4.88	2.83
53-98	1.28	3.18	0.74	0.87	1.61	3.2	3.12	1.81
98-136	0.65	3.88	0.38	1.06	1.44	4.0	1.60	0.93
Section 13								
0-9	2.60	1.10	1.51	0.30	1.81	6.3	6,34	3.68
9-45	1.32	1.34	0.76	0.37	1.13	12.2	2,50	1.45
45-85	1.14	1.32	0.66	0.36	1.02	14.5	2,78	1.61
85-136	0.84	1.49	0.49	0.41	0.90	11.6	2,07	1.20
Section 1								
0-8	1.19	1.05	0.59	0.29	0.98	12.6	2.90	1.68
8-38	1.02	1.22	0.59	0.33	0.92	13.5	2.48	1.44
40-69	1.00	2.02	0.58	0.55	1.13	14.8	2.45	1.42
69-105	0.92	1.95	0.53	0.53	1.06	16.3	2.22	1.29

It is known that organic (C_{org}) and inorganic (C_{carb}) carbon constitute a single pool of soil carbon (Tan et al., 2014). In arid regions, the proportion of C_{carb} often dominates, while in more humid regions, its proportion is lower in the upper leached strata and increases in deeper ones, which may also be due to the influence of carbonate soil-forming rocks (Tan et al., 2014). In the brown soils we studied, the assessment of the organic and inorganic carbon content showed that the latter is present in an amount of about 12-76% of the total carbon (Table 2). At the same time, the vertical profile of C_{carb} repeats the general distribution of carbonates by depth. The level of carbonate in brown soils varies from low to medium (2.5-9% in terms of $CaCO_3$). The vertical distribution is eluvial with a uniform increase in content down the profile, or with a sharp increase in the carbonate horizon. The soils are weakly leached, with carbonate content above 1% in the surface horizon. The granulometric composition of the studied soils is middle-loamy. The proportion of clay fraction varies from 2.9 to 18.3% (Table 2). The vertical distribution is relatively uniform with indistinct accumulation in the middle part of the profile, which can be explained by illuvial processes. In connection with the fact that the clay fraction in soils is an important depot of organic matter (Hassink, 1997), organic and mineral interactions cause the

concentration of large amounts of C_{org} in the fraction of this dimension (<0.001mm) (Table 2.) which, as we observe, is much higher than the total amount of soil carbon.

Indicators of potassium and phosphorus in the researched soils are the following (Table 3). The total potassium content in the sod horizon of the brown soils studied varies from 1.240 to 1.685%, and phosphorus–from 0.141 to 0.350%. Exchangeable K varies from 265 to 1028 mg kg^{-1} of soil, which depends on erodibility and humus content of the soil, so in the humus layer of moderately eroded soils of sections 13, 66the indicator is from 559 to 708 mg 100 g^{-1} in slightly eroded soils of sections 40, 54, 28, 74 – from 265 to 1028 mg 100 g^{-1} (Table 3). The percentage of exchangeable potassium from the total content of potassium, in the sod horizon, varies from 15.72 to 45.56%. The mobile phosphorus varies from 12.50 to 54.00 mg kg^{-1} of soil. The percentage of mobile P from total phosphorus content varies from 6.98 to 38.30%. The tendency of decreasing total and mobile P content to lower horizons of the soil profile is noticeable (Table 3).

Table 3. The total content of potassium, phosphorus, exchangeable potassium, and mobile phosphorus in the mountain brown soils of Uzbekistan

Depth, (cm)	Total, (%)		Exchangeable K and mobile P, $Mgkg^{-1}$		Exchangeable K and mobile P from the total, (%)	
	K	P	K	P	K	P
			Section 40.			
0-7	1.685	0.248	265	21.17	15.72	8.54
7-11	1.470	0.145	241	11.10	16.39	7.66
11-27	1.120	0.098	241	13.96	21.52	14.24
27-50	0.924	0.074	217	7.84	23.48	10.59
50-80	1.032	0.065	188	5.90	18.72	9.08
			Section 13.			
0-9	1.560	0.151	559	26.23	35.83	17.37
9-45	1.461	0.143	568	16.71	38.88	11.68
45-85	1.442	0.127	442	9.12	30.65	7.18
			Section 66.			
0-5	1.554	0.164	708	43.20	45.56	26.34
5-29	1.476	0.157	686	28.80	46.48	18.34
29-63	1.464	0.107	648	16.00	44.26	14.95
63-90	1.050	0.090	600	11.20	57.14	12.44
90-122	0.930	0.037	578	8.00	62.15	21.62
0-5	1.554	0.164	708	43.20	45.56	26.34
			Section 54.			
0-9	1.442	0.350	310	48.05	21.50	13.16
9-31	1.412	0.241	304	20.48	21.53	8.50
31-52	1.288	0.215	262	12.14	20.34	5.65
52-85	1.035	0.200	213	9.21	20.58	4.61
85-121	1.005	0.152	194	8.17	19.30	5.40
			Section 28.			
0-8	1.321	0.179	271	12.50	20.51	6.98
8-53	1.488	0.103	240	8.45	16.12	8.20
53-98	1.474	0.059	228	4.31	15.47	7.31
98-136	0.967	0.052	180	0.96	18.61	1.85
			Section 1.			
0-8	1.270	0.279	443	35.14	34.88	12.60
8-38	1.165	0.209	271	25.25	23.26	12.08
40-69	1.120	0.214	190	12.46	16.96	5.82
69-105	0.930	0.145	100	7.13	10.75	4.92
			Section 74.			
0-7	1.240	0.141	1028	54.00	32.90	38.30
7-26	1.280	0.124	539	14.09	42.11	11.32
26-75	0.960	0.128	424	18.00	44.16	14.06

The numerical values for potassium and phosphorus reserves are shown in Table 4. As indicated, the direct reserve of potassium and phosphorus is available for microorganisms and plants. The vertical distribution of the direct reserve of both elements depends on slope exposure and soil erodibility, as it

is mobile in a mildly alkaline environment along the soil profile. In the studied soils, the share of potassium direct reserve varies from 10 to 103 mg per 100 g^{-1}, and the share of total phosphorus direct reserve from total phosphorus content varies from 1.56 to 8.31% (Table 4).

Table 4. Changes in the content of potassium and phosphorus reserves, taking into account the clay fraction in the mountain brown soils of Uzbekistan

Depth, (cm)	Content of the clay fraction, %	In the clay fraction, %		Potassium reserves, from the total content, %			Phosphorus reserves, from the total content, %		
		K	P	Direct	Near	Potential	Direct	Near	Potential
				Section 40					
0-7	12.7	3.02	0.379	2.12	2.79	75.61	8.47	19.35	72.18
7-11	13.5	2.63	0.222	1.63	4.15	74.22	7.59	20.69	71.72
11-27	12.7	2.00	0.150	2.14	2.68	75.18	14.29	19.39	66.33
27-50	11.9	1.65	0.113	2.38	1.21	76.41	10.81	17.57	71.62
50-80	11.1	1.34	0.100	1.84	4.44	83.72	9.23	16.92	73.85
				Section 13					
0-9	6.3	2.79	0.234	3.60	11.28	85.13	5.96	9.76	84.11
9-45	12.2	2.62	0.231	3.90	21.90	74.20	17.22	18.54	64.24
45-85	14.5	2.58	0.219	3.05	25.94	71.01	11.89	22.38	65.73
				Section 66					
0-5	5.0	2.78	0.251	4.57	8.88	86.55	26.2	7.68	65.86
5-29	5.2	2.64	0.240	4.67	9.28	86.04	18.47	7.96	73.25
29-63	3.6	2.62	0.164	4.37	6.42	89.21	14.95	5.51	79.44
63-90	4.0	1.88	0.138	5.71	7.14	87.14	12.72	6.11	81.11
90-122	2.9	1.66	0.057	6.24	5.16	88.60	21.62	5.41	72.97
				Section 54					
0-9	11.1	2.58	0.558	2.15	19.83	78.16	13.15	16.99	69.86
9-31	17.5	2.53	0.369	2.12	31.37	66.50	8.75	27.08	64.17
31-52	18.3	2.31	0.329	2.02	32.84	65.14	5.58	27.91	66.51
52-85	18.3	1.86	0.306	2.03	32.85	65.12	4.4	28.0	67.5
85-121	17.5	1.31	0.233	1.89	22.79	75.32	5.3	26.97	67.76
				Section 28					
0-8	4.8	2.36	0.274	2.04	8.55	89.40	7.26	7.26	85.47
8-53	4.0	2.66	0.158	1.61	7.12	91.20	8.74	5.83	85.44
53-98	3.2	2.63	0.090	1.56	5.70	93.0	6.78	5.08	88.14
98-136	4.0	1.26	0.080	1.86	5.17	92.96	1.92	6.15	92.31
				Section 1					
0-8	12.6	2.27	0.427	3.46	22.52	74.01	12.55	8.99	68.46
8-38	13.5	2.09	0.320	2.32	24.21	73.48	11.96	0.57	67.46
40-69	14.8	2.00	0.322	1.70	26.43	71.86	5.61	7.43	71.96
69-105	16.3	1.66	0.221	3,.46	22.52	74.01	4.83	4.84	70.34
				Section 74					
0-7	3.0	2.22	0.216	8.31	5.40	86.29	38.30	9.86	82.14
7-26	8.3	2.29	0.189	4.22	14.84	80.94	11.29	7.90	75.81
26-75	8.6	1.88	0.230	4.38	16.88	78.75	14.06	5.62	70.31

The near reserve was defined as potassium in the clay fraction, taking into account the content of the clay fraction of <0.001 mm (%). The vertical distribution of near-reserve potassium is not uniform, with an uneven decrease in content down the profile from 1.84 to 4.57%, with a poor accumulation in the middle and lower parts of the profile, which can be explained by illuvial processes. In percentage correlation in the sod horizon of soils, it ranges from 5.40 to 32.85% of the total potassium content. The maximum accumulation of near reserve in the sod horizon of brown soils in the section is 40 - 384 mg 100 g^{-1}, and the minimum in the section is 28 - 50 mg 100 g^{-1}. In contrast to potassium, the vertical distribution of near-reserve phosphorus is homogeneous, with a uniform decrease in content down the

profile from 62.0 to 2.0 mg 100 g^{-1}. In percentage correlation of the upper soil horizon, it ranges from 7 to 30% of total phosphorus content. The maximum accumulation of near reserve in the humus horizon of brown soils in section 54 is 62.0 mg 100 g^{-1}, and the minimum in section 66 is 12.60 mg 100 g^{-1}. On the slopes of the southern and eastern exposition (sections 40, 13, 1) the distribution of potassium and phosphorus of the near-reserve across the profile is non-uniform since it is the southern and eastern slopes that receive less precipitation (Table 3).

In the studied brown soils potential reserve is dominating in the total content and varies on a profile - potassium from 65.12 to 93.0% **(**Table 4), phosphorus from 60.0 to 90.0%, it was observed that sharp decrease of indicators of phosphorus of potential reserve in sub-sod horizon of a profile and in general its vertical distribution is not uniform at both elements. The maximum share of potassium potential reserve in the humus horizon of brown soils was recorded in section 66 - 1345 mg 100 g^{-1}(up to 89% of total potassium), phosphorus in section 54 - 255 mg 100 g^{-1}(up to 70% of total phosphorus). Minimum potassium potential reserve in section 1 - 940 mg 100 g^{-1}(up to 74% of total potassium), phosphorus in section 74 - 59 mg 100 g^{-1}(up to 82.14% of total phosphorus) (Table 3).

The hydromicas (hydromuscovite) mineral is hydromorphic, as a result of which, under the influence of high humidity in the upper layers of the soil, the process of montmorillitization of hydromicas proceeds - the formation of the montmorillonite mineral. In this process, potassium is lost from the mineral structure of hydromica, which can be seen from the given crystallochemical reaction:

$$K<Al_2(SiAl)_4O_{10}(OH)_2n\ H_2O + Al_2O_3\ 2SiO_2 2H_2O + 5MgO + 2Fe_2O_3 + 2SiO_2 + MgCl_2 = 2Mg_3(Si_4O_{10})(OH)_2\ (Al_2Fe)_2\ (Si_4O_{10})(OH)_2 + H_2O + 2KCl \quad (3)$$

This process occurs in the lower horizons of sections 54, 40, 13, 1, and 74, indicating that due to the high moisture content in the lower horizons of the soil profile, the process of montmorillonization of the hydrosludite mineral is more pronounced than in the humus horizon, leading to a decrease in total potassium content. Evidence of this process is the fact that the amount of exchangeable potassium in the sod horizon was 1028 mg per 100 kg^{-1} in the example of section 74.

The content of phosphorus, introduced with fertilizers and released during the decomposition of phosphorus-containing primary minerals, is typically higher in the clay fraction than in larger fractions. Under similar conditions, loamy and clayey soils have higher phosphorus content compared to soils with a lighter granulometric composition. Additionally, the phosphorus content in humus and sub-humus horizons is 1.5-2.0 times greater than in other soil horizons (Akhatov and Murodova, 2020). Phosphorus compounds undergo changes under the influence of irrigation duration and increased soil. In regions with hot and dry climates, applied phosphorus fertilizers constantly precipitate in insoluble forms due to their chemical absorption capacity (Muindi, 2019). An increasing amount of fulvic acid in the humus composition accelerates the dissolution of phosphorus precipitation, resulting in lower chemical absorption of phosphorus. For example, in order to obtain higher yields, the annual rate of phosphorus fertilizers in light grey soils under cotton is 180 kg per hectare, of which about 30% is taken up by plants, 30-35% is converted into insoluble forms, and 30% is lost through leaching (Akhatov et al., 2022).

The profile and exposure distribution of total humus in brown soils, as well as its fractions, are influenced by both the peculiarities of humus formation and erosion processes (Aliyev, 2017; Pulatov et al., 2020; Aliyev et al., 2022) The processes of humus formation occurring under generally favorable conditions have contributed to the accumulation of up to 6% of humus in the upper soil horizon, with a deep humus content noted in the profile. Erosion processes lead to the dehumidification of brown soils, and soil erosion also obscures the pattern of humus exposure distribution, reducing the general diversity and mottling of the mountain slope soil cover (Aliyev, 2017). Highly eroded soils also lose their ability to deposit carbon and lose part of it, fixed in humus, due to leaching, dissolution, and mineralization. All sections are characterized by a sharp vertical decrease in the total content of potassium and phosphorus, indicating their deep penetration. The vertical distribution of potassium and phosphorus reserves is characterized by an indistinct accumulation in the middle part of the profile, which can be explained by illuvial processes. Thus, the problem of erosion impact on soils in the region is quite acute. Additionally, the manifestation of erosion on slopes is aggravated by intensive economic use. Regular cattle grazing prevents the restoration of vegetation cover and sodding of the soil surface.

Conclusion

The studied brown soils of the mountain pastures in Uzbekistan have a loamy granulometric composition, with the clay fraction not exceeding 20%. The carbonate content is low, ranging from 2.5% to 9%. The distribution of total humus, potassium, and phosphorus, as well as their fractions, in the profile and exposure, is influenced by both the formation peculiarities and erosion processes.

The total humus content in brown soils varies widely, from 1% to 6.6%. In soils undisturbed by erosion, the vertical distribution of humus follows a regressive-accumulative pattern. Soils on northern and western slopes contain more humus than those on southern and eastern slopes. The total potassium content varies depending on the slope's exposure and erodibility. In the top horizon of the studied brown soils, the total potassium content is up to 1.685%, and phosphorus is up to 0.350%. The decrease in total potassium content is influenced by the montmorillonitization process occurring in the lower, more humid horizons. Illuvial processes explain the unclear accumulation of potassium and phosphorus reserves in the middle part of the profile. The direct reserve of potassium and phosphorus is less than one-third of the total content, with the potential reserve predominating.

The depletion of potassium and phosphorus in brown soils mainly occurs through leaching of the immediate reserve, with this process being more active on southern, less sodded slopes. The problem of erosion impact on the soils in the studied regions is significant. Erosion processes lead not only to dehumidification but also to a decrease in the content of potassium and phosphorus. Regular grazing and intensive economic use hinder the restoration of vegetation cover and sodding of the soil surface, increasing erosion on the slopes and reducing the overall diversity of the soil cover of mountain slopes. Further study of the peculiarities of humus, potassium, and phosphorus, their accumulation, and restoration in brown soils is essential for developing recommendations for their rational use, anti-erosion protection, and increasing the productivity of mountain pastures in Uzbekistan.

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Research Article

Assessment of Residual Impacts of Poultry Manure on Nutrient, Sucrose, Fructose and Glucose Content of Second Crop Onion (*Allium cepa* L.)

Funda YOLDAŞ*[1], Şafak CEYLAN[2], Nilgün SAATÇI MORDOĞAN[3]

[1,2]Organic Agriculture Programme, Odemis Vocational School, Ege University, 35750 Izmir
[3]Soil Science and Plant Nutrition Department, Faculty of Agriculture, Ege University, 35040 Izmir

[1]https://orcid.org/0000-0001-6205-9751, [2]https://orcid.org/0000-0001-7333-1482, [3]https://orcid.org/0000-0003-1881-6318

*Corresponding author e-mail: fundayoldas@hotmail.com

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Abstract: This study, conducted at Ege University, Odemis Vocational School, aimed to investigate the influence of poultry manure applications on the nutrient, sucrose, fructose, and glucose content of second crop onions. In this research, three onion varieties (Burgaz, Snow White, Champion), three different doses of poultry manure (20, 40, 60 t ha^{-1}), and mineral fertilizer were employed. An unfertilized plot was employed as the control. The experiment was designed using a split-split plot arrangement and replicated three times. The analysis comprised the assessment of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) contents in samples collected from both onion leaves and bulbs. In addition, sucrose, fructose, and glucose levels in the bulb were determined. The Snow White variety contains the highest level of fructose, sucrose, and glucose compared to other varieties. In the exploration of the impact of macro-micro element contents in bulbs, the Champion variety exhibited significantly higher levels of N, Ca, Fe, and Cu compared to other varieties. Additionally, P, K, and Na contents were significantly higher in Champion and Snow White varieties compared to Burgaz bulbs. The difference between the applications of these nutrients was insignificant except for N, P, Na, and Fe. The Nitrogen, Potassium, Sodium, and Iron contents of soils were the lowest control, while the highest dose application had the highest value at 60 t ha^{-1} dose. The difference among the applications in these nutrients was significant except for Mg and Cu. It may be recommended to use 40 t ha^{-1} dose in onion cultivation.

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1. Introduction

Currently, onion is produced as one of the most important crops in the world (Kumar et al., 2003 and 2007; Sharma, 2003; Yoldas et al., 2011; Sekara et al., 2015; Yoldas et al., 2020). Onion is one of our special foods, with great importance in human nutrition and its place in Turkish cuisine and a special nutritious unit.

In Türkiye, fresh onion production is 126 185 tons, and dry onion production is 2 500 000 tons (Anonymous, 2022). The cultivated area is 87 393 ha for spring onions (Anonymous, 2018) and 69 897

ha for dry onions (Anonymous, 2022). The best onion cultivation is done in sandy-clay or clay-sandy, organic-rich soils. Heavy clay or sandy soils and excess moisture are unsuitable for growing onions (Ekinci, 1971; Günay, 1983; Vural et al., 1987 and 2000).

Soils are generally poor in organic matter in Türkiye. Animal fertilizers with high organic matter content regulate and also improve soil properties (physical, chemical and biological, etc.). These materials are also storage for plant nutrients. High fertilizer doses used in onion cultivation harm human health and the environment; therefore, it is important to create conscious fertilization programs for sustainable production and sustainable living according to plant varieties. Crop production has increased with the increasing world population, and increasing inputs.

The studies discussed various aspects of onion production and fertilization methods. Yoldas et al. (2019) highlighted the positive impact of poultry manure, indicating a significant increase in yield, especially with a 60 t/ha application. Mallanagouda et al. (1995) found that the highest onion yield resulted from combining the recommended NPK amount with animal manure. Vural et al. (1987) observed that the combination of animal manure with NPK yielded the highest marketable yield under favorable moisture conditions, while low manure alone produced better results than NPK alone. In another study, different animal manure applications, mineral fertilizers (NPK), and their combinations were examined, with NPK showing effectiveness primarily in low humidity conditions. Akoun (2004) emphasized the impact of animal fertilizers on onion production.

Combining mineral and organic fertilizers significantly increased crop yields, as demonstrated by Kuldkepp (1997), Ellmer et al. (2000), Saleh et al. (2000), and Sady et al. (2008). Blay et al. (2002) investigated the effects of poultry manure and inorganic fertilizers on onion production, noting increased product yield but reduced dry matter.

High yield and quality in onion production depend on nutrient content, soil fertility enhancement, pollution prevention, and the conscious use of organic materials (Syed et al., 2000). These findings collectively underscore the importance of proper fertilization practices for optimizing onion production.

The study aimed to achieve three objectives: (i) to investigate the effects of poultry manure doses on nutrient content in onion bulbs, (ii) to explore the impact of organic manure doses on nutrient content in onion leaves, and (iii) to assess the residual effect of poultry manure applications on sucrose, fructose, and glucose content in second crop onions.

2. Material and Methods

This research was performed to expose the effects of poultry manure on content of nutrient content of second crop onion grown after lettuce. This study was done in the research and application field of Ege University, Ödemiş Vocational School. Three onion varieties - Burgaz, Snow White, and Champion (*Allium cepa* L.) - were utilized in the study.

Control (0), 20, 40, 60 t ha^{-1} poultry manure applications were made to the plots for lettuce production in the previous production period. In addition, mineral fertilizer NPK (120:100:150 kg ha^{-1}), 15:15:15, K_2SO_4, and Ammonium Nitrate were applied to the lettuce plant. The lettuce was harvested on 04.04.2017, and then the planting of onion seedlings was conducted on the same trial plan.

Seeds were cultivated on January 23 to produce seedlings of onion varieties used in the experiment. Onion seedlings were planted in their places on April 05 and harvested on August 02. In the study, after harvesting lettuce and before planting onion seedlings, each plot's soil samples were taken for analysis. The planting distance was 30×15 cm in the experiment, and the distance between the parcel and the block was 1 m. The study was established with three replications according to the split-split plot design.

The composition of poultry manure was analyzed following the method outlined by Kacar (1995) and El-Sheref et al., (2023), and the results are presented in Table 1.

Table 1. Poultry manure's properties

pH	8.55	**C/N**	12.1
Total Salt (ms/cm)	2.47	**P (%)**	0.70
Ash 550ºC (%)	79	**K (%)**	1.02
Organic Matter (%)	19.8	**Ca (%)**	1.37
Organic Carbon (%)	11.51	**Mg (ppm)**	3729
Total N (%)	0.95	**Na (ppm)**	1248

Soil samples were taken from 0-20 cm depth in each plot (15 samples) at the beginning of onion vegetation. They were dried, grounded, and then passed through a 2 mm sieve to determine chemical properties. The soil analysis involved the determination of pH following the method outlined by Jackson (1967), total soluble salt content based on the procedure by Anonymous (1951), $CaCO_3$ content analyzed using the method by Kacar (1995), and organic matter content determined according to Reuterberg and Kremkurs (1951). Additionally, total nitrogen (N) was analyzed (Bremner, 1965), available potassium was determined by extracting with 1 N NH_4OAc using a flame photometer (Atalay et al., 1986), and available phosphorus was measured using a colorimeter, following the method by Olsen et al. (1954).

Leaf samples were taken as the youngest leaves for chemical analyses (Jones et al., 1991). Before the onion bulbs reached maturity. The leaf and bulb samples were dried at 70°C for analyses using the method by Kacar, 1972. In the study, manure, bulbs, and leaf samples underwent wet digestion (nitric acid (HNO_3): perchloric acid ($HClO_4$); 4:1) for P, K, Ca, Mg, Na, Fe, Cu, Zn, and Mn analyses. Phosphorus was quantified using the colorimetric method, while K, Ca, and Na were analyzed by flame photometer. Mg, Fe, Cu, Zn, and Mn were determined by AAS (Atomic Absorption Spectroscopy) following the methods outlined by Moore (1992) and Campbell and Plank (1992). Total nitrogen in plant samples was analyzed using the modified Kjeldahl method as described by Baker and Thompson (1992).

2.1. Sugar analysis methods

Extraction of samples and HPLC analysis conditions were done according to Camara et al. (1996). First, fruit samples (10 g per each) were taken. The samples were mixed with 50 ml of distilled water and homogenized by crushing in a homogenizer. Subsequently, the homogenized samples underwent centrifugation at 6000 rpm and were filtered through the Whatman No. 42 filter paper. The final volume was adjusted to be acetonitrile: filtrate (6:2, v/v). Samples were kept at −18°C. A Refractive index detector (RID) was used for sugar analysis. Conditions for HPLC analysis were adjusted as follows. The method was modified as needed.

The chromatographic analysis was conducted using a Supelco column (300mmx4.1mm ID) at room temperature (18-22°C). The mobile phase consisted of acetonitrile and distilled water (75:25), with a flow rate of 1.8 m/min. Detection was performed by a refractive index (RI) detector at 30°C, and the injection amount was 20 µL.

2.1.1. Evaluation of data or statistical analyses

The data were subjected to statistical analysis using the TARIST software package, as described by Açıkgöz et al. (1993).

3. Results and Discussion

3.1. The effect of poultry manure applications on physical and chemical properties of the soil

At the onset of onion vegetation, pH, organic matter, and lime contents of field soils at 0-20 cm depth were determined and are presented in Table 2. According to this, soil pH is 6.98-7.08; organic matter is between 0.66% to 0.89%, and lime is between 0.63% to 0.84%. Thus, field soils are neutral (6.6-7.3), humus ($p<0.01$), and $CaCO_3$ (0-2.5) poor (Table 2).

Table 2. The chemical properties of the soils at the onset of onion production

Treatments ha^{-1}	pH	Organic Matter (%)	$CaCO_3$ (%)
0	7.06	0.66	0.63
NPK	7.00	0.89	0.84
20 t	7.08	0.83	0.69
40 t	6.98	0.72	0.72
60 t	7.02	0.79	0.66
LSD	Ns	Ns	Ns

**: $p < 0.01$, *: $p < 0.05$, Ns: not significant.

Onion vegetation and field soils' element contents at 0-20 cm depth were determined and are given in Table 3. Total N: 0.056% - 0.110%, available P: 24.16%-35.50%, K: 97.4%-106.7%, Ca: 891%-1089%, Mg: 210%-224%, Na: 16.26 mg kg^{-1}-42.90 mg kg^{-1} was found. The micro elements are Fe: 3.49 mg kg^{-1}-3.75 mg kg^{-1}, Zn: 1.03 mg kg^{-1}-3.03 mg kg^{-1}, Mn: 3.68 mg kg^{-1}-4.15 mg kg^{-1}, Cu: 0.63 mg kg^{-1}-0.67 mg kg^{-1} (Table 3).

Table 3. Contents of macro and micro elements of experiment soils (initiation of production)

Treatments ha^{-1}	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg^{-1})
0	0.056 c	24.16 b	100.6	1056	224	16.26 d
NPK	0.090 b	34.56 a	98.0	1023	215	27.96 bc
20 t	0.076 b	27.59 b	97.4	986	210	23.90 cd
40 t	0.081 b	24.72 b	80.9	891	220	32.13 b
60 t	0.110 a	35.50 a	106.7	1089	218	42.90 a
LSD	0.018**	5.92**	Ns	Ns	Ns	8.15 **

Treatment ha^{-1}	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
0	3.38 b	1.06	3.68	0.64
NPK	3.75 a	1.19	4.03	0.63
20 t	3.49 ab	1.16	4.13	0.67
40 t	3.49 ab	3.03	4.04	0.65
60 t	3.75 a	1.09	4.15	0.63
LSD	0.368*	Ns	Ns	Ns

**: $p < 0.01$; *$p < 0.05$; ns: not significant.

The difference between applications in these nutrients was insignificant except for N, P, Na, and Fe. In control plots, N, P, Na, and Fe contents of the soils were determined as low values. At the application dose of 60 tons, the highest value was observed for poultry manure. 60 t treatment and mineral application gave statistically the same results for the Fe content of soils. Poultry manure had a positive impact on enhancing the nutritional status of the soil, generally up to organic matter mineralization. Similarly, Ceylan et al. (2020) stated that the application of poultry manure under greenhouse conditions significantly positive effect on soil nitrogen content. Mordoğan et al. (2013) found that organic manure applications on olive-growing soils at 0-20 depths N, K, Ca, Mg, Cu, and Na content. P, K, Ca, Mn, Cu, and Na contents were affected. Dikinya and Mufwanzala (2010) observed a significant increase in nitrogen and phosphorus with poultry manure.

When the fertility of the trial soils was evaluated, N was found to be moderate (0.05–0.1 %); P rich (greater than 3.26 ppm); K -<150 mg kg^{-1}- and Ca -715-1430 ppm- poor; Mg (>114 ppm) well; Fe (2.5-4.5 ppm) deficiency possible; Zn ->1 ppm-, Mn ->1 ppm-, Cu ->0.2 mg kg^{-1}- appears to be adequate (Bergmann, 1993).

3.2. The residual effect of applications on the nutrients of second crop onion leaves

The residual effects of manure applications on the leaf nutrient content of second crop onion are given in Table 4.

Table 4. The effect of poultry manure on onion's leaf macro and microelement content.

Treatment ha^{-1}	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg^{-1})
0	2.09 d	0.14 c	1.89 c	0.93 c	0.51 b	922.9 b
NPK	2.69 a	0.19 ab	2.48 ab	1.25 ab	0.63 a	948.8 b
20 t	2.29 c	0.18 b	2.19 b	1.11 b	0.59 ab	1071.2 ab
40 t	2.39 bc	0.19 ab	2.53 a	1.34 a	0.67 a	1155.0 a
60 t	2.47 b	0.22 a	2.64 a	1.29 a	0.61 a	1193.7 a
LSD	0.126**	0.028**	0.337**	0.124**	0.099**	199.4**

Treatment ha^{-1}	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
0	86.36 b	16.78 b	23.20	5.58
NPK	102.36 a	19.07 a	24.51	5.99
20 t	97.72ab	19.92 a	20.99	7.02
40 t	100.51ab	19.75 a	25.06	6.22
60 t	93.57ab	19.89 a	20.40	6.10
LSD	14.89*	1.25**	Ns	Ns

**: $p < 0.01$, *: $p < 0.05$; Ns: not significant.

N content in leaf is between 2.09%-2.69%, P is between 0.14%-0.22%; K is between 1.89%-2.64%; Ca is between 0.93%-1.34%; Mg is between 0.51%-0.67% Na is between 922.9 mg kg^{-1}-1193.7 mg kg^{-1}; Fe is between 86.36 mg kg^{-1}-102.36 mg kg^{-1}; Zn is between 16.78 mg kg^{-1}-19.92 mg kg^{-1}; Mn is between 20.4 mg kg^{-1}-25.06 mg kg^{-1}; Cu is between 5.58 mg kg^{-1}-7.02 mg kg^{-1} (Table 4).

The difference among the applications in these nutrients was significant except for K, Na, Mn, and amounts. The quantities of nutrients analyzed include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) of the leaves were at the lowest level in the control plots, while the highest doses (40 and 60 t ha^{-1}) of application had the best value. However, Nitrogen and Mn contents of the leaves were statistically at the same level with the highest applications of poultry manure and NPK ($p < 0.01$).

When the sufficiency levels of the nutrient content of onion leaves are investigated, it is seen that P, Zn, Mn, and Cu values are at the deficiency level, although N, Ca, and Mg amounts are sufficient. K values in leaves were determined at a sufficient level only in plots where 40 and 60 t ha^{-1} poultry manure doses were applied (Bergmann, 1993).

In the research, it is noteworthy that the P, Zn, Mn, and Cu nutrient contents of onion leaves are insufficient, while the P content of the soils is high before planting. This may be due to converting available phosphorus in the soil into a fixed form that plants cannot take up during vegetation. On the subject, Kırmızı (1990) reported that the phosphorus fixation capacity in alluvial soils of the Aegean region was between 36-89%. It is thought that the important positive correlation that the researchers determined between soil organic matter and exchangeable Mg X phosphorus fixation explains this situation (Kırmızı, 1990; Ceylan et al., 2003).

The soil Ca content is poor, but it is seen that the Ca amount in the leaves is sufficient (Table 3, 4). The outcome could be associated with the gradual release of organic manure and its impact on subsequent yields. Although P, Zn, Mn, and Cu sufficient in the soil, these elements are found in insufficient amounts in the leaves. This situation can be explained by the antagonistic effects between P and Zn, P and Mn, and P and Cu (Kılınç et al., 1991). Thus, Lee and Lee (2014) stated that the excess nutrient content in the soil does not benefit plants or may even depress the uptake of nutrients and crop growth and yield.

The study showed that the nutrient content of the leaf varies significantly according to onion varieties except for Fe, Mn, and Cu amounts (Table 5).

Table 5 shows that the contents of N, P, and Zn of the Burgaz variety are significantly higher than other varieties. In addition, Burgaz and Snow White varieties had significantly higher K content than Champion varieties; Snow White and Champion varieties had significantly higher level Ca than Burgaz varieties; the Mg content of the Champion variety was higher than others.

The differences weren't significant between the Champion and Snow White in terms of Mg content; in terms of Fe, Mn, and Cu content. There were no significant differences between the varieties based on the applications, as indicated in Table 5.

Table 5. Effect of varieties on the onion leaves macro-micro element content

Varieties	N	P	K	Ca	Mg
	(%)	(%)	(%)	(%)	(%)
Burgaz	2.56 a	0.20 a	2.45a	1.08b	0.55 b
Snow White	2.48 b	0.19 ab	2.40a	1.19a	0.61 ab
Champion	2.12 c	0.17b	2.19b	1.29a	0.65 a
LSD	0.049**	0.026**	0.185**	0.118**	0.096**

Varieties	Fe	Zn	Mn	Cu
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Burgaz	92.37	20.37 a	21.50	6.48
Snow White	94.55	18.68 b	23.74	6.01
Champion	101.39	18.21 b	23.25	6.06
LSD	Ns	1.227**	Ns	Ns

**: $p < 0.01$,*: $p < 0.05$, Ns: not significant.

3.3. The residual effect of applications on the nutrients of second crop onion bulbs

Macro and micronutrient contents of onion bulbs are given in Table 6. According to Table 6, N is 2.10% to 2.93%, P is between 0.16% to 0.23%; K is between 1.37% to 1.73%; Ca is between 0.10% to 0.15%; Mg is between 0.156% to 0.183%; Na is between 283.9 mg kg^{-1} to 477.7 mg kg^{-1}; Fe is between 26.97 mg kg^{-1} to 43.19 mg kg^{-1}; Zn is between 20.19 mg kg^{-1} to 25.65 mg kg^{-1}; Mn is between 12.37- mg kg^{-1} to 14.89 mg kg^{-1}; Cu is between 4.64 mg kg^{-1} to 5.96 mg kg^{-1}. The order of nutrient elements, determined by their measured quantities in the analyzed onion bulbs, was N > K > P > Mg > Ca > Na > Fe > Zn > Mn > Zn.

Difference among applications, these nutrients were significant except for Mg and Cu. The contents of N, P, K, Ca, Na, Zn, and Mn in the bulbs were lowest in the control plots given in Table 6. Conversely, K, Ca, Na, and Fe's highest contents in bulbs were confident by the residual effect of 40 t ha^{-1}.

Yoldas et al. (2011) found that similar result in the first year. The potassium content in bulbs exhibited a significant increase with the application of cattle manure. Nevertheless, manure applications did not significantly affect N, P, Ca, Mg, Fe, Zn, Cu, Mn, and Na contents in bulbs. Coolong et al. (2004) explained that N and P content in bulbs was increased by the application of N. Although Mn, Fe, and Zn contents increased, K, Cu, and Mn contents were not affected. Abdelrazzag (2002) revealed a noteworthy correlation: elevating the rate of sheep and poultry manure led to a significant increase in the nitrogen content of onions, whereas phosphorus and potassium levels remained relatively low. Mahmoud et al. (2013) noted a significant improvement in the chemical components, including nitrogen (N), phosphorus (P), potassium (K), and total protein, as compost application levels increased. This enhancement was particularly notable, reaching significance at levels up to 180 kg N ha^{-1} in sandy soil. These findings suggest that the influence of soil organic matter could contribute to various functional soil properties, encompassing physical, chemical, and biological aspects, and play a pivotal role in nutrient cycling, as highlighted by Murphy (2014).

Table 6. Effect of poultry manure on bulbs macro-micro element content

Treatments	N	P	K	Ca	Mg	Na
ha^{-1}	(%)	(%)	(%)	(%)	(%)	(mg kg^{-1})
0	2.10 e	0.16 b	1.37 c	0.10c	0.156	283.9 d
NPK	2.93 a	0.23 a	1.59 ab	0.12b	0.183	448.4 ab
20 t	2.32 d	0.20 a	1.49 bc	0.1bc	0.182	402.9 bc
40 t	2.54 c	0.22 a	1.73 a	0.14a	0.178	477.7 a
60 t	2.68 b	0.21 a	1.52 bc	0.15a	0.178	390.8 c
LSD	0.128**	0.033**	0.174**	0.014**	Ns	57.038**

Treatments	Fe	Zn	Mn	Cu
ha^{-1}	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
0	32.29 c	20.19 b	12.37 b	5.25
NPK	41.94 ab	25.65 a	14.89 a	5.78
20 t	26.97 abc	22.49 ab	13.71 ab	5.42
40 t	43.19 a	23.99 ab	13.56 ab	5.96
60 t	36.11 bc	21.86 ab	13.33 ab	4.64
LSD	6.823**	5.024*	1.863*	Ns

**: $p < 0.01$; *: $p < 0.05$; Ns: not significant.

Onion varieties cultivated in the same environment, sharing similar soil and climatic conditions, display notable differences in the mineral composition of their bulbs. This variance is attributed to genotypic factors, as emphasized by Chope and Terry (2009).

Upon investigating the impact of varieties on the nutrient content of onion bulbs, it was evident that the Champion variety exhibited significantly higher levels of nitrogen (N), calcium (Ca), iron (Fe), and copper (Cu) compared to other varieties ($p < 0.01$) (Table 7). Furthermore, Champion and Snow white bulbs demonstrated elevated phosphorus (P), potassium (K), and sodium (Na) content.

Table 7. Effect of variety on the nutrient content of bulb

Varieties	N	P	K	Ca	Mg	Na
	(%)	(%)	(%)	(%)	(%)	(mg kg^{-1})
Burgaz	2.37 c	0.17 b	1.34 b	0.12 b	0. 171	325.2 a
Snow White	2.47 b	0.22 a	1.61 a	0.12 b	0.175	501.2 b
Champion	2.71 a	0.23 a	1.67 a	0.13 a	0.179	375.9 b
LSD	0.065**	0.029**	0.099**	0.008**	Ns	69.055**

Varieties	Fe	Zn	Mn	Cu
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Burgaz	26.94 c	21.19 b	11.61 b	4.78 b
Snow White	39.19 b	22.69 ab	16.83 a	4.73 b
Champion	48.18 a	24.61 a	12.28 b	6.73 a
LSD	3.721**	2.09**	0.845**	0.825**

**: $p < 0.01$, *: $p < 0.05$, Ns: not significant.

Furthermore, there were no significant differences between the varieties depending on the applications in terms of Mg content.

3.4. The residual impact of applications on the sucrose, fructose, and glucose content of second crop onions.

The residual effects of poultry manure applications on the sucrose, fructose, and glucose content in bulbs as the second crop are presented (Table 8).

Table 8. Effect of poultry manure on sucrose, fructose, and glucose contents in bulbs

Treatment ha^{-1}	Sucrose (g 100 ml^{-1})	Fructose (g 100 ml^{-1})	Glucose (g 100 ml^{-1})
0	3.33 a	17.71 a	22.13 a
NPK	2.29 b	12.09 b	11.70 c
20 t	2.56 b	16.89 a	19.68 a
40 t	2.69 ab	12.16 b	13.93 bc
60 t	2.12 b	13.03 b	15.55 b
LSD	0.715**	1.752**	2.670**

**: p < 0.01, *: p < 0.05, Ns: not significant.

3.4.1. Sucrose

Bulbs sucrose contents changed significantly depending on the applications (p<0.01). The highest sucrose content in the control plots, with no application, was 3.33 g 100 ml^{-1} (Table 8). Sucrose content in the bulbs decreased with applications. Also, Dehkordi et al. (2019), reported that treatment of sheep manure had the lowest sucrose amount in sugar beet compared to the control. Nitrogen causes sugar percentage reduction, influencing sucrose percentage. Therefore, the negative impact of manure on sucrose is due to its increasing impact on N content.

3.4.2. Fructose

Residual effects of poultry and mineral fertilizer applications significantly affected the fructose content of second crop onion bulbs grown after lettuce (p<0.01) (Table 8). Fructose values were determined in onion bulbs from the control plots (17.71 g 100 ml^{-1}) and 20 t ha^{-1} poultry manure applications as highest (16.89 g 100 ml^{-1}).

3.4.3. Glucose

The glucose contents of onion bulb samples changed significantly depending on the applications (p<0.01). Similar to fructose contents, the highest glucose content was analyzed in the control plots, where no treatment was performed (Table 8). This was followed by onion bulb samples taken from the parcels with a 20 t ha^{-1} poultry manure residual effect. The lowest glucose values were determined in the parcels where the residual effect of mineral fertilizer was applied. Likewise, high nitrogen (N) application resulted in a reduction of glucose and fructose in cabbage leaves, as reported by Yano et al. (1981). Conversely, a decrease in nitrogen application led to an increase in sugar content, as observed in the study by Takebe et al. (1995). This situation can be considered the amount of nitrogen applied to the plant to increase the vegetative part and product and the dilution of glucose, sucrose, and fructose amounts.

When the effect of varieties on sucrose, fructose, and glucose content of onion bulbs was investigated, it was determined that the highest sucrose content was in Burgaz (3.53 g 100 ml^{-1}). Conversely, the highest fructose (18.95 g 100 ml^{-1}) and glucose (20.35 g 100 ml^{-1}) content were in the Snow White variety (Table 9).

Table 9. Variety effect on sucrose, fructose, and glucose content of onion bulbs as the second crop

Variety	Sucrose (g 100 ml^{-1})	Fructose (g 100 ml^{-1})	Glucose (g 100 ml^{-1})
Burgaz	3.53 a	14.61 b	18.30 b
Snow White	2.37 b	18.95 a	20.35 a
Champion	1.89 b	9.57 c	10.55 c
LSD	0.472**	0.774**	1.373**

**: p < 0.01; *: p < 0.05; Ns: not significant.

Conclusion

As a result, poultry manures' residual effect and varieties on nutrient content in leaves, bulbs, sucrose, fructose, and glucose levels in the bulb of second crop onion grown after the lettuce were significantly affected.

In general, the highest nutrient contents in both leaves and bulbs were observed with the residual effect of 40 tha^{-1} poultry manure applications. Notably, the Burgaz onion variety exhibited a more pronounced response to organic manure in leaves, while The Champion variety exhibited elevated nutrient values in its bulbs.

Organic fertilizers, renowned for their slow-release attributes, influence soil fertility over successive years and crops. Our study emphasizes the importance of embracing an environmentally conscious approach, considering the observed long-term effects of organic fertilizers.

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Research Article

Evaluation of Aggregate Stability Using the Slaking Index Method with Soil Physical Approach in Keduang Sub-Watershed, Indonesia

Nanda Mei ISTIQOMAH*[1], Ongko CAHYONO[2], Mujiyo MUJIYO[3], Dwi Priyo ARIYANTO[4]

[1,2,3,4]Department of Soil Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Indonesia

[1]https://orcid.org/0000-0002-0263-8206, [2]https://orcid.org/0000-0001-5781-7538, [3]https://orcid.org/0000-0002-6161-7771
[4]https://orcid.org/0000-0001-6605-9599

*Corresponding author e-mail: meimei2017_99@student.uns.ac.id

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Watershed

Abstract: The Keduang Sub-Watershed area has faced multiple natural disasters like landslides, erosion, and flooding because of the poorly managed terrain in the area. This study examines the distribution of the slaking index on agricultural land in the Keduang Sub-Watershed, analyzes the impact of soil type on it, and identifies the soil physical elements that have the most significant influence on it. The study took place in the Keduang Sub-Watershed, Indonesia, utilizing agricultural land from woods, plantations, drylands, and paddy fields with Andisols, Alfisols, Inceptisols, and Entisols soil types. This survey research was supported by laboratory analysis of the soil's physical and chemical properties and used GIS for data interpretation. Soil samples were collected from 22 Land Map Units (LMUs) with 3 replications each, resulting in 66 samples. The SLAKES software assesses the primary parameter, the slaking index. The supporting parameters analyzed were aggregate stability, bulk density, texture, structure, pH, organic C, and Cation Exchange Capacity (CEC). The research showed that soil types in the Keduang Sub-Watershed significantly affect the slaking index value. The slaking index ranged from 0.13-11.63, with the highest values for Andisols in a forest, while the lowest values were Inceptisols in a plantation. The allophane mineral in Andisols was causing the high slaking index. The soil factors determining the slaking index were bulk density and exchangeable K. The lower the bulk density, the higher the slaking index. Meanwhile, the lower the exchangeable K, the lower the slaking index. The land management recommendations based on determinant factors are adding organic material and reducing soil cultivation practices.

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1. Introduction

A natural disaster occurs in the sub-watershed due to improper land use, decreasing soil resistance to landslides and erosion. Due to poor land management, several disasters occurred, including landslides, erosion, and flooding in the Keduang Sub-Watershed. In 2014–2020, the Wonogiri Regency Central Statistics Agency reported 165 flood disasters, which 5 of them occurred in the Keduang Sub-Watershed, and 431 landslides, which 164 of them occurred in the Keduang Sub-Watershed (Central Bureau of Statistics Wonogiri Regency, 2021). Seventy-nine landslides struck from 2014 to 2021 in

Jatiyoso District, Karanganyar Regency, and the Keduang Sub-Watershed (Karanganyar Regency Government, 2021). Landslides are common due to unstable soil aggregates (Sun and Zhou, 2017). Natural and human factors include increased rainfall intensity, mountainous or hilly topography, land use, and land conversion (LULC), which induce landslides (Rofiq et al., 2022). Keduang Watershed is a water catchment in Wonogiri and Karanganyar Regencies. In the Keduang Sub-Watershed, significant cases of land degradation are caused by erosion and sedimentation. In 2016, the Keduang Sub-Watershed had an erosion rate of 1 829 554.91 ha ton^{-1} year^{-1}; in 2021, it was 1 128 910.89 tons ha^{-1} year^{-1}. Despite a 700 644.02 tons ha^{-1} year^{-1} erosion rate drop from 2016-2021, this number is still significant (Aisy et al., 2023).

Climate change and anthropogenic land degradation worsen soil erosion, a significant challenge to environmental sustainability and agricultural productivity (Zhu et al., 2019; Sengupta and Thangavel, 2023).Due to housing and infrastructure development, tremendous population growth reduces agricultural land, threatening water availability, biodiversity, and biomass production (Wahyuti et al., 2023; Widhiyastuti et al., 2023). The population pressure value on agricultural land in the Keduang Sub-Watershed is 28 978.16, indicating an imbalance between population growth and agricultural land availability, causing extensive erosion and land degradation (Wuryanta and Susanti, 2015). Wonogiri Regency in Central Java has significant agricultural potential, especially for food crops covering 53.80% of the area. Intensive rice cultivation without sustainable agricultural methods could harm the environment (Mujiyo et al., 2023; Romadhon et al., 2023; Şenyer et al., 2023). Furthermore, soil characteristics like soil aggregate stability, which regulates water availability and air circulation, and soil colloids, which affect plant growth, also affect erosion events. Massive deforestation, intensive agricultural practices, temperature, wind, rainfall intensity, human activities, and climate change threaten soil sustainability and productivity by causing erosion. Erosion causes soil degradation by removing topsoil containing humus, changing soil depth, and reducing soil nutrient status, reducing agricultural land productivity. The Keduang Sub-Watershed medium class has the highest soil degradation potential at slope slopes >40% (Istiqomah et al., 2023). Higher slopes increase deterioration risk (Mujiyo et al., 2022; Romadhon et al., 2023). The Keduang Watershed has minimal land cover, especially forest, which causes floods and landslides (Nugrahanto et al., 2022; Romadhon and Aziz, 2022). The impact is that during the dry season, the water source discharge in Wonogiri Regency decreases from 8 dm^3 s^{-1} to 6 8 dm^3 s^{-1} (Radarsolo, 2023). The results of the evaluation carried out by the Bengawan Solo River Basin Center (BBWS) stated that the handling of sedimentation from the Keduang Sub-Watershed still needs to be improved, causing damage to the function of the reservoir (Solopos, 2022).

The slaking index has the most significant impact on aggregate degradation. Slaking assessment is a new method to develop erosion management and soil stability faster and more efficiently. According to Xiao et al. (2017), the results show that slaking causes more than 50% of aggregate disturbance when rainfall kinetic energy is between 50 and 800 J m^{-2} h^{-1}. Lower aggregate stability and greater slaking values increase soil erosion (Nciizah and Wakindiki, 2015). Previous research by Jones et al. (2021) shows that slaking value affects soil type and agricultural land use where the slaking index in dry land on mixed crops of wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.), cotton (*Gossypium hirsutum* L.), and chickpeas (*Cicer arietinum* L.) in vertisol soil with irrigation is higher than in pasture and forested areas. We investigated the slaking index in the Keduang Sub-Watershed, which has diverse soil types and agricultural land uses. The slaking index is assumed to be affected by soil type. This research intends to investigate the distribution of the slaking index on agricultural land along the Keduang Sub-Watershed, how soil type affects it, and what soil physical and chemical factors most affect it. To reduce erosion risk, to offer soil and land management solutions for agricultural land around river watersheds. By guiding conservation efforts, surveying the Keduang Sub-Watershed slaking index can help reduce erosion and soil damage.

2. Material and Methods

2.1 Research site

The research was conducted in the Keduang Sub-Watershed in Karanganyar and Wonogiri Regencies, Central Java Province, Indonesia, from February to August 2023. Keduang Sub-Watershed

is found at 7°42'29.65"-7°55'27.97"S and 111°13'23.51"-110°56'54.61"E. The total area of the research site is 29 242.98 ha. Soil samples have been analyzed in the Soil Laboratory of Universitas Sebelas Maret. Its land use includes forests (1 284.11 ha), moorland (5 947.54 ha), paddy fields (15 380.78 ha), and plantations (6 630.55 ha). Soil types in the research area consist of Andisols (4 380.23 ha), Alfisols (9 540.85 ha), Inceptisols (12 538.92 ha), and Entisols (2 782.98 ha). Those data were obtained from the Indonesian Center for Agricultural Land Resources Research and Development (ICALRD) (ICALRD, 2020). Research area's slope characterized as flat (1-8%) of 12 471.94 ha, sloping (9-15%) of 8 366.16 ha, fairly steep (16-25%) of 5 859.99 ha, and severe (26-40%) of 2 544.89 ha. Low (1 709.4 mm $year^{-1}$), medium (2 074 mm $year^{-1}$), and high (3 359.2 mm $year^{-1}$) rainfall are found in the research area (Minister of Environment, 2009).

2.2 Soil sampling and analysis

The working map for observation and sampling points is in the form of a Land Mapping Unit (LMU), made at a scale of 1:50 000 and includes the results of a base map overlay, namely a rainfall map (Figure 1), a soil-type map (Figure 2), a slope map (Figure 3), and a land use map (Figure 4) determination of sample points using purposive sampling. The number of LMUs obtained from the overlay results was 22 LMUs, with sampling repeated 3 times, so 66 samples were taken. The thematic maps used to create land unit maps are administrative maps (Indonesian Earth Map, Geospatial Information Agency, 2014), soil type maps (ICALRD, 2020), rainfall maps (Data from the Bengawan Solo River Region Center (BBWS) in 2021), a land use map (Peta Rupa Bumi Indonesia), and a slope map (Digital Elevation Model (DEM) of Indonesia) with a scale of 1:50 000 using ArcGIS 10.4 software.

Soil samples were taken in the tillage layer of the soil, namely a depth of 1-20 cm from the soil surface. The results of the thematic map overlay show the LMU of 22 with a total of 66 sampling points, then each sample taken was ±1 kg (presented in Figure 5). Three points repeated the number of samples taken at 22 LMUs with 66 sampling points. Several parameters of soil physical and chemical properties were analyzed in this research (Table 1).

Table 1. Parameters and methods of soil analysis

Parameters	Units	Methods
Soil Physical Properties (Center for Research and Development of Agricultural Land Resources, 2022)		
Aggregates Stability	%	Double Sieve
Bulk Density	g cm^{-3}	Ring Sample
Texture	%	Pipette
Structure		Qualitative
Soil Chemical Properties (Center for Research and Development of Agricultural Land Resources, 2009)		
pH		Electrometry
Organic-C	%	Walkley & Black
Cation Exchange Capacity (CEC)	cmol (+) kg^{-1}	NH_4OAc N pH 7

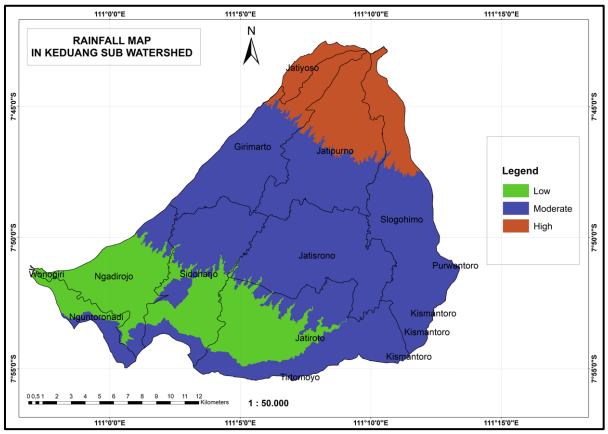


Figure 1. Rainfall map in Keduang Sub-Watershed.

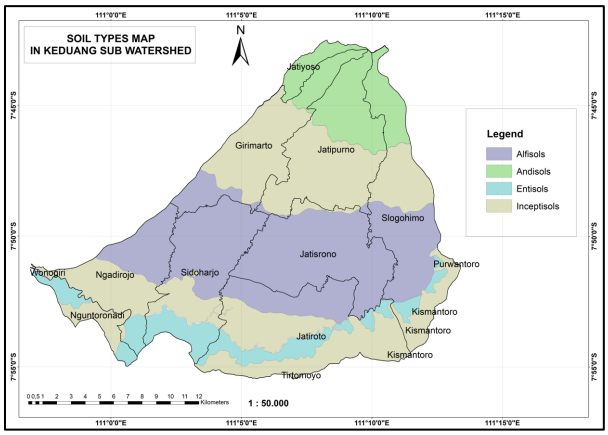


Figure 2. Soil type map in Keduang Sub-Watershed.

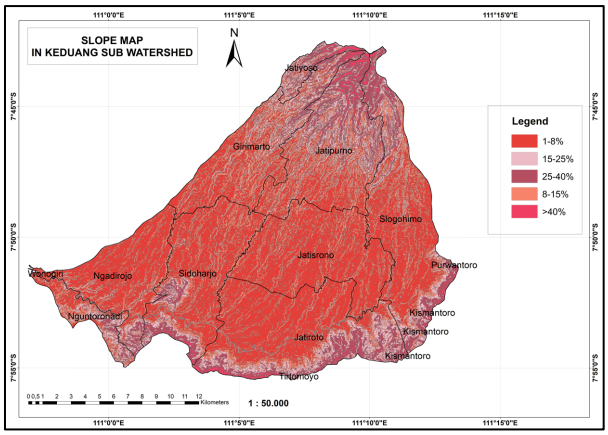


Figure 3. Slope map in Keduang Sub-Watershed.

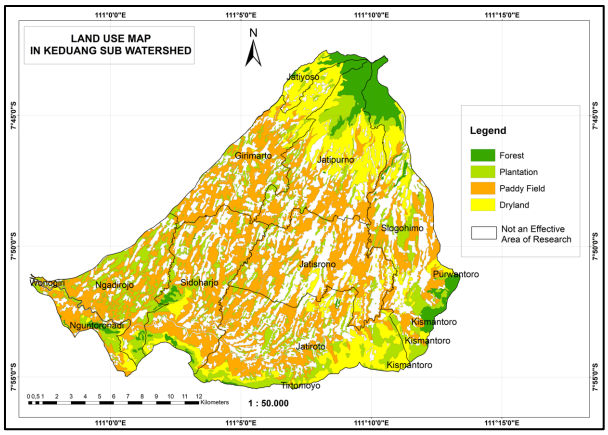


Figure 4. Land use map in Keduang Sub-Watershed.

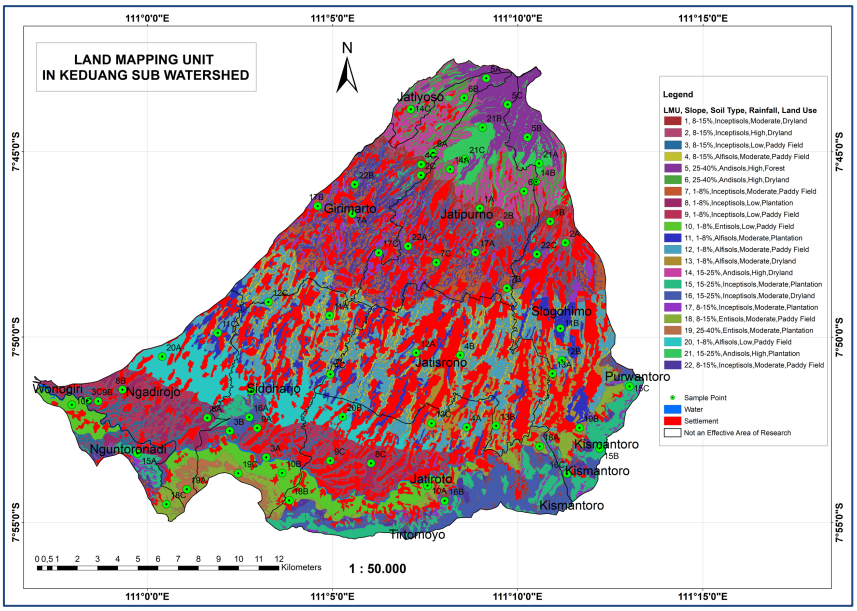


Figure 5. The LMU in Keduang Sub-Watershed.

2.3 Slaking index analysis

The soil samples used were three aggregates measuring 2-15 mm. (1) The first stage for analyzing with the SLAKES application is to place three soil aggregates into the first petri dish and then take a photo of the initial soil condition by pressing the "reference image" button; (2) the first petri dish is then replaced with a second petri dish of the same diameter (9 cm) filled with 3/4 of distilled water; (3) the soil samples in the first petri dish were transferred to the second petri dish in a spread out position (not stuck together); (4) then press the "start" button on the SLAKES application and wait for processing for 10 minutes, then the slaking index value will be displayed on the smartphone application screen (Fajardo and McBratney, 2019). The slaking index obtained from analysis using the SLAKES application was input and then mapped using ArcGIS 10.4 software.

The SLAKES application uses an image segmentation approach to calculate the footprint area of an aggregate expressed as several pixels. Then, it tracks the relative increase in the area of individual aggregates as they decompose over a certain period (Fajardo et al., 2016). The aggregate slaking index within a certain period after the dyeing process is calculated using a number formula (1)

$$SI_t = \frac{At - A0}{A0} \text{ (Jones et al., 2021)} \quad (1)$$

Where A0 is the initial site area of the aggregate; At is the aggregate site at time t; SI = 0 means the aggregate site area does not change; SI = 1 means the footprint area increases by 100%, etc. The SI value obtained from the SLAKES application is the average of parameters calculated individually for each aggregate. The SI values obtained were then classified based on the slaking index coefficient (Table 2). The higher the SI value, the lower the aggregate stability, and vice versa.

Table 2. Slaking index coefficient (Fajardo and McBratney, 2019)

Slaking Index Coefficient	Aggregate Stability Class
<3	High
3-7	Moderate
>7	Low

2.4 Statistical analysis

Research data obtained from field surveys and laboratory analysis carried out using One-way Analysis of Variance (ANOVA) to determine the effect of soil type on the slaking index, and if there any significant value ($\alpha \leq 0.05$; $\alpha \leq 0.01$), it continued further by Duncan Multiple Range Test (DMRT) to

determine whether the mean value. Pearson Correlation Analysis with p-value <0.05 using the SPSS ver. 25.0 to find the determining factors of the relationship between soil parameters and the slaking index. The series of statistical analyses aims to develop recommendations for appropriate soil management in managing erosion risk by controlling the research area's determining factors.

3. Results

3.1 Physical and chemical characteristics of soil in the Keduang Sub-Watershed

The results of analyses on the physical and chemical (Table 3) parameters of the soil were analyzed to determine which soil properties support the interpretation of the slaking index data and which parameters are related. Aggregate stability in Keduang Sub-Watershed, analyzed using the double sieve method, is included in the unstable classes, not very stable, pretty stable, durable, and very stable (Center for Research and Development of Agricultural Land Resources, 2022). The less stable class is only at SPL 9, while the LMUs included in the less stable class are LMU 1 and 19. Aggregate stability is relatively stable at LMU 6, 11, 12, 13, 14, 15, 16, 18, 20, 21, and 22. The bulk density in the Keduang Sub-Watershed is in the range of 1.03 g cm^{-3} up to 1.66 g cm^{-3}. The lowest BD value is at LMU 5, and the highest is at LMU 10. The soil's clay content in the Keduang Sub-Watershed ranges between 5.95% to 75.01%. The texture in the Keduang Sub-Watershed consists of clay loam, clay, silt clay, silt clay loam, loam, silt loam, and silt. Clay textures are the most dominant textures in the research area. pH value is in the range of 6.56 to 7.25, where the highest value is at LMU 8 while the lowest is at LMU 10. Organic C in the study area is 0.68-2.15%, with an average of 1.27%. The organic C grade is included in the low, low, and medium classes, with the average being in the typical class. The Cation Exchange Capacity (CEC) in the Keduang Sub-Watershed is 54.83-92.02 cmol(+) kg^{-1}, included in the very high class. Na^{+} is in the value range of 0.19-1.71. The K^{+} value is in the value range of 0.24-1.29. The Ca^{2+} is in the value range of 10.69-45.40, and Mg^{2+} values are 1.45-19.36.

Table 3. Soil physical and chemical characteristics in the Keduang Sub-Watershed

LMU	Aggregate Stability (%)	Bulk Density (g cm^{-3})	Clay (%)	pH	Organic-C (%)	CEC (cmol(+) kg^{-1})	Exc. Na^{+} (cmol(+) kg^{-1})	Exc. K^{+} (cmol(+) kg^{-1})	Exc. Ca^{2+} (cmol(+) kg^{-1})	Exc. Mg^{2+} (cmol(+) kg^{-1})	Slaking Index
1	44.92±4.82	1.25±0.13	68.27±32.84	6.96±0.16	1.23±0.06	54.83±10.03	0.46±0.05	0.26±0.54	11.71±5.44	2.55±2.16	1.00±0.53
2	75.49±16.73	1.35±0.13	60.96±7.96	6.84±0.10	1.47±0.59	72.13±9.24	0.55±0.23	0.41±0.05	32.27±6.28	6.13±1.57	1.20±0.80
3	88.18±18.18	1.53±0.03	51.11±8.29	6.97±0.04	1.12±0.02	54.93±1.33	0.75±0.33	0.36±0.10	35.70±2.22	12.08±4.03	1.30±0.87
4	125.80±10.66	1.26±0.10	69.72±11.20	6.97±0.07	0.74±0.45	66.06±7.96	0.59±0.10	0.60±0.22	22.25±2.04	5.86±0.20	0.30±0.20
5	73.73±13.44	1.03±0.21	39.68±10.71	6.78±0.06	2.09±1.10	62.56±5.07	0.68±0.08	1.12±0.36	18.25±2.52	1.45±0.95	11.63±0.12
6	62.04±13.89	1.20±0.17	60.72±21.43	6.76±0.33	1.47±0.79	55.98±21.45	0.64±0.07	0.42±0.17	10.69±2.66	2.14±1.57	0.33±0.15
7	96.14±9.36	1.38±0.14	59.10±4.95	6.88±0.03	1.37±0.50	69.09±10.82	1.71±0.96	0.38±0.17	28.27±4.57	6.10±3.45	0.20±0.10
8	73.74±10.88	1.56±0.17	62.85±10.70	7.12±0.06	0.83±0.51	83.39±15.61	0.79±0.26	0.54±0.46	23.14±8.88	6.69±0.99	0.13±0.06
9	34.31±12.08	1.57±0.07	57.13±25.91	6.77±0.34	0.97±0.49	79.44±4.03	1.03±0.38	1.29±0.48	31.27±2.25	9.62±1.18	0.80±0.52
10	104.43±33.46	1.66±0.19	46.98±11.74	6.56±0.40	1.23±0.55	92.02±5.33	0.69±0.40	0.40±0.08	35.85±1.48	11.71±1.04	0.37±0.31
11	64.09±7.13	1.42±0.17	75.01±7.63	6.98±0.07	1.38±0.32	83.82±2.15	0.19±0.11	0.61±0.39	11.33±2.11	3.86±0.33	0.40±0.36
12	62.91±5.30	1.48±0.12	64.23±13.50	6.98±0.19	2.15±1.53	79.67±3.26	1.00±0.18	0.31±0.24	25.96±5.98	5.51±1.58	0.17±0.12
13	50.67±11.93	1.38±0.15	45.90±22.23	6.83±0.02	0.68±0.16	72.84±14.07	0.89±0.29	0.36±0.06	22.20±0.73	6.41±1.60	0.20±0.10
14	50.97±14.33	1.46±0.18	61.47±14.61	6.97±0.03	1.20±0.51	70.12±5.54	0.74±0.41	0.44±0.22	25.57±5.18	6.76±7.33	1.13±0.35
15	60.13±13.12	1.46±0.17	17.59±15.06	7.25±0.02	1.36±0.12	84.57±4.22	0.97±0.22	0.62±0.20	45.40±4.19	15.65±1.66	0.17±0.06
16	50.19±5.26	1.50±0.21	20.27±9.17	7.08±0.09	0.70±0.39	72.73±6.90	0.43±0.27	0.66±0.31	30.65±4.40	15.13±5.64	0.33±0.15
17	81.12±12.43	1.59±0.20	64.03±12.90	6.62±0.18	1.79±0.40	65.74±5.92	0.60±0.23	0.24±0.04	24.65±1.50	4.10±1.95	0.23±0.15
18	52.92±8.96	1.15±0.02	14.88±10.77	7.17±0.16	0.90±0.39	82.45±8.68	1.35±0.69	0.39±0.15	43.95±4.32	15.88±3.13	0.23±0.15
19	40.48±2.89	1.36±0.19	5.95±3.03	7.11±0.12	0.95±0.37	77.46±10.48	0.30±0.04	0.77±0.25	38.37±2.93	19.36±2.85	0.27±0.06
20	61.65±17.96	1.54±0.13	40.93±18.71	7.04±0.19	0.84±0.36	72.57±6.09	0.34±0.02	0.57±0.17	27.11±2.73	4.87±1.28	0.47±0.15
21	61.87±4.07	1.42±0.19	21.09±19.11	6.96±0.03	2.04±1.39	64.12±8.94	1.04±0.34	0.38±0.28	14.49±1.69	2.21±0.41	0.33±0.15
22	60.09±10.93	1.20±0.20	22.33±12.42	6.81±0.03	1.48±0.37	58.69±9.67	0.27±0.05	0.33±0.18	19.13±3.05	3.87±0.96	0.23±0.15

3.2 The distribution of slaking index under different soil types

The slaking index value in the Keduang Sub-Watershed varies from 0.13 to 11.63, as shown in Table 3. The results were classified according to the slaking index coefficient class (Table 2), with the aggregate stability in the Keduang Sub-Watershed falling into low and high classes. The SLAKES provides a slaking index converted into an aggregate stability class and displayed on a map (Figure 6). The low aggregate stability class, covering 1284.11 hectares, is situated in the northern or upstream section of the Keduang Sub-Watershed and is represented by a brown color. The yellow color represents the high aggregate stability class covering 27 958.87 hectares, predominating most of the Keduang Sub-Watershed area.

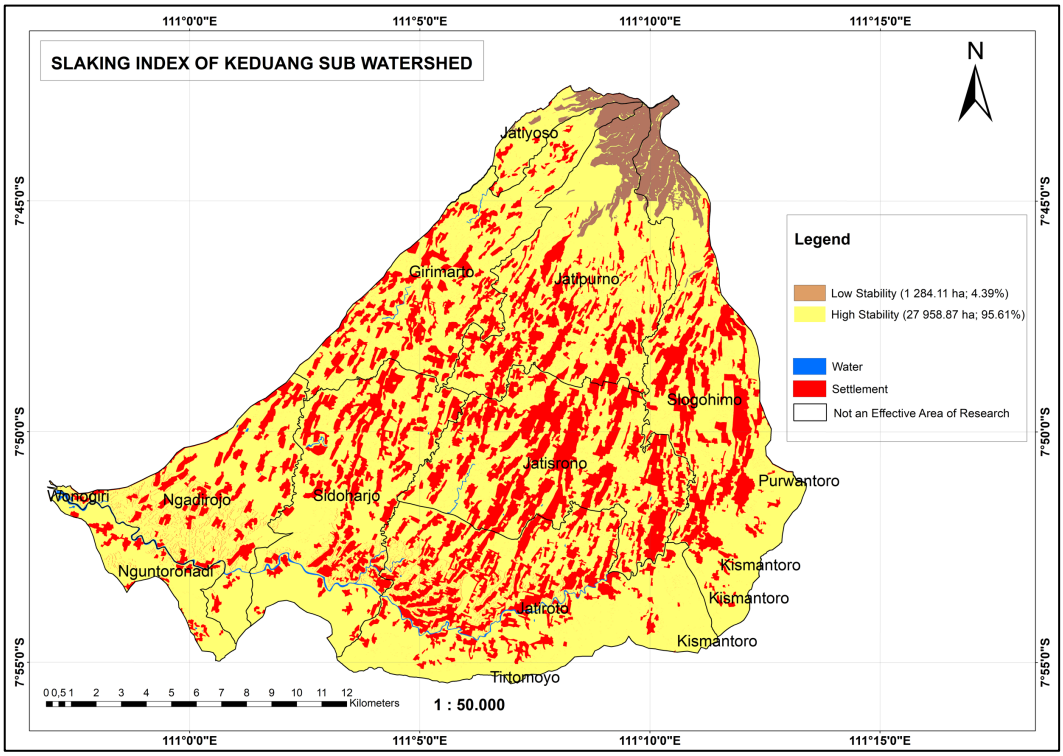
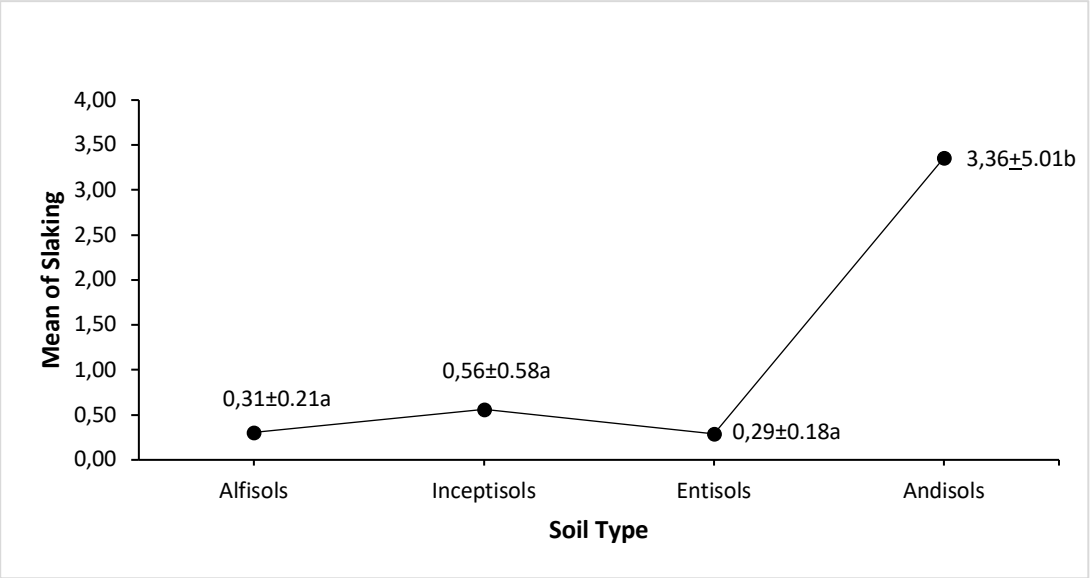


Figure 6. Aggregate stability on a slaking index.

The ANOVA was done to determine the effect of soil type on the slaking index. The variability of soil type is significantly affected by the slaking index in the Keduang Sub-Watershed (F-count = 6.082; p-value = 0.001; N = 66). The DMRT was conducted to determine whether the mean values differed significantly. Based on DMRT between soil types on the slaking index, Andisols are significantly different from Entisols, Alfisols, and Inceptisols (Figure 7). This condition shows that different types of soil cause other slaking indices. The Andisol soil type, which is located upstream of the sub-watershed and located at an altitude of > 1000 meters above sea level, has the characteristics of a more crumbly soil and low bulk density, so it is very easily destroyed during the soaking process for slaking index analysis.



*.Numbers followed by the same letter notation indicate there is no significant difference based on the DMRT test at the 5% level

Figure 7. Average index value of slaking under different soil types.

3.3 Determinant Factors

Defining factors can be used as a reference to provide recommendations for land management in the research area. The statistical analysis to help determine the determining factors is the test between sources of diversity and the slaking index. The correlation test results (Table 4) can be used as a supporting basis for the test to provide land management recommendations.

Table 4. Relationship between aggregate stability, soil physical characteristics, soil chemistry, and slaking index

	Agr Stability	BD	Clay	pH	Org-C	CEC	Exc. Na	Exc. K	Exc. Ca	Exc. Mg	Slaking
Agr Stability	1										
BD	.036	1									
Clay	.281*	.102	1								
pH	-.242*	.067	-.337**	1							
Org-C	.082	.047	.066	-.186	1						
CEC	.011	.339**	-.079	.194	-.216	1					
Exc. Na	.144	-.101	-.028	-.026	.043	.155	1				
Exc. K	-.240	-.056	.024	-.038	-.112	.012	-.130	1			
Exc. Ca	.023	.233	-.406**	.243*	-.133	.496**	.281*	-.064	1		
Exc. Mg	-.157	.109	-.473**	.359**	-.349**	.369**	.136	.042	.742**	1	
Slaking	.031	-.374**	-.016	-.146	.237	-.190	-.049	.394**	-.166	-.242	1

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

4.1 Soil characteristics in the research site

Soil characteristics, especially aggregate stability, are closely related to soil sensitivity to erosion (Liu et al., 2023; Yao et al., 2020). Aggregate stability is influenced by management strategies and soil type, typically rising with higher levels of soil organic matter, clay surface area, and CEC (Temgoua et al., 2019). This is in line with the results of our research, which show that the average CEC value is high, with the most dominant texture being clay, so the aggregate stability class based on the

slaking index is dominated by high stability. A high slaking index with low aggregate stability occurs in forest land use with Andisols soil type, 26-40% slope, and high rainfall. Areas with steep elevations usually have high erosion on the same slope. Forests on Andisol soils with lower crop cover have higher erosion because infiltration is low (Henry et al., 2013; Neris et al., 2013).

4.2 The distribution of slaking index in various soil types

Slaking is the process of clay disintegration upon contact with water, leading to erosion and stability issues (Gautam and Shakoor, 2016). The LMU 5 is the only one classed in the low aggregate stability class. The rest are classified as high. The LMU 5 is a land map unit characterized by heavy rainfall and Andisols-type forest land use on a slope ranging from 26% to 40%. The LMU 5 indicates a slaking index value of 11.63, signifying a 1 163% expansion of the sample area after a 10 minute soaking period. Andisol soil is characterized by a crumb structure with easily disintegrating particles. Additionally, according to Hanifa and Suwardi (2022), soil on steep slopes has low stability and high erosion susceptibility due to its coarse texture.

The aggregate of LMU 5 is significantly different than others, caused by the mineralogical properties of Andisol soil in the form of allophane. Allophane is a non-crystalline mineral with many fine pores that is soft when wet, has a high water binding capacity, and does not have a defined crystal structure, making it extremely soluble (Suryani et al., 2015). Allophane minerals found in Andisols are likewise less stable than crystalline minerals. Andisols are young volcanic ash containing organic glass, so they dissolve easily and quickly weather in humid conditions into non-crystalline minerals (Suratman et al., 2018). This condition causes soil with Andisols aggregate to have a high slaking index because the aggregate easily expands and disintegrates when soaked. The expansive capacity of soil depends on the type and amount of minerals and their cation exchange capacity (Schäbitz et al., 2018). Differences in clay content in each soil type cause the slaking index results to vary. The clay-dominated fraction will tend to be dispersed moderately, but if it is bound by organic material, it will be slightly dispersed (Karahan and Yalim, 2022; Umam et al., 2022).

Other LMUs have better aggregate stability since fewer soils expand during soaking than LMU 5. The lowest slaking index is 0.13 at LMU 8: Inceptisols with plantation land use, 1-8% slopes, and low rainfall. The clay content at LMU 8 (62.85%) is higher than at LMU 5 (39.68%). Even though the total clay is higher, the different soil types cause LMU 8 to be more stable, and Inceptisols do not have allophane. Inceptisol soil colloids are included in crystalline silicates. Clay 2:1 smectite type can expand and contract, containing illite and montmorillonite minerals. The ability to develop each soil aggregate depends on the amount of montmorillonite mineral content (Li et al., 2020).

The LMU 9 (Inceptisols on paddy fields) has the lowest aggregate stability in the unstable class. Processing the land as rice fields with a hoe or agricultural machinery damages the aggregate at LMU 9, making it unstable. Continuous planting damages paddy aggregates, reducing aggregate stability (Gu et al., 2023). The source of diversity in the form of soil type significantly affects the slaking in the Keduang Sub-Watershed. Andisols are pretty different from other soil types, while Alfisols, Inceptisols, and Entisols are closely alike. This condition means that the Andisols experience aggregate disintegration and the highest slaking index value compared to others. Andisols have the highest slaking value, with a clay content of 39.68%, BD 0.12 g cm^{-3}, and CEC 62.56 cmol(+) kg^{-1}. Research by Jones et al. (2021) shows that land use and soil type are correlated with the slaking, with vertisol soil having a clay content of >25% and a CEC: clay ratio of >0.5, it has the highest slaking index value with land use, and differences in soil type cause different slaking indexes. This condition matches our studies showing that clay concentration >25% has a high slaking index. The slaking index of dry land in this study is similar to that of rice fields and plantations; however, it tends to be high. With vertisol soil having a clay content of >25% and a CEC: clay ratio of >0.5, it has the highest slaking index value with land use, and differences in soil type cause different slaking indexes. Intensive tillage on moorland causes a decrease in the physical quality, including aggregate damage (Mamta et al., 2023).

Organic C is one of the fundamental parameters in sustainable agriculture (Alaboz et al., 2022; Rahayu et al., 2024; Romadhon et al., 2024) and plays a role in nutrient availability, improving soil's physical, chemical, and biological properties (Farrasati et al., 2019; Meilani et al., 2023; Smith et al., 2013). The results of organic C analysis show that organic C at the research location is in the very low to medium category (Center for Research and Development of Agricultural Land Resources, 2009). The

medium class, organic C content, is at LMU 5, 12, and 21 (forests, rice fields, and plantations). The organic C content varies depending on land use (Romadhon et al., 2024), with higher levels found in plantation land use (Dadgar, 2018; Supriyadi et al., 2021), while research by Eleftheriadis et al. (2018) shows the highest average organic C in forests, other research states that rainfed paddy fields have the organic C content is higher than in plantations (Rekwar, 2022). Rainfall affects the organic C content (F-count = 3.946; p-value = 0.024; N = 66). This condition can occur because decreased rainfall causes reduced accumulation of soil organic matter, resulting in poor soil fertility (Gong et al., 2013).

4.3 Defining factors

Based on the results of the correlation analysis, it was found that bulk density had a significant negative correlation with slaking and a significant positive correlation with exchangeable K^+. This interrelated relationship shows that improving bulk density and exchangeable K determinants can improve the slaking index. The positive correlation between the slaking index and BD (Table 4) shows that the smaller the BD, the higher the slaking index value. The large number of micropores in Andisols makes the BD low at 1.03 g cm^{-3}. Bulk density will affect the pore space of the soil; the lower the BD value, the higher the pore space will be (Juarti, 2016). Andisols, which is generally located at an altitude of >1,000 meters above sea level, has a low bulk density value, so the slaking index value is high, in line with research results where LMU 5 with the Andisol soil type in the upstream sub-watershed has the highest slaking index value.

The CEC value supports the bulk density, which can be seen from the analysis results and has a significant positive correlation. Andisols are characterized by low density, friable to very friable consistency, mineralogical composition dominated by allophane, and high P retention, organic matter content, and cation exchange capacity (Yatno et al., 2016). In line with the results of this research, which show that the CEC on Andisols soil is in the very high category. Apart from CEC, the slaking index has a significant positive correlation with other soil chemical properties, namely exchangeable K^+ cations, meaning that the higher the SI, the higher the K cation. The exchangeable K content in the soil is influenced by clay minerals, namely smectite, texture, organic C, and CEC (Volf et al., 2017; Toprak and Seferoğlu, 2023). Vertisols, Alfisols, and Inceptisols contain the mineral smectite. It means that the minerals contained in the soil tend to consist of illite and a small amount of montmorillonite, and the low increase in aggregate area proves this. Illite and vermiculite are 2:1 type clay minerals that can bind potassium, which plays a role in determining the availability of K in the soil (Bilias et al., 2022). Illite has bonds with K cations, which can be substituted by H^+ ions when H_2O is present. The amount of exchangeable K^+ at the research location is correlated with the slaking index. It means that the minerals found in the soil tend to consist of illite and a small amount of montmorillonite, and the low increase in aggregate area proves this.

Aggregate stability is strongly linked to soil characteristics that enhance aggregation, such as CEC and the percentage of polyvalent cations (Ca^{2+}, Al^{3+}). These properties support disaggregation, such as quartz content and monovalent cations (especially K^+), while clay dispersion is closely related to pH, power content, texture, and Na^+ adsorption ratio (Almajmaie et al., 2017). Based on the correlation test results, aggregate stability is supported by clay content, pH and is indirectly related to exchanged Ca and exchanged Mg. Clay content was significantly negatively correlated with exchangeable Ca and Mg, while pH was positively correlated with exchangeable Ca and Mg. It means that the higher the clay content, the higher the aggregate stability, while the amount of Ca and Mg that can be exchanged is lower. The low availability of polyvalent cations (Ca^{2+} and Mg^{2+}) causes soil to be more susceptible to dispersion due to the repulsive force between negatively charged clay particles. Polyvalent cations such as Ca^{2+} and Mg^{2+} function to react with clay or organic material to strengthen soil aggregates. The Ca and Mg content can be exchanged in soil pH, resulting in higher stability due to the negative bonds of clay particles, transforming them into more stable microparticles.

4.4 Erosion risk management strategy through management of soil slaking determinants

Reducing soil bulk density will reduce the slaking index. Adding soil organic matter can reduce the bulk density and soil compaction, thereby increasing the total porosity and infiltration rate (Brar et al., 2015; Syamsiyah et al., 2023). In general, returning plant residues to the soil can increase the organic matter content of the soil, thereby helping to improve the nutrient content in the soil, especially in the

tillage layer (Stošić et al., 2020; Mujiyo et al., 2021; Dewi et al., 2022). The organic solid waste from sugar factories, called *blotong* can reduce the bulk density to a reduction of 0.103 g cm^{-3} in the tillage layer (Hartono et al., 2018) and increase the soil pore space. Apart from that, conservation practices can be carried out by cultivating contours and improving terraces (Dewi et al., 2023), as well as using organic mulch as core reinforcement has been proven to be able to reduce erosion by 15, 51% in Andisol (Suyana et al., 2017). The aggregate stability is correlated to soil properties such as sand/quartz content smectite. It is highly correlated with Ca^{2+} exchange capacity, indicating that soil hardening can be reduced by applying calcium products such as gypsum and dolomite (Almajmaie et al., 2017; Fitria and Soemarno, 2022; Cahyono et al., 2023).

Conclusion

The slaking index is evaluated using the software as a unique, basic, cost-effective, and practical approach to ascertain aggregate stability and formulate erosion control strategies in the study site. Andisols in the forest have the highest slaking index value. The minimum value is associated with Inceptisols in the planting. The slaking index is significantly influenced by soil type, with Andisols having the greatest value. In Andisols, aggregate rises due to the mineral allophane, which is non-crystalline and less stable than the minerals and swells of other soil types and disintegrates when moistened. The key factors are the soil's bulk density and exchangeable potassium levels. Soil with lower bulk density has a higher slaking index, and a decrease in exchangeable potassium is directly related to a fall in the slaking index. To reduce erosion risk in the Keduang Sub-Watershed, incorporate organic material and limit intense tillage methods. The research on slaking index assessment in different soil types in the area reveals its ability to analyze the distribution of slaking index across diverse soil types. The determinant factors can be a recommendation for creating erosion risk management in the Keduang Sub-Watershed. The slaking index will evaluate erosion estimates, saving time, effective analysis, and efficient economics.

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Short Communication

Bioprospecting of Fragrant Ginger (*Zingiber aromaticum*) Endophytic Bacteria from Enggano Island, Indonesia as Antimicrobial Compounds Producer

Risky HADI WIBOWO*[1], Sipriyadi[2], Welly DARWIS[3], Eddy SUKMAWINATA[4], Masrukhin[5], Mashudi[6], Muhammad ASRIL[7], Thoriqul HIDAYAH[8], Aldy TRIANDA[9]

[1,2,3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu, Indonesia, 38112, Indonesia
[1,2,8]Master Study Program of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Jl. W.R. Supratman, Bengkulu, 38371, Indonesia
[4]Research Centre for Veterinary Science, Research Organization for Health, National Research and Innovation Agency, Bogor, West Java, 16911, Indonesia
[1]Research Centre of Sumatera Natural Products and Functional Materials, Universitas Bengkulu, Jl. W.R. Supratman, Bengkulu, 38371, Indonesia
[5]Biosystematics and Evolution Research, Cibinong Science Center, Cibinong, Indonesia, 16911, Indonesia.
[6]Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia, 60111, Indonesia
[7]Department of Biology, Institut Teknologi Sumatera, Way Hui, Jati Agung, Lampung Selatan, Lampung, 35365, Indonesia
[9]Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu, 38112, Indonesia

[1]https://orcid.org/ 0000-0002-5917-4625, [2]https://orcid.org/ 0000-0003-1042-2576, [3]https://orcid.org/0000-0002-5982-8983
[4]https://orcid.org/0000-0002-8749-0270, [5]https://orcid.org/0000-0001-8853-6034, [6]https://orcid.org/0009-0001-4819-505X
[7]https://orcid.org/0000-0001-8637-4190, [8]https://orcid.org/0009-0005-2719-3046, [9]https://orcid.org/0009-0004-2608-7527

*Corresponding author e-mail: riskyhadiwibowo80@gmail.com

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Abstract: Fragrant Ginger or Lempuyang wangi (*Zingiber aromaticum* Val.) is one of the plants from the Zingiberaceae family that Indonesians widely use as traditional medicine. Endophytic bacteria living in the healthy plant are potentially carrying antimicrobial properties and good secondary metabolites. This study aims to determine the potential of endophytic bacteria from fragrant ginger plants from Enggano Island as antimicrobial. Antimicrobial activity was analyzed using the disc diffusion method from pallets and supernatant of bacteria. The results showed that five of 44 isolates consisting of *Providencia* strain LWERG 29, *Stenotrophomonas* strain LWERG 30, *Bacillus* strain LWEBG 39, *Bacillus* strain LWEBG 41, and *Pseudomonas* strain LWEBG 42 isolates were able to suppress pathogenic bacteria such as *B. subtilis*, *P. aeruginosa*, and *E. coli*. Interestingly, those selected species could show their ability to inhibit tested pathogens with a strong category. This is the first study that showed the potential of endophytic bacteria as antimicrobial agents isolated from fragrant ginger (Lempuyang Wangi) in Enggano Island, Indonesia.

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1. Introduction

Lempuyang Wangi (*Zingiber aromaticum*), or fragrant ginger, is a Zingiberaceae family plant widely cultivated in the tropics, including Indonesia, but its origin is still uncertain (Leong-Skornickova et al., 2019). Enggano Island is an island located in the Indian Ocean, Indonesia. Geological data show that this island is oceanic and unique regarding its biogeography and evolution. Four genera of Zingiberaceae were reported from Enggano Island: Amomum, Etlingera, Zingiber, and Curcuma, mostly found along the rivers in Kuala Besar and Kali Jangkar districts (Hamidy et al., 2017). Lempuyang Wangi or fragrant ginger rhizome is commonly applied as a remedy for asthma, diarrhea, malaria, flu, anthelmintics, and appetite stimulants. Secondary metabolites found in Lempuyang Wangi include zerumbone, α-humulene, β-selinene dan (-)-caryophyllene oxide, saponins, tannins, flavonoids, and essential oils (Aji and Zakkiyah., 2021). The isolation of endophytic bacteria, which live inside plant tissues without causing harm to plants, and benefit their host by producing substances that regulate growth and disease resistance, is considered to have bioactive compounds such as antibiotics similar to those produced by the host plants (Achika Rori et al., 2020; Duhan et al., 2020; Fani et al., 2022; Uçar et al. 2023).

Since drug resistance has emerged, the discovery and development of new antibiotics have been challenging. Several genera of endophytic bacteria were known to produce secondary metabolites and potential sources of novel antimicrobials, such as *Streptomyces* sp. Tc022 was isolated from Zingberaceae (*Alpinia galanga*) as an actinomycin producer (Nurjannah et al., 2023). A recent study showed the potency of Zingiberaceae endophytic bacteria isolated from North Sumatera, Indonesia to inhibit pathogens such as Enteropathogenic *Escherichia coli*, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* BTCC B693, *Methicillin-Resistant S. aureus* ATCC 43300, *S. epidermidis* ATCC 12228 and *Proteus vulgaris* ATCC 13315 (Mamangkey et al., 2020). Recently, we successfully isolated 44 fragrant ginger endophytic bacteria from Enggano Island and grouping into the genera *Bacillus*, *Micrococcus*, *Amphibacillus*, *Pseudomonas*, and *Azotobacter* based on morphology, Gram-staining, and biochemical tests. (Andeas et al., 2023). Nevertheless, the detailed information on endophytic bacteria species from Indonesian fragrant ginger is poorly explored. In this study, we explored the potential of collected fragrant ginger endophytic bacteria, including their species, as a producer of antimicrobial compounds against pathogenic microbes.

2. Material and Methods

2.1. Bacteria isolates

In this study, 44 endophytic bacterial isolates from the collection of the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu were screened to see the potential of antimicrobial activity. Previously, those collections were isolated from fragrant ginger (*Zingiber aromaticum* Val.; local name: Lempuyang Wangi) from Enggano island, Bengkulu Province, Indonesia. Gram Staining and biochemical tests of those isolates have been done in our previous study (Andeas et al., 2023). All isolates were recultured to Nutrient Agar (NA; Oxoid CM0003B, UK) supplemented with nystatin (0.01% w/v) and incubated at 30 °C for 48-72 h (Andeas et al., 2023).

2.2. Antimicrobial test of endophytic bacteria against pathogenic microbes

The antimicrobial activity of endophytic bacterial isolates was tested against four pathogens consisting of three bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and a fungi *Candida albicans* based on Wibowo et al. (2023). First, overnight cultures of pathogen bacteria and fungi at 30 °C were prepared using tryptic soy broth (TSB; Oxoid CM0129B, UK) and potato dextrose broth (PDB; Oxoid CM0962, UK), respectively. Then, we prepared plate agar containing 1:100 of each pathogen culture mixed with tryptic soy agar (TSA; Oxoid CM0131, UK) for bacteria and potato dextrose agar (PDA; Oxoid CM0139, UK) for fungi. Subsequently, an inoculating loop of endophytic bacterial cultures was spotted on the agar plate containing the pathogen and incubated for 24 h at 30 °C. The endophytic bacterial isolates that were able to inhibit the growth of pathogens were observed from a clear zone surrounding the endophytic bacterial isolate and selected as antimicrobial producers.

To confirm and quantify the antimicrobial activity, 1.5 ml overnight culture from selected endophytic isolates was centrifuged at 10000 rpm for 5 min, and the pellets and supernatant were separated for use in the antagonist test against pathogenic microbes. The pallet from endophyte bacteria was resuspended with 150 µl supernatant to get a concentrate culture. Then, to observe the antimicrobial activity from bacteria and its metabolite, 20 µl of each supernatant and concentrate culture were dripped onto different paper discs, placed on the plate agar containing the pathogen, and incubated for 24 h at 30 °C. Chloramphenicol 30 µg (Oxoid CT0013B) and Nystatin 100 international units (Oxoid CT0073B) were used as a positive control for bacteria and fungi susceptibility, respectively. Antimicrobial activity was evaluated by measurement of the clear zone surrounding the paper disc. Then, the degree of intensity from the antimicrobial zone of inhibition (ZOI) was classified into four categories: very strong (>20 mm); strong (10-20 mm), medium (5-10 mm), and weak (<5mm) (Ouchari *et al.,* 2019). The assay was conducted in two independent experiments. The data analysis was conducted using the statistical data management application Statistical Product and Service Solution (SPSS) 25 with Analysis of Variance (ANOVA). If there were significant differences in the data, the analysis was continued with Duncan's multiple range test (Dahlan, 2014).

2.3. Species identification

The isolate that showed antimicrobial activity was identified to the species using sequencing analysis. Genomic DNA was extracted by using Presto™ Mini gDNA Bacteria Kit (Genaid, GBB100, Taiwan) based on manufacture protocol. All isolates were amplified using 16S rRNA to investigate the bacterial species based on minor modifications from Anggraini et al., (2018). All amplicon products were then sent to Genetika Sains Indonesia in Jakarta/Indonesia for sequencing. The construction of a phylogenetic tree based on the 16S rRNA coding gene was carried out using the Neighbor-Joining method with a bootstrap value of 1000 replications using MEGA 7.

3. Results

In this study, we found five species with antimicrobial activities that consisted of *Providencia* sp. LWERG29, *Stenotrophomonas rhizophila* LWERG30, *Bacillus altitudinis* LWEBG39, *Bacillus amyloliquefaciens* LWEBG41, and *Pseudomonas* sp. LWEBG42. Isolate identification is shown in Table 1 and Figure 1.

Table 1. Sequence alignment of 16S rRNA gene from Lempuyang Wangi isolates

Isolate	Source*	Homology	Query Cover (%)	E-Value	Similarity	Accession Number
LWERG29	Rhizome	*Providencia rettgeri* strain HSC-49S18	100 %	0.0	99.76 %	MK640700.1
LWERG30	Rhizome	*Stenotrophomonas rhizophila* strain KR2-13	100 %	0.0	100 %	MN753976.1
LWEBG39	Stem	*Bacillus altitudinis* strain P5.15	100 %	0.0	99.68 %	QQ295976.1
LWEBG41	Stem	*Bacillus amyloliquefaciens* strain K2-2	100 %	0.0	99.76 %	MH265986.1
LWEBG42	Stem	*Pseudomonas* sp. strain PAMC 27353	100 %	0.0	99.81 %	MT555388.1

*Bacteria were isolated by grinding method (Andeas et al., 2023).

In general, the antimicrobial activity showed by the inhibition zone from the pellet was higher than supernatants. Four species (*Providencia* sp. LWERG29, *S. rhizophila* LWERG30, *B. amyloliquefaciens* LWEBG41, and *Pseudomonas* sp. LWEBG42) in both pellet and supernatant had inhibitory activity against three pathogenic bacteria, *E. coli*, *P. aeruginosa*, and *B. subtilis*. Only *B. altitudinis* LWEBG39 did not have antimicrobial activity against *P. aeruginosa*. However, the *B.*

altitudinis LWEBG39 strain revealed antimicrobial activity against *C. albicans* in pellet form, while antimicrobial activity was not found in supernatant. Specifically, the highest antimicrobial activity of *Z. aromaticum* isolates pellet to *E. coli*, *P. aeruginosa*, and *B. subtilis* were shown by *S. rhizophila* LWERG30, *B. amyloliquefaciens* LWEBG41, and *Providencia* sp. LWERG29, respectively. Consistently, *S. rhizophila* LWERG30 and *B. amyloliquefaciens* LWEBG41 supernatants also showed the highest antimicrobial activity to *E. coli* and *P. aeruginosa,* respectively. When we categorized them based on their strong ability to inhibit bacteria from species pallets, *E. coli* inhibition was only shown by *S. rhizophila* LWERG30. Near all species showed strong intensity to inhibit *P. aeruginosa* and *B. subtilis,* except for *B. altitudinis* LWEBG39 which couldn't inhibit *P. aeruginosa* and *Pseudomonas* sp. LWEBG42 weakly inhibit *B. subtilis*. Interestingly, *B. amyloliquefaciens* LWEBG41 supernatant also revealed strong intensity to inhibit *P. aeruginosa*. The results of the antimicrobial activity test using the disc diffusion method of pellets and supernatant are summarized in Table 2, Table 3, and Figure 2.

Table 2. Antibacterial activity of endophytic *Zingiber aromaticum* pellet by disc diffusion method

Sample	Inhibition Zone (size ± s.d. in mm)			
	E. coli	***P. aeruginosa***	***B. subtilis***	***C. albicans***
LWERG 29	$3.25^{b} \pm 0.35$	$14.5^{d} \pm 0.15$	$16.80^{e} \pm 0.30$	$0.00^{a} \pm 0.00$
LWERG 30	$10.20^{f} \pm 0.10$	$13.95^{c} \pm 0.15$	$12.25^{c} \pm 0.05$	$0.00^{a} \pm 0.00$
LWEBG 39	$5.95^{c} \pm 0.75$	$0.00^{a} \pm 0.00$	$13.70^{d} \pm 0.10$	$4.80^{b} \pm 0.40$
LWEBG 41	$7.15^{d} \pm 0.55$	$16.75^{e} \pm 0.15$	$16.60^{e} \pm 0.10$	$0.00^{a} \pm 0.00$
LWEBG 42	$8.50^{e} \pm 0.20$	$11.45^{b} \pm 0.05$	$8.15^{b} \pm 0.05$	$0.00^{a} \pm 0.00$
Chloramphenicol	$28.00^{g} \pm 0.70$	$20.60^{f} \pm 0.85$	$29.45^{f} \pm 0.5$	NT
Nystatin	NT	NT	NT	$19.15^{c} \pm 0.5$

*Different superscripts indicated a significant difference in the group with $p<0.05$. s.d.: Standard deviation. NT: Not tested.

Table 3. Antibacterial activity of endophytic *Zingiber aromaticum* supernatant by disc diffusion method

Sample	Inhibition Zone (size ± s.d. in mm)			
	E. coli	***P. aeruginosa***	***B. subtilis***	***C. albicans***
LWERG 29	$5.70^{d} \pm 0.30$	$4.05^{c} \pm 0.35$	$5.90^{d} \pm 0.10$	$0.00^{a} \pm 0.00$
LWERG 30	$8.10^{e} \pm 0.30$	$8.55^{d} \pm 0.15$	$7.85^{f} \pm 0.05$	$0.00^{a} \pm 0.00$
LWEBG 39	$1.55^{b} \pm 0.45$	$0.00^{a} \pm 0.00$	$1.40^{b} \pm 0.20$	$0.00^{a} \pm 0.00$
LWEBG 41	$4.25^{c} \pm 0.05$	$14.95^{e} \pm 0.15$	$4.55^{c} \pm 0.25$	$0.00^{a} \pm 0.00$
LWEBG 42	$4.05^{c} \pm 0.35$	$1.55^{b} \pm 0.05$	$6.35^{e} \pm 0.05$	$0.00^{a} \pm 0.00$
Chloramphenicol	$28.00^{f} \pm 0.70$	$20.60^{f} \pm 0.85$	$29.45^{g} \pm 0.5$	NT
Nystatin	NT	NT	NT	$19.15^{b} \pm 0.5$

*Different superscripts indicated a significant difference in the group with $p<0.05$. s.d.: Standard deviation. NT: Not tested.

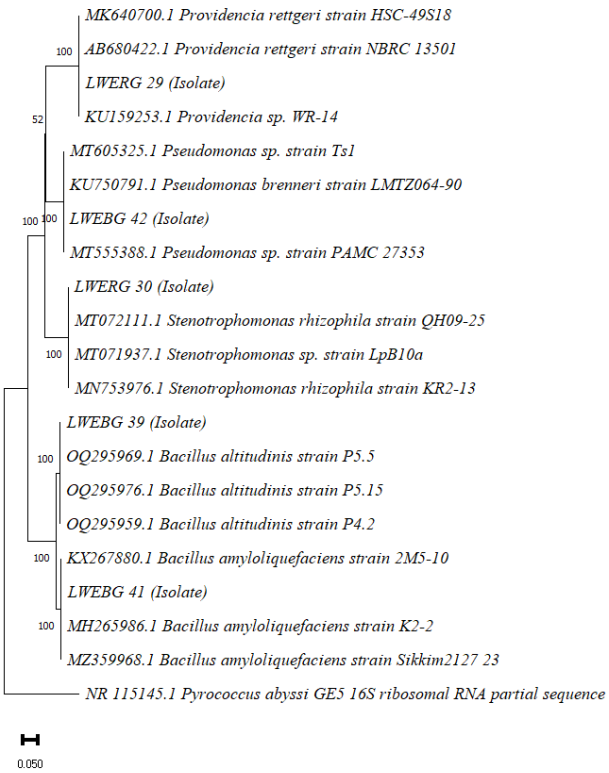

Figure 1. The phylogenetic tree of five sequenced isolates are LWERG29, LWERG30, LWEBG39, LWEBG41, and LWEBG42.

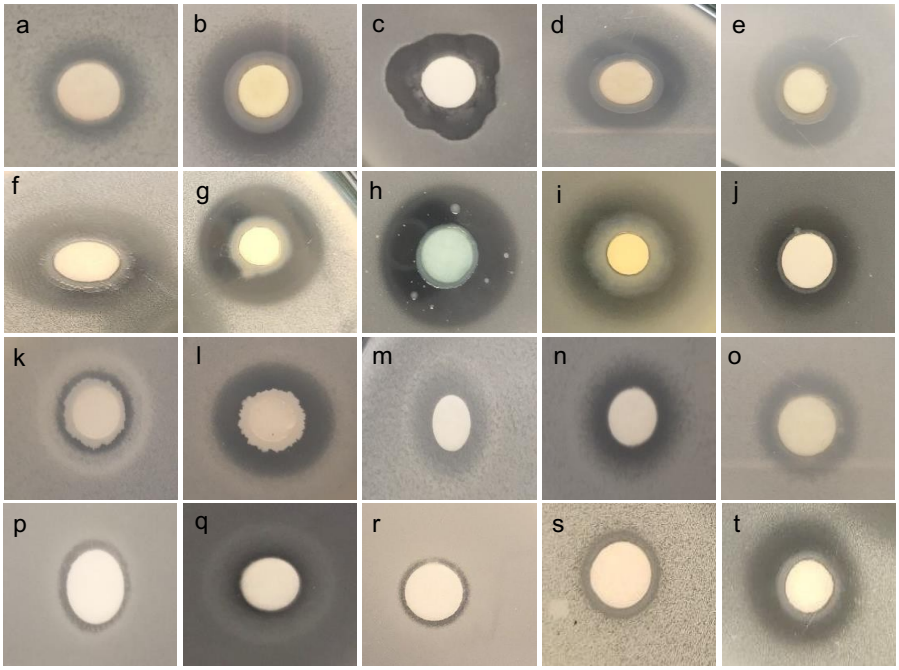

Figure 2. Antibacterial and antifungal activities of endophyte bacterial pellets using disc diffusion method sequentially from (a)-(j): (a) LWERG 29, (b) LWERG 30, and (c) LWEBG 39 against *E. coli,* (d) LWEBG 41, and (e) LWEBG 42 against *P. aeruginosa,* (f) LWERG 29, (g) LWERG 30, and (h) LWEBG 39 against *B. subtilis,*(i) LWEBG 39 and (j) LWEBG 41 against *C. albicans*, and supernatant sequentially from (k)-(t) : (k) LWERG 29, (l) LWERG 30, and (m) LWEBG 41 against *E. coli*, (n) LWERG 30 (o) LWEBG 41, and (p) LWEBG 42 against *P. aeruginosa,* (q) LWERG 29, (r) LWERG 30, and (s) LWEBG 39 against *B. subtilis*, (t) LWEBG 41 against *C. albicans.*

4. Discussion

The ability of the antimicrobial compounds produced by selected species to limit the growth of the pathogenic microorganisms differed between species. The inhibition zone resulting from supernatant administration proved that tested bacteria excreted antimicrobials into the medium (extracellular) by bacterial isolates, while bacterial pallets produced bioactive compounds and secreted intracellularly (Xie et al., 2021). The ability to create an inhibition zone varies among five potential species in pellet assays. In the stationary phase of bacterial growth, there is competition to obtain nutrients and defend themselves so that bacteria will produce bioactive compounds, such as antimicrobial compounds. (Cappucino and Welsh., 2018).

Providencia strain LWERG29, *Stenotrophomonas* strain LWERG30, *Bacillus* strain LWEBG39, *Bacillus* strain LWEBG41, and *Pseudomonas* strain LWEBG42 isolates were able to suppress nearly all pathogenic bacteria, including *B. subtilis*, *P. aeruginosa*, and *E. coli*. Some modes of action from antimicrobial compounds include suppressing bacterial cell wall formation, bacterial protein synthesis, folate synthesis, DNA synthesis, and modifying cell membrane permeability (Reygaert, 2018). On the other side, the pathogenic fungus *C. albicans* was suppressed by *Bacillus* strain LWEBG39. There are numerous mechanisms of antifungal action, including cell membrane damage, inhibition of ergosterol production, inhibition of protein synthesis, and inhibition of enzyme action (Hossain et al., 2022).

In our study, *Stenotrophomonas* strain LWERG30 is close to *S. rhizophila* species. Previously, this species was known to have antifungal activity against *Botrytis cinerea* infection in tomato leaves (Raio et al., 2023), while *Stenotrophomonas* LWERG30 had strong intensity in inhibit *E. coli* and *B. subtilis.* Only *Bacillus* strain LWEBG39 which is close to *B. altitudinis*, showed antifungal and antibactertia activities in this study. Consistent with our findings, *B. altitudinis* and *B. amyloliquefaciens* have also been reported as bacteriocins producers (Abednego et al., 2023; Hanafy et al., 2023). In addition, *Pseudomonas* strain LWEBG42 was able to inhibit *E. coli* and *B. subtilis* might be due to the activity of pyocins that are commonly produced by *Pseudomonas* as a previous report (Ghequire et al., 2023).

Conclusion

In conclusion, this study confirmed that a total of five best potential species of fragrant ginger endophytic bacteria successfully inhibit four testing pathogenic microbes using their cultures, supernatant, and pellets. Interestingly, selected species could show their ability to inhibit pathogens with a strong degree of intensity and that potential to develop as new antimicrobial compounds. These results also suggested that Enggano Island, Bengkulu, and Indonesia are sources of potential endophytic bacteria isolated from fragrant ginger (Lempuyang Wangi) that can be used as antimicrobial agents. This study was limited to identifying fragrant ginger endophytic bacteria that showed their potential to produce antimicrobial compounds. Based on the 16s rRNA gene, those 5 isolates were highly homolog to *Providencia, Stenotrophomonas, Bacillus,* and *Pseudomonas* genera. Further analysis is necessary to continue to identify the genes responsible for antimicrobial activities using whole genome sequencing.

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Research Article

Distribution and DNA Barcoding of Anomalini Beetles (Coleoptera: Scarabaeidae: Rutelinae) in Wheat Fields of Van, Türkiye

Mehmet Salih ÖZGÖKÇE*[1], Gamze GÖKSUGÜZEL[2], Hilmi KARA[3], Mehmet Ramazan RİŞVANLI[4] Merve DOĞAÇ[5]

[1,2,3,4,5]Van Yüzüncü Yil University, Faculty of Agriculture, Plant Protection Department, 65090, Van, Türkiye

[1]https://orcid.org/0000-0002-6777-9149, [2]https://orcid.org/0000-0002-1795-5052, [3]https://orcid.org/0000-0003-0580-0464
[4]https://orcid.org/0000-0002-5882-0533, [5]https://orcid.org/0009-0008-4316-1951

*Corresponding author e-mail: msozgokce@yyu.edu.tr

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Abstract: Anomalini beetles (Coleoptera: Scarabaeidae: Rutelinae) constitute an important group of pests causing significant crop losses in wheat cultivation areas worldwide, including Türkiye. The aim of this study was to comprehensively evaluate the phylogeny, diversity, abundance, and distribution of Anomalini beetles in wheat fields of Van province, Türkiye. Surveys were conducted between April and August 2021, involving monthly sample collection at predetermined locations within six districts: Başkale, Çaldıran, Erciş, Gevaş, İpekyolu, and Tuşba. A Standard sweepnet with a diameter of 35 cm was used to collect samplings. In molecular studies, the mitochondrial COI gene region has been amplified and sequenced using universal primers. Anomalini beetles were detected in all sampling areas except Çatak district. Seven species were identified: *Anisoplia austriaca, A. signata, A. lata, Brancoplia leucaspis, Blitopertha nigripennis, Chaetopteroplia segetum*, and an unidentified *Anisoplia* sp. *Chaetopteroplia segetum* emerged as the most prevalent and abundant species across all districts. Notably, all identified Anomalini species represent the first records for Van province and its environs. While Anomalini beetles were present in the region, their population densities were not considered high enough to cause economic damage.

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1. Introduction

The tribe Anomalini are ecologically and agriculturally important beetles that wide-range around the world, contain one of the largest genera in the animal kingdom (genus *Anomala* with approximately 1000 species), and have over 2000 species (Jameson et al., 2003; Morón and Ramírez-Ponce, 2012). The life history of species of the tribe Anomalini, some of which are agricultural pests, is highly variable (Jameson et al., 2003). The larvae of many species feed on the subsoil parts of a wide

variety of plants. While the adults of some species feed little or not at all, the adults of others feed on the leaves, flower parts, and fruits of angiosperms and gymnosperms, causing severe damage to fruits and leaves (Jameson et al., 2003; Filippini et al., 2016; Vittum, 2020).

In 1918, Ohaus established a comprehensive worldwide classification for the Anomalini tribe, which involved the division of the tribe into four subtribes, namely the Anisopliina, Anomalina, Popilliina, and Isopliina (Jameson et al., 2007). The Anisopliina subtribe (Scarabaeidae: Rutelinae: Anomalini) inhabits a large geographical area spanning the Palaearctic, Oriental, Ethiopian, Nearctic, and Neotropical biogeographical regions, with about 100 species belonging to nine different genera (Jameson et al., 2007). Anisopliinae beetles, also known as grain beetles or grass-feeding beetles, feed on grass pollen and seeds as adults and grassroots as larvae. This dietary preference has shaped their life cycle and ecological niche within grassland ecosystems. Their adults exhibit a diversified feeding strategy, consuming a wide range of non-cultivated grasses as adults, with some species also adapted to feed on cultivated plant species such as oats, wheat, rye, and corn (Hurpin, 1962; Mico et al., 2001; Jameson et al., 2007).

In the Old World, where Anisopliina's species richness is highest, species inhabit a wide variety of grassy habitats, including scrub forests, grasslands, meadows, riparian areas, and roadsides. New World species of Anisopliina are distributed in the dry, desert zone of southern Arizona and northwestern Mexico, the pine-oak forests of central Mexico, and the tropical oak and deciduous forests of central Mexico (Morón et al., 1996). Although larvae of many Anisopliina species are recognized as pests of various crops (Bogachev, 1946), little is known about larval morphology or adult biology. The genus *Anisoplia* is the most abundant and widespread genus of Anisopliina, with more than 50 species (Machatschke, 1961). In various regions of Türkiye, 28 different *Anisoplia* species have been identified (Lodos, 1989). *Anisoplia syriaca, A. austriaca* and *A. segetum* are important *Anisoplia* species in Türkiye (Lodos, 1989). The species of the genus *Anisoplia* found in Türkiye are *Anisoplia* (*Anisoplia*) *agnata* Reitter., *A. lata* Erichson., *A. agricola* Poda, *A. lanuginosa* Erichson., *A. aprica* Erichson., *Brancoplia leucaspis* (Castelnau, 1840) ., *A. austriaca* (Herbst), *A. mülleri* Pilleri., *A.* (*Anisoplia*) *clypealis* Reitter *A.* (*Anisoplia*) *nohai* Petrovitz, *A.* (*Anisoplia*) *dispar* Erichson., *A. parva* Kraatz., *A. egregia* Petrovitz, *A. petrovitzi* Machatschke, *A. faldermanni* Reitter, *A.* (*Anisoplia*) *reitteriana* Semenov, *A. farraria farraria* Erichson., *Chaetopteroplia segetum* (Herbst, 1783), *A. flavipennis* Brullé, *A.* (*Anisoplia*) *signata* Faldermann, *A. hebes* Reitter, *Chaetopteroplia syriaca* (Burmeister, 1844) , *A.* (*Anisoplia*) *hirta* Zaitzev, *A. tenebralis* Burmeister., *A. imitatrix* Apfelbeck, *A. thessalica* Reitter, *Chaetopteroplia inculta* (Erichson, 1847) ., and *A. tritici* Kieswetter (Anonymous, 2021a). Anisoplia spp are widespread in areas where cereals are cultivated in Türkiye. *Anisoplia* larvae, which cause damage to many gramineas, especially wheat, barley, oats, and rye, cause the main damage by eating the root of the plant, and the adults cause the main damage by eating the wheat grains and the presence of 3-4 adult individuals per m^2 in the fields cause economic damage (TAGEM, 2008).

In this study, we determined the faunistic presence, diversity, density, and DNA Barcoding of a tribe of Anomalini beetles in wheat growing areas in and around Van province. No comprehensive phylogenetic analysis has been conducted, but the DNA sequences of the species were recorded by DNA barcoding. DNA barcoding has become an indispensable tool in faunistic studies of insects, primarily due to its capacity to offer precise species identification and assist in the detection of new species. The vast diversity of insects and their ecological and economic significance have positioned them as a primary focus for DNA barcoding (Jinbo et al., 2011; Wilson et al., 2017). This approach has been particularly beneficial in expediting species identification and description, particularly in scenarios where traditional taxonomic methods pose challenges. It has facilitated the identification of agriculturally significant insects, thereby contributing to pest management and control. Additionally, DNA barcoding has played a pivotal role in unveiling concealed biodiversity and delineating species within intricate taxa, such as parasitoid species (Jinbo et al., 2011; Ferreira et al., 2020; Li et al., 2021). With this study, the presence of the relatively weakly volatile Anomalini (Mico et al., 2001; Micó and Galante, 2002; Bekircan and Tosun, 2021) species in the study area will be important in terms of understanding the species adapted to the region and revealing the regional inventory by determining the DNA records.

2. Material and Methods

The study was conducted in the wheat-growing areas of Van province and its districts (Bahçesaray, Başkale, Çaldıran, Çatak, Edremit, Erciş, Gevaş, Gürpınar, İpekyolu, Muradiye, Özalp, Saray and Tuşba) from April to August 2021 (Figure 1). Surveys were carried out in each sampling district at two-week intervals starting from the tillering period of wheat plants. At least 3 fields were selected in each district and 200 samples were collected in each field using a standard sweepnet with a diameter of 35 cm. The location of each sampled field was marked on the map with the help of a GPS device (Garmin) and marked on the map (Figure 1).

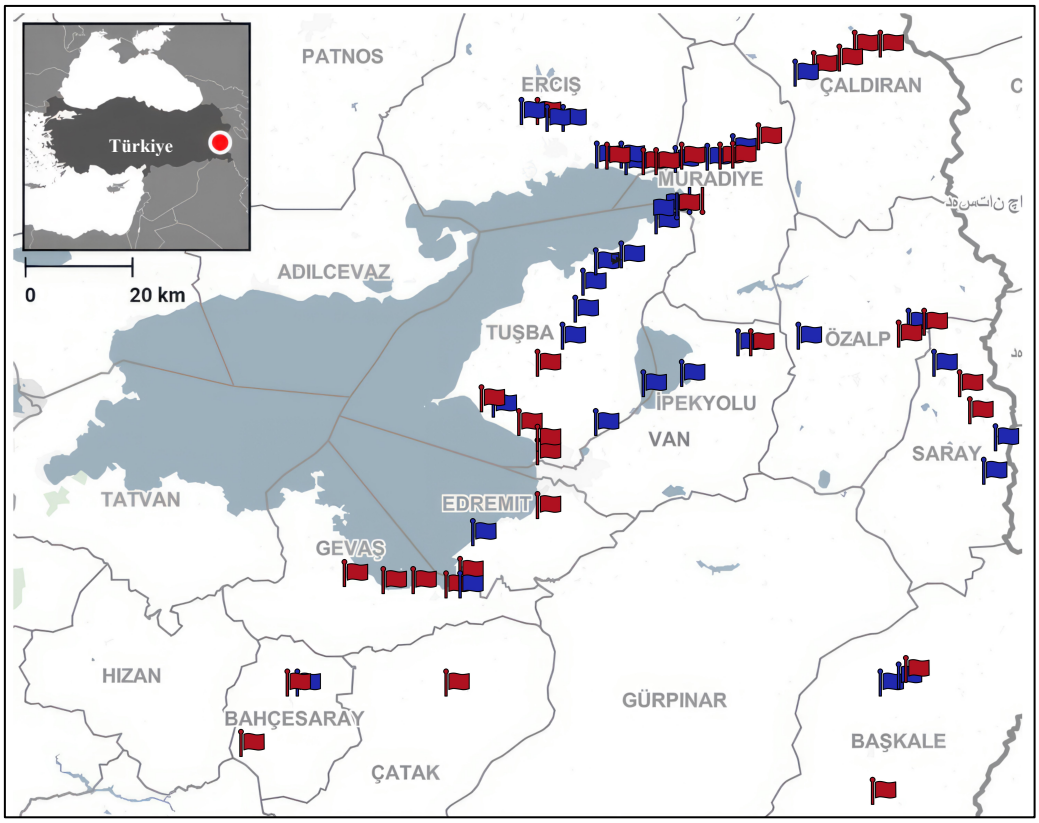

Figure 1. GPS locations of the sampled fields (blue and red flags), where insects were detected (red flag) (Garmin BaseCamp).

2.1. Morphologic identification

The collected individuals were separated according to their location and morphological characters, and the specimens that were very similar to each other were counted and recorded as the same species. Genital organ preparations of male individuals were made for species identification. For this purpose, specimens were soaked in 10% potassium hydroxide for 1-2 hours, and genital organs were removed. Identification keys were used for species identification and also confirmed with the opinions of Dr Denis Keith (Muséum d'Histoire Naturelle et de Préhistoire, France), an expert on these beetles.

2.2. Molecular studies

After the morphological identification procedures, DNA barcoding of the samples was carried out according to the following methods; thus, the genetic sequences of the species were obtained.

DNA isolation was carried out by removing the samples in 99% ethanol from their media stored at -20 °C, firstly the legs of each sample were dissected and washed with distilled water, and DNA isolation procedures were started. DNA isolation was performed using PureLink™ Genomic DNA Mini Kit (Invitrogen). For isolation, 180 µl Genomic Digestion Buffer and 20 µl Proteinase K were added to the insect legs, which were thoroughly crushed with metal pliers, and incubated at 55 °C for half an

hour. After centrifugation at the highest speed for 2-3 min, the pellet formed in the tube was removed and 20 µl RNase, 200 µl PureLink™ Genomic Lysis/Binding Buffer, and 200 µl 96-100% ethanol were added to the clear liquid and vortexed thoroughly to form a homogenous mixture. After these procedures, genomic DNA was obtained by washing twice.

Polymerase Chain Reaction (PCR)-Agarose Gel Electrophoresis The universal primers LCO1490 5′-GGTCAACAAACAAATCATAAAGATATATTGG-3′ and HCO 2198 5′TAAACTTCAGGGTGACCAAAAAATCA-3′ were used for the mitochondrial COI regions of the isolated DNAs. PCR reactions were prepared in a volume of 25 µl and tubes were prepared by adding 5 µl of Genomic-DNA, 2.5 µl of 10X reaction buffer (100 mm Tris-HCl, pH 8.8, 500 mm KCl, 0.8% Nonidet P40), 3 µl of 25 mM MgCl2, 10 mM each dNTP (thermo scientific), 0.5 µl of each of the primers diluted to 10 pmol (Sentebiolab, Ankara). The PCR reaction was prepared by adding 0.625 U Taq DNA polymerase (Thermo Scientific) into the prepared 25 µl final volume reaction. The PCR process was carried out in a VWR brand PCR device with initial denaturation at 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and then 72°C for 5 min as the final elongation to amplify the targeted region. The amplicons obtained after the reaction were run on a 1% agarose gel at 100 volts for 60 min.

2.3. Phylogenetic analysis

COI sequences of 6 species were obtained from specimens collected in Van and its districts. Two taxa, *Phyllognathus dionysius* Fabricius (Scarabaeidae: Dynastinae: Pentodontini) and *Cyclocephala atripes* Bates (Scarabaeidae: Dynastinae: Cyclocephalini) with MZ664295.1 and KX298196.1 NCBI accession numbers, respectively, were used as outgroup. In addition, 4 species belonging to the subtribes Anisopliina and Anomalina with MF706436.1, AY090506.1, AY090507.1, and MH115532.1 NCBI accession numbers were used for comparison with the in-groups. COI sequences of the species selected as outgroups were retrieved from GenBank for phylogenetic analysis. A total of 654 bp nucleotide sequences of the mitochondrial COI region of all individuals (including the outgroup) were aligned and visually checked with ClustalW using MEGA 11 (Tamura et al., 2021). The evolutionary history of beetles was elucidated through the implementation of the Maximum Likelihood (ML) method and the Tamura-Nei (T92) model (Tamura and Nei, 1993). The optimal tree generated by this method is presented in the figure. The bootstrap test, which involved 1000 replicates, was employed to assess the robustness of the inferred tree topology.

3. Results and Discussion

Anomalini beetles were found in 42 of 85 sampling points in Van province, and a total of 149 individuals were collected. Among these specimens, a total of seven species were identified from wheat cultivation areas in Van province (Table 1). Six of the identified species (*Anisoplia* (*Autanisoplia*) *austriaca, Anisoplia* (*Anisoplia*) *lata, Anisoplia* (*Anisoplia*) *signata, Anisoplia* sp., *Brancoplia leucaspis, Chaetopteroplia segetum*) belong to the Anisopliina subtribe of the Anomalini tribe, and one species (*Blitopertha nigripennis*) belongs to the Anomalina subtribe. All of the identified specimens are new records for Van Province.

In the study areas, *C. segetum* was the most common species (28%), followed by *Br. leucaspis* (24%), *A. austriaca* (21%), *Bl. nigripennis* (12%), *A. signata* (9%), *A. lata* and *Anisoplia* sp. (3%). The most abundant species was *C. segetum* 38%, the second most abundant species was *Br. leucaspis* 32%, followed by *A. austriaca* 18%, *Bl. nigripennis* 8%, *A. signata* 2%, and *A. lata* and *Anisoplia* sp. 1%. In previous studies, 28 different *Anisoplia* species were identified in various regions of Türkiye, and *A. syriaca, A. austriaca,* and *A. segetum* were reported as the most important species (Lodos, 1989).

Table 1. Anomalini beetles collected from wheat fields in Van and its districts

Subfamily	Tribe	Subtribe	Genus	Species	Location, number of individuals
Rutellinae	Anomalini	Anisopliina	Anisoplia	*Anisoplia* (*Autanisoplia*) *austriaca*	1* (4**), 3 (6), 5 (3), 6 (3), 7 (2), 10 (1), 11 (2), 12 (29)
				Anisoplia (*Anisoplia*) *lata*	2 (1)
				Anisoplia (*Anisoplia*) *signata*	1 (1), 2 (1), 3 (1)
				Anisoplia sp.	9 (1)
			Brancoplia	*Brancoplia leucaspis*	1 (10), 3 (1), 6 (2), 7 (3), 9 (3), 10 (2), 11 (1), 13 (5)
			Chaetopteroplia	*Chaetopteroplia segetum*	1 (23), 3 (4), 5 (16), 6 (1), 7 (3), 8 (3), 9 (1), 10 (1), 13 (4)
		Anomalina	Blitopertha	*Blitopertha nigripennis*	2 (3), 3 (2), 10 (1), 11 (5)

*Locations: 1: Bahçesaray, 2: Başkale, 3: Çaldıran, 4: Çatak, 5: Edremit, 6: Erciş, 7: Gevaş, 8: Gürpınar, 9: İpekyolu, 10: Muradiye, 11: Özalp, 12: Saray, 13: Tuşba. **Number of individuals.

3.1. The subtribe Anisopliina beetles in Van Province and its surroundings

3.1.1. Anisoplia (*Autanisoplia*) *austriaca* (Herbst, 1783)

The adults of *Anisoplia* (*Autanisoplia*) *austriaca*, also known as crop grain beetle or wheat grain beetle, cause damage to crops such as wheat, maize, rye, and oats (Hurpin, 1962). Regarding the morphological characteristics of the insect, Baraud (1991) reported that the body length is 13-20 mm, the head, and pronotum are sometimes green or bluish-black, and the elytra, which does not completely cover the body, is brown-yellow or brown-red. Baraud (1991) further adds that there is a rectangular black spot surrounding the scutellum, the body is almost glabrous or with a small amount of pseudo-pubescence, the large front claw of males is very long and slightly curved or not curved, and this shape of the claw is similar to the shape observed in *Brancoplia leucaspis* (Figure 2). Baraud (1991), noted another important feature: the metasternum has very long, very dense, and erect bristles in the shape of a brush (Schoonhoven et al., 1998; Schoonhoven et al., 2005). This observation is in line with the study by (Jones et al., 2016), which collected morphological data on insect wings, indicating the significance of bristles in insect morphology. Additionally, the study by Jameson et al. (2007) provides further context by categorizing *Brancoplia* within a specific clade, emphasizing the importance of understanding its unique morphological features. Furthermore, the work of Engels et al. (2021) discusses the concept of ptiloptery, which refers to wings with long bristles attached to a narrow membranous section, providing insights into the potential aerodynamic implications of such bristles.

Figure 2. *Anisoplia austriaca,* adult (a: dorsal, b: ventral).

Distribution in and around Van: *Anisoplia austriaca* was found in Bahçesaray (at N38° 08.940' E42° 49.202' and 1874 m, 1 individual; N38° 08.954' E42° 49.173' and 1880 m, 1 individual; N38° 08.906' E42° 49.520' and 1847 m, 2 individuals), Çaldıran (N39° 08.091' E43° 56.024' and 2048 m, 4 individuals; N39° 07.296' E43° 52.714' and 2254 m, 2 individuals), Erciş (N39° 05.007' E43° 15.264' and 1755 m, 3 individuals), Gevaş (N38° 19.631' E42° 55.980' and 1667 m, 1 individual; N38° 18.302' E43° 08.754' and 1691 m, 1 individual), Muradiye (N38° 57.605' E43° 41.457' and 1673 m, 1 individual), Özalp (N38° 39.789' E44° 01.418' and 2024 m, 1 individual; N38° 39.807' E44° 05.587' and 2080 m, 1 individual), and Saray (N38° 35.701' E44° 13.212' and 2254 m, 9 individuals; N38° 37.966' E44° 11.461' and 2152 m, 20 individuals).

Distribution in Türkiye: Distribution to Ankara, Adana, Adıyaman, Antalya, Bayburt, Bilecik, Bitlis, Çankırı, Çorum, Denizli, Diyarbakır, Erzincan, Erzurum, Eskişehir, Isparta, İzmir, Kars, Konya, Muğla, Sivas, and Yozgat in Türkiye (Rezaei, 2015; Polat et al., 2018).

Distribution in Worldwide: It was recorded in Austria, Bulgaria, Czechoslovakia, South Germany, Hungary, Romania, Russia, Ukraine, Baraud (1991), cited by Porta (1932), Azerbaijan, Armenia, Georgia, Iraq, Iran, Israel, Switzerland, Lebanon, Syria, Türkiye, and Greece (Rezaei, 2015).

3.1.2. Anisoplia lata (Erichson, 1847)

Wilhelm Ferdinand Erichson described the beetle *A. lata* in 1847. *Anisoplia lata* is a member of the *Anisoplia* genus and the Rutelidae family, with the subspecies *A. l. lamiensis* also recognized (Anonymous, 2021b). Baraud (1991) noted that this species is represented by two forms: *Anisoplia lata lata* Erichson and *A. lata lamiensis* Apfelbeck. Baraud (1991) provided morphological details, stating that the body length ranges from 11-14 mm, the elytra are brownish-yellow, mostly black, and the pronotum is nearly hairless. Males have straight-sided pronotums, while females have pronotums parallel to the body's rear and sides curved forward from the base (Figure 3). Additionally, males have long, pointed, and highly curved front tarsi. Zazharska et al. (2019) described the beetle's head and pronotum as highly glossy, black, and greenish, with the elytra being uniformly brownish-red, brown, or black. They also noted that the setae near the scutellum are not prominent in males' elytra, and the abdominal setae are scattered. These details provide a comprehensive understanding of the taxonomy and morphological characteristics of *A. lata* and its subspecies.

Figure 3. *Anisoplia lata,* adult.

Distribution in and around Van: *Anisoplia lata* was collected in Başkale at N37° 50.640' E44° 06.646' and 1847 m 1 individual.

Distribution in Türkiye: Ankara, Çanakkale, Eskişehir (Rezaei, 2015).

Distribution in Worldwide: It was found in Albania, Austria, Hungary, Macedonia, Romania, Yugoslavia, and Greece (Baraud, 1991). It has been observed in Southern and Southeastern Europe, the European part of Russia, and Moldova (Mico et al., 2001).

3.1.3. Anisoplia signata (Faldermann, 1835)

Only 3 female individuals of this species were found. Since there were no male individuals, genital preparations could not be obtained, but Denis Keith reported that the specimens may belong to *A. signata* based on the morphological characters of the female individuals (Figure 4).

Distribution in and around Van: *Anisoplia signata* was found in Bahçesaray (at N38° 08.787' E42° 50.269' and 1891 m, 1 individual), Başkale (N37° 50.640' E44° 06.646' and 1847 m, 1 individual), and Çaldıran (N39° 07.293' E43° 52.717' and 2046 m, 1 individual).

Distribution in Türkiye: Ardahan, Artvin, Bingöl, Erzurum, Iğdır, Mersin, Rize, Trabzon (Baraud, 1991).

Distribution in Worldwide: Armenia, Siberia (Pilleri, 1954), Syria (Baraud, 1991).

Figure 4. *Anisoplia signata,* adult.

3.1.4. Anisoplia sp.

Only 1 specimen of this species belonging to the genus *Anisoplia* could be collected. Morphological measurements of the species are as follows: Total body length was 11.1 mm, elytra 6.57 mm, thorax 2.89 mm, head 1.64 mm. The width of the head was 2.35 mm, the width of the thorax was 4.48 mm, and the width of the elytra was 6.06 mm. The body is generally black, especially the abdomen and lower parts of the thorax are covered with dense, long yellow hairs. The upper part of the thorax is glossy black and glabrous, while the abdomen is mustard yellow or yellowish brown on a glossy black background (except around the scutellum and the outer edges of the elytra and where the two wings meet). Neither the thorax nor the upper part of the elytra have any hairs (Figure 5).

Distribution in and around Van: *Anisoplia* sp. was found in İpekyolu (at N38° 30.212' E43° 19.380' and 1657 m, 1 specimen).

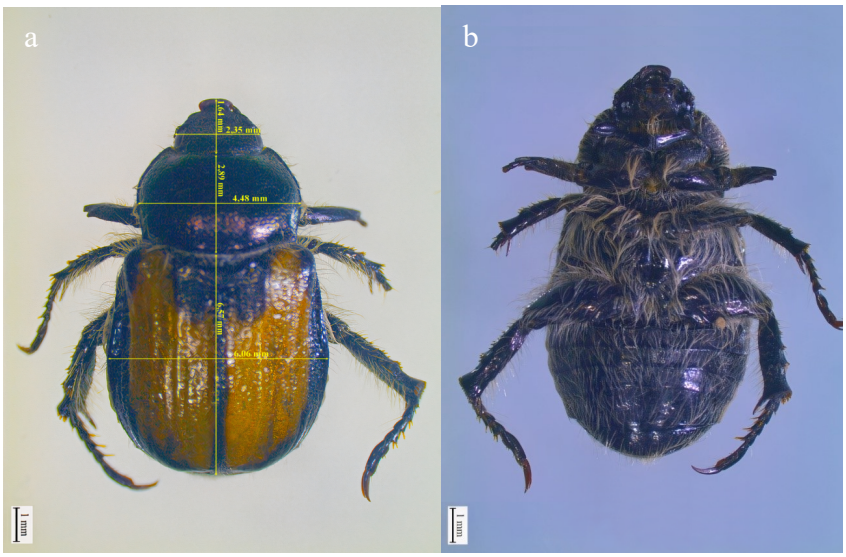

Figure 5. *Anisoplia* sp., adult (a; dorsal, b; ventral).

3.1.5. Brancoplia leucaspis (Laporte, 1840)

According to current knowledge, *Brancoplia* has six species found exclusively or predominantly in the Middle East (Rössner, 2016). These species are *Br. leucaspis* (Laporte, 1840), *Br. umila* (Marseul, 1878), *Br. vseteckai* (Pilleri, 1951), *Br. mesopotamica* (Pilleri, 1954), *Br. klapperichi* (Petrovitz, 1971) and *Br. waitzbaueri* (Rössner, 2017; Rössner and Sabatinelli, 2020). Elytra are densely pubescent, male genitalia have a long and chitinized canal, and the parameres are absent or slightly S-shaped curved when viewed from the side (Anonymous, 2021c) (Figure 6). Two groups of species (*Br. leucaspis* group and *Br. pumila* group) can be distinguished based on external morphology and genital morphology (Rössner, 2016).

Distribution in and around Van: *Brancoplia leucaspis* was collected in Bahçesaray (at N38° 08.787' E42° 50.269' and 1891 m, 1 indvidual; N38° 08.940' E42° 49.202' and 1874 m, 5 individuals; N38° 08.906' E42° 49.520' and 1847 m, 1 individuals; N38° 06.051' E42° 45.073' and 1837 m, 3 indviduals), Çaldıran (N39° 08.583' E43° 57.412' and 2049 m, 1 individual), Erciş (N39° 00.131' E43° 29.005' and 1685 m, 2 individuals), Gevaş (N38° 19.851' E43° 11.378' and 1695 m, 1 individual; N38° 18.590' E43° 10.577' and 1704 m, 1 individual; N38° 18.467' E43° 00.078' and 1666 m, 1 individual), İpekyolu (N38° 30.961' E43° 18.945' and 1656 m, 3 individual), Muradiye (N38° 59.170' E43° 44.636' and 1688 m, 1 individual; N39° 05.206' E43° 47.939' and 1936 m, 1 individual), Özalp (N38° 39.453' E43° 44.702' and 1883 m, 1 individual), and Tuşba (N38° 34.625' E43° 16.335' and 1706 m, 3 individuals; N38° 40.126' E43° 18.236' and 1731 m, 1 individual; N38° 36.660' E43° 13.666' and 1729 m, 1 individual).

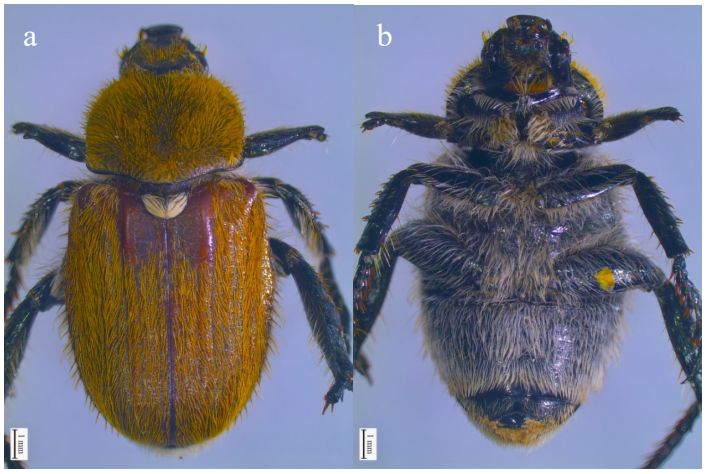

Figure 6. *Brancoplia leucaspis,* adult (a: dorsal, b: ventral).

Distribution in Türkiye: Adıyaman, Bingöl, Diyarbakır, Erzurum, Kars (Polat et al., 2018).

Distribution in Worldwide: The range of the *Brancoplia leucaspis* extends from the Crimean peninsula and the Caucasus in the north to Turkmenistan and south through the Zagros Mountains in Iran to northern Egypt (Zaitzev, 1917; Rössner, 2016). Azerbaijan, Georgia, Türkiye; (Medvedev, 1949): Iraq, Iran, Russia; (Pilleri, 1954): Azerbaijan, Armenia, Iran, Russia; (Rössner, 2016; Polat et al., 2018).

3.1.6. Chaetopteroplia segetum (Erichson, 1847)

Harold (1869) initially named the beetle known as *Melolontha segetum* as *C. segetum*, and this name was later accepted by subsequent authors (Mulsant, 1871; Bedel, 1911). During the period between Fabricius' 1787 publication and Harold's 1869 publication, the names *M. fruticola* Fabricius 1787 and *M. segetum* Herbst 1783 were used for the species now known as *C. segetum* (Anonymous, 2021d). Regarding the morphological characteristics of the beetle, Machatschke (1961) reported that the body length was 9-13 mm, the body was broad and oval, the head and pronotum were blackish green or black covered with dense yellowish hairs, the elytra were brownish and the hairs were sparser (Figure 7). In addition, males had longer antennae and thickened front claws, while females had paler elytra.

Figure 7. *Chaetopteroplia segetum,* adult.

Distribution in and around Van: *Chaetopteroplia segetum* was found in Bahçesaray (at N38° 08.940' E42° 49.202' and 1874 m, 7 individuals; N38° 08.954' E42° 49.173' and 1880 m, 3 individuals; N38° 08.906' E42° 49.520' and 1847 m, 1 individual; N38° 06.051' E42° 45.073' and 1837 m, 11 individuals; N38° 08.744' E42° 50.310' and 1921 m, 1 individual), Çaldıran (N39° 07.293' E43° 52.717' and 2046 m, 1 individual; N39° 05.995' E43° 50.018' and 2012 m, 3 individuals), Edremit (N38° 25.572' E43° 15.787' and 1646 m, 16 individuals), Erciş (N39° 05.007' E43° 15.264' and 1755 m, 1 individual), Gevaş (N38° 19.851' E43° 11.378' and 1695 m, 1 individual; N38° 19.631' E42° 55.980' and 1667 m, 1 individual; N38° 18.585' E43° 03.427' and 1677 m, 1 individual), Gürpınar (N38° 19.198' E43° 20.110' and 1729 m, 1 individual; N38° 17.270' E43° 49.978' and 2030 m, 1 individual; N38° 19.084' E43° 24.731' and 1773 m, 1 individual), İpekyolu (N38° 30.961' E43° 18.945' and 1656 m, 1 individual), Muradiye (N38° 57.662' E43° 38.086' and 1646 m, 1 individual), and Tuşba (N38° 34.625' E43° 16.335' and 1706 m, 4 individuals).

Distribution in Türkiye: It was distributed to Konya (Venieraki et al., 2017), Adana, Afyonkarahisar, Ankara, Antalya, Artvin, Bitlis, Erzincan, Erzurum, Hatay, Iğdır, Kars, Mersin, Rize, and Trabzon (Polat et al., 2018).

Distribution in Worldwide: It was a widespread beetle that occurred in a broad area from eastern France to western Siberia (Baraud, 1992). Its distribution range includes Poland (Pawłowski et al., 2002), Anatolia, Belgium, central, eastern, and southeastern Europe, Greece, Syria, Siberia (Rezaei, 2015, Anonymous, 2021d), and Iran (Venieraki et al., 2017).

3.2. The subtribe Anomalina beetle in Van and its surroundings

3.2.1. Blitopertha nigripennis (Reitter 1888)

Blitopertha nigripennis, which is reported to be very dense and widespread in the regions where it was found in Türkiye (Yıldırım et al., 2018), was collected in four different regions in and around Van. The body length of *Bl. nigripennis* is 8-13 mm; head, pronotum, and scutellum are black; dorsum has 2 longitudinal black lines; elytra is yellow or light brown, upper wings are slightly hairy; pygidium is longer in males (Figure 8) (Rezaei, 2015).

Figure 8. *Blitopertha nigripennis,* adult.

Distribution in and around Van: Distributed to Başkale (at N38° 04.772' E44° 06.193' and 2099 m, 3 individuals), Çaldıran (N39° 08.091' E43° 56.024' and 2048 m, 2 individuals), Muradiye (N38° 57.531' E43° 38.272' and 1670 m,1 individual), and Özalp (N38° 40.598' E44° 03.685' and 2076 m, 5 individuals).

Distribution in Türkiye: It was collected in Adana, Adıyaman, Ağrı, Antalya, Edirne, Eskişehir, Gaziantep, Hatay, İçel, Kahramanmaraş, Kastamonu, Kars, Kayseri, Osmaniye, Sakarya, Sinop, Yozgat (Lodos et al., 1999; Rozner and Rozner, 2009; Şenyüz and Şahin, 2009), Bingöl, Bursa, Erzurum (Polat et al., 2018).

Distribution in Worldwide: Azerbaijan, Armenia, Georgia, Iran, Israel, Cyprus, Russia: Southern Europe, Lebanon, Syria, Türkiye, Turkmenistan, Jordan (Löbl and Löbl, 2016).

3.3. Phylogenetic analyses

The phylogenetic tree was constructed using 12 sequences, two from the outgroup, four from the subtribes Anisopliina and Anomalina, and 6 from the ingroup. Sequences of species other than the inner group were obtained from GenBank (with species names and accession numbers given in Figure 9). Seven different species were identified in Van and its surroundings. Unfortunately, DNA isolation of *Anisoplia* sp. found just one in İpekyolu and not identified morphologically could not be performed

due to unsuitable waiting conditions. Therefore, sequences of only 6 species were used as ingroups in the phylogenetic tree.

The tree with the highest log likelihood (-3273.26) is shown. The percentage of trees in which the associated taxa clustered together is shown below the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.2268)]. This analysis involved 12 nucleotide sequences.

When the phylogenetic tree was analyzed, the internal groups were divided into two important clades. While 5 in-group species and 3 reference species belonging to the Anisopliina subtribe were clustered in the first clade, *Bl. nigripennis* and *Bl. lineolata* (reference species) belonging to the Anomalina subtribe was included in the other clade (Figure 9). The reference sequence of *Anisoplia austriaca* and the sequence obtained from this study were found to be on the same branch in the phylogenetic tree with a very high bootstrap rate (99%). The reference species belonging to the genus *Blitoperta* in the second clade and *Bl. nigripennis* morphologically identified in this study were located on the same branch with a bootstrap rate of 94%.

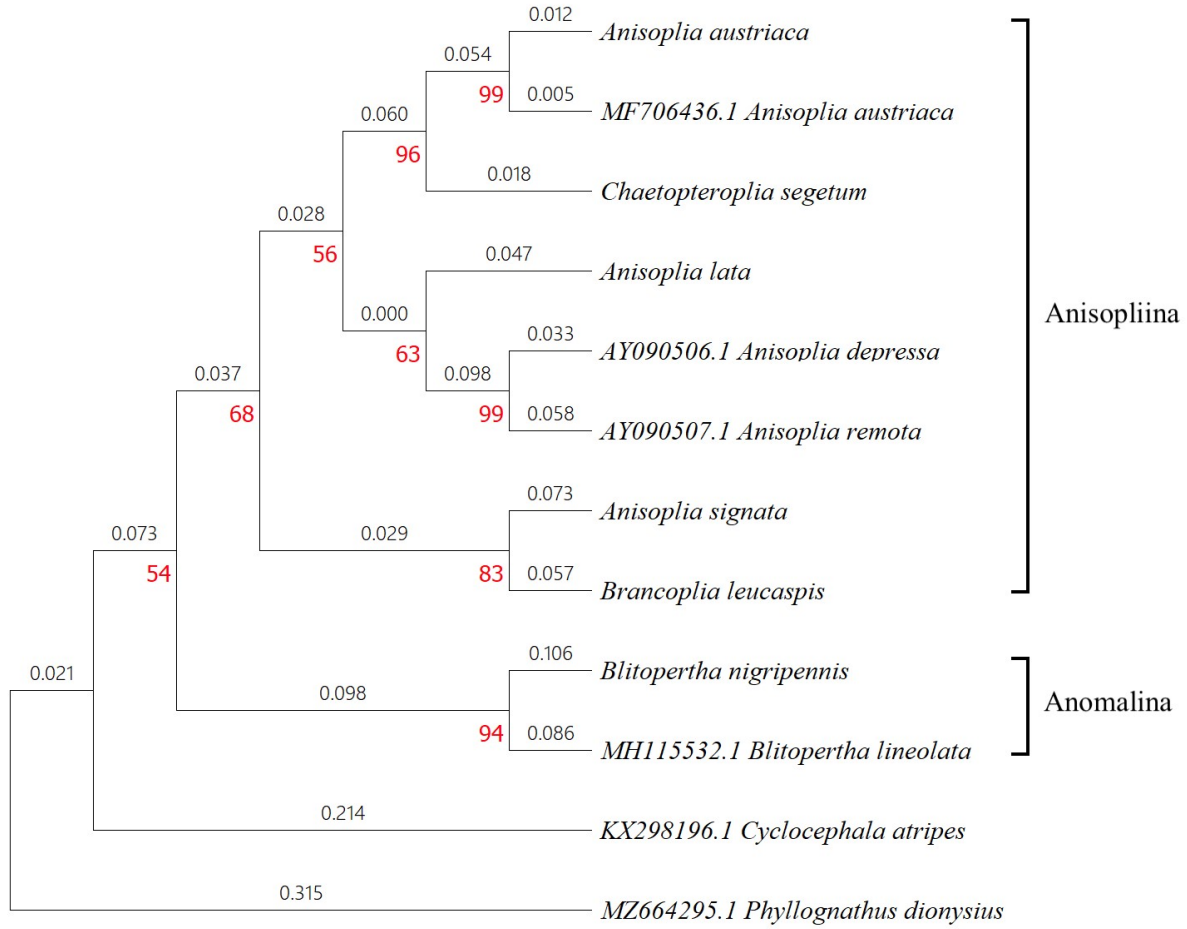

Figure 9. The phylogenetic tree of the Anomalinii species in Van and surroundings was constructed by MEGA 11 using the Maximum Likelihood (ML) method and 1000 bootstrap replicates.

The study results conducted in the Van region provide valuable insights into the population dynamics and species composition of the Anomalini tribe in wheat fields. Species of the Anomalini tribe, which may cause economic losses in their high populations, were identified in wheat fields in Van and its surroundings at low densities. Among the collected species, 6 species (*Anisoplia* (Autanisoplia) *austriaca*, *Anisoplia* (*Anisoplia*) *lata, Anisoplia* (*Anisoplia*) *signata, Brancoplia leucaspis,*

Chaetopteroplia segetum, and Anisoplia sp.) Anisopliina subtribe, while one species (*Blitopertha nigripennis*) belongs to the Anomalina subtribe. All of the detected species are the first records for the Van region.

When the sampling points where the tribe Anomalini beetles were collected were analyzed, the lowest altitude was 1646 m (Edremit-Muradiye) and the highest altitude was 2254 m (Çaldıran-Saray). Anomalini larvae started to pupate at the end of May and adults were collected towards the end of June. Towards the end of August, the adults started to disappear again. Individuals were observed to be denser at the edges of the wheat field than in the center. Crop bumblebees, which are described as weak fliers (Mico et al., 2001; Micó et al., 2003) are easily recognized by the eye due to their large body structure when disturbed and flown. Since adult individuals generally prefer to feed and mate on the stems, they were easily caught especially during the hot hours of the day. No individuals were found in the study areas between 22.05.2021 and 10.06.2021 when the surveys were carried out regularly. The first individuals, albeit at a very low density, started to be seen on 18.06.2021 and increased on 28.06.2021, and reached the highest population density on 20.07.2021. From this date onwards, the population density has decreased steadily until harvest.

Chaetopteroplia segetum was found to be the most common and densely populated Anomalini species. It was observed that the insect population was denser in irrigated wheat fields than in non-irrigated wheat fields. However, it was concluded that the population densities of this species and other species were not economically significant. The main reasons for this may be the effectiveness of climatic factors or natural enemies. Agricultural product production in the region is carried out in limited areas due to climatic conditions and the use of chemical pesticides against disease and pest populations is almost non-existent. These results, the study highlights the prevalence of *C. segetum* and its population density variations in irrigated and non-irrigated wheat fields, shedding light on the impact of agricultural practices on insect populations.

In addition to the morphological identification of the collected species, the study undertook phylogenetic analyses of the Anomalini species. The importance of phylogenetic analyses in identifying, classifying, and ecologically characterizing insects has been better understood with research conducted in recent years. Phylogenetic studies underline their fundamental role in advancing our knowledge of evolutionary patterns, dynamics, ecological relationships, species diversity, gene expression, and gene and genome duplications among insects (Lewinsohn et al., 2005; Procheş et al., 2009; Yu et al., 2009; Trautwein et al., 2012; Moriyama et al., 2015; Li et al., 2018). The results of the phylogenetic analyses, particularly the sister branch relationships of the identified species with those registered in GenBank, provide valuable genetic insights into the evolutionary relationships and taxonomic affiliations of these insects. In the phylogenetic analyses of Anomalini species, the sequences of the species collected in this study and the closest species registered in GenBank were analyzed together as an ingroup. As a result of the analysis, the species *A. austriaca*, registered in GenBank with accession number MF706436.1, and the specimen morphologically identified with the same name in this study were in the sister branch with a 99% bootstrap ratio. In addition, the species diagnosed as Bl. nigripennis in this study and the species Bl. lineolata with accession number MH 115532.1 was in the sister branch with a 96% bootstrap ratio. In the analyses, the other species were included in the clade from which the subtribe they belonged was separated. However, the study also acknowledges the limitations posed by the unavailability of DNA sequences for all identified species in GenBank, emphasizing the need for comprehensive genetic reference databases. Since the DNA sequences of all species identified in this study were unavailable in GenBank, a complete comparison could not be made.

The findings of this study underscore the importance of integrating phylogenetic analyses, such as DNA barcoding, into entomological research. DNA barcoding has emerged as a powerful tool for species identification and discovery, particularly in cases where morphological identification may be challenging or inconclusive (Meyer and Paulay, 2005). Furthermore, the study's emphasis on the potential economic impact of the identified species and the influence of climatic factors and natural enemies on population densities underscores the practical implications of entomological research for agricultural management and pest control strategies. In conclusion, the entomological study in the Van region not only contributes to the knowledge of Anomalini species composition and population dynamics but also highlights the significance of phylogenetic analyses, such as DNA barcoding, in advancing entomological research and its practical applications in agricultural contexts.

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Research Article

The Individual and Combined Effects of *Cystoseira compressa* Extracts and Inoculation of Arbuscular Mycorrhizal on Growth and Yield of Wheat under Salinity Conditions

Sami Mohammed SALIH*[1], Ahmed Amrajaa ABDULRRAZIQ[2]

[1,2]Department of Biology, Faculty of Education, Omar Al-Mukhtar University, Al-Beida, Libya

[1]https://orcid.org/ 0000-0001-7644-2380, [2]https://orcid.org/ 0000-0003-3722-4836

*Corresponding author e-mail: sami.mohammed@omu.edu.ly

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Abstract: Combined treatments are a successful way to overcome salinity damage in an environmentally safe and cost-effective method. So this experiment aimed to study the individual and combined effects of a seaweed extract of *Cystoseira compressa* (SWE) and Arbuscular Mycorrhizal Fungi (VA-M) on the growth and yield of *Triticum aestivum L.* cultivar (ACSAD 1398), under salinity conditions. In general, the study showed a significant decrease in morphological and biochemical parameters of the wheat under salinity levels. On the contrary, the results showed that all treatments significantly increased shoot and root length, number of leaves /plant, leaf area, seedling length, fresh and dry weight seedlings, spike length, fresh and dry weight spike, chlorophyll (a b), carotenoids, total pigments, Ca, Mg, P, K, Cu, N, crude protein, and total soluble sugars. As caused a decrease in proline content. The findings revealed that the (SWE+VA-M) combined treatment was superior to the foliar individual application of (SWE), and (VA-M) individual inoculation.

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple foods (cereal) in terms of global production and use, providing about 55% of starch and more than 20% of food calories(Gupta et al., 2021). Salinity is one of the most dangerous environmental phenomena threatening the growth and productivity of crops (Salih and Abdulrraziq, 2023), where wheat is exposed to losses of about 60% of the total crop production as a result of salt stress (Chaurasia and Kumar, 2023). This abiotic major stress condition causes yield reductions, due to changes in morpho-physiological and biochemical activity (Ramzan et al., 2023), in a negative way, such as increasing oxidative stress, osmotic stress, ionic toxicity, ion homeostasis conditions, and reduction of nutrient mobilization (Riseh et al., 2021). Also, hormonal imbalance, disruption of cellular homeostasis, deterioration of photosynthesis, protein synthesis, amino acid biosynthesis, lipid metabolism, nucleic acid damage, and reactive oxygen species ROS (Sharaya et al., 2023).

In recent years, the use of biological applications (Mycorrhizal symbiosis) and seaweed extracts has received greater attention as a method to alleviate abiotic and biotic stresses as an environmentally friendly tool (Rana et al., 2023; Wahab et al., 2023).

Previous studies have demonstrated the beneficial effect of Arbuscular mycorrhizal fungi such as improved uptake of phosphorus and other essential minerals (such as zinc, copper, and nitrogen) from the soil, by increasing the root surface area, nitrogen fixation, promoting soil fertility, and stress modulation (Ortas, 2023). Huang et al. (2023) found that arbuscular mycorrhizal fungus (AMF) increased in root and shoot length, fresh and dry weight, chlorophyll a, b, total, and carotenoids in the wheat under salt stress conditions. Also, inoculation with Funneliformis mosseae showed salt tolerance of wheat cultivars by improving osmoregulation, antioxidant enzyme activity, and reducing lipid peroxidation (Fayaz and Zahedi, 2021). On the other hand, natural seaweed extracts, are a substitute for synthetic fertilizers, that promote root growth, and improve increasing crop yields (Prajapati et al., 2023). They contain micro and macronutrients, amino acids, and organic matter, as well as vitamins, polysaccharides, auxins, gibberellins, and cytokinins, used either by adding them directly to the soil or spraying them on the vegetative parts of plants (Al-Ealayawi and Al-Dulaimy, 2023). Laboratory experiments showed that *Ulva linza* extract application in low concentrations (5, 10 and15%) improves physiological features (germination rate, total plant length, total fresh and dry mass, chlorophyll, carotenoids, sugars, proteins, lipids, proline, and alkaloids) in wheat seedlings, and mitotic and abnormality indices of wheat root cells (Hamouda et al., 2022).

There are many reports of applications of mycorrhizal fungi and seaweed extracts on the growth and productivity of wheat, but there are no studies on the application of arbuscular mycorrhiza (VA-M) inoculation within the combination of seaweed extract (SWE), under salinity conditions. The purpose of this research was to determine the individual and combined effects of a seaweed extract of *Cystoseira compressa* (SWE) and Arbuscular Mycorrhizal Fungi (VA-M) on the growth and yield response of the wheat cultivar (Acsad 1398), under salinity conditions**.**

2. Material and Methods

2.1. Seed selection

The genotype of bread wheat (ACSAD 1398), is one of the genotypes that have not been tested in Lybia. was obtained from The Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), Cleaned of impurities, and tested for viability by soaking in distilled water to remove empty seeds floating on the surface, were soaked in 1% sodium hypochlorite solution for 3 minutes, and washed with distilled water (Dafaallah et al., 2019).

2.2. Arbuscular Mycorrhizal Fungi (VA-M) source

Glomus mosseae strain kindly supplied by the Department of Microbiology, Faculty of Agriculture, El-Minia University, Egypt. The endomycorrhizal species (*Glomus mosseae* Nicol and Gerd.), Gerdman and Tappe, a representative species from a temperate agrosystem was obtained from a stock pot culture of *Allium cepa L*. as a host plant to produce a rhizosphere soil containing spores mycelia and mycorrhizal root. Each pot holding 5kg of soil received 20g of autoclaved soil containing mycorrhiza hyphae.

2.3. Seaweed extract collected and preparation

Fresh brown seaweed *Cystoseira compressa* were collected from the coastline of Al-Hamamah/ Al-Jabal Al-Akhdar/ Libya, and classified in the Department of Biology/ Faculty of Education/ Omar Al-Mukhtar University, They were washed and rinsed with distilled water in order to eliminate sand and plankton, drained, cut into small pieces and stored at -20 $^{\circ}$C until further use. Fresh seaweed (1 kg) was crushed and suspended in (1L) boiling distilled water for 1 h and filtered through double-layered muslin tissue (Sivasankari et al., 2006). The obtained filtrate was chosen as a 100% concentrated seaweed extract. A 5% concentration of *Cystoseira compressa* solution was prepared by adding distilled water. Later, the application of extract was through foliar spraying twice a week during the morning for two weeks (14 days) after sowing.

2.4. Preparation of salt solutions

The brine was prepared using sodium chloride salt (0, 100, and 200 mM NaCl), regional agricultural conditions as follows:

1 mMol = molecular weight of the solute / 1000 * concentration
100 mMol= molecular weight of the solute /1000 *100
100 mMol=58.5 / 1000* 100
100 mMol=5.85g/L

Take weight 5.85 g of NaCl salt, then dissolve it in a standard flask of capacity 1000 ml and complete the volume with distilled water to the mark and the same steps were followed for the concentration of 200 mM, according to(Salih and Abdulrraziq, 2023).

2.5. The pot experiments

The pot experiment was carried out in greenhouse conditions, the soil samples were sterilized at (90 ºC for 48 h). Five kilograms of sterilized clay-sandy soil were put into pots, a ratio of 2:1 (w/w). Ten seeds of wheat (ACSAD1398) were sown in each pot. The seedlings were removed and adjusted to five plants/pot after the plant reached a height of 12 cm each/pot. In greenhouse conditions, the experiment was conducted using 36 pots, 75 days after sowing (spike maturity stage), and with inon-applied chemical fertilizers. The experiment was set up in a completely random arrangement with twelve treatments and three repetitions as follows:
Treatments:

NaCl	SWE	VA-M	SWE+VA-M
0, 100, 200 mM NaCl	0, 100, 200 mM NaCl	0, 100, 200 mM NaCl	0, 100, 200 mM NaCl

2.6. Morphological Parameters

In the study, the roots were washed very carefully and care was taken not to break the capillary roots. seedling length, shoot and root length, and spike length (cm) parameters were measured by using a graduated ruler. The leaf area (cm^2) of each plant was measured and calculated according to the equation given below;

$$\text{Leaf Area / Plant (cm2)} = K\ (y*m) \quad (1)$$

Where: K=0.7, y=leaf length, m=leaf width, according to (Mokhtarpour *et al*., 2010).

- Fresh and dry weight of seedlings and spikes (g): fresh weights were determined (seedlings and spikes), and dried in an oven at 70 C^0 for 72h.

2.7. Biochemical parameters

2.7.1. Photosynthetic pigments

The following equations were used to calculate photosynthetic pigments (carotenoids, chlorophyll a, and b) spectrophotometrically (based on the Metzner et al., 1965):

$$Chlorophyll\ a = 10.3 \times 663 - 0.918 \times 644 = mg/ml \quad (2)$$

$$Chlorophyll\ b = 19.7 \times 644 - 3.87 \times 663 = mg/ml \quad (3)$$

$$Carotenoids = 4.2 \times 452.5 - 0.0264 \times chl.a + 0.4260 \times chl.b \quad (4)$$

2.7.2. Estimation of minerals

Oven-dry samples of seedlings were finely ground and assayed for mineral ion content by the wet digestion method. Minerals (Ca, Mg, P, K, and Cu) were determined using an atomic absorption spectrophotometer and flame photometer expressed based on dry weight (Humphries, 1956).

2.7.3. Estimation of total nitrogen(N)

0.2 g powder plant (stem of leaves) was digested by using 2 ml concentrated sulfuric acid and 1 ml of H_2O_2 (50%), the solution was then completed with H_2O or distilled water to a fixed volume. Absorption was measured by spectrophotometer at 420 nm according to Nessler's method as described by Hesse (1971).

2.7.4. Estimation of crude protein

The percentage of crude protein in spike tissues (including grain) was found by multiplying the total nitrogen content (%N) by factor 6.25 (A.O.A.C., 1975).

2.7.5. Estimation of Total soluble sugars (TSS)

The total soluble sugars were measured in an ethanolic extract of wheat plants (stem, leaves, and spike), using phenol-sulfuric with the using Pure glucose as standard (Dubois et al., 1966).

2.7.6. Estimation of proline

Proline in all leaves *w*as extracted using the ninhydrin reagent method and assayed according to (Bates et al., 1973).

2.8.Statistical Analysis

The complete random design (CRD) was used in the creation of the study experience. ANOVA variance analysis tables and the Minitab 17 application were used for statistical analysis. Tukey's test was used to compare the averages at $P < 0.05$.

3. Results

3.1.The effect of salinity levels on morphological and biochemical parameters

Current work shows in Tables (1, and 2) an effect of salinity levels (0, 100, and 200 mM) on some morphological parameters after 75 days of sowing. The results showed NaCl application decreased Morphological parameters by increasing salt concentration compared to the control. The concentration of 100mM caused a decrease in (shoot and root length, number of leaves /plant, leaf area, seedling length, fresh and dry weight seedlings, spike length, fresh and dry weight spike), from (100%) of control to (81.4, 95.2, 93.0, 83.4, 85.2, 66.5, 69.0, 83.3, 86.5 and 63.6%) respectively. The adverse effect of saline stress was obtained, (in the concentration of 200 mM), which recorded the highest rates of decline in all evaluated parameters in general, with (72.0, 82.3, 69.7, 69.1, 74.8, 50.8, 52.3, 70.2, 65.8 and 45.4%), for all parameters respectively. The results also showed that in Table 3 the results of salinity levels' effect on photosynthetic pigments, where the concentration of 100 mM caused a decrease of chlorophyll (a, b), carotenoids, and total pigments from (100%) of control to (70.8 and 91.1%) of chlorophyll (a, b), (79.5%) of carotenoids and total pigments by up to (76.5%). As salinity rose, so did the rates at which photosynthetic pigments decreased. Showed a concentration of 200 mM a significant decrease for chlorophyll (a, b), carotenoids, and total pigments with (60.9, 83.0, 61.,3 and 66.4 %) respectively. The data recorded in Table 4 showed the effect of salinity levels on the content of minerals in the seedling of the wheat, recorded a concentration of 100 mM decrease from (100%) of control to (91.3, 86.9, 92.5, 76.9, and 88.2%), while recorded a concentration of 200 mM largest rates the decrease of (84.7, 95.6, 85.1, 48.2, and 52.9%), for (Ca, Mg, P, K, and Cu) respectively. Figure 1 presents the effect of salinity levels on the total nitrogen (N) content, and crude protein (%). According to the observed values the a decrease in the contents of the N, contents from (1.3%) of the control to (0.4%), and a decrease in crude protein in spike tissues, from (1.8%) of the control to (2.5%), for concentration 200 mM, while concentration 100 mM had no significant effect on the content of percentage total nitrogen and crude protein. Data indicated in Figure 2 to the effect of salinity levels on the total soluble

solids (TSS), and proline of the wheat. The contents of total soluble solids in the seedling fresh weight decreased, according to the results, from (22.5 mg/g) in the control to (19.8, and 13.2 mg/g), at concentrations of 100 and 200 mM, respectively. Proline content in leaves also increased, from (34.0mg/g) of control to (56.4, and 89.7 mg/g), for concentrations (100 and 200 mM) respectively.

3.2. Effect of foliar application of *Cystoseira compressa* extract on some morphological and biochemical parameters under salinity levels

The data presented in Tables 1 and 2 show the individual effect of the foliar application of *C. compressa* extract on wheat growth. Results indicated a positive effect of the treatment (SWE + 100 mM NaCl) increase in (shoot and root length, number of leaves/plant, seedling length, fresh and dry weight seedlings, spike length, fresh and dry weight spike) by (4.5, 7.7, 13.9, 3.0, 5.3, 5.8, 7.1, 11.9, 4.9 and 4.5%) respectively. Furthermore, treatment (SWE + 200 mM NaCl), showed an increase of (3.9, 4.7, 23.3, 2.5, 4.2, 1.3, 7.2, 4.7, 15.9, and 9.1%), respectively for the same previous parameters, compared to the untreated plant. Findings in Table 3 demonstrate that in comparison to the untreated plant, the contents of the photosynthetic pigments have slightly increased. Pigments, which increased by (2.2, 3.0, 13.6, and 3.3%) of treatment (SWE + 100 mM NaCl), (5.4,1.5, 11.4, and 4.9%) of treatment (SWE + 200 mM NaCl), of chlorophyll contents (a, b), carotenoids, and total pigments respectively, compared to the untreated plant. Table 4 showed an increase in the content of minerals compared to the untreated plant, by (2.2, 13.0, 7.4, 27.3, and 23.5%) of treatment (SWE + 100 mM NaCl). Also by (6.5, 17.4, 18.6, 15.4, and 14.7%) of treatment (SWE + 200 mM NaCl) of (Ca, Mg, P, K, and Cu) respectively. The findings displayed in Figure 1 demonstrated that there were no discernible variations in the total nitrogen (N) level, and crude protein between concentrations 100 mM and treatment (SWE + 100mM NaCl), while the increase occurred in treatment (SWE + 200 mM NaCl), by (0.9 and 5.6 %) of total nitrogen (N) content, and crude protein respectively, compared to the untreated plant. The results showed in Figure 2, an increase in the contents of the total soluble solids in the seedling fresh weight by (12.4 and 11.1%) of treatment (SWE + 100 and 200 mM NaCl), respectively, compared to the untreated plant. Also, a decrease of proline by (31.7 and 11.5%) of treatment (SWE + 100 and 200 mM NaCl), respectively, compared to the untreated plant.

3.3. Effect of Arbuscular Mycorrhizal Fungi (VA-M) inoculation on some morphological and biochemical parameters under salinity levels

Tables 1 and 2 presented an effect of the individual Arbuscular Mycorrhizal Fungi (VA-M) inoculation on some morphological parameters of the wheat under salt stress conditions. Inoculation with (VA-M) showed there were highly significant differences, compared with foliar application of *Cystoseira compressa* extract of all parameters. The increase was obvious in (shoot and root length, number of leaves/plant, leaf area, seedling length, fresh and dry weight seedlings, spike length, fresh and dry weight spike) by (11.7, 18.9, 23.2, 8.0, 13.6, 19.0, 19.0, 66.7, 23.2, and 36.4%) respectively, of treatment (VA-M + 100mM NaCl), compared to the untreated plant. In addition, treatment (SWE + 200 mM NaCl), showed an increase of (12.1, 23.5, 39.6, 7.4, 15.2, 16.1, 26.2, 25.0, 37.8, and 45.5%) respectively for the same previous parameters, compared to the untreated plant. Tables (3) reveal that the application of Arbuscular Mycorrhizal Fungi (VA-M) enhanced a significant increase in the pigments in wheat leaves under NaCl stress, which were found to increase by (15.9, 8.1, 13.6, and 34.1%) of treatment (VA-M + 100 mM NaCl), and (15.3,5.9, 13.7, and18.1%) of treatment (VA-M + 200 mM NaCl), of chlorophyll contents (a, b), carotenoids, and total pigments respectively, compared to the untreated plant. The results in Table 4 showed a significant increase in the content of minerals by (38.7, 34.8, 37.1, 78.3, and 38.2%) of treatment (VA-M+100 mM NaCl), and (50.0, 56.5, 29.7, 46.9, and 35.3%) of treatment (VA-M+200 mM NaCl) of (Ca, Mg, P, K, and Cu) respectively. Figure 1 shows that there were no discernible variations in the total nitrogen (N) content between the concentration of 100 mM NaCl and treatment (VA-M+100 mM NaCl), while the increase occurred in treatment (VA-M+200 mM NaCl), by (0.8 %) of Total nitrogen (N) content. The increase appears in crude protein by (0.6 and 5.0%), of treatment (VA-M+100 and 200 mM NaCl) respectively, compared to the untreated plant. Also, Figure 2 a significant increase in the contents of the total soluble solids by (27.1 and 30.7%) of treatment (VA-M+100 and 200 mM NaCl) respectively, compared to the untreated plant. Data also

indicated a decrease of proline by (47.9 and 53.0%)of treatment (VA-M+100 and 200 mM NaCl) respectively, compared to the untreated plant.

3.4. Effect of combined treatment *Cystoseira compressa* extract +Arbuscular Mycorrhizal Fungi (SWE+VA-M) on some morphological parameters and biochemical under salinity levels

Data regarding the treatment (SWE+VA-M), in Tables 1, 2, 3, and 4 indicated that there were highly significant differences, compared with the individual foliar application of SWE, and individual (VA-M) inoculation. Showed the highest registered increments of the shoot and root length by (15.2, and 22.4%). The number of leaves/plant (30.2%). Leaf area (20.6%). Seedling length, fresh and dry weight seedlings (17.2, 29.3, and 23.6%). Spike length, fresh and dry weight spike (59.5, 36.6 and 45.4%). respectively, of treatment (SWE+VA-M +100 mM NaCl), compared to the untreated plant. On the other hand, the treatment showed (SWE+VA-M +200 mM NaCl), increments in shoot and root length by (15.2 and 24.7%). The number of leaves/plant (34.9%). Leaf area (22.1%). Seedling length, fresh and dry weight seedlings (17.8, 27.2, and 23.8%). Spike length, fresh and dry weight spike (40.5, 36.6, and 50.0%) respectively, compared to the untreated plant. Also caused an increase in the contents of the photosynthetic pigments (chlorophyll a, b carotenoids, and total pigments), which reached (14.1, 8.1, 34.1, and 15.3%) of treatment (SWE+VA-M +100 mM NaCl). Likewise (9.7, 8.9, 11.4and 9.6%) of treatment (SWE +VA-M +200 mM NaCl), respectively, compared to the untreated plant. Moreover, recorded increase in the content of minerals of (Ca, Mg, P, K, and Cu) by (50.0, 65.2, 40.8, 73.4, and 23.5%) of treatment (SWE+VA-M +100 mM NaCl). Likewise (52.2, 39.1, 26.0, 38.5, and 41.2%) of treatment (SWE+VA-M +200 mM NaCl) of (Ca, Mg, P, K, and Cu) respectively. Figure 1 reveals significant differences in the increase in the Total nitrogen (N) by (0.2 and 0.9%) of treatment (SWE+VA-M+100 and 200 mM NaCl) respectively, compared to the untreated plant. while crude protein recorded an increase of (1.2 and 5.6%), in treatment (SWE+ VA-M+100 and 200 mM NaCl), respectively, compared to the untreated plant. Also, Figure 2 a significant increase in the contents of the total soluble solids TSS by (28.4 and 32.5%) of treatment (SWE+VA-M+ 100 and 200 mM NaCl) respectively, compared to the untreated plant. The decrease of proline by (50.0 and 18.3%)of treatment (SWE+VA-M+ 100 and 200 mM NaCl) respectively, compared to the untreated plant.

Table 1. Effect of different treatments on shoot length, root length, leaves/plant, leaf area, and seedling length of the wheat cultivar ACSAD under salinity levels

Treatments		Shoot length		Root length		Leaves/plant		Leaf area		Seedling length	
		cm	%	cm	%	N	%	cm^2	%	cm	%
	0	45.4 d	100	17.0 def	100	4.3 cd	100	52.5 c	100	62.4 e	100
NaCl	100	37.0 g	81.4	16.2 efg	95.2	4.0 de	93.0	43.8 f	83.4	53.2 h	85.2
	200	32.7 h	72.0	14.0 g	82.3	3.0 e	69.7	36.3 h	69.1	46.7 j	74.8
	0	48.2 c	106.1	17.9 cde	105.2	5.3 abc	123.2	56.0 b	106.6	66.1 c	105.9
SWE	100	39.0 f	85.9	17.5 cdef	102.9	4.6 cd	106.9	45.4 e	86.4	56.5 g	90.5
	200	34.5 h	75.9	14.8 fg	87.0	4.0 de	93.0	37.6 h	71.6	49.3 i	79.0
	0	52.8 b	116.2	21.0 ab	123.5	6.4 a	142.2	64.2 a	122.2	73.8 b	118.2
VA-M	100	42.3 e	93.1	19.4 bcd	114.1	5.0 bcd	116.2	48.0 d	91.4	61.7 e	98.8
	200	38.2 fg	84.1	18.0 cde	105.8	4.7 cd	109.3	40.2 g	76.5	56.2 g	90.0
	0	55.0 a	121.1	22.3 a	131.1	6.0 ab	139.5	65.5 a	124.7	77.3 a	123.8
SWE+VA-M	100	43.9 de	96.6	20.0 abc	117.6	5.3 abc	123.2	54.6 b	104.0	63.9 d	102.4
	200	39.6 f	87.2	18.2 cde	107.0	4.5 cd	104.6	47.9 d	91.2	57.8 f	92.6

Different letters in each column denote statistical differences at $P < 0.05$.

Table 2. Effect of different treatments on fresh weight and dry weight seedling, spike length, fresh weight and dry weight spike of the wheat cultivar ACSAD under salinity levels

Treatments		Freshweight Seedling		Dry weight seedling		Spike length		Fresh weight spike		Dry weightspike	
		g	%	g	%	cm	%	g	%	g	%
	0	24.2 cd	100	4.2abcd	100	8.4 cde	100	0.82 f	100	0.22 c	100
NaCl	100	16.1 g	66.5	2.9 cd	69.0	7.0 ef	83.3	0.71 gh	86.5	0.14 de	63.6
	200	12.3 h	50.8	2.2 d	52.3	5.9 f	70.2	0.54 i	65.5	0.10 e	45.4
	0	25.9bc	107.0	4.4 abc	104.7	10.3 bc	122.6	0.93 d	113.4	0.23 bc	104.5
SWE	100	17.5 fg	72.3	3.2 bcd	76.1	8.0 def	95.2	0.75 g	91.4	0.15 d	68.1
	200	13.6 h	56.1	2.5 cd	59.5	6.3 ef	75.0	0.67 h	81.7	0.12 de	54.5
	0	28.2 ab	116.5	5.2 ab	123.8	12.0 ab	142.8	1.11 b	135.3	0.27 ab	122.7
VA-M	100	20.7 e	85.5	3.7 abcd	88.0	12.6 a	150.0	0.90 de	109.7	0.22 c	100
	200	16.2 g	66.9	3.3 bcd	78.5	8.0 def	95.2	0.85 ef	103.6	0.20 c	90.9
	0	29.8 a	123.1	5.5 a	130.9	13.6 a	161.9	1.17 a	142.6	0.28 a	127.2
SWE+VA-M	100	23.2 d	95.8	3.9 abcd	92.6	12.0 ab	142.8	1.01 c	123.1	0.24 abc	109.0
	200	18.9 ef	78.0	3.2 bcd	76.1	9.3 cd	110.7	0.84 f	102.4	0.21 c	95.4

Different letters in each column denote statistical differences at P <0.05.

Table 3. Effect of different treatments on photosynthetic pigments of the wheat cultivar ACSAD under salinity levels

Treatments		Chlorophyll a		Chlorophyll b		Carotenoids		Total pigments	
		mgg^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%
	0	3.71 b	100	1.36 d	100	0.44 de	100	5.51 b	100
NaCl	100	2.63 d	70.8	1.24 fg	91.1	0.35 f	79.5	4.22 ef	76.5
	200	2.26 f	60.9	1.13 h	83.0	0.27 g	61.3	3.66 g	66.4
	0	3.79 b	102.1	1.42 bc	104.4	0.47 cd	106.8	5.68 b	103.0
SWE	100	2.71 d	73.0	1.28 e	94.1	0.41 e	93.1	4.40 de	79.8
	200	2.46 e	66.3	1.15 h	84.5	0.32 f	72.7	3.93 fg	71.3
	0	4.07 a	109.7	1.45 b	160.6	0.53 b	120.4	6.05 a	109.8
VA-M	100	3.22 c	86.7	1.35 d	99.2	0.50 bc	113.6	5.07 c	92.0
	200	3.12 c	76.2	1.21 g	88.9	0.33 f	75.0	4.66 d	84.5
	0	4.18 a	112.6	1.49 a	109.5	0.58 a	131.8	6.25 a	113.4
SWE+VA-M	100	3.15 c	84.9	1.41 c	103.6	0.50 bc	113.6	5.06 c	91.8
	200	2.62 d	70.6	1.25 ef	91.9	0.32 f	72.7	4.19 ef	76.0

Different letters in each column denote statistical differences at P <0.05.

Table 4. Effect of different treatments on the content of minerals in the Seedling of the wheat cultivar ACSAD under salinity levels

Treatments		Ca		Mg		P		K		Cu	
		mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%
	0	4.6 abc	100	2.3 ab	100	2.7 bc	100	14.3 c	100	3.4ab	100
NaCl	100	4.2 bc	91.3	2.0 b	86.9	2.5 bc	92.5	11.0 d	76.9	3.0 ab	88.2
	200	3.9 c	84.7	2.2 ab	95.6	2.3 bc	85.1	6.9 e	48.2	1.8 b	52.9
	0	5.3 abc	115.2	2.9 ab	126.0	3.2 abc	118.5	18.5 b	129.3	3.9 ab	114.7
SWE	100	4.5 abc	97.8	2.4 ab	104.3	3.0 abc	111.1	13.2 c	92.3	3.5 ab	102.9
	200	4.0 c	86.9	2.5 ab	108.6	2.5 bc	92.5	10.8 d	75.5	2.6 ab	76.4
	0	6.3 ab	136.9	3.5 a	152.1	4.0 a	148.1	21.2 a	146.8	4.5 a	132.3
VA-M	100	6.0 abc	130.4	2.8 ab	121.7	3.5 abc	129.6	22.2 a	155.2	4.3 a	126.4
	200	6.2 ab	134.7	3.5 a	152.1	3.1 abc	114.8	13.6 c	95.1	3.0 ab	88.2
	0	5.9 abc	128.2	3.3 ab	143.4	4.2 a	155.5	23.1 a	161.5	4.8 a	141.1
SWE+VA-M	100	6.5 a	141.3	3.5 a	152.1	3.6 ab	133.3	21.5 a	150.3	3.8 ab	111.7
	200	6.3 ab	136.9	3.1 ab	134.7	3.0 abc	111.1	12.4 cd	86.7	3.2 ab	94.1

Different letters in each column denote statistical differences at P <0.05.

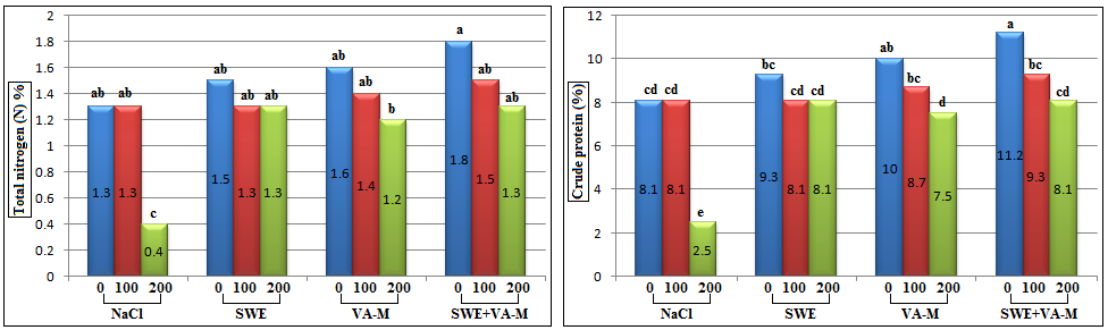

Figure 1. Effect of different treatments on the Total nitrogen (N) content, and crude protein of wheat cultivar ACSAD under salinity levels.

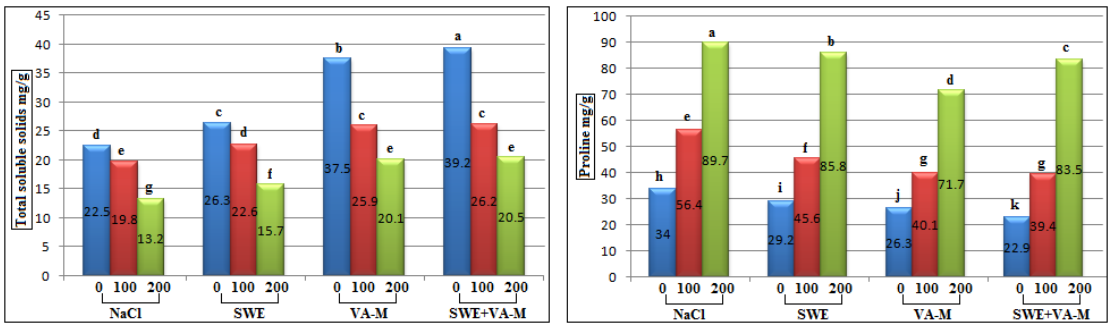

Figure 2. Effect of different treatments on the Total soluble solids (TSS), and Proline of wheat cultivar ACSAD under salinity levels.

4. Discussion

Salinity is the most environmentally important obstacle facing the productivity and quality of wheat crops (Salih et al., 2023), by disrupting the vital physiological, morphological, and biochemical parameters of plants (Ghonaim et al., 2023). This was clear from the results of this study which showed highly significant differences when ($P<0.05$) in reducing all morphological and biochemical parameters of wheat. Similar findings were reported for wheat by(Fairoj et al., 2023; Salih and Abdulrraziq, 2023; Singh, 2023; Paudel et al., 2023). This negative effect may be attributed to Increased Na+ disorganizing ionic balance in cells, disrupting the cell cycle and redistribution of cells in the phases of the cell cycle, inhibiting cell expansion, higher cellular membrane damage, an increased rate of lipid peroxidation, altering the nutrient level, inhibiting apical growth, inhibiting protein synthesis, and enhancing the generation of reactive oxygen species (Amin et al., 2023; Bogoutdinova et al., 2023; Sarkar and Sadhukhan, 2023). Furthermore, salinity stress limits CO_2 fixation in the leaves by stomatal closure, downregulation of the Calvin cycle, as well as increased proteolytic enzymes chlorophyllase responsible for the degradation of chlorophyll, and decreased activity of ribulose bisphosphate (Sadak and Ahmed 2016; Kwon et al., 2019; Sharma et al., 2020). The results of the data analysis showed that with increasing salinity, proline accumulation increases in wheat, this result agreed with many studies that confirmed that proline accumulation is evidence of plants' resistance to salt stress (Hussain et al., 2023; Zulfiqar and Ashraf, 2023). The reason is that proline preserves membrane structure, creating osmotic compatibility, and protecting plant cells from oxidative stress by stopping the synthesis of hydrogen peroxide, superoxide ions, and ROS hydroxyl ions (Chakraborty and Kumari, 2024). The individual foliar application of *Cystoseira compressa* extract alleviated the adverse effects of salinity levels in most morphological and biochemical parameters of wheat, compared to the untreated plant. Our results are consistent with many studies that showed seaweed extract treatments successfully increase the productivity of wheat crops (Latique et al., 2021; Sayyari Zahan et al., 2022). The increase in growth resulting from the effect of Foliar spray seaweed extract under salinity, was due to the content of important micronutrients, vitamins, and plant hormones, such as auxins, cytokinins, and gibberellins in seaweed (Al-Saif et al., 2023). Moreover, Seaweed polysaccharides increase plant resilience to abiotic stress and encourage crop growth (Zou et al., 2019). Data regarding the individual treatment inoculation

of wheat plants with Arbuscular Mycorrhizal Fungi (VA-M) individually indicated that there were highly significant differences when ($P<0.05$) in increasing all morphological and biochemical parameters of salt-stressed wheat compared to the untreated plant and foliar individual application treatment of Cystoseira compressa extract. Our result agrees with previous studies that reported beneficial changes in wheat due to Mycorrhizal inoculation in saline environments (Huang et al., 2023; Puccio et al., 2023). Mycorrhiza fungus provides many benefits through improving nutrient uptake and water, production of plant hormones, improvement of soil structure, and Mycorrhizal penetrates root skin cells and forms structures called vesicles and arbuscules, increasing the level of metabolic content (Bayanati et al., 2023). In addition, caused an increase in the biosynthesis of osmoprotectants, maintaining the integrity of plasma membranes and stability under salt stress by enhanced expression of genes related to signal transduction, vesicle trafficking, RNA processing, trehalose metabolism, and cell wall organization (Chandra et al., 2023; Puccio et al., 2023). On the other hand, mycorrhizae regulate the functions of a few important proteins involved in the metabolism of lipids, amino acids, and glutathione in root tissue (Chang et al., 2023). Also, mycorrhizae increase photosynthesis by increasing Rubisco activity, electron transport rates, and adenosine triphosphate (ATP) synthesis (Kaschuk et al., 2009). This was clear from the results of a study in terms of increased contents of photosynthetic pigments under conditions of salt stress.

In the current study, the obtained results showed that combined treatment (SWE+VA-M), was superior in recording the best indicators studied, compared to the untreated plant, foliar individual application of (SWE), and (VA-M) individual inoculation. Our results are consistent with many studies that showed combined application treatments successfully increased the productivity of wheat crops. For example, the combined application of biochar and arbuscular mycorrhizal fungi is a promising way to reduce the harmful effects of salt stress in wheat production observed by (Ndiate et al., 2022), and Rashed and Hammad (2023) that the interaction application between vinasse treatment and compost tea was efficient in improving the soil's bulk density and porosity, and availability of N, P, and K, this helped to promote the vegetative growth of the wheat.

According to Setta et al. (2018), foliar application of *Cystoseira compressa* extract decreased salt stress and enhanced wheat growth by excreting chemicals that promote plant growth, such as gibberellic acid (GA3) and indole acetic acid (IAA). Moreover, an extended mycelial network facilitates the growth of plant roots beyond the zone of root depletion, enabling the plant to absorb more water and mineral nutrients from the soil. It also offers a direct route for the translocation of carbon derived from photosynthetic processes to soil microsites and a sizable surface area for microbial interactions (Finlay, 2008).

Conclusion

With in light of the results obtained from this study, NaCl resulted in a progressive decrease in morphological parameters and biochemical of the (*Triticum aestivum L.*). The 200 mM NaCl level was the most toxic to the plant. All the treatments that were tested played a role in mitigating the detrimental impacts of salinity. The combined treatment (SWE+VA-M) had a superior effect to all tested treatments, followed by the (VA-M) treatment and finally the (SWE)treatment. Therefore, we suggest the co-application to improve the parameters and biochemical parameters of the wheat cultivars different under salinity levels, as a safe, easy-to-prepare, and inexpensive method.

Conflict of Interest

The authors declare the contribution of the authors is equal. The authors have declared no conflict of interest.

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Research Article

Morphological and Physiological Changes under NaCl Stress in Some *Pyrus* and *Quince* Rootstocks

Melih AYDINLI*[1], Fatma YILDIRIM[2], Emel KAÇAL[3], Mesut ALTINDAL[4], Halit YILDIZ[5]

[1,3,4]Fruit Research Institute, 32500, Isparta, Türkiye
[2]Isparta University of Applied Science, Faculty of Agriculture, Horticulture Department, 32200, Isparta, Türkiye
[5]Provincial Directorate of Agriculture, 48000, Muğla, Türkiye

[1]https://orcid.org/0000-0002-1166-5791, [2]https://orcid.org/0000-0001-7304-9647, [3]https://orcid.org/0000-0003-4834-5510
[4]https://orcid.org/0000-0002-0332-6677, [5]https://orcid.org/0000-0001-7922-9366

*Corresponding author e-mail: melih.aydinli@tarimorman.gov.tr

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Salinity stress,
Tolerance

Abstract: In this study, the aim was to determine some morphological, physiological, and biochemical changes in non-grafted plants of OHxF 97, OHxF 333, Fox 11, and BA 29 rootstocks under NaCl stress. NaCl (0 mM, 20 mM, 40 mM, and 80 mM) was applied to the rootstocks planted in 18-liter pots with irrigation water repeated over two years. Under NaCl stress, plant height, plant diameter, and leaf area decreased in all rootstocks. Additionally, Fox 11 and BA 29 rootstocks were more adversely affected by NaCl stress to leaf necrosis. The amounts of chl a, chl b, and total chl decreased in Fox 11 rootstock with moderate and severe stress treatments. Carotenoid content in the leaves, especially under severe stress conditions, showed a decrease in *Pyrus* rootstocks. Under NaCl stress, the leaves of Fox 11 were rich in proline. MDA content generally increased with NaCl stress compared to the control in Fox 11 and BA 29. Although significant changes in plant nutrients were generally not observed with NaCl, a significant decrease in the amount of K^+ in the leaves of Fox 11 was identified. Consequently, Fox 11 and BA 29 rootstocks exhibit sensitivity to NaCl stress, whereas OHxF rootstocks demonstrate greater tolerance.

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1. Introduction

Salinization is a significant abiotic stress factor that adversely affects available agricultural lands. 7% of the world's terrestrial areas, 20% of arable lands, and half of irrigable agricultural lands are facing salinity (Gupta and Huang, 2014; Kumar et al., 2015). Furthermore, the growing apprehension among scientists regarding the escalating incidence of salinity stress, particularly in arid and semi-arid regions, is propelling extensive research into salt stress in the foreseeable future.

Salinity exerts direct effects on plants by inducing osmotic and ion stress, while its indirect effects (secondary effects) manifest through oxidative stress (Petridis et al., 2012; Wei et al., 2017). Osmotic stress is the initial stress resulting from an increase in salt concentration in the root rhizosphere (Tuteja, 2007). As a result of osmotic stress, factors such as a reduction in leaf water potential (LWP)

and stomatal conductance (Shabala and Munns, 2012; Navada et al., 2020), photosynthesis inhibition (Meloni et al., 2003), and elevated production of reactive oxygen species (ROS) (Jia et al., 2019) contribute to growth interruption (Munns and Tester, 2008). Ion stress induced by toxic ions such as sodium (Na^+) and chloride (Cl^-) (Sofy et al., 2020), particularly the excessive accumulation of Na^+ ions (Niu et al., 2017), contributes to the indirect effects. Oxidative stress arises from the excessive generation of harmful ROS (Arif et al., 2020). To withstand these stress factors, plants employ tolerance mechanisms and develop various physiological and biochemical adaptations. Important tolerance mechanisms include a) ion homeostasis and partitioning of ions, b) ion transport and uptake, c) synthesis of osmoprotectants and compatible solutes, d) activation of antioxidant enzymes and synthesis of antioxidant compounds, e) synthesis of polyamines, f) production of nitric oxide, g) hormone modulation (Gupta and Huang, 2014).

Plants are classified into halophytes and glycophytes based on their adaptation abilities to salt, with most agriculturally important species falling into the glycophyte class (Gupta and Huang, 2014). Pear (*Pyrus* spp.), one of the important temperate climate species, shows sensitivity when exposed to prolonged relatively low salinity (Okubo et al., 2000). Additionally, growth retardation and leaf damage occur under osmotic and ionic stress (Hasegawa et al., 2000)

Although pear cultivation in Türkiye is currently conducted in non-saline soils, according to Musacchi et al. (2006) the use of drip irrigation methods, the inclusion of fertigation systems in practices, and cultivation in areas close to the coast may lead to secondary salt stress and, consequently, salt damage. Minimizing this damage is seen as a priority and developing salt-tolerant rootstocks or determining the tolerance/sensitivity levels of existing plant materials used in production are important considerations. Against this backdrop, the current study aims to determine the tolerance mechanisms of OH x F 97, OH x F 333, Fox 11, and BA 29 rootstocks, against salt stress induced by irrigation water containing different concentrations of NaCl. The study generates data on the responses of rootstocks and their tolerance/sensitivity status based on various morphological, physiological, and biochemical parameters.

2. Material and Methods

2.1. Plant material

The study included one-year-old non-grafted plants of rootstocks OHxF 97 (Old Home x Farmingdale 97), OHxF 333 (Old Home x Farmingdale 333), Fox 11, and BA 29, which are used as rootstocks in pear growing. Clonal rootstocks of pear (*Pyrus communis* L.) were propagated through tissue culture, while the quince (*Cydonia oblonga* L.) clonal rootstock was propagated using the stool-bed technique. At the onset of the vegetation period, the plants were transplanted into a cultivation medium consisting of garden soil (sieved) + sand + peat (2:1:1) in 18-liter containers. In June, the plants were moved into a climate-controlled greenhouse where the treatments would take place.

2.2. NaCl treatments

The NaCl treatments were initiated in mid-July. The study included four different NaCl concentrations, including a control (irrigation water ~ 3 mM NaCl), light stress (20 mM), moderate stress (40 mM), and severe stress (80 mM). The amount of water given to the plants was determined based on the previously established field capacity. The irrigation interval was set at 4-5 days in the research. To prevent osmotic shock in the plants, NaCl doses of 20 mM were gradually applied. After approximately sixty days of NaCl treatments, damage related to NaCl stress was observed in the leaves, and the trial was concluded. For biochemical analyses, fully developed leaves from the middle part of each plant were collected at the end of the study, immersed in liquid nitrogen, and stored at -80°C until the analyses were conducted. Following this process, each plant was cut at the root collar.

The study was designed as a randomized complete factorial experiment with three replicates, with each replicate consisting of five plants, following a factorial design in the experimental plots. This research was repeated for two years.

2.3. Measurement of morphological parameters

Plant heights were measured in meters from the graft point to the top of the leading shoot. Shoot diameter was measured with calipers from the midpoint of the shoots. Leaf area measurements were conducted on 10 randomly selected leaves from each replicate at the end of the experiment. The surface areas of the samples were recorded in "cm^2" using a digital planimeter (Koizumi KP-90 N). The scale developed by Sivritepe et al. (2008) was employed to assess the damage occurring in the leaves and shoots of plants exposed to NaCl stress.

2.4. Measurement of physiological parameters

LWP measurements were conducted using a pressure chamber (PMS Instrument Company, Model 1000) between 12:00 and 14:00 on at least two fully mature leaves randomly selected from a plant in each treatment. To ensure the samples reached a stable state, leaves were wrapped in aluminum foil before measurements (Küçükyumuk et al., 2015).

2.5. Biochemical analyses

The total chlorophyll (total chl), chlorophyll a (chl a), chlorophyll b (chl b), and carotenoid (car) concentrations in the leaves were determined according to the method described by Arnon (1949). Fresh leaf samples were subjected to extraction with 80% (v/v) acetone. A portion of the extracted sample was taken, and the absorbances of chl a at 663 nm, chl b at 645 nm, and car at 470 nm were measured using a spectrophotometer (Shimadzu, UV-1800). The following equations were used in the calculations (Lichtenthaler and Wellburn, 1983):

$$\text{chl a} = (11.75 \times A663 - 2.23 \times A645) \times 20 / \text{mg sample weight} \quad (1)$$

$$\text{chl b} = (18.61 \times A645 - 3.96 \times A663) \times 20 / \text{mg sample weight} \quad (2)$$

$$\text{car} = ((1000 \times A470 - 2.27 \times \text{chl a} - 81.4 \times \text{chl b}) / 227) \times 20 / \text{mg sample weight} \quad (3)$$

The proline content in freeze-dried leaf samples was determined according to Bates et al. (1973). Readings were taken at 520 nm using a spectrophotometer, and the results were expressed as µmol proline per g dry weight.

Lipid peroxidation, expressed as malondialdehyde (MDA) content, was assessed according to Hernandez and Almansa (2002). Readings at 532 nm and 600 nm were recorded on the spectrophotometer, and the results were presented as nmol per g.

For macro-nutrient analysis of leaves, the Kjeldahl method (Gerhardt Vapodest 40) with a live burning technique was used for N^{2+}. Microwave live burning methods were employed for P^{4+}, K^{+}, Ca^{2+}, and Mg^{2+} analyses in dried leaf samples. Measurements of prepared samples were conducted using an ICP-AES (Spectro Arcos Blue2) device (Kaçar and İnal, 2008).

2.6. Statistical analyses

The study was conducted with a factorial experimental design in randomized complete blocks, with three replications, and five plants were used in each replication. Statistical analyses were performed using JMP 11 software. The Shapiro-wilk test was used to determine whether the data showed normal distribution. Non-parametric tests, including the Kruskal-Wallis test, were applied to data that deviated from a normal distribution to evaluate the results. Differences between treatments were determined using the LSD Multiple Comparison Test ($P<0.05$; $P<0.01$; $P<0.001$).

3. Results

3.1. Effect of NaCl stress on morphological variables

In all *Pyrus* rootstocks exposed to NaCl stress, plant height generally decreased over both years, depending on genotype and stress level (Figure 1A and Figure 1B). Particularly, the plant height of the Fox 11 was significantly restricted in severe stress treatments in both years of the study. Specifically, when compared to control, the largest percentage decreases were determined to be 71.91% and 69.31% in Fox 11 in successive years, as shown in Figure 1A and Figure 1B. For the BA 29, representing the *Quince* species in the study, significant changes in plant height were not observed in both years under NaCl. Typically, the impact of NaCl treatments on plant height revealed noticeable decreases, with variations observed across years and a consistent decline noted with increasing stress levels (Figure 1A and Figure 1B).

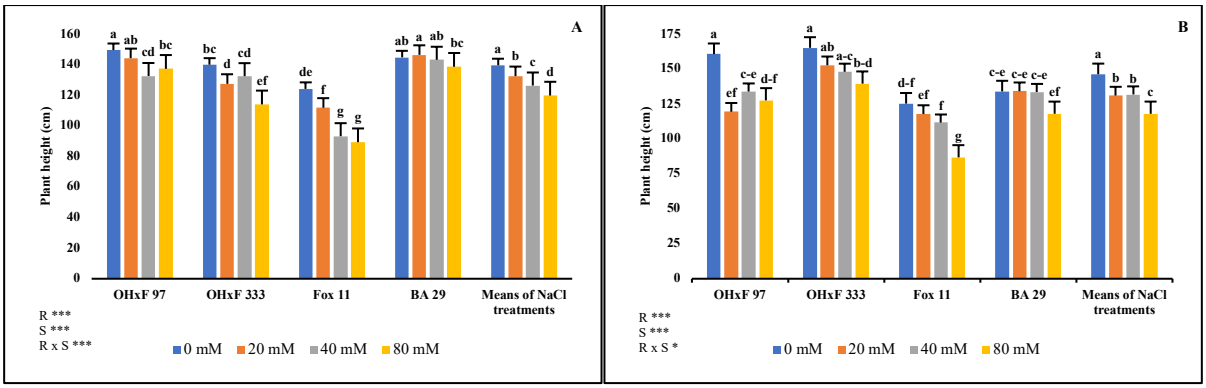

Figure 1. Plant height of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=15). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. *** and * represent the significance level of differences at p<0.001, p<0.05, and non-significant, respectively.

In the first year, the plant diameter of the OHxF 97 rootstock significantly decreased in the moderate (6.47 mm) and severe stress (6.52 mm) compared to the control (7.52 mm) and light stress (7.52 mm) treatments (Figure 2A). The plant diameter of the OHxF 333 rootstock decreased significantly at all stress levels compared to the control. However, the plant diameter in Fox 11 and BA 29 rootstocks was not affected by NaCl. In the second year, it was determined that NaCl stress led to a proportional decrease in plant diameter in all *Pyrus* rootstocks (Figure 2B).

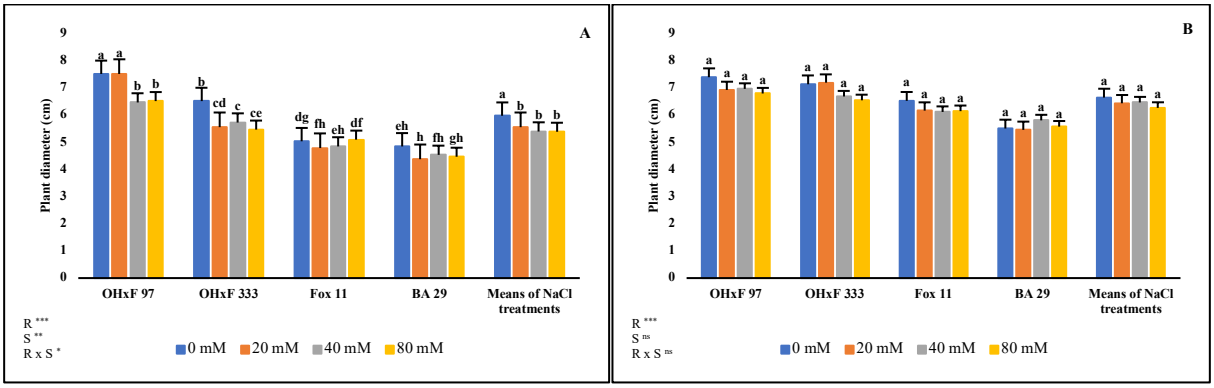

Figure 2. Plant diameter of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=15). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. ***, **, *, and ns represent the significance level of differences at p<0.001, p<0.01, p<0.05, and non-significant, respectively.

In the first year, the leaf area was affected by NaCl stress in all rootstocks except Fox 11 (Figure 3A). Especially in the BA 29 rootstock, leaf area significantly decreased at all stress levels (light stress - 27.13 cm^2; moderate stress - 26.93 cm^2; severe stress - 23.47 cm^2) compared to the control (30.4 cm^2). In the second year of the study, moderate and severe NaCl stress reduced leaf area in all rootstocks (Figure 3B), with the highest percentage decreases observed in the severe stress treatments of OHxF 333 (31.48%) and OHxF 97 (23.32%), respectively. The study revealed a significant reduction in leaf area due to NaCl stress, and the smallest leaves were observed because of severe stress.

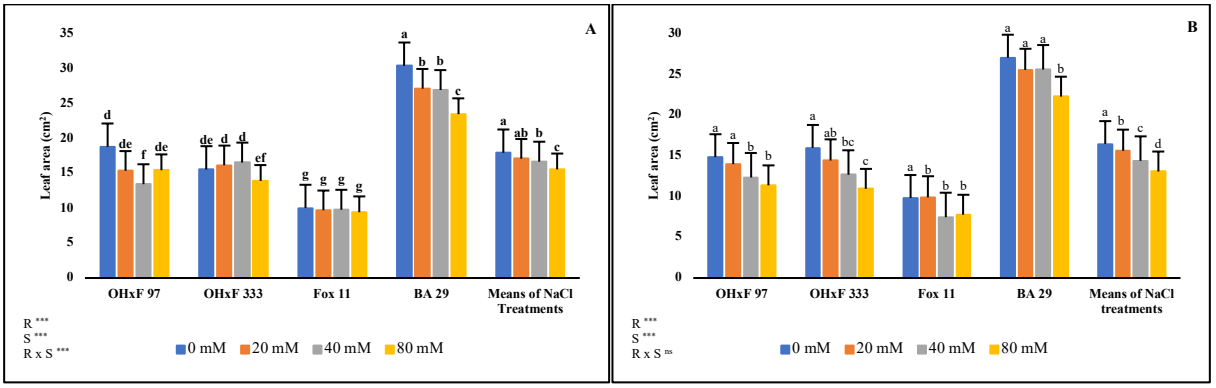

Figure 3. Leaf area of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=30). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. *** and ns represent the significance level of differences at $p<0.001$ and $p<0.05$, respectively.

The leaf necrosis observed in plants exposed to NaCl stress is presented in Figure 4A and Figure 4B. According to this, no signs were observed with light stress treatments in plants treated with NaCl for approximately sixty days. Additionally, there was no indication of NaCl damage at moderate stress levels in OHxF rootstocks. However, Fox 11 and BA 29 suffered significantly under moderate and severe NaCl stress. For instance, in the case of the Fox 11 rootstock, the leaf necrosis, which was 1.87 and 1.33 in moderate stress treatments in successive years, was determined as 3.0 and 2.67 in severe stress treatments. In BA 29, the leaf necrosis, which was 0.67 and 0.80 in successive years, was measured as 2.0 and 1.60 under severe stress.

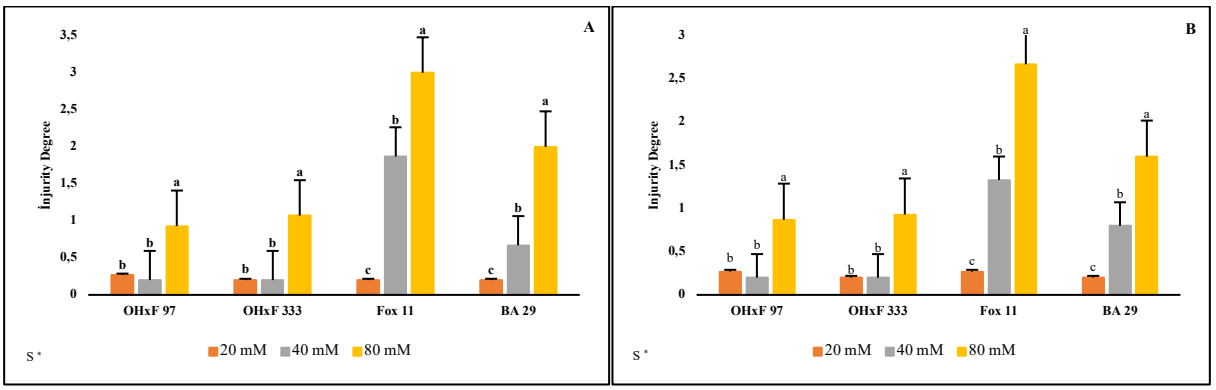

Figure 4. Injurity degree of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=3). Letters represent the effect of NaCl treatment on rootstocks. *, represents the significance level of differences at $p<0.05$.

3.2. Effect of NaCl stress on physiological variables

The effect of NaCl treatments on LWP is presented in Figure 5A and Figure 5B. LWP values at the end of the first year trial reveal that is not affected by NaCl treatments in all rootstocks. However, in the treatment averages, LWP increased under moderate and severe stress conditions. In measurements taken at the end of the second year trial, the LWP significantly decreased in severe stress treatments for

OHxF 333 (-4.61 MPa) and Fox 11 (-4.13 MPa) rootstocks. In BA 29, the treatments did not affect the LWP.

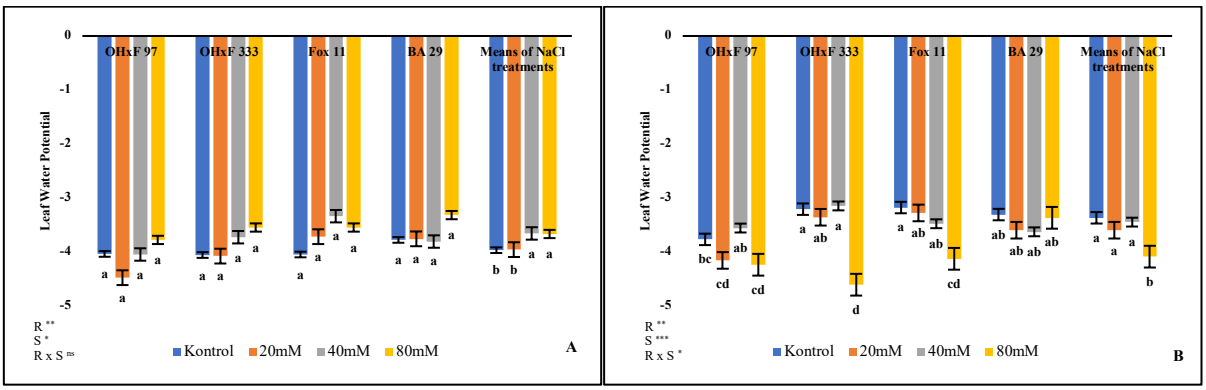

Figure 5. Leaf water potential of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=6). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. ***, **, *, and ns represent the significance levels of $p<0.001$, $p<0.01$, $p<0.05$, and non-significant, respectively.

3.3. Effect of NaCl stress on biochemical variables

In the first year, the amounts of chl a, chl b, total chl, and carotenoid in the leaves were not affected by the treatments (Table 1). In the second year, however, the mentioned parameters were influenced by the rootstock x treatment interaction. While the amount of chl a generally remained unchanged in OHxF and BA 29 under stress treatments, it significantly decreased in plants exposed to moderate (2.87 mg g^{-1}) and severe (2.93 mg g^{-1}) stress in the Fox 11. A similar situation was observed in the amounts of chl b and total chl, where a decrease was found in plants subjected to moderate (chl b; 1.01 mg g^{-1} – total chl; 3.88 mg g^{-1}) and severe (chl b; 1.07 mg g^{-1} – total chl; 3.99 mg g^{-1}) stress in the Fox 11. The amount of carotenoid in the leaves significantly increased in the OHxF rootstocks (OHxF 97; 4.17 mg g^{-1}; OHxF 333; 4.09 mg g^{-1}) at the moderate stress level compared to other stress levels. The lowest carotenoid amount in *Pyrus* rootstocks occurred in plants exposed to severe stress (OH x F 97; 1.64 mg g^{-1} - OH x F 333; 1.51 mg g^{-1} - Fox 11; 1.94 mg g^{-1}) and was significantly different from other treatments.

Table 1. The influence of NaCl treatments on the concentration of photosynthetic pigments in leaves

	Photosynthetic Pigment Concentration							
NaCl (mM)	**chl a (mg g^{-1})**		**chl b (mg g^{-1})**		**total chl (mg g^{-1})**		**car (mg g^{-1})**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
OH x F 97								
0	2.97	2.80bc	0.91	1.03b	3.89	3.83b	3.37	3.49abc
20	4.05	2.02de	1.31	0.81c	5.36	2.83cd	4.67	3.06bcd
40	3.73	2.63bc	1.18	1.00bc	4.91	3.63bc	4.25	4.17a
80	3.42	2.31cde	1.15	0.89bc	4.57	3.20bc	4.05	1.64f
OH x F 333								
0	3.01	2.54bcd	0.90	0.90bc	3.91	3.45bc	3.34	2.82cd
20	3.37	2.52bcd	1.04	0.89bc	4.41	3.41bc	3.83	3.07bcd
40	2.82	2.65bc	0.86	0.91bc	3.68	3.55bc	3.10	4.09a
80	3.04	2.72bc	0.97	0.93bc	4.01	3.65b	3.53	1.51f
Fox 11								
0	2.92	4.17a	0.98	1.38a	3.90	5.55a	3.49	3.58ab
20	3.44	3.93a	1.15	1.33a	4.59	5.26a	4.04	3.08bcd
40	2.92	2.87bc	0.98	1.01bc	3.90	3.88b	3.46	3.10bcd
80	3.18	2.93b	1.14	1.07b	4.32	3.99b	3.95	1.94ef
BA 29								
0	3.28	1.18fg	0.98	0.38d	4.26	1.56ef	3.58	2.97bcd
20	3.74	1.07g	1.12	0.32d	4.86	1.39f	4.17	3.34bc
40	3.65	1.35fg	1.08	0.42d	4.73	1.77ef	4.06	3.26bcd
80	2.91	1.75ef	0.85	0.53d	3.76	2.28de	3.30	2.56de

Table 1. The influence of NaCl treatments on the concentration of photosynthetic pigments in leaves (continued)

	Photosynthetic Pigment Concentration							
NaCl (mM)	**chl a (mg g^{-1})**		**chl b (mg g^{-1})**		**total chl (mg g^{-1})**		**car (mg g^{-1})**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
Means of treatments								
0	3.04b	2.67	0.94b	0.92	3.99b	3.60	3.45b	3.22b
20	3.65a	2.39	1.16a	0.84	4.80a	3.22	4.18a	3.14b
40	3.28ab	2.37	1.02ab	0.84	4.30ab	3.21	3.72b	3.66a
80	3.14b	2.43	1.03ab	0.85	4.17b	3.28	3.71b	1.91c
Means of rootstocks								
OH x F 97	3.54	2.44b	1.14a	0.93b	4.68	3.37b	4.08	3.09
OH x F 333	3.06	2.61b	0.94b	0.91b	4.00	3.52b	3.45	2.87
Fox 11	3.12	3.47a	1.06ab	1.20a	4.18	4.67a	3.73	2.92
BA 29	3.39	1.34c	1.01ab	0.41c	4.40	1.75c	3.78	3.03

Letters represent the interaction between rootstock and NaCl stress and the variation among treatment averages.

The proline levels in the leaves increased significantly or relatively in all rootstocks under NaCl stress (Figure 6A and Figure 6B). In both years, the highest proline levels were observed in the severe stress treatment of the Fox 11 (182.3-232.15 µmol g^{-1}), showing a significant difference compared to other treatments. OHxF 97 followed Fox 11 in terms of proline amount in both years, and different results were observed among treatments for this rootstock over the years.

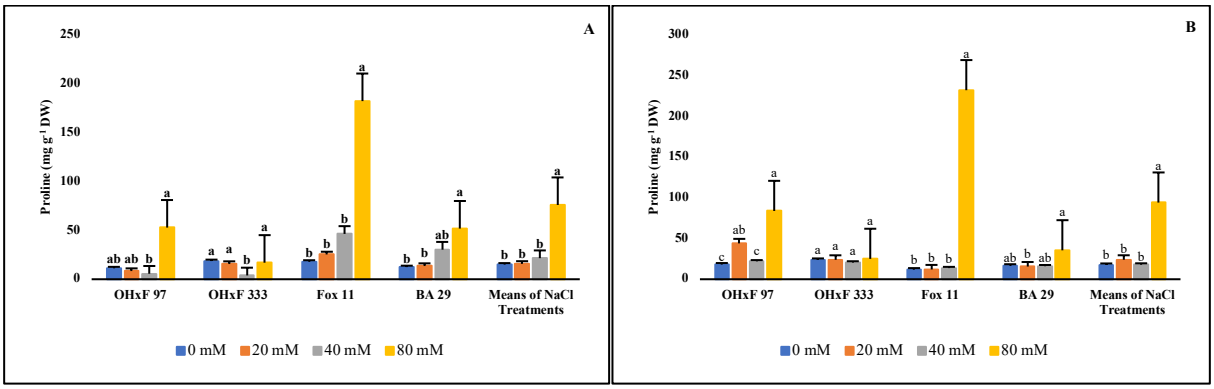

Figure 6. Proline content of rootstocks leaves under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=3). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. The significance level of $p<0.05$.

In the first year of the study, the highest MDA levels were observed in OHxF 333 under light stress (2.95 nmol g^{-1}) and in BA 29 under both moderate and severe stress (3.3-2.92 nmol g^{-1}, respectively) (Figure 7A). In the second year, BA 29 exhibited the highest MDA levels across all stress treatments (2.33-2.36-3.12 nmol g^{-1}) (Figure 7B). Particularly, the MDA content significantly increased in Fox 11 and BA 29 rootstocks under severe stress compared to the control plants in the second year. A similar trend was observed in the overall averages of NaCl treatments in the second year. While no significant difference was found between the control and light to moderate stress treatments, the MDA content substantially increased in plants subjected to severe stress.

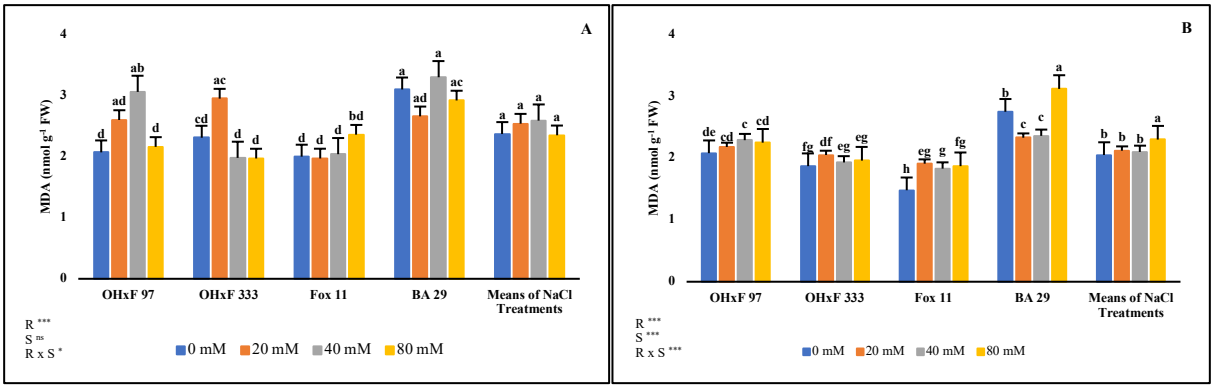

Figure 7. MDA content of rootstocks leaves under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=3). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. ***, * and ns denote the significance level of $p<0.001$ and $p<0.05$, respectively.

The N^+ content in the leaves ranged from 2.09% to 2.38% in the first year, respectively for Fox 11 under moderate stress and OHxF 97 under severe stress. In the second year, it varied between 2.24% and 2.72%, with OHxF 97 under moderate stress and OHxF 333 in the control having the highest and lowest values, respectively (Table 2). While N^+ content was unaffected by NaCl treatments in the first year, there was a significant decrease in the second year due to NaCl treatments. Additionally, Fox 11 exhibited lower N^+ content (2.16%) compared to other rootstocks. For P^{4+} content, it ranged from 0.12% to 0.16% in the first year, with Fox 11 under moderate stress, OHxF 97, and BA 29 in the control showing variations. Moreover, P^{4+} content significantly decreased in the leaves of OHxF 333 under NaCl stress. In the second year, P^{4+} content fluctuated between 0.23% and 0.33%, with OHxF 333 under severe stress, OHxF 97, and Fox 11 under light stress having the highest values (Table 2). Particularly, under severe stress, a significant decrease in P^{4+} content in the leaves was observed.

NaCl treatments on the K^+ content in the leaves revealed a significant reduction in Fox 11 rootstock under both light and severe stress compared to the control. Furthermore, while the leaf K^+ content was unaffected by NaCl stress, Fox 11 (1.50%) had a higher K^+ content than other rootstocks (Table 2). Ca^{2+} content in the leaves ranged from 1.93% to 2.45% in the first year, with Fox 11 in the control group and OHxF 97 under severe stress having the highest values, respectively. In the second year, Ca^{2+} content varied between 1.61% and 2.34%, with Fox 11 and BA 29 under moderate stress showing the highest values. The Ca^{2+} content in the leaves increased significantly or relatively due to NaCl stress. However, throughout both years of the study, Fox 11 consistently exhibited the lowest Ca^{2+} content (2.14% to 1.76%) among the rootstocks (Table 2). Mg^{2+} content in the leaves ranged from 0.27% to 0.53% in the first year, with Fox 11 in the control and BA 29 under severe stress showing variations. In the second year, Mg2+ content fluctuated between 0.12% and 0.35%, with OHxF 333 under severe stress and BA 29 in the control having the highest values. Particularly, Mg^{2+} content was significantly affected by severe stress treatments (0.43%). Additionally, BA 29 consistently had the highest Mg^{2+} content in both years of the study (0.47% to 0.29%) among the rootstocks (Table 2).

Table 2. The influence of NaCl treatments on the concentration of specific macro nutrients in leaves

	Macro nutrients									
NaCl (mM)	**(%) N^{2+}**		**(%) P^{4+}**		**(%) K^+**		**(%) Ca^{2+}**		**(%) Mg^{2+}**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
OH x F 97										
0	2.22^{ns}	2.67^{a}	0.16^{ns}	0.32^{ns}	1.31^{df}	1.42^{ns}	2.21^{ns}	1.80^{ns}	0.33^{ns}	0.16^{ns}
20	2.31	2.42^{b}	0.15	0.33	1.31^{de}	1.44	2.35	1.75	0.39	0.23
40	2.24	2.24^{b}	0.15	0.26	1.33^{de}	1.23	2.30	2.28	0.32	0.24
80	2.38	2.28^{b}	0.15	0.27	1.43^{bd}	1.35	2.45	1.98	0.47	0.17

Table 2. The influence of NaCl treatments on the concentration of specific macro nutrients in leaves (continued)

	Macro nutrients									
NaCl (mM)	**(%) N^{2+}**		**(%) P^{4+}**		**(%) K^{+}**		**(%) Ca^{2+}**		**(%) Mg^{2+}**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
OH x F 333										
0	2.35	2.72^{ns}	0.15^{a}	0.31	1.30^{df}	1.46	2.01	1.62	0.38	0.13
20	2.32	2.60	0.14^{b}	0.29	1.14^{fg}	1.54	2.29	1.88	0.34	0.15
40	2.23	2.38	0.13^{b}	0.31	1.10^{g}	1.47	2.38	1.80	0.31	0.19
80	2.18	2.35	0.13^{b}	0.23	1.25^{eg}	1.36	2.29	1.94	0.31	0.12
Fox 11										
0	2.11	2.54^{ns}	0.14^{ns}	0.26	1.62^{a}	1.40	1.93	1.98	0.27	0.25
20	2.19	2.39	0.14	0.33	1.43^{bd}	1.43	2.20	1.79	0.36	0.24
40	2.09	2.33	0.12	0.26	1.53^{ac}	1.45	2.09	1.61	0.32	0.22
80	2.23	2.42	0.13	0.24	1.43^{bd}	1.42	2.35	1.64	0.41	0.21
BA 29										
0	2.15	2.49^{ns}	0.16^{ns}	0.30	1.22^{eg}	1.34	2.33	2.08	0.49	0.35
20	2.36	2.27	0.15	0.29	1.21^{eg}	1.31	2.36	1.98	0.40	0.24
40	2.31	2.30	0.15	0.29	1.55^{ab}	1.36	2.27	2.34	0.46	0.31
80	2.34	2.33	0.13	0.28	1.37^{ce}	1.28	2.42	1.96	0.53	0.26
Means of treatments										
0	2.21^{ns}	2.60^{a}	0.15^{a}	0.30^{a}	1.36^{ns}	1.40^{ns}	2.12^{b}	1.87^{ns}	0.37^{b}	0.22^{ns}
20	2.30	2.42^{b}	0.14^{b}	0.31^{a}	1.27	1.43	2.30^{a}	1.85	0.37^{b}	0.21
40	2.22	2.31^{b}	0.14^{b}	0.28^{ab}	1.38	1.38	2.26^{a}	2.01	0.35^{b}	0.24
80	2.29	2.35^{b}	0.14^{b}	0.26^{b}	1.37	1.35	2.38^{a}	1.88	0.43^{a}	0.19
Means of rootstocks										
OH x F 97	2.29^{a}	2.41^{ns}	0.15^{a}	0.29^{ns}	1.34^{b}	1.36^{ns}	2.33^{a}	1.95^{ns}	0.38^{b}	0.20^{b}
OH x F 333	2.27^{a}	2.51	0.14^{b}	0.29	1.20^{c}	1.46	2.24^{ab}	1.81	0.33^{b}	0.15^{c}
Fox 11	2.16^{b}	2.42	0.13^{b}	0.27	1.50^{a}	1.42	2.14^{b}	1.76	0.34^{b}	0.23^{b}
BA 29	2.29^{a}	2.35	0.15^{a}	0.29	1.34^{b}	1.32	2.34^{a}	2.09	0.47^{a}	0.29^{a}

Letters represent the interaction between R × S and the variation between treatment averages.

4. Discussion

Salt stress, one of the most significant abiotic stress factors, adversely affects almost all physiological and biochemical processes of plants (Misra and Gupta, 2005), thus imposing limitations on plant growth and productivity (Kotagiri and Kolluru, 2017). As known, rootstocks are used by plants to gain tolerance against soil-related stress factors (Tavallali and Karimi, 2019). The role of rootstocks is crucial in determining the performance of trees under saline conditions (Grattan and Grieve, 1999), and it has been suggested that the accumulation of salt in leaves is primarily controlled by the rootstock genotype (Santa-Cruz et al., 2002). Proper rootstock selection may reduce the adverse effects of salinity or enhance salt tolerance (Colla et al., 2010; Huang et al., 2013; Karimi and Nasrolahpour-Moghadam, 2016).

In woody plants, shoot development is hindered by high concentrations of NaCl in the growing medium (Gong et al., 2013). In our study, we observed a decrease in plant height in the *Pyrus* rootstocks under NaCl stress, with the highest percentage decrease found in the Fox 11 exposed to 80 mM NaCl. The findings are consistent with previous salt stress studies in pears (Okubo and Sakuratani, 2000; Okubo et al., 2000; Musacchi et al., 2006). A noteworthy aspect was observed in the BA 29 rootstock. Despite intense necrosis in its leaves due to NaCl stress, significant changes in plant height were not on this rootstock. According to Greenway and Munns (1980), one of the reasons for the decrease in growth under salt stress is the restriction of cytokinin transport from roots to shoots. The main regions where

cytokinin's are synthesized in plants are root apical meristems (Taiz and Zeiger, 2008). The BA 29, compared to other *Pyrus* rootstocks in the study, has a more extensive lateral root system and, consequently, more meristematic regions. Based on current insights, it is hypothesized that the root anatomical structure of the BA 29 rootstock plays a role in promoting longitudinal growth under NaCl stress.

Vegetative growth parameters in plants are adversely affected under saline conditions, and the decrease in stem/shoot diameter represents one of the most crucial criteria. Indeed, studies conducted on different species to date have shown that shoot diameter is influenced by salt stress (Nassar et al., 2016; Mehdi-Tounsi et al., 2017). In our study, shoot diameter is generally affected by NaCl stress. On the other hand, the shoot diameter of the Fox 11, which we thought could be sensitive to NaCl stress, was not affected by the stress. Under stress, the apical bud stops growing longitudinally but continues to grow laterally. According to our observations, NaCl stress first appeared in the Fox 11 rootstock, and the apical bud formed earlier. The lack of changes in stem diameter in the Fox 11 is associated with this situation.

The initial rapid response by plants to salt stress is a reduction in leaf surface area (Wang and Nii, 2000), and this is considered a mechanism to minimize water loss (Ribeiro et al., 2006). Previous studies have consistently shown that leaf area is adversely affected by salinity (Munns and Tester, 2008; Singh et al., 2016). In our study, we also found that the leaf area was negatively affected by NaCl stress, and this interaction was particularly significant in the BA 29 in both years.

When the accumulation of harmful ions such as Na^+ and Cl^- reaches toxic levels in tissues, plants experience ionic stress (Munns and Passioura, 1984), leading to toxic symptoms such as chlorosis and necrosis in mature leaves (Tester and Davenport, 2003; Munns et al., 2006). The tolerance level of a plant to salt stress can be determined by the damage index. This assessment reflects the overall response of plants to salinity (Wang et al., 2015). In our study, the highest damage occurred in the severe stress treatments of the Fox 11 and BA 29 rootstocks. Additionally, in each stress treatment, the highest damage was observed in the Fox 11, while the lowest damage was observed in the OHxF rootstocks. One of the significant tolerance mechanisms developed by plants against salt stress is the prevention of the transport of Na^+ and Cl^- ions to leaves (to toxic levels) by retaining them in woody tissues. Based on this, it is suggested that such a mechanism may exist, especially in the OHxF rootstocks, particularly OHxF 97.

At the initial stage of salt stress, the reduced water uptake capacity of root systems and the resulting osmotic stress due to high salt accumulation in the soil and plant occur (Munns, 2005; Munns and Tester, 2008). The decrease in LWP is considered a useful criterion for determining the water stress caused by salinity (Katerji et al., 2003). In their study exposing different *Pyrus* species to NaCl stress, Okuba et al. (2000) observed a decrease in LWP values until the ninth week. After the ninth week, they determined that the control LWP values were lower, and they stated that this condition did not arise from mechanism differences induced by salt stress.

Leaf chlorophyll content is an indicator of the overall health of plants (Zhang and Kirkham, 1994). A decrease in chlorophyll pigments in stressed plants is a significant indicator of oxidative stress, oxidation, and pigment breakdown (Sarker and Oba, 2018). Additionally, a decrease in photosynthetic pigments under salt stress has been directly associated with salt tolerance (Ashraf and Sarwar, 2002). In our study, the content of chl a, chl b, and total chl generally remained unchanged in OHxF and BA 29, while it significantly decreased in Fox 11, especially under moderate and severe stress conditions.

Carotenoids play an important protective role against lipid peroxidation in plants under abiotic stress (dos Santos et al., 2019). In our study, a significant decrease in carotenoid levels was observed in *Pyrus* rootstocks exposed to severe NaCl stress. However, an interesting result emerged in OHxF rootstocks, where the carotenoid content increased under moderate NaCl stress. This situation in OHxF rootstocks is thought to be related to the protective role of carotenoids against photooxidation under salt stress (Barthod et al., 2007; Zrig et al., 2011).

The presence of salt in the growing medium generally results in the accumulation of compounds with low molecular weight (Hasegawa et al., 2000). Simultaneously, one of the most important of these compounds, also known as compatible solutes, is proline (Ghoulam et al., 2002; Girija et al., 2002). There are significant differences in the types of accumulated soluble compounds and their relative contributions to low osmotic potential across plant species and varieties (Larher et al., 2009). Moreover, it has been noted that, depending on plant species and varieties, the accumulation of proline does not

play a highly significant role in cellular osmotic adjustment (Ghars et al., 2008; Bendaly et al., 2016). Salt-sensitive genotypes accumulate more proline in various species (Kim et al., 2016). In our study, the highest amount of proline was observed in Fox 11. Tuteja (2007) reported that salt-sensitive plants attempt to maintain osmotic balance by synthesizing compatible solutes.

MDA is a product of lipid peroxidation and tends to accumulate in large quantities in plants exposed to salt stress (de Azevedo Neto et al., 2006). Additionally, it is used as a marker in determining plant sensitivity to oxidative damage (Xu et al., 2016). On the other hand, it has been suggested that salt-sensitive plants contain higher amounts of MDA compared to tolerant or resistant plants (Ahmed et al., 2013). In our study, it can be said that the amount of MDA is affected by NaCl stress, and the impact is more pronounced in rootstocks we characterized as sensitive to stress. For example, in Fox 11, an increase in MDA levels in response to NaCl stress was observed in both years. A similar situation was observed in BA 29 under severe stress conditions. In this regard, the results obtained in the study are consistent with the findings of Wu and Zou (2009) in *Pyrus betulafolia*.

Potassium (K^+), an essential element for plants, plays a significant role in plant development (Kaçar, 1984). Due to the similar physicochemical structure of Na^+ and K^+, under salt stress, the transportation of Na^+ in high concentrations causes K^+ deficiency, leading to a significant decrease in K^+ levels in most glycophyte species (Greenway and Munns, 1980; Maathuis and Amtmann, 1999). K^+, Ca^{2+} and Mg^{2+} alleviate the adverse effects of salt stress on plant growth (Ahmad and Prasad, 2012; Sarwat et al., 2013). In the study, NaCl stress did not have a negative effect on Ca^{2+} and Mg^{2+}, but N^+ levels in OHxF 97, P^{4+} levels in OHxF 333, and K^+ levels in Fox 11 rootstock significantly decreased under NaCl stress. Additionally, it was generally determined that Fox 11 had the least nutrient content.

5. Conclusions

When the data obtained in the study is evaluated as a whole, it can be stated that the Fox 11 rootstock is sensitive to NaCl stress. Even at around the threshold salinity value in the soil of 4 dS m^{-1}, damage occurred in the Fox 11 rootstock due to NaCl stress, suggesting that the use of this rootstock in areas under salt threat may be problematic. Although not as much as Fox 11, it was determined that the BA 29 rootstock also showed sensitivity to NaCl stress. According to the results obtained in our study, it would not be wrong to say that the rootstocks that are more tolerant to NaCl stress are the OHxF rootstocks. It can be said that the tolerance parameters to NaCl stress work more effectively, especially in the OHxF 97 rootstock. The salinity level in the soil, especially below the threshold salinity value, indicates that OHxF 97 rootstock can be used in pear cultivation. However, considering characteristics such as growth vigour and early fruit setting, OHxF 333 rootstock is also considered an alternative to the mentioned rootstock in pear production.

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Research Article

Innovative Approaches to Rice (*Oryza sativa*) Crop Health: A Comprehensive Analysis of Deep Transfer Learning for Early Disease Detection

Utpal BARMAN*[1], Dulumani DAS[2], Gunikhan SONOWAL[3], Mala DUTTA[4]

[1,2,3,4]Faculty of Computer Technology, Assam Down Town University, Panikhaiti, Assam, India, 781026

[1]https://orcid.org/0000-0002-2000-5007, [2]https://orcid.org/0000-0001-9211-2314, [3]https://orcid.org/0000-0001-5626-2411
[4]https://orcid.org/0000-0001-9560-0751

*Corresponding author e-mail: utpalbelsor@gmail.com

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Abstract: In this research, the primary objective is to tackle the pressing issue of identifying and effectively managing diseases in rice plants, a problem that can results in substantial crop losses and pose a severe threat to food security. The study employs Convolutional Neural Networks (CNNs), a type of deep learning model widely used for image analysis, to conduct an extensive investigation using a sizable dataset comprising 5,932 RGB images. These images represent four distinct disease classes in rice plants: Bacterial Leaf Blight (BLB), Blast, Brownspot, and Tungro. To conduct this research, the dataset is split into two subsets: a training set, which comprises 80% of the data, and a testing set, which makes up the remaining 20%. This division allows for a systematic evaluation of the performance of four different CNN architectures: VGGNet, ResNet, MobileNet, and a simpler CNN model. The results of this study consistently show that ResNet and MobileNet outperform the other CNN architectures in terms of their ability to accurately detect diseases in rice plants. These two models consistently achieve remarkable accuracy in identifying these diseases. The research findings not only emphasize the potential of deep learning techniques in addressing the critical issue of rice crop diseases but also highlights the significant role that ResNet and MobileNet play in strengthening crop protection efforts and contributing to global food security.

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1.Introduction

Rice (*Oryza sativa* L.) is the supreme crop of many countries around the globe. It is the largest cultivated and consumed food all over the globe. In comparison to other crops, rice has more nutrients and is affordable to every earning individual. About 70% of the population in Asia has a diet of rice. In 2020 rice production in Assam is 5.1 million tonnes (Sandhya Keelery, 2022). Production of rice at Assam grew at an average rate of 2.73% from 4.73 million tonnes in 2017 to 5.1 million tonnes in 2020 (Pathak et al., 2018). However over time, nematodes, some non-insect pests, and diseases that affect insects have all increased in abundance (Prakash et al., 2014). These biotic stresses caused numerous epidemics to occur throughout the nation. There are several types of rice plants and hence many uncovered diseases are present regarding different rice varieties. Over time, the frequency and extent of

the harm have varied (Gowda et al., 2021). Depending on the region, varieties planted, weather circumstances, and production practices, these pests and diseases show concurrent transitions both in their minor and major status (Soren et al., 2020). Sheath rot, early seedling blight, grain discoloration, false smut, bakanae, and narrow brown spots are only a few examples of minor plant ailments that have now developed into serious issues. Among those diseases the most common and destructive diseases are Bacterial Leaf Blight (BLB) (*Xanthomonas oryzae pv. Oryzae*), tungro (*Rice tungro bacilliform virus*), blast (*Magnaporthe grisea*), and brownspot (*Cochliobolus miyabeanus*) which lead to some catastrophic loss of quality and quantity of rice plants yielded in several acres of land. The early identification and accurate diagnosis of these diseases are crucial for implementing timely and effective management strategies, ensuring minimal crop losses, and optimizing yield. Traditionally, agri consultants, agri experts, or farmers themselves would visually inspect the plants to detect any infections since they had the knowledge to do so. The other conventional approach used laboratory testing, which involved measuring soil properties including pH, moisture, and nitrogen. Other frequently used laboratory techniques include microscopic analysis and serological tests. However, these methods have certain drawbacks, such as being time-consuming, requiring staff monitoring, and being ineffective for large farms. Diseases were only discovered when they caused significant crop loss (Patil and Kumar, 2021). Traditionally, experienced professionals visually inspect plant tissue to gauge the severity of plant diseases. The expensive and ineffective evaluation of plant diseases hinders the rapid advancement of modern agriculture (Mutka and Bart, 2015). Automated disease detection models were employed more frequently in precision agriculture, high-throughput crop phenotyping, intelligent greenhouses, and other industries as a result of the expanding uptake of digital cameras and advancements in computer vision (Barman and Choudhury, 2019 and 2022; Barman et al., 2023). Deep learning models were recommended by many studies for image-based identification of diseases of plant (Voulodimos et al., 2018). Deep learning has recently become a potent tool in the field of machine vision and visual analysis, with astounding success in a wide range of fields. Its ability to automatically learn hierarchical representations from large-scale datasets has proven instrumental in solving complex visual recognition tasks. Utilizing previously trained models on a source task to enhance performance on a target task with little labeled data is known as transfer learning, a subset of deep learning. This approach has shown great promise in a wide range of applications, including agriculture and plant pathology. Liang et al. (2019) proposed a rice blast disease recognition using a Deep CNN. They used a dataset of 5808 images and developed a model for rice blast disease classification. Their model produced an accuracy of 95%. Wang et al. (2021) proposed a rice disease detection and classification using an attention-based neural network and bayesian optimization. They used an attention-based depthwise separable neural network with Bayesian optimization to create the model. They used a dataset of 2370 images and their model produced an accuracy of 94.65%. Latif et al. (2022) proposed rice plant diseases using an improved CNN Model. They used an improved CNN model specified as VGG19. They used a dataset with 6 different classes and their model produced an accuracy of 96.08%. The purpose of this study is to assess the efficiency of deep neural networks for the prompt identification of rice diseases such as BLB, tungro, blast, and brown spots. By exploiting the knowledge learned from large-scale image datasets, pre-trained deep neural networks can extract meaningful features from rice disease images, enabling accurate classification and identification. The transfer learning paradigm allows us to leverage the knowledge gained from other related tasks, such as general object recognition or plant disease identification, and adapt it to the specific context of rice diseases.

The primary objectives of this study are twofold.

- First, to evaluate the performance of deep transfer learning models in detecting and classifying common rice diseases.
- Second, to compare their effectiveness against conventional machine learning algorithms. By conducting a comprehensive assessment, we aim to provide valuable insights into the potential of deep transfer learning for early disease identification of rice.

Furthermore, this research will contribute to the development of automated and cost-effective disease monitoring systems that can be deployed in real-world agricultural settings. Early disease detection can facilitate timely interventions, such as targeted pesticide application, disease-resistant crop selection, or site-specific disease management, thereby reducing the risk of yield losses and improving overall crop health. In summary, this paper seeks to demonstrate the potential of deep transfer learning in revolutionizing the early identification and management of rice diseases. By leveraging the power of

deep learning, we can pave the way for sustainable and resilient rice production systems, ensuring food security for the growing global population.

2. Material and Methods

The research methods of the current study are presented below.

i. To gather a rice dataset comprising 5932 RGB images and subsequently pre-processing [resizing of images] the dataset for model training and testing.
ii. To develop a CNN-based model capable of detecting common rice leaf diseases accurately, including BLB, blast, brown spot and tungro.
iii. Investigate the performance of different CNN architectures, including transfer learning models such as VGGNet, ResNet, MobileNet.
iv. Finally evaluate the model's accuracy and effectiveness in identifying and classifying the four target rice disease classes.

2.1. About the dataset

The dataset has been collected from a study reported by Sethy et al. (2020). A total of 5932 numbers of RGB images, comprising 4 diseases namely BLB, blast, brownspot, and tungro are considered for this experiment.In this recognition, the total number of images taken for BLB, blast, brownspot,and tungro are 1584, 1440, 1600, and 1308, respectively.

BLB: It is a bacterial disease and one of the most serious diseases of rice. The crop loss due to this disease can be as high as 75%. It thrives in warm, humid environments. Leaf blight can be identified in the initial stage when there are yellow stripes on the leaf, the stripes can be in the middle or parallelly on the leaves eventually leading to the drying of the leaves. A sample image of bacterial leaf blight is shown in the Figure 1.

Blast: Blast can be identified by noticing the rice leaf plants that have eye-shaped spots, initially having a yellow appearance and later leading to a dark brown color. The fungus that causes blast is "*Magnaporthe oryzae*".It can occur when there are blasts pores are present. It occurs in rice plants at all stages of growth. The environment in which disease occurs includes drastic temperature differences in day and night, basically cool temperatures during the daytime. A sample image of the blast is shown in Figure 1.

Brownspot: Brownspot is a fungal disease infecting the leaf sheath, panicle branches, leaves, and spikelets. It is caused by the fungus "*Cochliobolus miyabeanus*". When the leaf is wet for more than 8 – 24 hours only then the infection can occur. This infection mostly occurs during the ripening stages of the crop. The initial stage of the disease is a small circular brown-purple color spot in the leaf. In Figure 1, a sample image of brownspot is depicted.

Tungro: The main cause of this disease is leaf hoppers that transmit the virus from plant to plant. It is the combination of two viruses, one of them is an RNA virus named "*Rice Tungro Spherical Virus*" and the other one is a DNA virus named "*Rice Tungro Bacilliform Virus*". In Figure 1, a sample image of Tungro is depicted.

Figure 1. Sample image of a) BLB, b) blast, c) brownspots, and d) tungro.

2.2. About method

2.2.1. Deep Convolutional Neural Network (DCNN)

Better recognition of images, the process of segmentation, and image retrieval have all been made possible by DCNN's presentation of a functional group of models for better comprehending the information contained in an image. The well-known trained networks of DCNN utilize this dataset after being trained over thousands of thousands of images in the datasets of the CIFAR 100 and Image-Nets, improving the effectiveness of categorization. Our work's main addition is the presentation of detection of object techniques utilizing various trained neural network architectures, where, according to Sharma et al. (2018), modern models perform differently for test photos compared to trained images.

2.2.2. Transfer learning

Transfer learning is a successful technique to develop robust classification networks with little information by adjusting the parameters of a machine learning network that has already been pre-trained on a large dataset, like ImageNet. Even if it was not trained on the dataset of crop leaves, the model can still be triggered by the area of the crop spots, leaves, and backgrounds. There are numerous transfer learning architectures, including ResNet50 (He et al., 2016), Inception-v3 (Szegedy et al., 2016), and VGGNet (Simonyan and Zisserman, 2015) which were used in the area of Agri-informatics. The pre-trained models, such as ResNet and MobileNet, have learned to extract high-level features from images. These features can be highly relevant for detecting diseases in rice plants. Transfer learning allows us to use these well-learned features as a starting point and fine-tune them for the specific task of rice disease recognition.

2.2.3. DCNN-based rice leaf disease recognition model

DCNNs are similar to conventional Artificial Neural Networks (ANN), where neurons are optimized in learning. The proposed DCNN-based model for this experiment has nine layers. The layers are convolution1, pooling1, convolution2, pooling2, convolution3, pooling3, flatten, dense layer1, dense layer2 (Table 1). The first layer of the DCNN is convolution, which considers the input images to perform the convolution operation on the image pixel before sending the results to the pooling layer. In this model, three convolution layers and a few filters have been used. Each filter recognizes specific aspects of the image of the rice leaf disease and is trained spatially, considering its position in the volume it is applied. In Table 1, a description of convolution layers and filters has been given. To make it simple to learn complex relationships in the data, the nonlinear activation function ReLU has been used. For Convo1, Convo2, and Convo3, 16, 32 and 64 filters have been employed, respectively. As pooling lowers variance and computational complexity, there are fewer parameters to learn in this model. The feature map's dimensions are decreased along with the spatial dimensions by downsampling. It also describes the features that may be seen in a section of the convolution's feature map. The results of the very last max pooling layer get flattened into a vector with one dimension and placed into a fully linked dense layer. To identify rice leaf disease, a one-dimensional vector was finally generated by the final max polling layer and provided into the dense layer. Two dense layers with 64 and 4 hidden neurons each were added to the model.

This experiment reported a total of 3 710 308 parameters and among them all are trainable and 0 non-trainable parameters. To learn and tune the network parameters in the convolution, pooling,and dense layers to condense the features into a 1x64 vector, it is required to input the pictures to our model in batches. These characteristics are then transferred to another thick layer to create a vector of 1x5. The images are processed through a series of iterations called epochs and use the collection of validation images to verify the model and its associated parameters.

Table 1. Architecture summary of the DCNN for rice disease dataset classification

Layers	Function	Kernal size	Pool-size	Filter	Output	Parameters
Input					256 x 256	0
Convo1	Convolution	3 x 3		16	16x254x254	448
Pooling	Max Pooling		2,2	16	16x127x127	0
Convo2	Convolution	3x3		32	32x125x125	4640
Pooling 2	Max Pooling		2,2	32	32x62x62	0
Convo3	Convolution	3x3		64	64x60x60	18496
Pooling 3	Max Pooling		2,2	64	64x30x30	0
Flatten	Flatten				57600	0
Dense	Dense				1x64	3686464
Dense_1	Dense				1x4	260

2.2.4 VGG16 (Visual Geometry Group) based rice disease detectionmodel

VGG -16 is 16 layers DCNN which was trained with over 1 million images from the ImgeNet database. The input size of the image for the network is 224x224 (Barman et al., 2020). The VGG 16 contains 5 sets of convolution layers followed by the maxpool layer. In VGG16, there are a total of 14 815 044 parameters; among them, there are 100 356 and 14 714 688 trainable parameters and non-trainable parameters, respectively. In Table 2, a brief description of the different parameters of VGG16 is given.

Table 2. Summary of parameters for VGG16 model training on rice disease dataset

Image		Parameter		Training	
Train set	4746	**Total**	14815044	**Epoch**	10
Validation set	1186	**Trainable**	100356	**Loss**	Categorical cross-entropy
Size	(244,244)	**Non-trainable**	14714688	**Optimizer**	ADAM
				Learning rate	0.0001

2.2.5. Residual Network 50 (ResNet50) based rice disease detection model

He et al. (2016) introduced the Residual Neural Network (ResNet) deep neural network framework. By introducing a novel ”residual” or ”skip connection” concept, was created to overcome the difficulty of training deep neural networks. A special variation of the ResNet architecture called ResNet-50 has 50 layers. ResNet50 is a deeper network compared to earlier versions like ResNet-18 or ResNet-34. It contains 50 layers, including convolutional, pooling, and fully connected layers. ResNet-50 utilizes a specific type of residual block called the bottleneck block. The bottleneck block decreases the number of parameters and computations, which lowers the computational cost of training deeper networks. The training set consists of 4746 images (Table 3). These are the images used to train the ResNet model and adjust its parameters based on the provided labels. The test set contains 1186 images. These images, which are distinct from the training set, are used to assess how well the trained ResNet model performed. The test set aids in evaluating the model’s ability to generalize to new data. In this study, the model has 6 744 164 trainable parameters, which are adjusted based on the training data to improve model performance (Table 3). The model has 18 067 328 non-trainable parameters. The Adam optimizer, a common optimization technique that is well-known for it’s success in training deep neural networks, is utilized in this instance.

Table 3. Summary of parameters for ResNet model training on rice disease dataset

Image		Parameter		Training	
Train set	4746	**Total**	24811492	**Epoch**	10
Test set	1186	**Trainable**	6744164	**Loss**	Categorical Cross Entropy
Shape	(244,244)	**Non-trainable**	18067328	**Optimizer** **Learning rate**	ADAM 0.001

2.2.6. MobileNet based rice disease detection model

The CNNs are used in mobile imaging applications like MobileNet. They are constructed using these compact deep neural networks with depth-wise separable convolutions that can have minimal latency for embedded and mobile devices (Barman et al., 2020). Compact deep neural networks with depth-wise separable convolutions are used in their construction, allowing for minimal latency for embedded and mobile devices (Barman et al., 2020).

In Table 4, a brief description of the different parameters of MobileNet has been given. In this study, the model has 2 228 996 trainable parameters, which are adjusted based on the training data to improve model performance. The model has 34112 non-trainable parameters. The model is trained for 10 epochs like VGG 16 and ResNet 50.

Table 4. Summary of parameters for MobileNet model training on rice disease dataset

	Image		Parameter		Training
Train set	4746	**Total**	2263108	**Epoch**	10
Test Set	1186	**Trainable**	2228996	**Loss**	Categorical cross entropy
Shape	(244,244)	**Non-trainable**	34112	**Optimizer** **Learning Rate**	ADAM 0.0001

3. Results

Table 5 shows the performance metrics (precision, recall, and F1 score) of different methods (CNN, MobileNet, VGG16, and ResNet 50) for detecting and classifying different rice diseases (Bacterial Leaf Blight, Blast, Brownspot, and Tungro).

Table 5. Comparison of CNN and its transfer learning models for rice disease detection

Methods		Diseases			
		Bacterial Leaf Blight	**Blast**	**Brown Spots**	**Tungro**
CNN	Precision	0.96	0.96	0.99	0.99
	Recall	0.98	0.95	0.97	1.00
	F1 score	0.97	0.95	0.98	0.99
MobileNet	Precision	1.00	0.98	1.00	0.98
	Recall	0.99	1.00	0.99	1.00
	F1 score	1.00	0.99	1.00	0.99
VGG16	Precision	0.98	0.93	0.98	0.99
	Recall	0.94	0.97	0.97	1.00
	F1 score	0.96	0.95	0.98	0.99
ResNet 50	Precision	0.99	0.99	1.00	1.00
	Recall	1.00	1.00	0.99	0.99
	F1 score	1.00	0.99	1.00	1.00

Precision is a measure of the accuracy of the model's positive predictions. It calculates the ratio of true positive predictions to the sum of true positive and false positive predictions. A higher precision indicates that the model has a lower false positive rate. In Table 5, MobileNet achieved high precision scores for most diseases, except for blast where it achieved a precision score of 0.98. This means that MobileNet had a relatively low false positive rate and made fewer incorrect positive predictions for most

diseases. Sethy et al., (2020) reported the 0.98 precision in ResNet 50 in their study which is lesser than this study.

Recall, also known as sensitivity, measures the ability of the model to correctly identify positive instances. It calculates the ratio of true positive predictions to the sum of true positive and false negative predictions. A higher recall indicates that the model has a lower false negative rate. In Table 5, MobileNet achieved high recall scores for most diseases, except for brownspot where it achieved a recall score of 0.99. This means that MobileNet correctly identified most positive instances and had a relatively low false negative rate for most diseases.

The F1 score is the harmonic mean of precision and recall. It provides a balance between precision and recall, considering both false positives and false negatives. A higher F1 score indicates a better overall performance of the model. In Table 5, MobileNet achieved high F1 scores for most diseases, except for blast and tungro where it achieved F1 scores of 0.99. This indicates that MobileNet had a good balance between precision and recall for most diseases.

4. Discussion

Overall, based on Table 5, MobileNet appears to be a strong performer for detecting and classifying rice diseases, as it achieved high scores in precision, recall, and F1 scores for most diseases. However, it's worth noting that VGG16 and ResNet 50 also performed well in some metrics and diseases, with generally high scores across the board. The choice of the best method depends on the specific requirements and priorities of the application. Sethy et al. (2020) reported the ResNet 50 as the best model in their study with 98% accuracy and 0.98 F1 score whereas the current study produced 99% accuracy with MobileNet. To compare the results, Table 6 demonstrates the comparative analysis of the different results of the previous study.

Table 6. Comparative analysis of different studies for rice disease detection

Author	Model	No of Rice Disease Class	Accuracy
Sethy et al. (2020)	ResNet 50	04	98%
Deng et al.(2021)	Ensemble Model	06	91%
Upadhyay and Kumar, (2021)	CNN	03	99%
CurrentStudy	MobileNet	04	99%

Table 6, shows a comparative analysis of different studies for rice disease detection, Sethy et al., (2020) used the ResNet 50 model to detect rice diseases. They worked with a dataset consisting of four different rice disease classes. The accuracy achieved by their model was 98%. Deng et al. (2021) employed an Ensemble Model for rice disease detection. Their study involved working with a dataset that consisted of six different rice disease classes. The accuracy achieved by their model was 91%. Upadhyay and Kumar (2021) utilized a CNN for rice disease detection. They worked with a dataset comprising three different rice disease classes. Their model achieved an accuracy of 99%. In this study, we used the MobileNet model for rice disease detection with an accuracy of 99%.

These studies highlight different approaches and models used for rice disease detection, with varying numbers of rice disease classes. The accuracy results suggest that all the models achieved high accuracy in detecting rice diseases, ranging from 91% to 99%. However, MobileNet can be considered one of the best models for rice disease detection. Deep learning models, such as ResNet and MobileNet, excel at automatically extracting intricate features from images. This feature extraction capability allows these models to detect subtle and early visual cues associated with the onset of rice diseases, including changes in leaf color, texture, and structure that may precede visible symptoms and lead to early identification of rice diseases. Again by integrating deep learning models into monitoring systems or deploying them on drones or cameras in the field, it becomes possible to continuously analyze rice plant images in real-time. Early signs of disease development can be detected swiftly, even before the human eye can discern them, enabling timely intervention.

Conclusion

One of the key diagnostic windows for rice diseases lies in the leaves, where distinct diseases manifest with varying impacts. Recognizing the nuanced differences in how these diseases affect the leaves represents a critical aspect of effective disease management. To address this challenge, our study embarked on a comprehensive exploration of various deep-learning algorithms. The overarching objective was clear: to achieve early diagnosis and intervention. Amidst this diverse algorithmic landscape, MobileNet emerged as the standout performer. Its exceptional capabilities in identifying and classifying rice leaf diseases signify a significant stride toward bolstering rice crop health.

Ethical Statement

Ethical approval is not required for this study.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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Research Article

In Silico Determination of The Antifungal Effect of Plant Active Molecules Against *Botrytis Cinerea* by Molecular Docking

Vildan Enisoglu ATALAY*[1], Beyza YILMAZ[2], Mehmet Emin URAS[3]

[1]Informatics Institute, Computational Science and Engineering, Istanbul Technical University, TR-34469 Istanbul, Türkiye
[2]Uskudar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, 34662, Istanbul, Türkiye
[3]Haliç University, Faculty of Sciences and Arts, Department of Molecular Biology and Genetics, 34060, Istanbul, Türkiye

[1]https://orcid.org/0000-0002-5086-7265, [2]https://orcid.org/0009-0007-5768-5070, [3]https://orcid.org/0000-0002-0444-9994

*Corresponding author e-mail: enisogluatalayv@itu.edu.tr

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Abstract: *Botrytis cinerea*, which has developed many strategies to infect plants, can survive in harsh environmental conditions, and has a wide host range, has become an important problem both economically and ecologically by causing tons of crop losses for many years. The residues in soil and crops caused by chemical pesticides used to get rid of agricultural pests pose serious threats to human and environmental health, such as hormonal abnormalities and acute respiratory poisoning, especially in children. The most critical step to avoid these hazards will be to replace chemical pesticides with plant-active molecules. At the same time, these studies primarily in silico will provide a return in terms of both time and cost. Inhibition of pectin methyl esterase, an important virulence factor of *B. cinerea*, will ensure the organism is controlled. In order to determine candidate biofungicide effector molecules, QSAR parameter values of 409 plant active molecules were calculated. Firstly, conformer distribution and geometry optimizations were performed with Spartan 14' software. Docking studies of the optimized molecules were carried out through Autodock Vina software, while visualization studies to make sense of the interactions between the target receptor structure and effector molecules were used by BIOVIA Discovery Studio software. As a result of all the analyses, the molecules that are alternatives to chemical pesticides as biofungicides were determined to be the following molecules: Podolactone B, Repin, Sandaracopimaradienediol, 6-Hydrogenistein, Artemisinin, Lycoricidine, 6-Methoxygossypol, Viscidulin, Ciprofloxacin, and 7,4'-Dihydroxyflavan.

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1. Introduction

Fungi, also referred to as plant pathogens due to the infections they cause on various plant species, have developed numerous strategies to infect plants and these relationships result in a series of processes ranging from beneficial interactions to the death of the host organisms (Williamson et al.,

2007). Pathogenic fungi cause a variety of diseases in plants, such as powdery mildew, root rot or blight, and gray mold in many vegetable and fruit species (tomatoes, cucumbers, onions, grapes, strawberries, pears, bananas, kiwifruit, etc.), which reduce crop productivity or have the potential to completely destroy the related crops (Mathew et al., 2021).

Botrytis cinerea and other *Botrytis* species are important plant pathogens with a wide range of host plants in temperate regions, especially nursery crops, vegetables, ornamentals, and field and orchard crops also store and transport agricultural products. *B. cinerea*, which is among the top 10 pathogens worldwide and ranks 2nd in terms of economic damage, has been reported to cause different diseases such as gray mold, root blight, and root and storage rot in more than 200 plant species, including most vegetable and fruit crops, trees and flowers (Dean et al., 2012). They can attack many parts of the host organism including leaves, stems, and fruit parts of infected plants as necrotrophs, and often cause heavy losses after harvest (Youssef et al., 2019). They can also be found as saprophytes on immunocompromised or dead plant material. Table 1 arranges information about the common organisms of B. cinerea.

Table 1. Hosts of *B. cinerea* pest

Scientific Name	Common Name	References
Vitis vinifera	Grape	(Latorre et al., 2015)
Solanum lycopersicum	Tomato	(Boukaew et al., 2017)
Pyrus communis	Pear	(Kurbetli et al., 2016)
Fragaria vesca	Strawberry	(Petrasch et al., 2019)
Actinidia deliciosa	Kiwifruit	(Karakaya and Bayraktar, 2009)
Rubus idaeus	Raspberry	(Dean et al., 2012)
Allium cepa	Onion	(Chilvers, 2006)
Solanum tuberosum	Potato	(Sun et al., 2017)
Cucumis sativus	Cucumber	(Sadek et al., 2022)
Ipomoea batatas	Sweet Potato	(Stahr and Quesada-Ocampo, 2019)
Cucurbita moschata	Yellow Squash	(Hawthorne, 1988)
Capsicum annuum	Bell Paper	(Wang et al., 2022)
Daucus carota	Carrot	(Stahr and Quesada-Ocampo, 2019)

B. cinerea is a highly successful pathogen due to its flexible infection strategies, high reproductive efficiency, wide host range, and ability to survive for long periods as small mycelial structures called conidia and/or sclerotia. (Abbey et al., 2019) reported that *B. cinerea* initiates invasion of host plants through damaged tissues or natural openings, enabling the fungus to establish infection. Although the initial infected tissue usually results in minor damage, there is a rapid spread of the fungus as a result of intensive conidia production (Viret et al., 2004). Researchers have described the primary infection step of *B. cinerea* as the formation and air transportation of asexual conidia spores from mature conidiophores. After the initial infection, *B. cinerea* enters a short phase in which it exists as a biotroph within the plant (Williamson et al., 2007), *B. cinerea* has been reported to enter an aggressive necrotrophic phase in maturing host tissues, which is suggested to be triggered by biochemical changes such as an increase in volatile organic compounds, sugar, and nitrogen content (Prusky and Lichter, 2007).

The effects of virulence factors on hosts are characterized by fruit rot resulting in the softening of the fruit section and brown, leathery skin (Xiao, 2006). A further pathophysiological consequence of infection is that *B. cinerea* itself undergoes rapid mycelial growth on plant surfaces and forms massive masses of gray conidia, causing necrosis of tissues and even loss of host organism viability.

Cell wall-degrading enzymes play a critical role in *B. cinerea* as they allow penetration through plant cell walls and degradation of host tissue after infection. Pectin methylesterase 1 (PME1) is secreted early in the penetration phase to hydrolyze pectin, an important plant cell wall component. Pectin methylesterases therefore catalyze the demethylesterification of homogalacturonan, rendering pectin degradable by polygalacturonases and pectate lyases (Nakajima and Akutsu, 2014). In mutant studies, the pme1 deletion mutant was reported to exhibit a fourfold decrease in PME activity. As a result, PME activity was found to be reduced by 75% in the Bcpme1 mutant (Valette-Collet et al., 2003).

Unfortunately, resistant varieties to *Botrytis* species are not available, chemical control remains the primary means of reducing the incidence of gray mold in major crops. However, due to the development of multidrug resistance in field strains of the fungus, this strategy is only partially successful. Researchers have made great efforts to understand the mechanisms of pathogenicity of this pathogen due to its wide host range and the severe damage it causes in agriculture. Anti-Botrytis products, used as a preventive measure against *Botrytis*, which can infect crops before or after harvest, have reached a market size of €1 billion in recent years. At the same time, Botrytis species have been reported to cause 10-70% crop losses before and after harvest (Elad et al., 2019).

Despite the availability of technologies for the early detection of gray mold infection developed in recent years (Sunil et al., 2023; Chun et al., 2024), the physical and financial damage to agricultural crops caused by the *B. cinerea* pathogen remains significant today (Rojas and Gilbert, 2024) due to limitations in their application in the field (Bock et al., 2020).

Using biofungicides containing plant active molecules instead of chemical fungicides to prevent plant damage caused by fungi, which are plant pathogens, plays an important role in agriculture and food protection due to their non-toxic nature, rapid biodegradability, absence of chemical residues, and focus on managing the host structure rather than destroying it. Through their distinct metabolic pathways, plants can synthesize chemicals, and these compounds have shown the potential to be employed in the treatment and prevention of diseases that are caused by microorganisms (Demirel et al., 2022). The development of new biological fungicide molecules prevent the damages caused by chemical-containing pesticides used to avoid these damages and to protect crop productivity for both the host organism and the soil structure will lead to the elimination of ecological threats to the environment and human health. Spending $1 on the use of pesticides for plant protection purposes generates a profit of $3-5 (Pimentel et al., 1978; Pimentel et al., 1991). In studies investigating the pesticidal activity of plant-derived molecules to replace chemical pesticide molecules, molecular docking method, which allows detailed analysis of the infection mechanisms of pathogens, is frequently used and these results overlap with their experimental data (Ez-Zoubi et al., 2023; Diab et al., 2024; Vivekanandhan et al., 2024).

In this study, calculations were made through in silico methods, similar to our previous fungi study (Atalay and Asar, 2024). In order to understand the structure-activity relationship and to analyze the action mechanism of the fungistic molecules, the physicochemical parameters of the active ones with candidate antifungals were determined, and according to the obtained results, established a relationship between molecular structures and protein targets. Considering the fact that the protein structure used in the study was selected for plant pests and the scope of the analyses performed, it is predicted that determining the active molecules primarily in silico will provide great progress in terms of time and cost in both experimental and commercialization studies.

2. Material and Methods

Through the Dr. Duke database (Duke, 2020), 600 plant active molecules with antifungal properties were identified. In order to make a comparison with these selected plant active molecules, 9 commercial fungicides (CFs) (Azoxystrobin, Boscalid, Cyprodinil, Fenhexamid, Fluazinam, Imazalil, Penthiopyrad, Pyraclostrobin, Pyrimethanil) molecules against *B. cinerea* pest were included in the study (TOB, 2023). The SwissADME database (Daina et al., 2017) was used to predict the drug-likeness and ADME (absorption, distribution, metabolism, and excretion) properties of plant active molecules to evaluate their potential as candidate biofungicide effector molecules. After 600 molecules were identified in the first studies, it was adjusted to 409 molecules based on their compatibility with Lipinski parameters (Lipinski et al., 1997). Conformer distribution and geometry optimizations of all molecules with the Molecular Mechanics/MMFF and Semi-Empirical PM6 methods, respectively, using Spartan'14 software (Hehre, 2003). Molecular weight (M_W) (amu); area (A^2); volume (A^3); partition coefficient (logP); dipole moment (μ) (debye) and polarizability (α) values were calculated and recorded for structure-activity relationship modeling.

In this study, in order to establish more comprehensive relationships between physicochemical parameters and biological activity to make detailed analyses, in addition to the parameters calculated in

Spartan software, electronegativity (χ), electrophilicity (ω) chemical properties were calculated using equations (1) and (2).

$$\chi = (E_{HOMO}+E_{LUMO})/2 \quad (1)$$

$$\omega = \chi^2 / 2*\eta \quad (2)$$

Researchers obtained the three-dimensional crystallographic structure of the protein with Uniprot accession number Q9C2Y1, which consists of 346 amino acids, through the AlphaFold database (Jumper et al., 2021). In molecular docking studies, the binding site coordinates of the protein were chosen as x = 17.673, y = 5.457, z = -3.031, and the grid box size 40x40x40 $Å^3$ with 0.375 Å grid space. Molecular docking studies were performed with Autodock Tools 1.5.6 (Morris et al., 2009) and Autodock Vina (Eberhardt et al., 2021) software. Table 2 abbreviates the binding energy values obtained from docking studies as BE. After the molecular docking studies, visualization was performed using the BIOVIA Discovery Studio program (Biovia, 2021).

3. Results

In order to perform more comprehensive analyses to establish meaningful relationships between the molecule set and protein structure, linear regression analyses were performed by keeping the BE values constant. The calculated physicochemical parameter values of 164 correlated plant active molecules and CFs also their the inhibition constants represented by K_i of these molecules in the target protein structure are given in Table 2 and the R^2 values obtained as a result of regression studies are given in Table 3.

Table 2. Computed physicochemical parameters and BE values

Effector Molecules	A^2	A^3	M_W	χ	ω	α	μ	logP	BE	K_i
CF-1 (Azoxystrobin)	426.7	402.1	403.4	9.9	439.1	72.0	3.71	0.91	-6.1	$3.4x10^{-5}$
CF-2 (Boscalid)	336.9	321.1	343.2	9.7	397.6	65.6	2.79	1.12	-6.7	$1.2x10^{-5}$
CF-3 (Cyprodinil)	273.1	250.9	225.3	8.6	310.4	59.8	1.63	2.19	-6.3	$2.4x10^{-5}$
CF-4 (Fenhexamid)	296.4	280.5	302.2	9.0	338.7	62.2	3.25	1.09	-5.6	$7.8x10^{-5}$
CF-5 (Fluazinam)	346.4	321.6	465.1	11.5	590.8	65.7	1.24	-2.75	-6.6	$1.5x10^{-5}$
CF-6 (Imazalil)	312.6	285.8	297.2	9.7	420.5	62.6	4.37	1.38	-5.2	$1.5x10^{-4}$
CF-7 (Penthiopyrad)	367.2	339.5	359.4	8.9	322.3	67.1	9.29	3.15	-6.4	$2x10^{-5}$
CF-8 (Pyraclostrobin)	399.6	375.1	387.8	9.1	340.2	70.0	2.25	1.29	-7.6	$2.7x10^{-6}$
CF-9 (Pyrimethanil)	242.6	220.9	199.3	8.6	308.8	57.4	1.78	1.47	-6.5	$1.7x10^{-5}$
L-323 (Podolactone B)	350.2	354.1	394.4	11.4	652.8	67.9	2.07	-1.76	-11.8	$2.24x10^{-9}$
L-56 (Aminolevulinic Acid)	162.2	130.7	131.1	9.6	452.3	49.7	3.04	-1.21	-10.7	$1.43x10^{-8}$
L-340 (Repin)	360.1	351.8	362.4	10.7	582.5	67.6	6.67	-0.37	-10.6	$1.7x10^{-8}$
L-353 (Sandaracopimaradienediol)	333.1	342.5	304.5	8.7	380.7	67.7	3.49	4.09	-10.2	$3.33x10^{-8}$
L-12 (3,8'-Biapigenin)	486.4	489.0	538.5	9.8	400.7	79.2	7.89	-5.73	-9.9	$5.53x10^{-8}$
L-277 (Narciclasine)	283.5	268.4	307.4	9.8	411.3	61.2	6.06	-4.94	-9.9	$5.53x10^{-8}$
L-28 (6-Hydroxygenistein)	272.2	260.5	286.2	9.5	377.1	60.7	1.23	-3.11	-9.9	$5.53x10^{-8}$
L-71 (Artemisinin)	281.0	277.0	282.3	9.8	457.8	61.7	6.99	2.86	-9.8	$6.54x10^{-8}$
L-145 (Cumambrin B)	277.1	270.1	264.3	9.7	435.5	61.1	4.87	0.63	-9.7	$7.75x10^{-8}$
L-162 (Desacetoxymatricarin)	267.4	257.6	246.3	10.1	492.9	60.0	5.58	2.28	-9.7	$7.75x10^{-8}$
L-259 (Lycoricidine)	275.1	261.2	291.3	9.7	405.9	60.7	5.81	-3.88	-9.7	$7.75x10^{-8}$
L-40 (Achillin)	266.1	257.1	246.3	10.0	490.9	60.0	5.34	2.28	-9.7	$7.75x10^{-8}$
L-29 (6-Methoxygossypol)	517.2	527.3	532.6	9.2	323.6	82.5	9.81	-4.02	-9.5	$1.09x10^{-7}$
L-57 (Ampelopsin)	297.1	280.4	320.3	9.6	391.7	62.2	1.35	-4.97	-9.5	$1.09x10^{-7}$
L-400 (Viscidulin C)	279.1	269.2	264.3	9.7	483.2	60.7	6.90	0.48	-9.4	$1.29x10^{-7}$
L-128 (Ciprofloxacin)	333.2	320.6	331.3	9.0	336.9	65.5	9.34	-1.63	-9.3	$1.52x10^{-7}$
L-31 (7,4'-Dihydroxyflavan)	264.9	249.6	242.3	8.7	326.0	59.6	1.88	-0.60	-9.2	$1.8x10^{-7}$
L-369 (Solstitialin)	289.0	278.2	280.3	10.1	522.6	61.5	3.78	-0.11	-9.2	$1.8x10^{-7}$
L-227 (Ilicic Acid)	284.9	274.8	252.4	10.3	530.2	61.3	1.96	2.75	-8.9	$2.99x10^{-7}$
L-80 (Avenalumin III)	319.5	299.7	291.3	9.6	376.0	63.9	1.43	1.21	-8.9	$2.99x10^{-7}$

Table 2. Computed physicochemical parameters and BE values (continued)

Effector Molecules	A^2	A^3	M_W	χ	ω	α	μ	logP	BE	K_i
L-151 (Daidzin)	407.9	386.9	416.4	9.3	362.2	70.9	3.23	-2.94	-8.8	$3.54x10^{-7}$
L-182 (Epigallocatechin Gallate)	427.9	407.4	458.4	9.4	374.8	72.6	1.63	-6.72	-8.8	$3.54x10^{-7}$
L-23 (6,6'-Dimethoxygossypol)	536.2	546.7	546.6	9.1	313.2	84.1	8.42	-3.92	-8.8	$3.54x10^{-7}$
L-63 (Anhydrotuberosin)	329.1	319.6	320.3	8.4	274.5	65.6	3.39	-2.62	-8.8	$3.54x10^{-7}$
L-203 (Glyceocarpin)	354.6	341.0	340.4	9.0	365.1	66.9	4.29	-1.89	-8.7	$4.19x10^{-7}$
L-81 (Azetidine-2-Carboxylic-Acid)	125.8	101.8	101.1	9.6	456.0	47.3	2.06	-0.68	-8.7	$4.19x10^{-7}$
L-136 (Coniferin)	334.8	321.2	342.3	9.0	331.4	65.6	11.86	-1.63	-8.6	$4.96x10^{-7}$
L-255 (Liquiritin)	416.6	392.1	418.4	9.8	436.3	71.1	5.53	-3.06	-8.6	$4.96x10^{-7}$
L-89 (Bayogenin)	598.3	649.4	650.9	9.0	387.4	91.7	3.64	3.72	-8.6	$4.96x10^{-7}$
L-201 (Genistein)	265.3	253.4	270.2	8.5	342.2	60.0	1.66	-2.03	-8.5	$5.87x10^{-7}$
L-204 (Glyceofuran)	353.6	340.3	354.4	9.1	359.9	67.0	2.50	-3.96	-8.5	$5.87x10^{-7}$
L-13 (3-Hydroxyuridine)	252.2	225.1	260.2	10.5	525.5	57.5	3.10	-2.19	-8.4	$6.95x10^{-7}$
L-160 (Demethylvestitol)	269.1	256.2	258.3	8.6	314.3	60.2	3.90	-1.81	-8.4	$6.95x10^{-7}$
L-215 (Hildecarpin)	312.8	300.9	330.3	8.9	330.5	63.9	2.39	-4.98	-8.4	$6.95x10^{-7}$
L-267 (Medicarpin)	279.2	267.6	270.3	8.8	335.1	61.0	0.78	-2.27	-8.4	$6.95x10^{-7}$
L-278 (Naringenin)	272.5	258.2	272.3	9.8	434.6	60.3	2.51	-2.15	-8.4	$6.95x10^{-7}$
L-314 (Pinnatin)	397.4	289.8	292.3	9.4	367.1	63.0	2.74	-1.28	-8.4	$6.95x10^{-7}$
L-389 (Trifolirhizin)	424.2	405.8	446.4	8.8	324.5	72.4	2.00	-5.21	-8.4	$6.95x10^{-7}$
L-82 (Baicalein)	266.0	253.7	270.2	9.6	386.9	60.1	3.89	-2.38	-8.4	$6.95x10^{-7}$
L-24 (6,7-Dihydroxyflavone)	261.6	247.7	254.2	9.7	404.6	59.6	2.55	-1.29	-8.3	$8.23x10^{-7}$
L-67 (Apiocarpin)	340.7	331.8	338.4	9.1	369.3	66.2	2.48	-2.84	-8.3	$8.23x10^{-7}$
L-7 (2,3-Dehydrokievitone)	357.7	345.9	354.4	9.4	365.8	67.6	3.37	-1.83	-8.3	$8.23x10^{-7}$
L-121 (Catechin)	288.4	271.5	290.3	9.2	375.5	61.4	3.77	-3.72	-8.2	$9.74x10^{-7}$
L-205 (Glyceollin-I)	341.0	331.5	338.4	9.0	354.5	66.3	2.93	-2.63	-8.2	$9.74x10^{-7}$
L-234 (Isoliquiritin)	423.4	396.8	418.4	9.9	426.0	71.6	3.35	-2.33	-8.2	$9.74x10^{-7}$
L-266 (Medicagol)	273.7	263.9	296.2	9.4	357.8	61.0	2.93	-3.97	-8.2	$9.74x10^{-7}$
L-61 (Anhydroglycinol)	254.9	243.2	254.2	8.7	297.1	59.3	0.63	-3.17	-8.2	$9.74x10^{-7}$
L-117 (Carnosol)	336.6	339.6	330.4	9.2	371.4	66.9	6.51	1.77	-8.1	$1.15x10^{-6}$
L-156 (Dehydromaackiain)	371.0	261.5	282.3	8.7	300.8	60.8	1.53	-4.01	-8.1	$1.15x10^{-6}$
L-158 (Demethylmedicarpin)	259.2	247.5	256.3	9.0	362.5	59.4	1.36	-2.38	-8.1	$1.15x10^{-6}$
L-285 (Nordihydroguaiaretic Acid)	331.3	320.0	302.4	8.5	300.2	65.4	5.36	0.11	-8.1	$1.15x10^{-6}$
L-34 (9-O-Methylcoumestrol)	278.7	265.7	282.3	9.3	347.5	61.2	4.52	-3.03	-8.1	$1.15x10^{-6}$
L-398 (Vestitol)	290.0	276.3	272.3	8.5	305.3	61.8	5.04	-1.70	-8.1	$1.15x10^{-6}$
L-45 (Agaroxin-A)	508.8	508.9	519.6	8.9	341.9	80.7	5.04	-4.09	-8.1	$1.15x10^{-6}$
L-79 (Avenalumin II)	305.4	287.3	279.3	9.7	400.3	62.8	2.72	0.80	-8.1	$1.15x10^{-6}$
L-147 (Curcumin)	409.2	378.3	368.4	9.3	356.5	70.2	1.05	-0.46	-8.0	$1.37x10^{-6}$
L-148 (Cyclokievitone)	350.6	341.1	354.4	8.9	342.5	67.1	4.50	-2.64	-8.0	$1.37x10^{-6}$
L-164 (Dianthalexin)	249.1	234.5	239.2	10.3	470.2	58.4	3.48	0.18	-8.0	$1.37x10^{-6}$
L-192 (Flavanone)	249.4	237.6	224.3	9.6	415.3	58.6	2.75	1.10	-8.0	$1.37x10^{-6}$
L-311 (Piceatannol)	267.3	245.0	244.2	9.1	343.8	59.4	2.01	-1.71	-8.0	$1.37x10^{-6}$
L-312 (Piceid)	397.1	376.4	390.4	9.0	342.4	60.0	1.00	-2.62	-8.0	$1.37x10^{-6}$
L-315 (Pinocembrin)	263.3	250.9	256.3	9.8	439.9	59.6	2.51	-1.06	-8.0	$1.37x10^{-6}$
L-36 (Acanthocarpan)	298.2	291.5	328.3	9.0	340.1	63.1	1.36	-4.84	-8.0	$1.37x10^{-6}$
L-379 (Tetrahydroxystilbene)	265.6	244.4	244.2	9.2	371.2	59.2	0.96	-1.71	-8.0	$1.37x10^{-6}$
L-77 (Astringin)	402.1	383.0	406.4	9.0	334.8	70.6	2.44	-3.70	-8.0	$1.37x10^{-6}$
L-306 (Phebalosin)	278.6	263.9	258.3	9.9	429.8	60.9	3.88	-0.41	-7.9	$1.62x10^{-6}$
L-338 (Quercetin)	281.2	267.9	302.2	9.5	365.6	61.3	0.89	-4.54	-7.9	$1.62x10^{-6}$
L-106 (Cajanol)	318.8	304.9	316.3	9.2	377.4	64.1	1.51	-2.97	-7.8	$1.91x10^{-6}$
L-25 (6-Alpha-Hydroxymaackiain)	283.1	273.4	300.3	9.2	356.2	61.6	2.54	-4.01	-7.8	$1.91x10^{-6}$
L-305 (Pheanthine)	618.7	644.4	622.8	8.1	264.6	91.8	6.03	-3.91	-7.8	$1.91x10^{-6}$
L-351 (Sakuranetin)	293.6	278.4	286.3	9.6	419.5	61.9	2.46	-2.04	-7.8	$1.91x10^{-6}$

Table 2. Computed physicochemical parameters and BE values (continued)

Effector Molecules	A_2	A^3	M_W	χ	ω	α	μ	logP	BE	K_i
L-385 (Tiliacorinine)	546.2	582.9	576.7	8.5	294.1	86.8	3.56	-4.02	-7.8	$1.91x10^{-6}$
L-46 (Aglafoline)	496.4	488.7	492.5	9.0	362.1	78.9	3.12	-2.70	-7.8	$1.91x10^{-6}$
L-62 (Anhydropisatin)	292.1	281.7	296.3	8.6	287.7	62.5	1.80	-3.90	-7.8	$1.91x10^{-6}$
L-105 (Cajanin)	290.7	279.6	300.3	9.4	366.5	62.2	3.79	-3.01	-7.7	$2.27x10^{-6}$
L-134 (Clandestacarpin)	340.2	328.6	336.3	9.1	356.1	66.1	1.98	-3.11	-7.7	$2.27x10^{-6}$
L-207 (Glyceollin-III)	343.5	332.7	338.4	8.9	348.7	66.3	3.55	-2.55	-7.7	$2.27x10^{-6}$
L-9 (2'-Hydroxydaidzein)	264.7	253.3	270.2	9.3	359.4	60.1	3.88	-2.03	-7.7	$2.27x10^{-6}$
L-110 (Canescacarpin)	340.8	330.3	336.3	9.4	386.4	66.2	5.11	-2.26	-7.6	$2.68x10^{-6}$
L-221 (Honokiol)	319.3	298.8	266.3	8.9	342.9	63.6	1.83	1.43	-7.6	$2.68x10^{-6}$
L-223 (Hordatine B)	622.4	593.1	580.7	9.0	340.9	87.6	5.57	-3.64	-7.6	$2.68x10^{-6}$
L-303 (Phaseolin)	333.4	324.0	322.4	8.8	333.0	65.7	1.46	-1.83	-7.6	$2.68x10^{-6}$
L-316 (Pinostrobin)	284.3	271.0	270.3	9.6	419.5	61.3	2.15	-0.96	-7.6	$2.68x10^{-6}$
L-342 (Resveratrol)	259.1	237.8	228.2	9.1	356.6	58.7	1.77	-0.62	-7.6	$2.68x10^{-6}$
L-382 (Theaflavin)	487.7	502.6	564.5	9.5	363.3	80.5	3.12	-7.56	-7.6	$2.68x10^{-6}$
L-386 (Trichocarpin)	402.2	385.1	406.4	10.0	446.6	70.7	2.68	-2.17	-7.6	$2.68x10^{-6}$
L-399 (Vestitone)	289.2	277.8	286.3	9.2	354.6	62.0	2.89	1.93	-7.6	$2.68x10^{-6}$
L-10 (2'-Hydroxygenistein)	269.7	259.4	286.2	9.5	378.3	60.6	2.52	-3.11	-7.5	$3.18x10^{-6}$
L-177 (Dolichin-B)	354.9	341.0	340.4	8.8	335.2	67.0	1.88	-2.14	-7.5	$3.18x10^{-6}$
L-254 (Liquiritigenin)	267.7	252.2	256.3	9.7	424.6	59.8	4.20	-1.06	-7.5	$3.18x10^{-6}$
L-318 (Pisatin)	304.2	293.6	314.3	9.1	346.9	63.3	1.83	-3.90	-7.5	$3.18x10^{-6}$
L-33 (8-Methoxypsoralen)	220.0	206.7	216.2	9.3	349.7	56.4	6.13	-1.65	-7.5	$3.18x10^{-6}$
L-65 (Anonaine)	269.4	268.4	265.3	8.9	327.0	61.2	1.09	-1.24	-7.5	$3.18x10^{-6}$
L-95 (Betagarin)	330.6	317.3	328.3	8.8	326.0	65.2	2.21	-2.77	-7.5	$3.18x10^{-6}$
L-170 (Dihydroresveratrol)	261.5	241.8	230.3	8.9	351.9	58.9	0.31	-0.30	-7.4	$3.76x10^{-6}$
L-228 (Integerrine)	600.4	619.9	593.7	8.5	302.9	89.7	4.47	-0.67	-7.4	$3.76x10^{-6}$
L-252 (Limacine)	608.0	626.0	608.7	7.9	244.3	90.3	8.36	-4.02	-7.4	$3.76x10^{-6}$
L-317 (Pinosylvin)	249.9	230.5	212.2	9.4	387.4	58.1	1.62	0.46	-7.4	$3.76x10^{-6}$
L-332 (Psoralidin)	344.8	331.7	336.3	9.3	350.4	66.5	5.61	-1.85	-7.4	$3.76x10^{-6}$
L-367 (Solasodine)	449.3	456.9	413.6	8.3	327.7	76.0	1.40	4.95	-7.4	$3.76x10^{-6}$
L-403 (Withaferin A)	462.0	480.2	470.6	10.2	499.6	78.1	7.91	3.46	-7.4	$3.76x10^{-6}$
L-44 (Afrormosin)	309.8	294.8	298.3	9.0	329.3	63.5	1.82	-1.82	-7.4	$3.76x10^{-6}$
L-6 (1-Tuliposide-B)	275.4	262.9	294.3	10.6	550.6	60.5	2.76	-2.71	-7.4	$3.76x10^{-6}$
L-208 (Glyceollin-IV)	376.5	361.2	354.4	8.7	329.4	68.6	2.31	-1.78	-7.3	$4.45x10^{-6}$
L-226 (Hydroxyphaseolin)	340.1	331.2	338.4	9.0	350.6	66.2	1.70	-2.63	-7.3	$4.45x10^{-6}$
L-104 (Caffeic Acid)	199.6	174.7	180.2	9.6	396.1	53.7	3.87	-0.86	-7.2	$5.27x10^{-6}$
L-176 (Dolichin-A)	355.0	341.1	340.4	8.8	336.3	67.0	0.70	-2.14	-7.2	$5.27x10^{-6}$
L-206 (Glyceollin-II)	340.0	331.1	338.4	8.9	332.7	66.3	2.96	-2.63	-7.2	$5.27x10^{-6}$
L-142 (Cristacarpin)	375.6	361.5	354.4	8.9	354.9	68.6	3.74	-1.78	-7.1	$6.24x10^{-6}$
L-292 (Oxypeucedanin)	297.4	281.3	286.3	9.7	404.6	62.3	7.44	-1.53	-7.1	$6.24x10^{-6}$
L-251 (Licoisoflavone A)	355.6	345.4	354.4	9.4	363.0	67.6	0.86	-1.83	-7.0	$7.39x10^{-6}$
L-66 (Antofine)	383.5	382.1	363.5	8.2	260.3	70.6	2.22	-1.69	-7.0	$7.39x10^{-6}$
L-86 (Batatasin-II)	305.0	288.4	274.3	8.3	288.8	62.8	2.91	-1.17	-7.0	$7.39x10^{-6}$
L-180 (Ellipticine)	266.5	264.2	246.3	8.5	279.0	61.1	3.43	-0.57	-6.9	$8.74x10^{-6}$
L-244 (Juglone)	178.3	166.9	174.2	10.8	505.0	53.1	0.66	0.57	-6.9	$8.74x10^{-6}$
L-281 (Nimbidin)	404.6	438.6	442.6	9.0	383.8	74.7	4.18	1.59	-6.9	$8.74x10^{-6}$
L-102 (Broussonin A)	293.2	279.1	258.3	8.6	323.2	62.0	0.86	0.22	-6.8	$1.04x10^{-5}$
L-84 (Batatasin V)	326.0	308.2	288.3	8.5	309.4	64.3	3.12	-1.07	-6.8	$1.04x10^{-5}$
L-249 (Lathodoratin)	215.8	199.0	206.2	9.9	450.1	55.5	1.92	-0.92	-6.7	$1.23x10^{-5}$
L-325 (Pogostone)	257.9	233.4	224.3	10.8	565.9	58.1	2.69	0.92	-6.7	$1.23x10^{-5}$
L-72 (Arvensan)	310.7	296.3	286.3	8.3	288.0	63.5	2.73	-1.59	-6.7	$1.23x10^{-5}$
L-194 (Frangufoline)	580.6	579.3	534.7	9.0	368.3	86.2	6.89	1.37	-6.6	$1.45x10^{-5}$

Table 2. Computed physicochemical parameters and BE values (continued)

Effector Molecules	A_2	A^3	Mw	χ	ω	α	μ	logP	BE	K_i
L-392 (Umbelliferone)	172.2	156.5	162.1	10.0	441.6	52.1	3.97	-0.58	-6.6	$1.45x10^{-5}$
L-124 (Chrysarobin)	246.1	239.4	240.3	9.8	422.4	58.8	5.21	-0.16	-6.5	$1.72x10^{-5}$
L-173 (Dihydrowyerone)	313.2	282.0	260.3	10.1	462.5	62.3	3.92	1.98	-6.5	$1.72x10^{-5}$
L-179 (Elemicin)	252.5	231.7	208.3	8.6	328.1	58.1	2.62	-0.64	-6.4	$2.03x10^{-5}$
L-276 (Myristicin)	224.3	203.2	192.2	8.6	322.1	55.8	1.23	-0.61	-6.4	$2.03x10^{-5}$
L-291 (Otobain)	334.9	330.2	324.4	8.5	312.9	66.1	1.39	-0.23	-6.4	$2.03x10^{-5}$
L-409 (Xyloidone)	256.0	244.4	240.3	10.0	420.3	59.4	0.92	1.12	-6.4	$2.03x10^{-5}$
L-52 (Allylpyrocatechol)	183.2	164.2	150.2	8.8	338.0	52.6	2.74	0.12	-6.4	$2.03x10^{-5}$
L-100 (Boa)	261.5	233.7	207.3	8.5	299.9	58.3	5.27	0.03	-6.3	$2.41x10^{-5}$
L-232 (Isoelemicin)	251.9	231.4	208.3	8.7	324.7	58.1	1.82	-0.69	-6.3	$2.41x10^{-5}$
L-83 (Batatasin III)	277.9	260.9	244.3	8.9	355.2	60.4	0.78	-0.20	-6.3	$2.41x10^{-5}$
L-195 (Frangulanine)	556.3	548.8	500.7	9.0	364.5	83.7	6.57	1.80	-6.2	$2.85x10^{-5}$
L-383 (Thujaplicin)	197.4	180.8	164.2	9.5	384.8	54.1	4.06	1.67	-6.1	$3.37x10^{-5}$
L-41 (Acoric Acid)	299.4	288.4	268.4	9.7	469.7	62.4	4.17	3.36	-6.1	$3.37x10^{-5}$
L-114 (Caprylic Acid)	206.0	172.4	144.2	10.9	688.2	52.6	2.13	2.43	-6.0	$4x10^{-5}$
L-289 (O-Methoxycinnamaldehyde)	200.6	180.1	162.2	9.3	391.9	53.9	4.88	0.07	-6.0	$4x10^{-5}$
L-212 (Helenalin)	270.0	264.9	262.3	10.6	558.8	60.6	4.92	2.00	-5.9	$4.73x10^{-5}$
L-384 (Thymol)	200.6	179.3	150.2	8.6	326.6	53.8	1.51	1.56	-5.9	$4.73x10^{-5}$
L-42 (Actinidine)	189.8	173.1	147.2	9.4	416.6	53.2	3.55	1.51	-5.9	$4.73x10^{-5}$
L-243 (Jodrellin A)	430.0	438.3	448.5	9.3	420.3	74.6	5.93	1.60	-5.8	$5.6x10^{-5}$
L-270 (Menthone)	206.7	187.4	154.3	9.3	438.8	54.1	3.49	3.07	-5.8	$5.6x10^{-5}$
L-70 (Arteannuin-B)	268.0	260.8	248.3	10.0	501.7	60.2	7.13	1.90	-5.8	$5.6x10^{-5}$
L-115 (Capsidiol)	276.4	268.0	236.4	9.4	446.3	60.6	1.79	2.37	-5.7	$6.63x10^{-5}$
L-224 (Humulone)	414.7	392.5	362.5	9.7	411.7	71.3	2.26	2.45	-5.7	$6.63x10^{-5}$
L-349 (Rugosal A)	272.1	273.3	266.3	9.7	415.6	61.5	4.43	1.41	-5.7	$6.63x10^{-5}$
L-377 (Terpinen-4-ol)	205.9	187.8	154.3	8.6	370.3	54.1	1.85	2.23	-5.7	$6.63x10^{-5}$
L-378 (Terpinolene)	194.2	175.7	136.2	7.9	296.6	53.2	0.49	2.81	-5.7	$6.63x10^{-5}$
L-131 (Citral)	219.2	191.8	152.2	9.4	414.6	54.7	5.21	2.35	-5.6	$7.85x10^{-5}$
L-132 (Citronellal)	223.6	196.1	154.3	9.1	396.1	54.9	3.25	2.36	-5.6	$7.85x10^{-5}$
L-401 (Warburganal)	266.4	267.6	250.3	10.0	467.2	60.9	4.49	1.71	-5.6	$7.85x10^{-5}$
L-126 (Cinnamaldehyde)	172.9	251.9	132.2	10.1	467.6	51.7	4.07	1.04	-5.5	$9.29x10^{-5}$
L-90 (Benzoic-Acid)	146.5	127.5	122.1	10.6	548.7	49.5	2.64	0.79	-5.5	$9.29x10^{-5}$
L-297 (Patchouli Alcohol)	247.8	254.2	222.4	8.3	383.7	59.0	1.76	3.85	-5.4	$1.1x10^{-4}$
L-394 (Undecylenic Acid)	261.7	223.6	184.3	9.8	502.5	57.0	1.75	3.42	-5.4	$1.1x10^{-4}$
L-93 (Benzyl-Isothiocyanate)	179.8	156.8	149.2	9.2	366.3	52.1	4.54	1.47	-5.4	$1.1x10^{-4}$
L-96 (Beta-Ionone)	252.0	232.6	192.3	9.3	400.7	58.0	3.82	3.43	-5.4	$1.1x10^{-4}$
L-113 (Capric Acid)	246.5	209.3	172.3	10.8	669.8	55.6	2.13	3.27	-5.3	$13x10^{-5}$
L-107 (Camphor)	188.8	176.4	152.2	9.1	418.5	53.2	3.44	2.92	-5.2	$1.54x10^{-4}$
L-37 (Acetophenone)	155.9	138.2	120.2	10.3	512.1	50.4	3.45	1.30	-5.2	$1.54x10^{-4}$
L-4 (1,8-Cineole)	195.3	182.1	154.3	8.0	345.8	53.3	1.60	1.86	-5.2	$1.54x10^{-4}$
L-133 (Citronellol)	230.4	201.1	156.3	8.3	339.4	55.2	1.66	2.82	-5.1	$1.83x10^{-4}$
L-368 (Solavetivone)	267.2	257.0	218.3	9.7	463.4	59.9	4.74	3.88	-5.1	$1.83x10^{-4}$

The R^2 values obtained as a result of linear regression analysis to identify candidate biofungicide active molecules are given in Table 3.

Table 3. Lineer regression results

Parameters	logP	ω	A^2	α	χ	Mw
R^2 values	0.8034	0.7677	0.7435	0.7359	0.7317	0.7188

4. Discussion

Several investigations using molecular docking programs have shown that using computational screening to rank the affinities of ligands binding to receptor proteins might lead to a greater enrichment of active molecules compared to random screening against plant pathogens such as viruses, fungi, and bacteria (Stahl and Rarey, 2001; Usta et al., 2023; Wyss et al., 2003).

Because of the large number of the studied molecules, the calculated parameter values followed a wide range of numbers within themselves. This allowed a detailed analysis of how and at what rate the binding energies change depending on the relevant parameters. For example; while the logP values of the CF used against *B. cinerea* were found to be in the range of $-2.75 \leq logP \leq 3.81$, the logP value ranges of the 409 plant active molecules studied were found to be $-7.56 \leq logP \leq 9.11$. In the linear regression analysis results, the highest correlation was observed in the logP parameter with the R^2 value of 0.8034 (Table 3). The logP value ranges of 172 candidate bioactive molecules that provide this correlation were determined as $-5.73 \leq logP \leq 4.95$. Plant active molecules with higher affinity than CFs had value ranges in the range of $-1.76 \leq logP \leq 2.86$ when evaluated together with BE values. As a result of these findings, it can be said that the determined target protein active site structure is lipophobic therefore hydrophilic. The recommended parameter value at the ranges in the studied PME inhibition mechanism was determined as $380 \leq \omega \leq 680$, $280 \leq M_W \leq 395$, $57 \leq \alpha \leq 68$, $250 \leq A^2 \leq 450$, $8.40 \leq \chi \leq 11.40$.

The interactions of the effector molecules with amino acids were analyzed by analyzing the active site of the selected receptor structure in the study for the inhibition of PME secreted by *B. cinerea* to break down the plant cell wall. For this, firstly, the interaction types and interaction distances of the effector molecules with the macromolecule in the interaction maps were collected as data (Supporting information). The conventional H bond interaction, which is considered the most critical interaction in intermolecular interactions, was taken as a reference in determining the critical amino acids in the active site. Figure 1 represents the interaction maps of proposed candidate molecules with active site amino acids. As a result of the information obtained, the amino acids expected to cause significant changes in protein structure and function in case of possible mutations in these amino acids in the receptor structure were determined as Gly301, Asn302, Thr303, Gly304, Ser307, Asn308, Ser309. The interaction distances of the critical amino acids in the active site with the effector molecules via conventional H bonds were observed to vary between 1.76-3.35 Å. Considering that the bond distance between intramolecular C-C atoms is 1.54 Å, it is possible to evaluate the observed conventional H bond interaction distances between the molecules as close interactions and this situation is closely related to the active site selectivity of the selected effector molecules. The contribution of this type of interaction to the BE values is quite high because the BE values increase as the interaction distances of the effector molecules with conventional H bond interactions shorten. In addition, when the chemical properties of the critical amino acids were analyzed, it was inferred that the receptor active site structure is mostly polar. This inference is consistent with the findings observed in the regression results of the logP parameter. Other essential results are the interaction maps, that the active site amino acids have remarkable interaction distances (2.70-3.37Å) with the candidate biofungicides, especially 6-Hydrogenistein, Artemisinin, and Lycoricidine. The named amino acids overlap above mentioned amino acids list as highlighted.

When the interaction tables are examined, after the conventional H bond interaction, π-alkyl, π-cation and π-π interactions were the most common interactions with apolar amino acids containing aliphatic R groups. Among these interactions, π-alkyl was observed with the amino acid Pro6 in the bond distance range of 3.45-5.21 Å, π-cation with the amino acid Leu4 in the bond distance range of 3.23-4.24 Å, and π-π interactions with the amino acid Ile5 in the bond distance range of 3.74-4.51 Å.

In order to verify the selectivity of the candidate biofungicide molecules in the active site of the target protein, they were compared with the amino acids interacting with the CFs included in the study as a reference. CFs interacted with Ile5, Pro6, Asn302, Ser307, and Ser309 amino acids in the active site. All the identified amino acids were also detected in candidate bio-fungicide molecules.

The BE values of CFs vary between -5.2 and -7.6 $kcal.mol^{-1}$, while the BE values of studied biofungicide active molecules vary between -5.1 and -11.8 $kcal.mol^{-1}$. Table 4 provides the coefficients of increase in binding affinity of alternative candidate bio fungicide active molecules against CF active molecules in the PME inhibition mechanism and the proposed molecules.

Table 4. Affinity differences between CFs and Candidate biofungicides

Candidate Biofungicide	Candidate Biofungicide BE (kcal.mol^{-1})	CFs	CFs BE (kcal.mol^{-1})	Affinity Difference	Number of Candidate Biofungicide≥CFs
L-323	-11.8	**CF-1**	-6.1	1.000-1.000.000	131
L-340	-10.6	**CF-2**	-6.7	300-126.000	118
L-353	-10.2	**CF-3**	-6.3	800-320.000	130
L-28	-9.9	**CF-4**	-5.6	4000-1.600.000	149
L-71	-9.8	**CF-5**	-6.6	400-160.000	121
L-259	-9.7	**CF-6**	-5.2	10.000-4.000.000	159
L-29	-9.5	**CF-7**	-6.4	600- 250.000	125
L-400	-9.4	**CF-8**	-7.6	40-16.000	78
L-128	-9.3	**CF-9**	-6.5	500-200.000	123
L-31	-9.2				

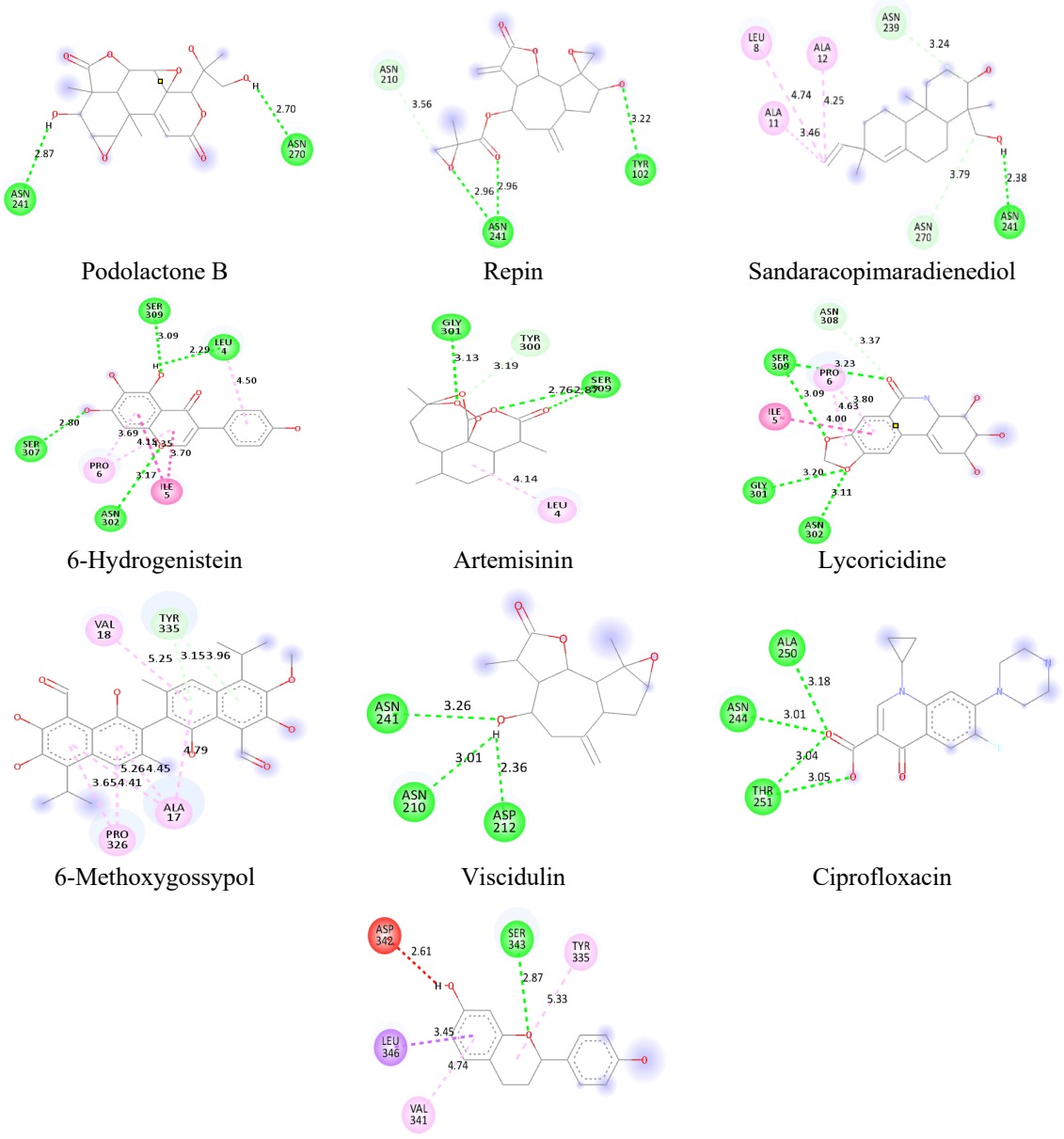

Figure 1. Interaction maps of the candidate biofungicides.

Conclusion

Pathogenic fungi cause various diseases in plants, reducing product productivity in many types of vegetables and fruits, or having the potential to completely destroy the product before or after harvest by causing powdery mildew, root rot or blight, and gray mold formation in the host organisms.

As a result of the studies and analysis of the findings, L-323 (Podolactone B), L-340 (Repin), L-353 (Sandaracopimaradienediol), L-28 (6-Hydrogenistein), L-71 (Artemisinin), L-259 (Lycoricidine), L-29 (6-Methoxygossypol), L-400 (Viscidulin), L-128 (Ciprofloxacin), L-31 (7,4'-Dihydroxyflavan) were determined as candidate biofungicide active molecules among 409 plant active molecules *in silico* characterization for PME inhibition of *B. cinerea* pest.

With the development of new strategies for the production of organic-based bio-pesticides in the biocontrol of plant pathogenic fungi, is expected to make significant contributions to the literature, especially with the increase in molecular-based studies of host adaptation and plant resistance aimed at maintaining plant resistance in a more sustainable manner. Initiating these researches within silico-based studies will contribute to experimental studies in terms of both time and cost.

Ethical Statement

Ethical approval is not required for this study.

Conflict of Interest

The Author(s) declare(s) that there are no conflicts of interest.

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Research Article

Spectrophotometric and Chromatographic Determination of Alkaloids and Nicotine Contents in Lebanese Tobacco Leaves

Sylvana ELCHAMIEH[1], Ali JABER*[2], Ghassan IBRAHIM[3], Edmond CHEBLE[4]

[1,2,3,4]Faculty of pharmacy, Laboratoire de Recherche et Développement des Médicaments et des Produits Naturels RDMPN, Lebanese University, Beirut, Lebanon.
[2]Department of Natural Sciences, School of Arts and Sciences, Lebanese American University, Beirut, Lebanon.
[3,4]Al-Kut University College, Department of Pharmacy, Wasit Governorate, Iraq.

[1]https://orcid.org/0009-0004-8143-6726, [2]https://orcid.org/0000-0002-9180-8384, [3]https://orcid.org/0000-0003-0231-3644
[4]https://orcid.org/0000-0001-9911-9025

*Corresponding author e-mail: ali.jaber.2@ul.edu.lb

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Abstract: *Nicotiana Tabacum*; the annual herb; known as Tobacco from the Solanaceae family was known for its alkaloids and especially nicotine (NCT) content; smoking products, insecticides, anthelminthic activity and clinically proven therapeutics are examples of its uses. Herein two different methods were applied in an attempt to quantify the total alkaloids and NCT content in *Nicotiana tabacum* cultivated in Lebanon. Total alkaloids were investigated through the formation of a complex with bromocresol green under a simple spectrophotometric method. Whilst HPLC-DAD was the choice for the quantitation of NCT levels. The column was Lichrospher select B (5 µm, 250x4 mm), the temperature was set at 29 ºC and the wavelength at 260 nm. The mobile phase consisted of 2 M *O*-phosphoric acid and methanol (60:40, *v*/*v*) using isocratic elution at 1 mL/min. A linear relationship was proved under both instruments. The extraction yield of alkaloid totum ranges between 2.1 ± 0.25 and 6.8% ± 0.58 and alkaloids contents range from 12.14 ± 2.01 to 53.12 ± 4.54 mg of AE/g of extract for Ghandouriyeh and Danniye samples respectively. On the other side among the different areas which cultivated Tobacco in Lebanon, Danniye was found to have the highest NCT concentration of dry weight (2.64%) while Al-Hissa possesses the lowest content (0.75%). Even if the results were generally similar to other countries, the study showed a difference in values from one region to another.

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1. Introduction

For years, investigating plants ranked the highest top level among researchers around the world. Secondary metabolites were one of many topics that researchers focused on due to their various uses in agricultures, flavorings, and medicinal purposes such as usefulness for monitoring compliance in smoking cessation programs (Lerman et al., 2015; Goettel et al., 2017).

Nicotiana Tabacum; the annual herb; known as Tobacco belonging to the Solanaceae family was first distributed in tropical America and described as the “Cinderella of plant biotechnology” which

evolved into an exemplary system for both tissue culture and genetic engineering (Ganapathi et al., 2004; Bhatia et al., 2015). Therefore, its cultivation is sought after in many countries around the world.

Among the two thousand five hundred secondary metabolites (Habib et al., 2023) present in *N. tabacum*, alkaloids are the predominant and quintessential responsible for its potential biological activities. These alkaloids occur predominantly in leaves with a total alkaloid concentration of 0.2 and 8% where the potent compound; Nicotine (NCT) occupies the primary alkaloids and identifies about 90-95% of the total alkaloids (Lewis et al., 2010; Shoji et al., 2010; Sun et al., 2018). *Nicotiana*'s roots and angiosperms also contain alkaloids but with less abundant concentrations.

Note that the usability of *N. Tabacum* in tobacco products is due mainly to NCT. The latter substance interacts with nicotinic-cholinergic receptors located in the autonomic ganglia, the adrenal medulla, and within the brain. (Tiwari et al., 2020). Other secondary metabolites are the less potent pyridine alkaloids known as nornicotine and anatabine which are the most considerable minor pyridine at approximately about 4 to 5 %. Besides, myosmine (0.1%), anabasine (0.5%), cotinine, 2,3'-bipyridyl, nicotine-1'-N-oxide, N-formylnornicotine, nornicotyrine, nicotyrine, and over 20 pyridyl-type alkaloids are with minimal concentration (Yang et al., 2002; Zhang et al., 2007; Murray, 2014).

Since NCT is classified with high concentration, total alkaloids analysis is required for the quality and usability qualitatively and quantitatively. Hence, both chromatographic and non-chromatographic methods are extensively employed due to the characteristics of pyridine alkaloids, along with the abundance of literature facilitating the identification of numerous alkaloids in the plant (Li et al., 2019; Perfetti et al., 2022).

Recently, the recommendations to reduce the NCT level in cigarettes make the low NCT content trait an interest to tobacco stakeholders. Therefore, it motivated breeders to evaluate different genetic variations to investigate their effect on reducing NCT levels (Lewis, 2019; Burner et al., 2022).

Tobacco plays a crucial role in Lebanon's economy, with tobacco leaf production totaling approximately eight thousand tons in 2015. Around 25000 families derive economic benefits from the cultivation and production of tobacco and its associated products, highlighting its crucial role in both the Lebanese economy and society (Jaber et al., 2020).

However, to date, the NCT content of *N. tabacum* cultivated in Lebanon has not yet been studied. Hence, the objective of this study was to measure the overall alkaloid and NCT content using spectrophotometric methods and HPLC-DAD, respectively. Extracts of *N. tabacum* from four different Lebanese regions were subjected to this study. The type of tobacco under examination was the authentic Saada Six. This designation originates from the Saadiyat Laboratory of the Regie. Developed through a combination of Bulgarian and Azmirly tobacco, cultivation of this variety commenced in 1973 (Jaber et al., 2020 and 2022).

2. Material and Methods

2.1. Chemicals and reagents

All chemicals were of analytical or HPLC grade. The water utilized in all processes was sourced from a system of water purification (TKA MICROMED, Germany), ensuring its ultrapure quality. Methanol was procured from Sigma Aldrich (USA); 85% orthophosphoric acid was obtained from Fisher Scientific Company (USA); Chloroform, aluminum chloride hexahydrate, toluene, sodium hydroxide, Folin-ciocalteu reagent, ascorbic acid, and ammonia solution were purchased from BDH (England). Ethyl acetate, gallic acid, rutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium carbonate anhydrous were purchased from Sigma Aldrich (USA). The samples were measured using both an analytical and a digital balance (Melter Toledo). The dried leaves were ground using a POLYMIX (PX-MFC 90 D) grind mill. The absorbance of the solutions was assessed using a VWR UV-6300PC double-beam spectrophotometer, and a HEIDOLPH rotavapor apparatus was used to concentrate the different extracts.

2.2 HPLC system

All measurements were conducted using an HP 1100 Series LC system (Hewlett Packard, Palo Alto, CA, USA) fitted, in the order, with a quaternary pump, degasser compartment, thermostatted column, and diode-array detector (DAD). The whole HPLC system was managed by the HP

Chemstation chromatography software. The stationary phase utilized was an Agilent Lichrospher select B (250 x 4 mm, 5 µm) column produced by Agilent (Germany). The pH adjustment required the use of the CG 820 (SCHOTT GERATE, made in West Germany) pH meter.

2.3. Plant material

The *N. tabacum* L. samples were collected from four different Lebanese regions on June 2018, more specifically from Hisah (34°35'47"N, 36°3'17"E, 33 m MSL), Yohmor El-Chkif (33°18′45″ N 35°31′3″ E, 530 m MSL), Ghandouriyeh (33°16′20″N 35°25′52″E, 530 m MSL), and Sir Al-Danniye (34°23'09" N 36°01'47" E, 898 m MSL). A voucher specimen (No. 1805, 1806, 1807, and 1808) has been deposited at the Pharmacognosy Department, Faculty of Pharmacy of the Lebanese University.

The specimens were conveyed to the laboratory and maintained at ambient temperature until processing. The harvested plant materials underwent an initial natural drying phase (shaded and at room temperature) for four weeks. Subsequently, the leaves were separated from the dried plant material. The leaves were then finely ground using a manual grinder. Lastly, the ground materials were stored in a tightly sealed container for future utilization.

2.4. Plant extraction

2.4.1. Ultrasound-assisted extraction (UAE)

Briefly, 150 mL of sulphuric acid (0.05 M) was added to 10 g of the powdered leaf samples. An ultrasonic cleaner bath USC100T (VWR, Malaysia) was used for the UAE. Ultrasonic-assisted extraction was conducted at room temperature for a duration of 30 minutes, employing a sonication power of 30 W and a frequency of 45 kHz. The samples were then filtrated and the aqueous solution was basified to pH 9-10 with a few drops of NH_4OH (25%, m/m). The obtained sample was extracted with chloroform (3 x 25 mL). After vigorous shaking the organic phases were collected and dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain the alkaloid totum (Jaber, 2017).

2.4.2. Extraction by maceration (ME)

Extraction by maceration was carried out with 10 g of powdered leaf samples in 150 mL of sulfuric acid (0.05 M) at room temperature for 24 hours. After maceration, the aqueous extracts were treated as described in Section 2.4.1.

2.4.3. Extraction yield

The extraction efficiency was determined using the Equal 1.

$$\text{Yield of leaves extract \%} = \frac{W_2}{W_1} \text{ x } 100 \quad (1)$$

Where W_1 represents the dry weight of the utilized material and W_2 is the weight of the alkaloid totum.

2.5. Total alkaloid content (TAC)

TAC estimation in the *N. tabacum* extract was carried out through the Bromocresol Green (BCG) spectrophotometry method, utilizing atropine as a standard (John et al., 2014). The procedure followed is as follows, in different separatory funnels, accurately measured aliquots (0.2, 0.4, 0.6, 0.8, 1 and 1.2 mL) of aqueous atropine standard solution (0.1 mg/mL) were added to 5 mL of phosphate buffer (Na_2HPO_4) (pH = 4.7, adjusted with citric acid 0.2 M) along with 5 mL of BCG solution (prepared by dissolving 69.8 mg of BCG in 3 mL of 2 M NaOH, and 5 mL of distilled water. The solution was gently heated, and then the volume was adjusted to 1000 mL with distilled water) shaken vigorously with 5 mL of chloroform. Following thorough mixing, the mixture was allowed to stand for 3 minutes. The extracts were then gathered in 10 mL volumetric flasks and subsequently diluted to reach the mark with chloroform. Likewise, solutions of *N. tabacum* extracts were determined using the same procedure. The absorbance of the complex in chloroform was measured at 417 nm against a reagent blank prepared as above without atropine. The calibration curve was plotted for the calculation of the content of total

alkaloids. The whole experiment was conducted in three replicates. The total alkaloid content was expressed as mg of AE/g of extract.

2.6. Quantitative analysis of nicotine by HPLC-DAD

The procedure was carried out at 29 °C using a Lichrospher select B (250 x 4 mm, 5 µm). The mobile phase consisted of 0.2 M orthophosphoric acid and methanol (60:40, v/v) in isocratic mode. The mobile phase was filtered before injection through a Whatman filter paper 0.45 µm (Whatman, Maidstone, UK), and delivered at a flow rate of 1 mL/min, and the injection volume was 20 µL. Quantification was done using signals detected at 260 nm.

The characteristics and procedures of the HPLC method were validated following the guidelines set forth by the International Conference of Harmonization (ICH). The validation encompassed various parameters, including specificity, linearity, recovery, precision, limit of detection (LOD), and limit of quantification (LOQ).

A stock solution of nicotine was prepared in methanol at 2 mg mL^{-1}. First of all, 20 µL of standard, sample, spiked sample, and mobile phase (blank) were injected separately in order to evaluate the specificity. The linearity response was established by injection (n = 3) of a series (six levels) of NCT work solutions ranging from 0.64 to 1200 mg mL^{-1}. The standard calibration curves were generated by plotting concentrations against the peak area of the analyte. The repeatability of the method was checked by analyzing six replicate samples of NCT and calculating the percent relative standard deviation (% RSD). The intermediate precision (ruggedness) was checked by repeating the linearity test for 3 consecutive days and calculating the RSD between 3 days for area, slope, and intercept. Additionally, the LOD and the LOQ for this method were determined based on the standard deviation (σ) of y-intercepts from regression analysis and the slope (m) of the calibration curve, as Equations 2 and 3, respectively.

$$\mathrm{LOD} = 3.3\frac{\sigma}{\mathrm{m}} \tag{2}$$

$$\mathrm{LOQ} = 10\frac{\sigma}{\mathrm{m}} \tag{3}$$

2.7. Statistical analyses

The experiments were performed in triplicates. The analysis of variance (ANOVA) followed by the Tukey test (p-value < 0.05) using the SPSS 21.0 software package (Chicago, IL, USA) was used to determine statistically significant differences between means. The coefficients of determination (r^2) were determined using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

3. Results and Discussions

Sample preparation is a vital part of any investigation of a biological analysis, regardless it is qualitative or quantitative. The importance of this step lies in the necessity to bring out the whole chemicals from the sample or only target substances in the selective extraction cases. In addition, the later application of chromatographic techniques needs a preliminary preparation giving a sample free of unwanted constituents that may hinder the analysis and ruin the column (Petruczynik, 2012).

Indeed, in this work, the first step was to establish the optimal selective extraction method of total alkaloid content from *N. tabacum*. The extraction procedure from an aqueous acidic medium was based on the basic properties of alkaloids. Then, the alkalinization (pH>7) by the addition of ammonia solution will stimulate the release of free alkaloids.

Leaf of *N. Tabacum* was collected from two fields from the north governorate (Danniye, Al-Hissa) and two from the south governorate (Ghandouriyeh, Yohmor El-Chkif). In order to choose the more appropriate extraction method sample undergoes two different extraction techniques, namely ultrasound-assisted extraction and maceration. The obtained extraction yields were 1.95 ± 0.15 and 1.19 ± 0.35 % for UAE and maceration respectively. Therefore, the % yield obtained by the UAE method nearly doubled that obtained by maceration, and UAE therefore was adopted for the subsequent works.

The extraction yield of alkaloid totum (Table 1) was found to range between 2.1±0.25 and 6.8 % ± 0.58 for Ghandouriyeh and Danniye samples respectively. The variance in extraction yield among the four examined samples is statistically significant ($p<0.05$).

To quantify total alkaloid content, a spectrophotometric method was applied. Atropine was used as standard, thus a range of different concentrations (2 and 14 mg mL^{-1}) was used for constructing the calibration curve. Upon mixing the different standard solutions with BCG, a yellow color complex was formed with maximum absorption at 417 nm. The results, as illustrated in Figure 1, indicate that the absorbance of the complex follows Beer's law within the concentration range of atropine (R^2=0.989).

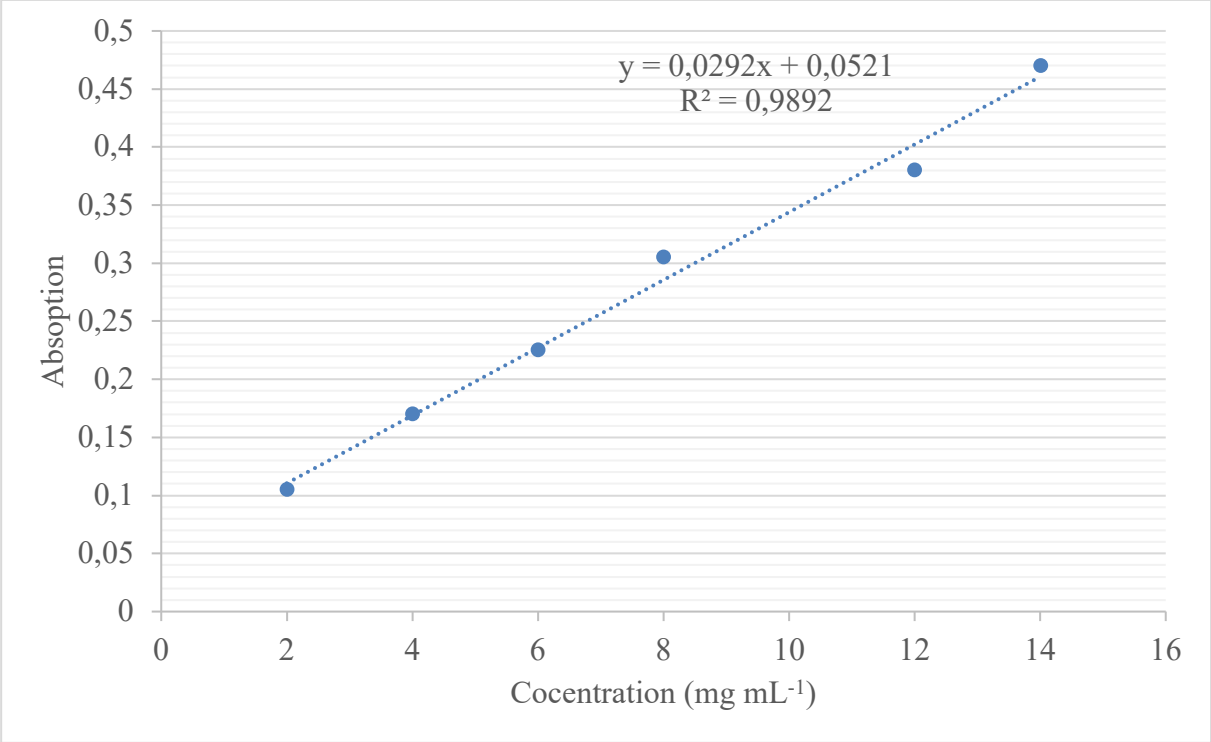

Figure 1. Calibration curve of standard atropine solutions.

At pH<7 most compounds containing tertiary amine groups or quaternary ammonium salts, form with BCG yellow complexes extractable by chloroform (Khoi, 1983). This spectrophotometric procedure is simple and sensitive, and BCG can react with the alkaloids having nitrogen inside their structure (Fazel et al., 2010; Ajanal et al., 2012; Liu and Liu, 2015; Salamah and Ningsih, 2017). After the protonation in an acidic medium, the protonated nitrogen (quaternary ammonium cations) is the target for the reaction with the BCG compound.

As above mentioned, tobacco is one of the richest plants in pyridine alkaloids (Figure 2). Among the tobacco-containing alkaloids, NCT is the major compound, besides many other minor alkaloids (Clemens et al., 2009; Jacob et al., 2022). Thus in their reaction to BCG, the NCT will be the most influential.

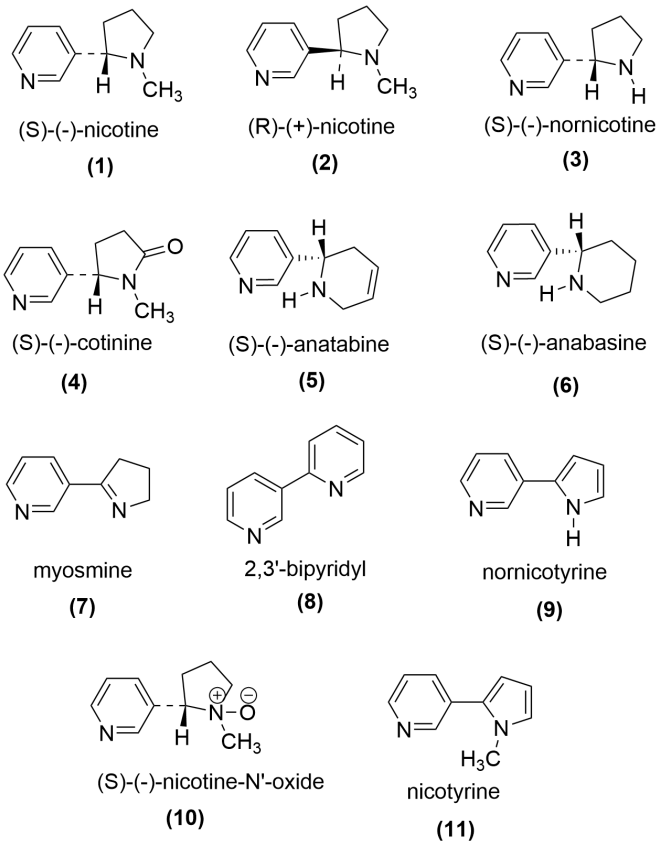

Figure 2. Nicotine and some related tobacco alkaloids chemical structures.

The nitrogen in the pyrrolidine cycle, holding a pka of 8, is the stronger basic site in the nicotine structure, and the pH of the used buffer solution is equal to 4.7 (pka>pH). Thereby, the nitrogen involved in the pyrrolidine will be protonated forming the ion-pair complex with BCG (Figure 3).

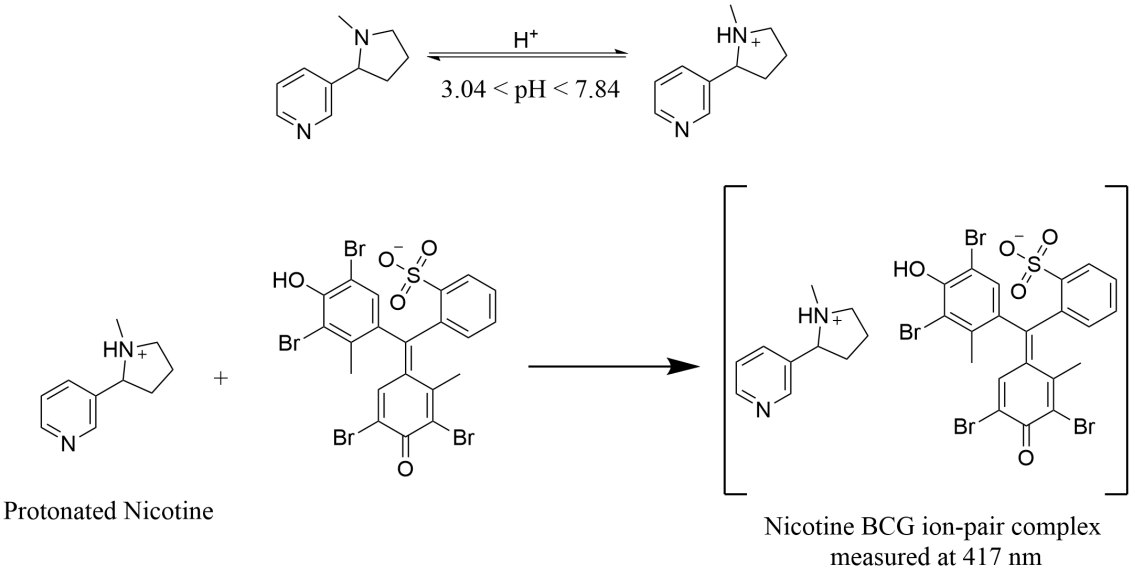

Figure 3. Structure of Nicotine and formed ion pairs.

The resulting reaction forms a yellow color complex, which indicates the mono-anionic form of BCG. The latter complex is the result of the electrostatic attraction between the mono-protonated alkaloids and the mono-anionic dye. The data obtained are presented in Table 1.

Table 1. Extractions yields (%) of alkaloids totum expressed; Alkaloid contents in the plant extracts expressed in terms of atropine equivalent (mg of AE/g of extract)

Samples	Extractions yields of alkaloids totum	mg of AE/g of extract
Danniye	6.80 ± 0.59^{a}	12.14 ± 2.01^{d}
Al-Hissa	4.13 ± 0.38^{b}	51.46 ± 5.44^{e}
Ghandouriyeh	2.10 ± 0.24^{c}	53.12 ± 4.54^{e}
Yohmor El-Chkif	4.93 ± 0.45^{b}	34.14 ± 3.98^{f}

Each value is the average of three analyses ± standard deviation, in each column values marked by the same letters (a-f) are not significantly different ($P < 0.05$), where AE is atropine equivalent.

The alkaloid contents were examined, a sample from Ghandouriyeh showed a higher content of alkaloids atropine equivalents, although it was the lowest extractive yield value. This observation applied to the three other samples, and the order of extractive values was reversed in the total alkaloid contents.

NCT is produced in the root of the tobacco plant before it moves to the leaves and the lateral parts (Baldwin et al., 1993). The NCT content depends on many factors such as the type of tobacco, nitrogen availability, temperature, light, and moisture (Yasinok et al., 2009). There's a relationship between the amount of nitrogen and the concentration in tobacco. In early development, N uptake is believed to be active so the concentration of NCT reaches its maximum at the late growth stage, especially after removing terminal buds in the middle and upper leaves (Wang et al., 2008). Therefore, the different bioclimatic levels in Lebanon, even in short distances, will probably lead to differences in the alkaloids and NCT contents.

Later, a simple and rapid HPLC–DAD method was developed for the quantification of NCT. The method validation was achieved according to the International Conference on Harmonization (ICH) (Singh, 2015). For specificity evaluation, as mentioned before, separately 20 µL from the standard, sample, spiked sample, and a mobile phase (blank) were injected into the chromatographic system. The chromatogram results showed that there are no interfering peaks at the retention time of NCT, which confirmed the method's specificity (Figure 4). Then a serial of different standard solutions (0.64 to 1200 mg mL^{-1}) was used for the linearity evaluation. A graph was constructed by plotting the peak area of NCT from each standard solution against the corresponding concentration. The regression analysis produced the linear equation $y= 30.177x+38.105$ with a correlation coefficient (R^2) of 0.9967, demonstrating a high linear relationship between the analyte's concentration and the peak's area. As a result, it has been found that the current analytical procedure is linear in the given range.

The LOD and LOQ values were calculated from the standard deviation of the y-intercepts of regression lines, and the calibration curve slope (Zahreddine et al., 2021; Beldar et al., 2022). The obtained LOD and LOQ were 132.207 mg mL^{-1} and 400.629 mg mL^{-1} respectively.

The precisions were tested by the evaluation of the repeatability of the adopted method. The intra-assay and inter-assay (expressed as % RSD) variations of retention times and concentrations were checked. Repeated measurements of standard solutions, 13 replicates (intraday) over three days (interday), were done. The intraday % RSD were 0.166 and 0.33% for retention times and concentrations respectively, while the interday % RSD were 0.27 and 0.71%.

The accuracy of the assay method was evaluated through recovery studies conducted at three concentration levels over three days. The percent recovery was determined by comparing the measured concentrations of NCT with the added concentrations. The percent recovery fell within the range of 91% to 112%, and the average percent relative standard deviation (% RSD) was 1.7%. These values are within the accepted limits, typically ranging from 80% to 120%, indicating the method's accuracy (Shikanga et al., 2012), and not more than 5% (Koetz et al., 2017), respectively. These values imply the applicability of the method for NCT analysis.

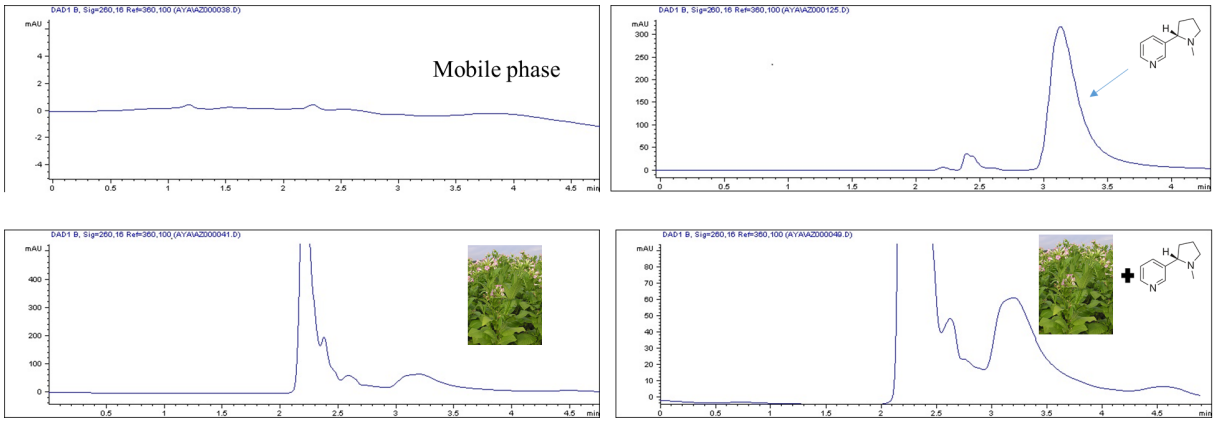

Figure 4. Chromatograms of the blank, the standard NCT, the N. tabacum extract, and spiked extract.

Table 2. Quantitation of nicotine in the different regions in Lebanon

Samples	% Nicotine in dry weight
Al-Hissa	0.75 ± 0.29^{a}
Yohmor El-Chkif	$1.76 \pm 0.42^{a,b}$
Danniye	2.64 ± 0.61^{b}
Ghandouriyeh	1.03 ± 0.22^{a}

Each value is the average of three analyses ± standard deviation, values marked by the same letters are not significantly different ($P < 0.05$).

Due to the variability in NCT content among tobacco plants, manufacturers have the option to substitute commonly used, nicotine-rich varieties such as *Nicotiana rustica* with lower-nicotine alternatives (Tengs et al., 2005). To offer consumers tobacco products with decreased health risks, companies employ genetic engineering and plant breeding techniques to cultivate tobacco with significantly lower NCT levels, which is then utilized in the manufacturing of cigarettes (Food and Drug Administration, 2018).

The findings herein show that NCT levels in the tobacco from north Lebanon, vary between 0.75% and 2.64%, while in the south 1.03 - 1.76%. In general, the level of NCT in Lebanese tobacco is comparable with that in the rest of the world.

Tobacco plants contain 2– 4% alkaloids of their total dry weight (Saitoh et al., 1985), while the NCT content varies from 0.3 to 3% in dry weight, in some cases, 5 to 7% are also reported (Tassew and Chandravanshi, 2015; Tayoub et al., 2016). Gonzalez-Coloma (Gonzalez-Coloma et al., 2010) report about 2-6% of NCT content of the leaves of *N. rustica* and *N. tabacum*.

In contrast, 6.7% of NCT content was found in Virginia variety, 4.9% in Burlip, 4.84% in Katrina, 4.67% in Shk al-bent, 4% in Zegrin, 3.3% in Basma of NCT in dry-weight leaves (Tayoub et al., 2016).

On the other hand, in Ethiopian tobacco leaves, levels of nicotine vary between the four varieties of Ethiopian tobacco including Burley tobacco (0.650%), Oriental tobacco leaves (≤0.05%), Virginia tobacco (3.26%), and native tobacco 'Gaya' (1.10%). Also, Tepecik and Ongun (2020) reported that NCT contents were greater in the second harvest (between 0.28-0.86%) than in the first harvest (0.19-0.74%) (Tepecik and Ongun, 2020). Moreover, the levels of NCT of the same species vary in different areas of cultivation (Tassew and Chandravanshi, 2015). A study carried out in China (Wang et al., 2008) reported that the NCT level depended on leaf position and different treatments, with levels ranging from 0.78 to 3.26%. The Bulgarian oriental tobacco was found to be 2.3% in the leaves (Popova et al., 2018).

Conclusion

To quantify the levels of total alkaloids and NCT content in dry weight Lebanese tobacco, extraction of total alkaloids was conducted. Then, spectrophotometric and chromatographic methods were used for quantification. The extraction yield of alkaloid totum ranges between 2.1 ± 0.25 and 6.8% ± 0.58 and alkaloid contents range from 12.14 ± 2.01 to 53.12 ± 4.54 mg of AE/g of extract for Ghandouriyeh and Danniye samples respectively. On the other side among the different areas which

cultivated Tobacco in Lebanon, Danniye was found to have the highest NCT concentration of dry weight (2.64%) while Al-Hissa possesses the lowest content (0.75%). This study of tobacco breeding in Lebanon shows that NCT levels are comparable with other countries.

Ethical Statement

Ethical approval is not required for this study.

Conflict of Interest

The Author(s) declare(s) that there are no conflicts of interest.

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Research Article

Effects of Hot Water Obtained by Solar Energy on the Weeds *Convolvulus arvensis* L., *Setaria viridis* (L.) P. Beauv. and *Amaranthus retroflexus* L.

Ayfer Güney SARITAŞ*[1], Ramazan GÜRBÜZ[2]

[1,2]Iğdır University, Faculty of Agriculture, Department of Plant Protection, Iğdır, Türkiye

[1]https://orcid.org/0000-0001-7641-9711, [2]https://orcid.org/0000-0003-3558-9823

*Corresponding author e-mail: ayferay4787@gmail.com

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Growth stages,
Hot water,
Solar method,
Weed

Abstract: This study was carried out to determine the effect of hot water obtained by the solar method (solar energy) on the different plant growth stages of *Convolvulus arvensis*, *Amaranthus retroflexus,* and *Setaria viridis* species, which are problematic in agricultural areas. Hot water at a temperature of 98 °C, obtained using the solar method, was applied at 15:00 pm. The hot water was applied in two different doses depending on the driving speed (1st speed: 4 km h^{-1}, 2nd speed: 2 km h^{-1}) of the tractor. The application was carried out at three stages of plant growth (20, 40, 60 days old plants). According to the BBCH scale, these periods correspond approximately to GS:19, GS:40, and GS:60. In the results of the study; it was found that hot water application was more effective in the of GS:19 2 km h^{-1} (77%) to the aerial parts of *C. arvensis* and GS:19 2 km h^{-1} (68%) to the underground parts of *A. retroflexus*. In the hot water speed, it was observed that the 2nd speed (2 km h^{-1}) was more effective on weeds than the 1st speed (4 km h^{-1}).

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1. Introduction

Ever since the advent of agriculture, man has been confronted with weeds and has been dealing with them ever since. These plants, which we define as plants that grow where we do not want them and whose harm outweighs their benefit, are dominant in the habitat where they occur as long as they are not affected by biotic factors (humans, animals, plants, micro-organisms, etc.). Weeds, whether they are native to the area where they occur or of foreign origin, can easily adapt to different environments, from agricultural areas to pastures, from parks to archaeological sites, from wet habitats to sports fields, from fields and roadsides to railways (Uygur and Uygur, 2010). The damage caused by weeds is not only limited to areas of agricultural production but is also a problem in many non-agricultural areas. The damage patterns vary depending on where they grow. Weeds cause various problems in non-agricultural areas such as airports, railways, historic areas, flammable and explosive storage areas, open materials highway and railway embankments, around buildings, factories, industrial areas, industrial estates, pipelines, canal banks, and slopes. They are a source of fire hazard, especially when the weeds are dry (Jodoin et al., 2008; Tepe, 2023). Weeds give non-agricultural areas a poor appearance and a feeling of abandonment in the areas where they grow. It enlarges the openings or cracks from which it grows and thus poses a fire hazard and plant particles cause new weeds to grow, block signs on highways, and

obstruct visibility on curves, leading to traffic accidents. By damaging the aesthetic appearance of the areas in which they are located, weeds shorten the life of historic monuments and sites (Rask, 2012; Gürbüz et al., 2019; Fracchiolla et al., 2022).

The types of weeds that are harmful vary between non-agricultural and agricultural areas, depending on the location. Many pathogens that damage crops allow insects and other creatures to thrive through weeds. (Tepe, 2023).

A widely used pesticide causes many health problems. A research of the scientific literature, it can be seen that pesticides are associated with a variety of negative health effects on humans, animals, wildlife, and the environment (Gürbüz and Koç, 2018; Kumar, 2023). Many methods are used to control weeds in agricultural and non-agricultural areas. Chemical control with herbicides is one of the most preferred methods because it is easy to apply and gives results in a short time. However, the harmful effects of these chemicals are increasing due to their excessive and unconscious use. To avoid these harmful effects, it is of great importance to use less environmentally harmful methods to control weeds. Thermal weed control is a non-chemical weed control method and can be used successfully in non-agricultural areas as well as in agricultural areas (Kitiş, 2010; Kitiş and Gürbüz, 2022).

Thermal weed control is the process of controlling weeds with high temperatures by transmitting heat energy from an energy source to the plant and increasing the temperature locally (Bauer et al., 2020). Here, methods such as flaming, hot water application, steam application, hot air, electric shock, microwave rays, infrared radiation, laser application, UV-rays, and freezing are used. Laser and optical radiation can be used effectively for thermal weed control, as the energy is absorbed by cellular pigments and water, converting light energy into heat (Zhang et al., 2024). In some of these methods (flaming, hot water application, steam application, etc.), heat energy is transmitted directly to the weeds, while in others (microwaves, UV rays, etc.) heat energy is transmitted indirectly (Rask and Kristoffersen, 2007).

Thermal weed control methods based on hot water are another alternative weed control method that causes less wear and tear on the treated surface than mechanical control methods such as hand pulling or hoeing. Many studies have shown that the above-ground parts of weeds can be destroyed by the application of hot water. The aim of the study was to use hot water to reduce the growth and spread of problematic weeds on hard surfaces without the use of herbicides. This includes the use of hot water generated by solar energy. The water is used to control weeds without harming the environment. On the other hand, this study was carried out to reduce the unnecessary use of herbicides and to prevent the possible development of resistance.

2. Material and Methods

This study was conducted in the greenhouse at Iğdır University Şehit Bülent Yurtseven Campus in 2018-2019. The aim of this study was to evaluate the effectiveness of solar-heated water as a non-chemical weed control method across various environments. To identify the most common problem weeds, surveys were carried out in non-agricultural areas (hard ground, pavements, asphalt and concrete).. As a result of the surveys, 2 dicotyledons (*Convolvulus arvensis* L.), (*Amaranthus retroflexus* L.) and 1 monocotyledon (*Setaria viridis* (L.) P. Beauv.) weed species with the highest density were used in the study.

2.1. Planting weed seeds in pots

The seeds of the weeds *C. arvensis, A. retroflexus,* and *S. viridis* were stored in a refrigerator at +4 °C to break the dormancy of the seeds for three months before use in the experiment. The soil medium was prepared by mixing garden soil, sand, and animal manure in a 1:1:1 ratio. In the experiment, 60 pots of 15x17 cm were used for each plant species were used. 10 seeds were planted in each pot to a depth of 5 mm. After planting, all pots were fertilized with 5 grams of Osmocote flower fertilizer at a 2:1:3 NPK ratio. When the plants grown from the seeds planted in the pots reached a size where they could compete with each other (3-4 leaves and 10 cm), they were thinned out leaving three plants in each pot. The thinned plants were watered regularly until they were 20 (1st GS/Growth Stages), 40 (2nd GS), and 60 (3rd GS) days old. The growth stages of the plants were determined according to the BBCH scale. These periods correspond approximately to GS:19, GS:40, and GS:60, each, according to the BBCH scale. GS:19 is the opening of 9 true leaves; GS:40 is the period in which the vegetative propagation organs begin to develop (rhizomes, stolons) and GS:60 is the period in which the first

flowers open (single).

2.2. Hot water application on weeds

The experimental design used was replicated 4 times; the application was made at third growth stages (1st GS, 2nd GS, and 3rd GS), two different doses (1st dose and 2nd dose) and at 15:00 pm, De Cauwer et al. (2016) the solar heated water (Güney Sarıtaş, 2019) temperature was 98 ºC. The plants were placed in individual strips 10 cm apart, on the concrete floor, and hot water was applied before application, the air temperature was measured at 37 ºC using a digital thermometer. The hot water tank, which was specially designed for hot water application and can maintain the temperature of the water for 24 hours, was used by connecting the trailer to the back of the tractor. The hot water pump will operate with electricity and is supplied with electrical energy from the electrical outlet of the tractor. Water was sprayed at a constant pressure of 3 atm through the hot water pump attached to the outlet of the hot water tank. During the applications, the boom height was kept as close to the pot/plant surface as possible (5 cm) to prevent loss of water temperature. During the application of the amount of water per unit area, the tractor speed was 2 km per hour for the 2nd dose application and 4 km for the 1st dose application. The approximate water and energy amount of two different doses applied was 66.6 kJ m^{-2} (1.8 L m^{-2}) and 33.3 kJ m^{-2}(0.9 L m^{-2}), respectively. The plants were kept for one week after the hot water application.

2.3. Determination of the effects of hot water application on weeds

In order to observe the effect of the applied hot water on the above ground and below ground parts of the plants, the above ground parts of the plants were removed with scissors, and the root parts were removed from the pots, washed in 0.2 mm sieves and placed in paper bags. The paper bags brought to the herbology laboratory were placed in the oven and kept at 70 ºC for 24 hours and then removed. Then, the dry weights of the above ground and below ground parts of the plants were weighed with a precision scale and the data were noted. When hot water is applied to weeds, it rapidly raises the temperature of the plant cells, causing thermal shock. This sudden increase in temperature can rupture cell walls and denature proteins, leading to cell death. Depending on the weather, the plants start to dry out after a short time (Figure 1).

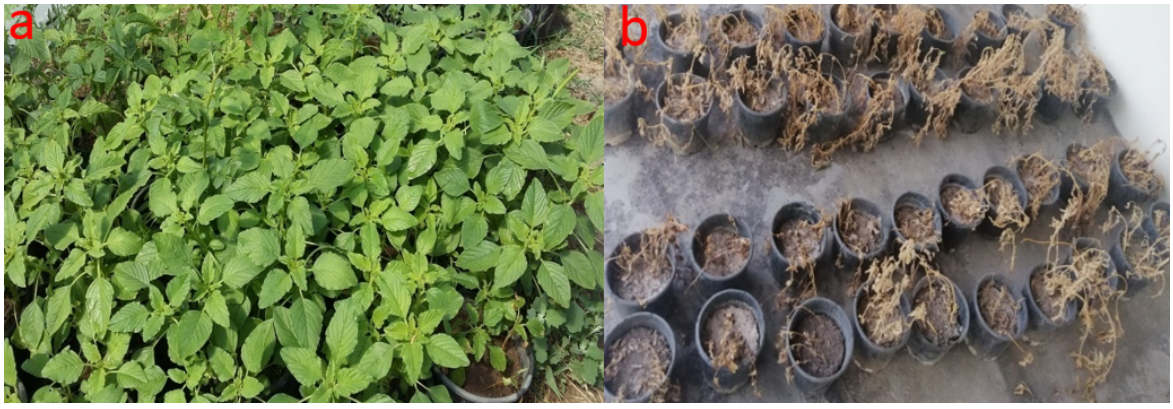


Figure 1. Visual comparison of plants before (a) and after (b) hot water application.

2.4. Statistical analysis

The obtained data were analyzed in the SPSS 20 statistical package program and subjected to analysis of variance (ANOVA). Differences between application doses and growth stages were determined by Duncan's multiple comparison test ($p<0.05$). Additionally, Microsoft Excel was used to show the percentage distribution of the findings.

3. Results

3.1. Determination of the effects of hot water application on weeds

In the study conducted, the results of the effects of hot water application on the above ground and below ground parts of the weeds *C. arvensis, S. viridis,* and *A. retroflexus* were evaluated by analysis of variance, and the differences between application doses and growth stages were determined by the Duncan multiple comparison test ($P<0.05$). The effects of different doses of hot water application on the growth stages of the above ground and below-ground parts of the *C. arvensis, S. viridis,* and *A. retroflexus* plants included in the experiment are presented in Figure 2.

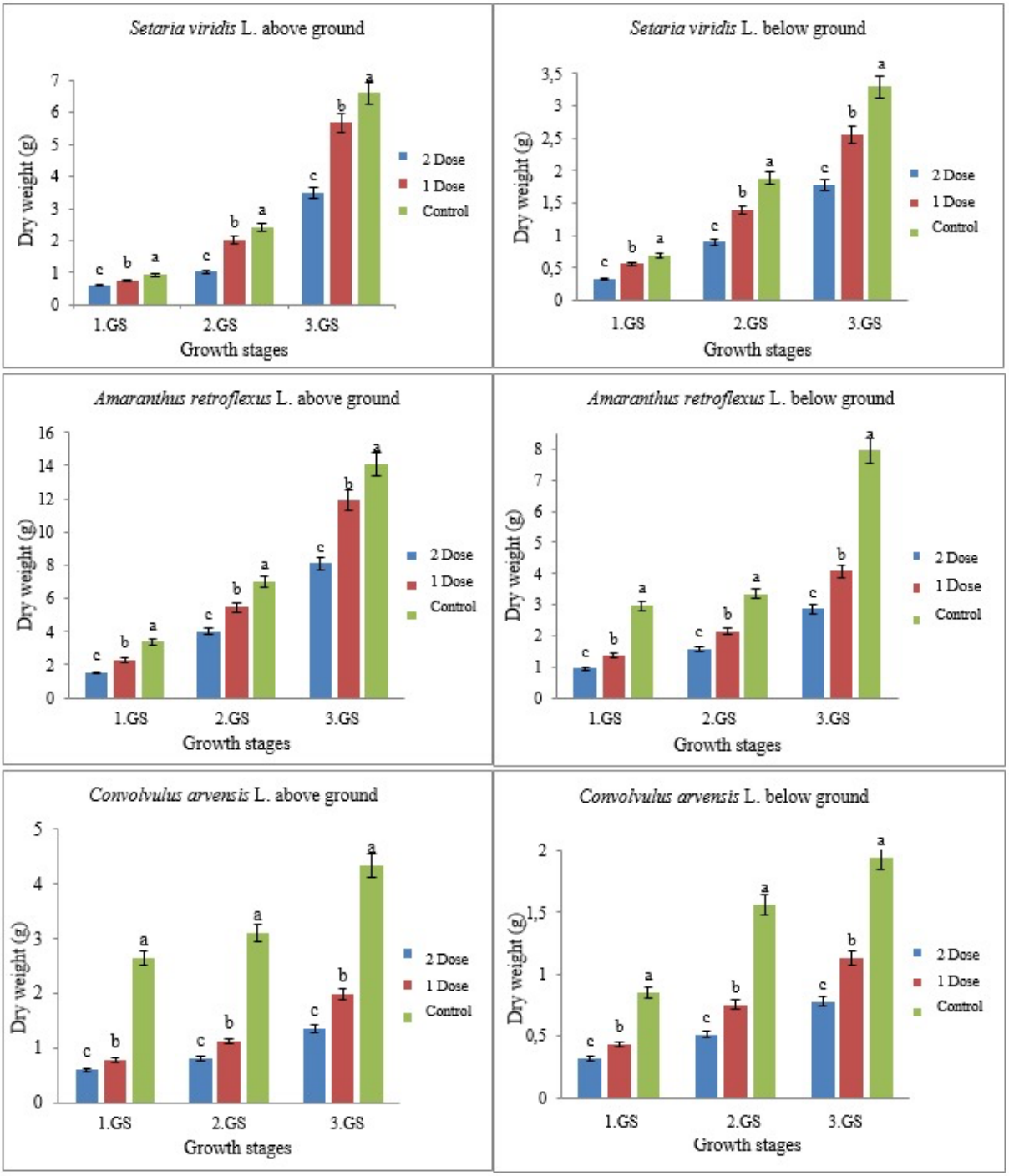


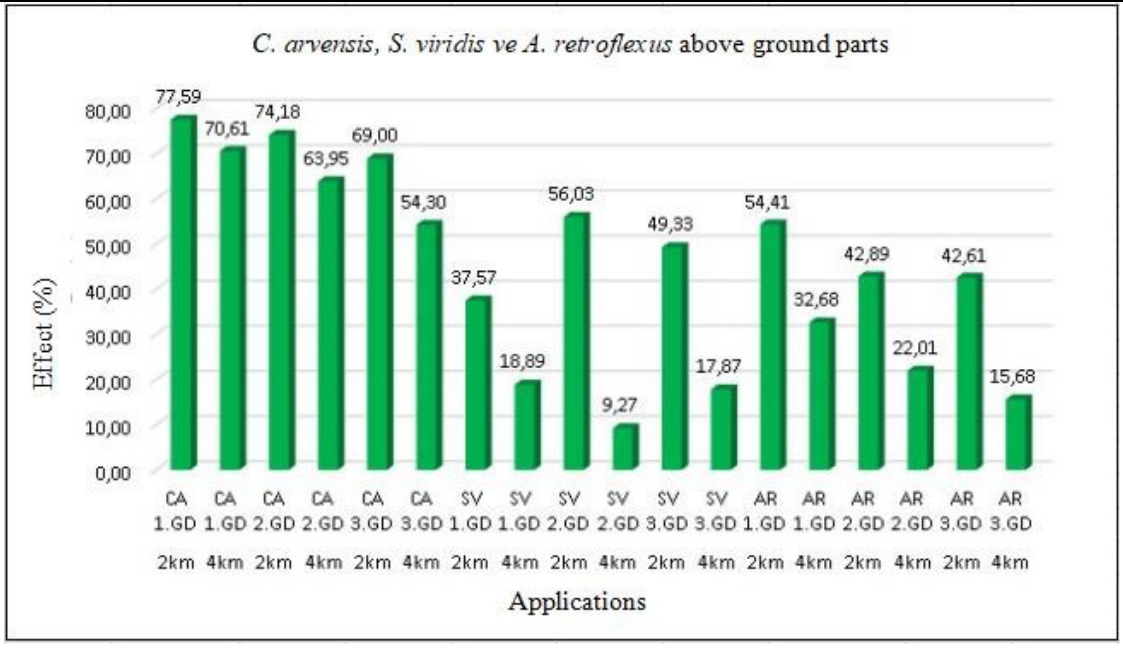
Figure 2. Effects of different doses of hot water application on the growth stages of the above ground and below ground parts of the *S. viridis, A. retroflexus,* and *C. arvensis.*

*Each growth stages were evaluated statistically within itself. Differences between means with the same letter are not significant at the 0.05 level. GS: Growth Stage.

As a result of the analyses, when we look at the growth stages for the above ground and below-ground parts of hot water application, *S. viridis, A. retroflexus,* and *C. arvensis* are the most effective in the first growth stage (1.GS), followed by the second growth stage (2.GS) and finally, it is also listed as the third growth stage (3rd GS). In general, it has been determined that 2 doses (66.6 kJ m^{-2}) are more effective than 1 dose (33.3 kJ m^{-2}). The same situation applies to the underground parts. When we compare the GS and doses of *S. viridis, A. retroflexus,* and *C. arvensis* aerial parts, 1st GS 1 dose applications show similar characteristics. As a result of the data obtained, it was seen that the decrease in the amount of dry matter was much more effective on the above ground and below ground parts of *C. arvensis*. As a result, the most effective one among the growth stages is the 1st GS, and it has been determined that 2 dose (66.6 kJ m^{-2}) application is more effective than 1 dose (33.3 kJ m^{-2}) application. Therefore, this comparison shows that hot water application is effective in controlling three weeds.

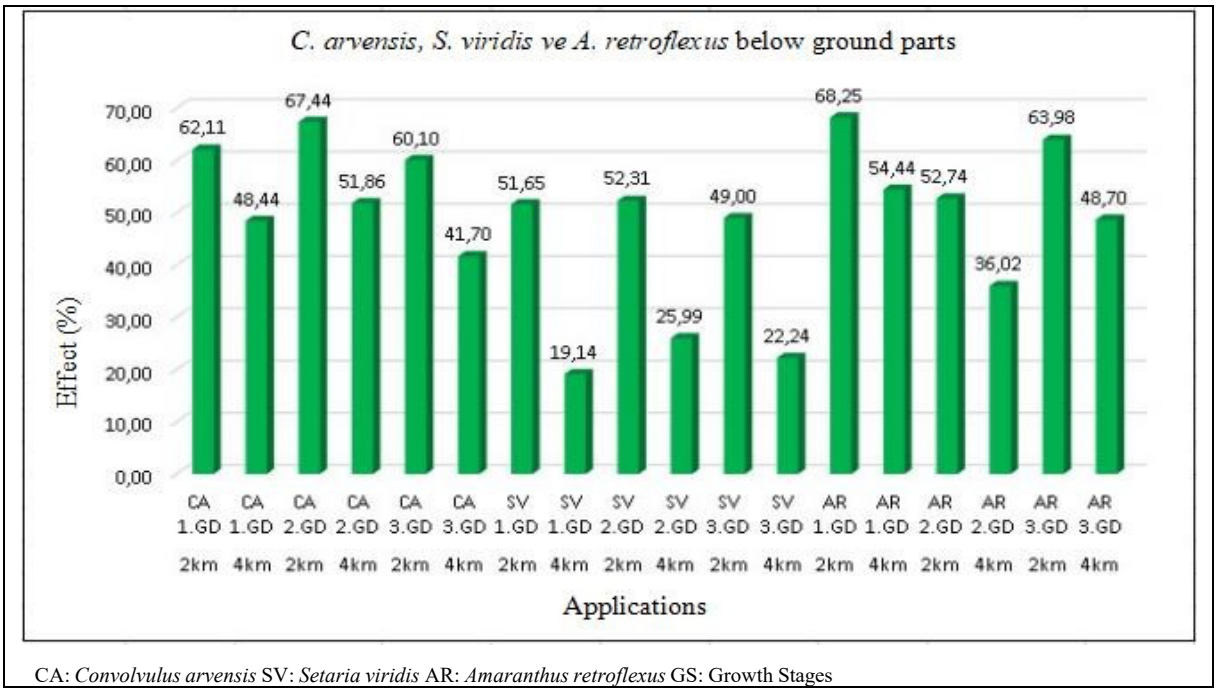
The percentage effect of hot water application on the dry weight of the above ground parts of the weeds is shown in Figure 3. The percentage effect of hot water application on the dry weight of above ground weed parts is shown in Figure 4.

The highest percentage effect of hot water application on the dry weight of the above ground parts of weeds is 77.59% on *C. arvensis* applied at a speed of 2 km in the 1st GS, and the lowest percentage effect is on GS, applied at a speed of 2 km in the 2nd GS with a rate of 9.27%. on *S. viridis* (Figure 3). The highest percentage effect of hot water application on the dry weight of the below ground parts of weeds is *A. retroflexus*, applied at a speed of 1st GS 2 km, with a rate of 68.25%, and the lowest percentage effect is 19.14% on *S. viridis*, applied at a speed of 1 st GS 4 km (Figure 4).



CA: *Convolvulus arvensis* SV: *Setaria viridis* AR: *Amaranthus retroflexus* GS: Grogwt Stages

Figure 3. Percentage effect of hot water application on the dry weight of above ground parts of weeds.



CA: *Convolvulus arvensis* SV: *Setaria viridis* AR: *Amaranthus retroflexus* GS: Growth Stages

Figure 4. Percentage effect of hot water application on the dry weight of the below ground parts of weeds.

4. Discussion and Conclusion

In our study, hot water was obtained using solar energy, whereas in a previous study (De Cauwer et al., 2015), hot water was obtained using diesel fuel. While the amount of energy delivered per unit area at the dose used effectively controls weeds, the number of applications can be reduced with an appropriate dose and excessive energy use can be avoided (Hansson and Ascard, 2002; De Cauwer et al., 2015). It is similar to the dosing practices we use. On the other hand, the growth stages of the weeds were also important for the effectiveness of the method (Hansson and Ascard, 2002; De Cauwer et al., 2015; Koç, 2019), and as the growth stages progresses, the amount of energy spent to control the weed also increases.

De Cauwer et al. (2015) obtained the best effect from the application of hot water at 98 ºC. It has been reported that broad-leaved plants have the highest sensitivity to hot water, while narrow-leaved plants have the lowest effect. In our study, it is similar in that hot water at 98ºC is used and *C. arvensis* is the most sensitive. De Cauwer et al. (2016) stated in their study that the plants they applied were more sensitive to hot water in the afternoon and that the sensitivity of plants to hot water was related to the change in leaf thickness and dry matter of the plants. In our study, it is parallel to our application of hot water at 15:00 in the afternoon.

In the research carried out by Hansson and Mattsson (2002), it was asserted that elevated temperatures generally resulted in a more significant reduction in the plant population. It is similar to our study. Similar to our study, Kristoffersen et al. (2008) used flame, steam, hot air, hot water, and brush applications in their study. They stated that the most effective application was hot water application. In our study, the air temperature was measured at 37 ºC with a digital thermometer before the application. Similar to our study, Hansson and Mattsson (2003) examined the effect of air temperature on the time when hot water was applied to the weed *Sinapis alba* L. in their study. Koç (2019) stated that hot water at 98°C obtained by thermal method for some weed species that cause problems in hard ground areas, the most effective result is at 15:00 and hot water is more effective on 1st GS. The results obtained in this study are parallel to the results we obtained in our study. While hot water application can control weeds, it has been reported that at much lower doses the seeds of *Impatiens glandulifera*, a problem in European countries, lose their ability to germinate. When comparing the effects of mowing and hot water in controlling *Impatiens glandulifera*, they reported that applying hot water at different stages of plant growth was more effective than mowing and that applying hot water in

the early and late stages could save 50% of the water used (Oliver et al., 2020). The results of this study parallel the results obtained in our study. In a study by Bayat et al. (2017), they stated that since there are no problems such as chemical resistance in thermal weed control, all weeds are controlled when sufficient temperature is reached in plant tissues. They also stated that since there is sufficient solar radiation in our country, there is the potential to use solar heated water in many thermal methods. The results of these studies are consistent with what we observed in our study. It is important to use methods that do not harm the environment when controlling weeds. One of these methods, the application of hot water, uses heat as an alternative to harmful chemicals to control weeds. They stated that this method is effective in reducing fire hazards that may arise from the use of flame (Hansson and Mattsson, 2002). The aboveground parts of weeds can be controlled by applying hot water. In this respect, it is similar to our study. However, the amount of energy required for this process may be higher compared to the use of herbicides (Hansson and Ascard, 2002). Compared to chemical weed control, the reapplication time of thermal methods is shorter (Reichel, 2003; Kristoffersen et al., 2004; Rask and Kristoffersen, 2007; Kristoffersen et al., 2008). However, problems caused by the effects of herbicides can be prevented with thermal methods. It is also important because it gives much faster results than chemical control. They stated that hot water application causes less wear compared to mechanical methods such as hand pulling or hoeing in non-agricultural areas and is a different, non-chemical alternative method for weed control (Gürbüz et al., 2019; Koç, 2019; Güney Sarıtaş, 2019; Gürbüz et al., 2024). The results obtained in these studies are parallel to the results we obtained in the present study.

As a result of the analysis, when we look at the growth stages for above ground parts of *C. arvensis, S. viridis,* and *A. retroflexus* with hot water application, the most effective is the first growth stage (1st GS), followed by the second growth stage (2nd GS) and finally the third growth stage (3rd GS). In general, it has been determined that 2 doses are more effective than 1 dose. The same situation applies to the below ground parts. It has been determined that 2 dose application in the third growth stage (3rd GS) and the second growth stage (2nd GS) is more effective than 1 dose application. However, no difference was found between doses in the first growth stages (1st GS). As a result of the study, it was generally observed that when 98 °C hot water was applied in the third growth stages of the plants, there was a significant decrease in the dry weight of the above ground and below ground parts. By using thermal methods instead of chemical methods used against weeds in non-agricultural areas, weeds will be controlled and the wrong and unnecessary use of herbicides will be prevented. In short, the use of hot water can be recommended as an alternative solution to chemical weed control.

Further research is necessary to optimize the use of hot water for weed control in non-agricultural areas, focusing on different weed species, varying water amounts, and different temperature settings.

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Review Article

Traditional, Phytochemical, Nutritional and Biological Importance of *Pithecellobium dulce* (Roxib.) Benth

Alaa A. ELHEWEHY*[1], Engy MOHSEN[2], Ahlam M. EL-FISHAWY[3], Marwa A. A. FAYED[4]

[1,4]Department of Pharmacognosy, Faculty of Pharmacy, University of Sadat City, Sadat City 32897, Egypt
[2,3]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Ainy Street, Cairo 11562, Egypt

[1]https://orcid.org/0000-0001-9622-2932, [2]https://orcid.org/0009-0007-5723-9476, [3]https://orcid.org/0000-0003-4925-7395
[4]https://orcid.org/0000-0001-5609-7436

*Corresponding author e-mail: alaa.ashraf@fop.usc.edu.eg

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P. dulce,
Phytochemistry,
Traditional uses

Abstract: *Pithecellobium dulce* (*P. dulce*) is described in this review in terms of its botanical features, traditional uses, phytoconstituents, biological activities, and nutritional value. The aril of the fruit is consumed raw as food in many countries like India for its sweet taste. The plant phytoconstituents possess anti-ulcerogenic, anti-microbial, anti-inflammatory, and anti-diabetic properties. The plant's different extracts contain a variety of bioactive phytochemicals, including flavonoids, saponins, and tannins. People have been paying attention to medicinal plants over the past few years due to their incredible significance in the medication discovery process, their effectiveness, safety, and lack of negative side effects. *P. dulce* is a highly regarded plant in traditional medicine because of its diverse biological and nutraceutical properties. This review covers information regarding traditional uses, nutritional values, phytochemicals, and pharmacological activities of the different extracts as well as the pure compounds isolated from *P. dulce's* different parts and extracts.

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1. Introduction

Medicinal plants are a great source of several natural components with various pharmacological properties. Nutraceuticals are nutritional supplements that have recently drawn much interest because of their profound physiological effects on the human body. Traditional natural medicine practices are gaining popularity, in rural to urbanized areas. Notably, substances derived from plants have enormously valuable benefits for maintaining good health and treating a wide range of diseases including diabetes, cancer, inflammation, etc. (Jamshidi et al., 2018).

Leguminosae family is one of the largest families of flowering plants, which contains 12000 species grouped into over 600 genera. Papilionoideae, Caesalpinioideae, and Mimosoideae are the three subfamilies subdivided into the family *Pithecellobium* is one of the genera belonging to the Mimosoideae subfamily.

P. dulce (Roxb.) Benth. is a widespread evergreen tree. It is one of the 100-200 *Pithecellobium* species and it is the only one that has spread beyond its origin. The Latin species name "*dulce*" refers to the sweet edible pulp of the pod, while the genius's name is derived from the "Pithekos" (ape) and

“Lobos" (pod) (Sneha et al., 2020). The aril is eaten raw, roasted, or combined with atole (a cornstarch-based hot beverage) or agua fresca (a cold tea). The seed may be used raw, cooked, or roasted as a coffee substitute or as a condiment (Kirthy et al., 2022).

P. dulce is locally known by various names in different regions. In Arabic, it is known as Showkat madras. In English, it is known as Quamachil, Madras thorn, Manila tamarind, Black bead tree, and Monkeypod. In French, it is known as Campeche (New Caledonia) and Cassie de Manille. In Spanish, it is known as Guamuchil, Guama americano, Quamachil, Huamuche, and Chiminango. In Hindi, it is known as Vilayati imli, Vilayati babul, and Jangle jalebi. In Chinese, it is known as Niu ti dou. In Bengali, it is known as Dekhani babul. In German, it is known as Camambilarinde. In Greek, it is known as Pithekos ellobion. In Gujarati, it is known as Bakhai Ambli, Goras ambli. In Japanese, it is known as Huamuche, Guamuche, and Asambelanda. In Javanese, it is known as Asem londo and Asam belanda. In Kannada, it is known as Seeme hunase. In Malayalam, it is known as Korukkapuli. In Marathi, it is known as Ingraji chinch. In Odia, it is known as Seema Kaiyan. In the Philippines, it is known as Camachile. In Sanskrit, it is known as Kodukkaapuli. In Tamil, it is known as Kodukkaapuli. In Telugu, it is known as Seema Chintakaya. In Thai, it is known as Makham-khong and Makham-tha. In Vietnamese, it is known as Gang Tay, Me nuoc, Keo Tay, and Me Keo (Orwa et al., 2009; Kulkarni and Jamakhandi, 2018; Srinivas et al., 2018; Sneha et al., 2020).

P. dulce has been utilized traditionally in treating many disorders in different countries by using the extracts of different parts of the plant (Kulkarni and Jamakhandi, 2018; Rao et al., 2018; Srinivas et al., 2018; Dhanisha et al., 2022b). The plant contains many biologically active phytoconstituents which may contribute to the various scientifically proven biological activities such as the anti-inflammatory, anti-diabetic, anti-diarrheal, anti-microbial, anti-convulsant, anti-ulcer, anti-oxidant, anti-cancer, hepatoprotective, cardioprotective and nephroprotective activities (Sneha et al., 2020; Dhanisha et al., 2022b). Also, *P. dulce* provides important vitamins, amino acids, critical minerals, and many fatty acids that contribute to its nutritive value (Murugesan et al., 2019; Dhanisha et al., 2022b).

2. Search Strategy

Due to the wide use of *P. dulce* in traditional medicine and the presence of a variety of phytochemicals that have been proven by different *in vitro* and *in vivo* studies to have many biological activities. This systematic review highlights these traditional uses and the biologically active phytoconstituents that may contribute to the various biological activities of *P. dulce* during the period (1994-2023). Several available scientific databases were searched like PubMed, Science Direct, Scopus, Web of Science, and Google Scholar using different keywords related to the topic discussed in this review.

3. Botanical Description

P. dulce is a medium-sized evergreen tree that grows to a height of 10 to 15 meters. Leaves (Figure 1A) are bipinnate compound leaves, with 2 pairs of 2 ovate-oblong apiculate (kidney-shaped) leaflets which are approximately 2-4 cm long. Usually, at the base of the leaflet thin, paired spines ranging from 2 to 15mm in length are present. Flowers are small (1 cm in diameter) white heads colored flowers, which possess a hairy corolla and about 50 thin stamens surrounded in the calyx in the form of a tube at the base. Pods (Figure 1B) are tightly coiled and irregularly shaped greenish brown to reddish pods, which measure approximately 10-15 cm long and 1.5 cm wide and dehiscent on both sides. Each pod has about 5-10 seeds. Seeds (Figure 1C) are shiny black (1 cm in diameter) and attached to the pods by a red funicle. The bark (Figure 1D) is gray and when gets matured it becomes rougher and starts peeling (Murugesan et al., 2019; Sneha et al., 2020).

Figure 1. Photo of *Pithecellobium dulce* (Roxib.) Benth. different organs. (A) Trunk, (B) Fruit, (C) Seeds, and (D) Leaf.

3.1. Taxonomy

Domain	Eukaryote
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order:	Fabales
Family	Leguminosae
Genus	*Pithecellobium*
Species	*dulce*
Binomial Name	*Pithecellobium dulce* (Roxb.) Benth

3.2. Distribution

P. dulce has spread widely outside its origin. It can be found in northern South America, along the Pacific coast, close to Mexico, Brazil, Argentina, Bolivia, Colombia, Central America, Huawei, and in India and Southeast Asia. Now it is common in tropical Africa, especially along coasts (Dhanisha et al., 2022b).

4. Traditional Uses

The various parts of the plant were utilized traditionally in treating many disorders summarized in Table 1. *P. dulce* fruit has numerous health and nutritional advantages. Owing to its delicious flavor and medicinal properties, these fruits are eaten raw, as a decoction, roasted, or combined with atole (a cornstarch-based hot beverage) or agua fresca (a cold tea) in many regions of India for gastrointestinal disorders, and to control diabetes. The seed may be used raw, cooked, or roasted as a remedy against peptic ulcers and diabetes mellitus. The leaf decoction is used to treat intestinal and gall bladder disorders also it is used for toothache, and earache. It has both emollient and astringent properties. The bark and the root decoctions are used to treat diarrhea and dysentery (Dhanisha et al., 2022b; Kirthy et al., 2022; Roselin and Parameshwari, 2022).

Table 1. Traditional uses of *P. dulce* different parts

Plant organ	Traditional uses	Reference
Bark	Prevent hemorrhage. Treatment of gum disorders and toothache. Treatment of dysentery, diarrhea, and constipation. Treatment of dermatitis and eye inflammation.	(Kulkarni and Jamakhandi, 2018; Rao et al., 2018;)
Fruits	As an astringent and for hemoptysis. (Fruit pulp). Treatment of gastrointestinal disorders such as peptic ulcer. To control diabetes (chewing raw fruit peel or as a decoction). Swellings treatment (fruit peel decoction).	(Srinivas et al., 2018; Dhanisha et al., 2022b)
Leaves	Abortifacient. Astringent. Emollient. In toothache and earache. Larvicidal. Treatment of intestinal disorders (as a decoction) and gall bladder disorder. To prevent miscarriages. Treatment of leprosy. Used for Venereal sores (as plasters).	(Shweta, 2013; Rao et al., 2018; Srinivas et al., 2018; Sneha et al., 2020)
Roots	Antipyretic. Treatment of dysentery and diarrhea.	(Srinivas et al., 2018; Dhanisha et al., 2022b)
Seeds	Anti-edematous (Seed Oil). Remedy against peptic ulcers. Spermicidal. Treatment of diabetes mellitus. To cleanse ulcers (grounded seed).	(Kulkarni and Jamakhandi, 2018; Rao et al., 2018; Dhanisha et al., 2022b)

5. Chemical Constituents

The different organs of *P. dulce* contain numerous bioactive substances summarized in Table 2 such as flavonoids, anthocyanin, tannins, coumarin, triterpenoids, saponins, alkaloids, sterols, and fatty acids.

Table 2. Different classes of chemical constituents of *P. dulce*

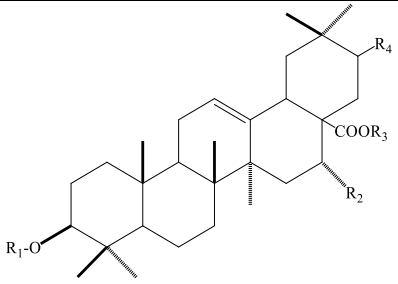
No.	Compound	Structure	Plant Organ	References
1.	**Terpenoids**			
	1.1. Triterpenoids			
				
1	Pitheduloside A	R_1= Glu.– Ara., R_2=OH, R_3=R_4=H.	Seed	(Nigam et al., 1997)
2	Pitheduloside B	R_1= Glu.– Ara.– Ara., R_2=H, R_3=R_4=H.		
3	Pitheduloside C	R_1= Glu.– Ara.– Xyl., R_2=H, R_3=R_4=H.		

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

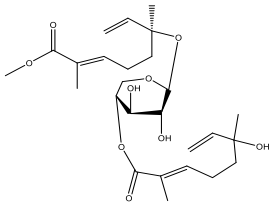
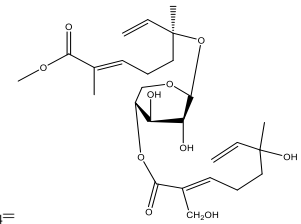
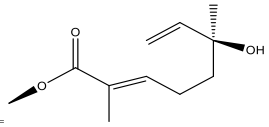
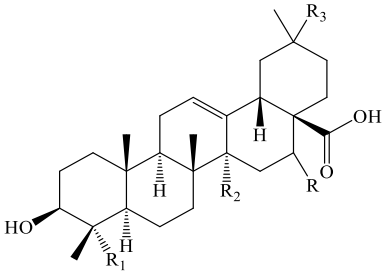
No.	Compound	Structure	Plant Organ	References
1.	**Terpenoids**			
	1.1. **Triterpenoids**			
4	Pitheduloside D	R_1= Glu. – Ara. – Ara., R_2=OH, R_3=R_4=H.		
5	Pitheduloside E	R_1= Glu. – Ara. – Xyl., R_2=OH, R_3=R_4=H.		
6	Pitheduloside F	R_1= Glu. – Ara. – Ara., R_2=H, R_3=R_4=H. | Glu		
7	Pitheduloside G	R_1= Glu. – Ara. – Xyl., R_2=H, R_3=R_4=H. | Glu		
8	Pitheduloside H	R_1= Glu. – Ara. – Ara., R_2=OH, | Glu R_3= Glu. – Ara. – Ara. | Glu  R_4=		
9	Pitheduloside I	R_1= Glu. – Ara. – Ara., R_2=OH, | Glu R_3= Glu. – Ara. – Ara. | Glu  R_4=	Seed	(Yoshikawa et al., 1997)
10	Pitheduloside J	R_1= Glu. – Ara. – Ara., | Glu R_2=OH R_3= Glu. – Rha.. – Ara. | Glu.  R_4=		
11	Pitheduloside K	R_1= Glu. – Ara. – Ara., R_2=OH, R_3= R_4=H | Glu 		
12	Oleanolic acid	R=H, R_1= R_2= R_3=CH_3	Seed	(Murugesan et al., 2019)
13	Hederagenin	R= H, R_1=CH_2OH, R_2=, R_3=CH_3		

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

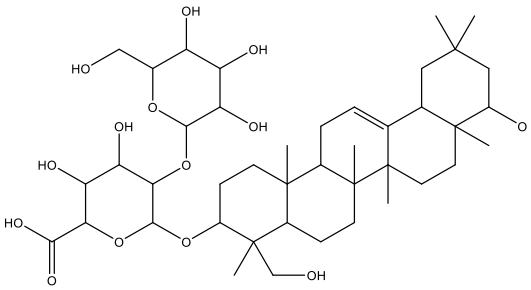
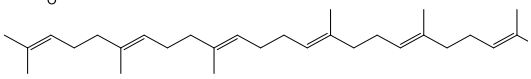
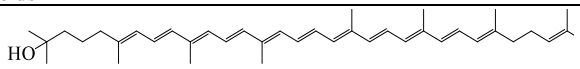
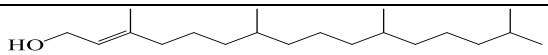
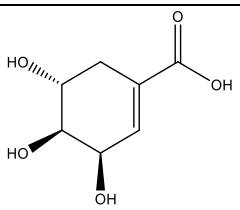
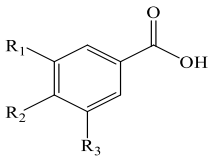
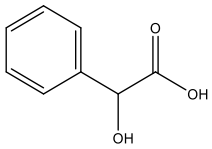
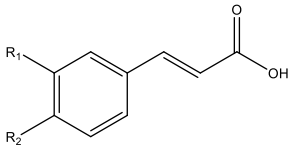
No.	Compound	Structure	Plant Organ	References
1. **Terpenoids**				
	1.1. **Triterpenoids**			
14	Echinocystic acid	R= OH, R_1= R_2= R_3=CH_3	Seed Stem Bark	(Katekhaye and Laddha, 2015)
15	Soyasaponin III		Seed	(Alhamed et al., 2023)
16	Squalene		Leaves	(Bobade, 2017)
	1.2. Tetraterpenoids			
17	Rhodopin		Leaf	(Bobade, 2019)
	1.3. Diterpenoids			
18	Phytol		Leaf	(Srinivas et al., 2018)
2. Phenolic compounds				
	2.1. Phenolic Acids			
19	Shikimic acid		Fruit	(Vargas et al., 2020)
				
20	Gallic acid	R_1=R_2=R_3=OH		
21	Vanillic acid	R_1=OCH_3, R_2=OH, R_3=H		
22	Mandelic acid		Fruit	(Murugesan et al., 2019)
				
23	Cinnamic acid	R_1=R_2=H		(Vargas et al., 2020)
24	Coumaric acid	R_1=H, R_2=OH	Fruit	(Murugesan et al., 2019)
25	Caffeic acid	R_1=R_2=OH		(Vargas et al., 2020)

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

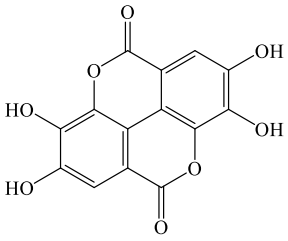
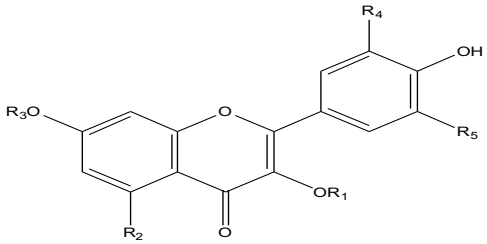
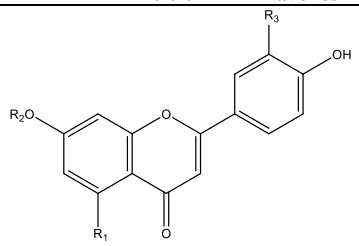
No.	Compound	Structure	Plant Organ	References
2. **Phenolic compounds**				
	2.1. **Phenolic Acids**			
27	Ellagic acid		Fruit	(Vargas et al., 2020)
	2.2. Flavonoids			
	2.2.1. Flavonols			
				
28	Kaempferol	R_1=H, R_2=OH, R_3=R_4=R_5=H	Leaf	(Srinivas et al., 2018)
29	kaempferol-3-rhamnoside (Afzelin)	R_1=Rha., R_2= OH, R_3= R_4= R_5 =H	Seed	(Alhamed et al., 2023)
30	Kaempferol 7-O-*β*-D-glucopyranoside	R_1=H, R_2=OH, R_3=Glu., R_4= R_5 =H	Leaf Fruit Fruit Peel	(Srinivas et al., 2018; Kulkarni and Jamakhandi, 2018)
31	Quercetin	R_1=H, R_2= R_3=H, R_4=OH, R_5=H	Fruit	(Murugesan et al., 2019)
32	Rutin	R_1=Glu–Rha., R_2= OH R_3= H, R_4=R_5= OH	Fruit	(Vargas et al., 2020)
33	Myricetin	R_1=Rha., R_2= OH, R_3= R_4= R5=H.		
	2.2.2. Flavones			
				
34	Apigenin	R_1=OH, R_2=R_3=H	Fruit	(Vargas et al., 2020)
35	Luteolin	R_1=OH, R_2=H, R_3=OH		(Vargas et al., 2020)
36	Prenylapigenine	R_1=OH, R_2=H, R_3= CH_2-CH-CH-$(CH_3)_2$	Stem	(Kulkarni and Jamakhandi, 2018)
37	3'-prenylapigenin-7-O-glucoside	R_1=OH, R_2=Glu., R_3= CH_2-CH-CH-$(CH_3)_2$	Stem Bark	(Katekhaye and Laddha, 2015; (Saxena and Singhal, 1999)
38	3'-prenylapigenin-7-O-rutinoside	R_1=OH, R_2=Glu. -Rha., R_3= CH_2-CH-CH-$(CH_3)_2$		

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

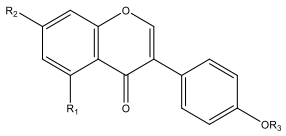
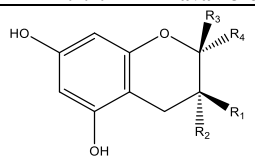
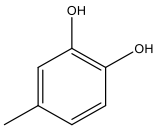
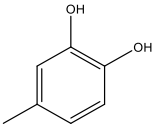
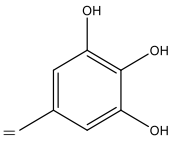
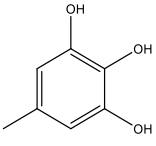
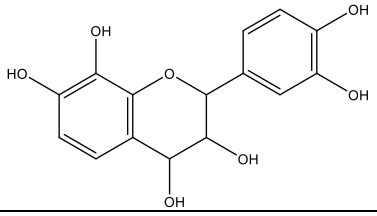
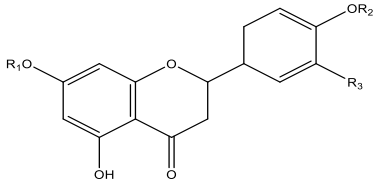
No.	Compound	Structure	Plant Organ	References
2. **Phenolic compounds**				
2.2. Flavonoids				
2.2.3. Isoflavonoids				
				
39	Genistein	R_1=OH, R_2= OH, R_3= H.	Fruit	(Vargas et al., 2020)
40	Genistein 4'-O-*α*-L-rhamnopyranoside	R_1=OH, R_2= OH, R_3= Rha.	Root	(Saxena and Singal, 1998)
41	Daidzein	R_1=H, R_2= OH, R_3= H.	Fruit	(Murugesan et al., 2019)
2.2.4. Flavan-3-ol				
				
42	Catechin	R_1=OH, R_2= H, R_3=H, R_4= 		
43	Epicatechin	R_1=H, R_2= OH, R_3=  R_4 =H.	Fruit	(Vargas et al., 2020)
44	Epigallocatechin	R_1=H, R_2= OH, R_3=H, R_4 = 		
45	3',4',5',7-tetrahydroxy flavan-3-ol (Robinetinidol)	R_1=OH, R_2= H, R_3=  , R_4 =H	Stem Bark	(Katekhaye and Laddha, 2015)
46	Melacacidin		Wood	(Murugesan et al., 2019)
2.2.5. Flavanones				
				
47	Naringin	R_1= Glu. -Rha., R_2= CH_3, R_3= OH	Fruit	(Murugesan et al., 2019)
48	Hesperetin	R1= Glu. -Rha., R2= H, R3= H		(Vargas et al., 2020)

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

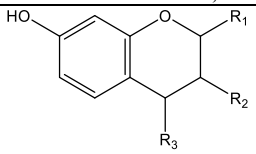
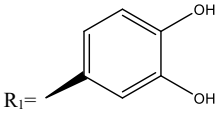
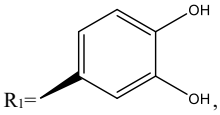
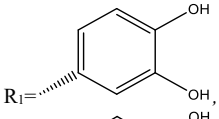
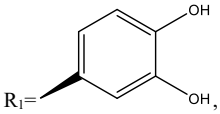
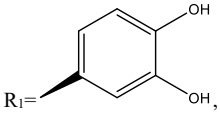
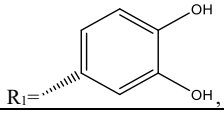
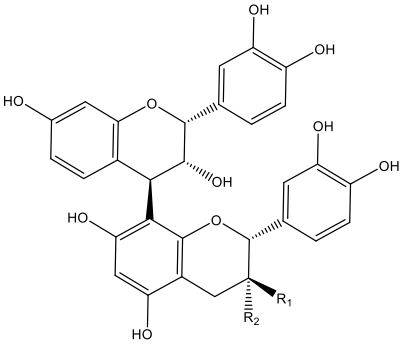
No.	Compound	Structure	Plant Organ	References
2. Phenolic compounds				
2.2. Flavonoids				
2.2.6. Flavan-3,4-diol				
49	3',4',7-trihydroxy flavan-3,4-diols	 R_1= , R_2=R_3=OH		
50	Epifisetinidol-4α-ol	 R_1= , R_2=R_3=α-OH		
51	Epifisetinidol-4β-ol	 R_1= , R_2=R_3=β-OH	Stem Bark	(Katekhaye and Laddha, 2015)
52	Fisetinidol-4α-ol	 R_1= , R_2= β-OH, R_3=α-OH		
53	Fisetinidol-4β-ol	 R_1= , R_2=R_3=β-OH		
54	Leucofisetinidin	 R_1= , R_2= α-OH, R_3=β-OH	Wood	(Murugesan et al., 2019)
2.3. Procyanidins and Proanthocyanidins				
				
55	Epifisetinidol-(4β,8)-catechin	R_1=OH, R_2=H	Stem Bark	(Katekhaye and Laddha, 2015)`
56	Epifisetinidol-(4β,8)-epicatechin	R_1=H, R_2= OH		

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

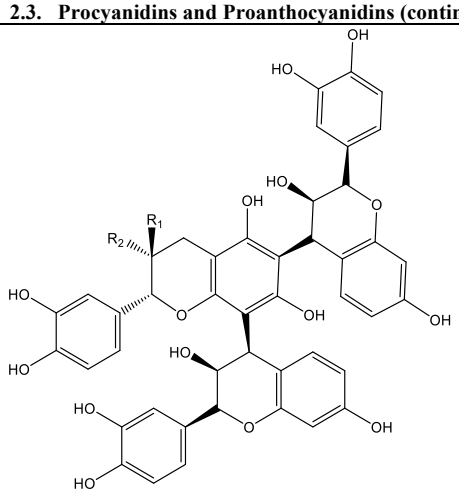
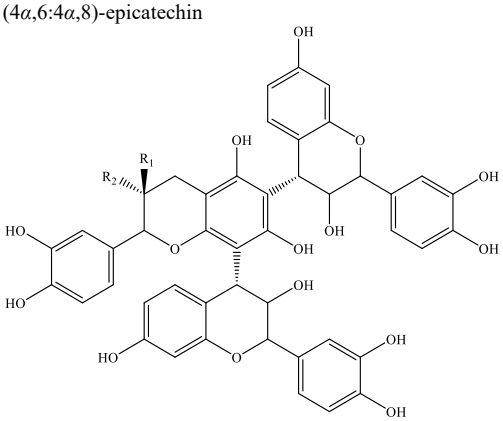
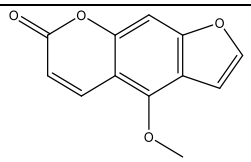
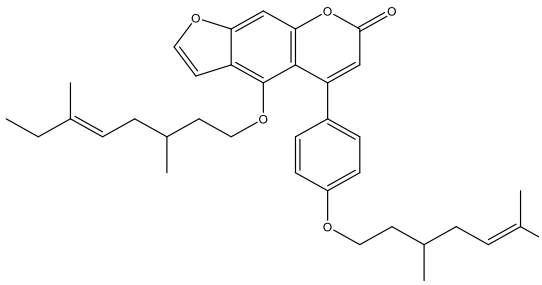
No.	Compound	Structure	Plant Organ	References
	2. Flavonoids			
	2.3. Procyanidins and Proanthocyanidins (continue)			
				
57	Bisepifisetinidol-(4*α*,6:4*α*,8)-catechin	R_1=OH, R_2=H	Stem Bark	(Katekhaye and Laddha, 2015)
58	Bisepifisetinidol-(4*α*,6:4*α*,8)-epicatechin	R_1=H, R_2=OH		
				
59	Fisetinidol-(4*α*,8)-catechin-(6,4*α*)-epifisetinidol	R_1=OH, R_2=H	Stem Bark	(Katekhaye and Laddha, 2015)
60	Fisetinidol-(4*α*,8)-epicatechin-(6,4*α*)-epifisetinidol	R_1=H, R_2=OH		
	2.4. Coumarins			
61	Bergapten.			
62	4(2,3-dihydro geranyl oxy)-5(4(2,3-dihydro geranyl oxy) phenyl bergapten		Stem Bark	(Katekhaye and Laddha, 2015)

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

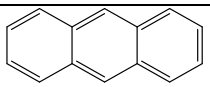
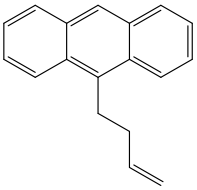
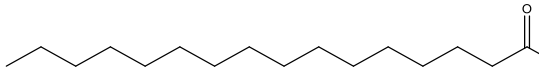
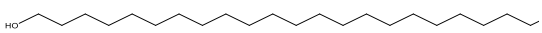
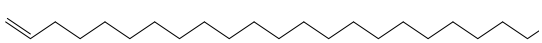
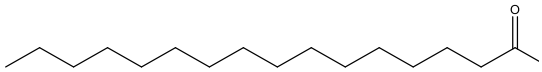
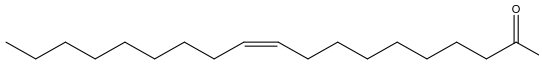
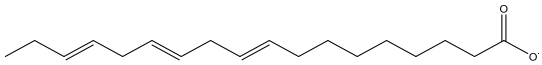
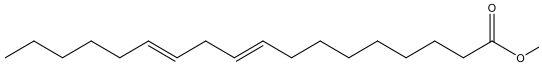
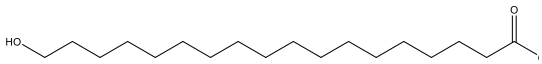
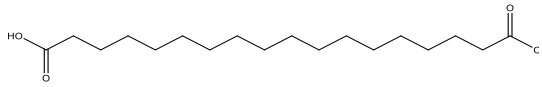
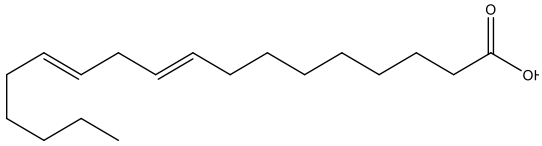
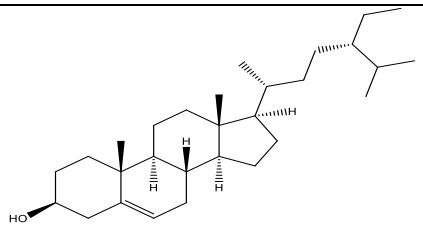
No.	Compound	Structure	Plant Organ	References
	3. Anthracenes			
63	Anthracene			(Srinivas et al, 2018)
64	9(3butenyl) anthracene		Leaf	(Vanitha and Manikandan, 2016)
	4. Fatty Acids			
65	Heptacosanoic acid			
66	Hexadecenoic acid (palmitic acid)		Fruit	(Kulkarni and Jamakhandi, 2018)
67	Tetracosanol			
68	22-tricosenoic acid			
69	Hexadecenoic acid methyl ester			(Bobade, 2019)
70	9 Octadecenoic acid (Z),			(Bobade, 2017)
71	9,17-octadecadienal		Leaf	
72	Ethyl 9,12,15-octadecatrienoate			(Vanitha and Manikandan, 2016)
73	9,12-octadecadienoic acid ethyl ester			
74	Hydroxystearic acid			
75	Octadecanedioic acid			
76	Linoelaidic acid		Seed	(Alhamed et al., 2023)
	5. Sterols			
77	β-sitosterol		Stem Bark	(Katekhaye and Laddha, 2015)

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

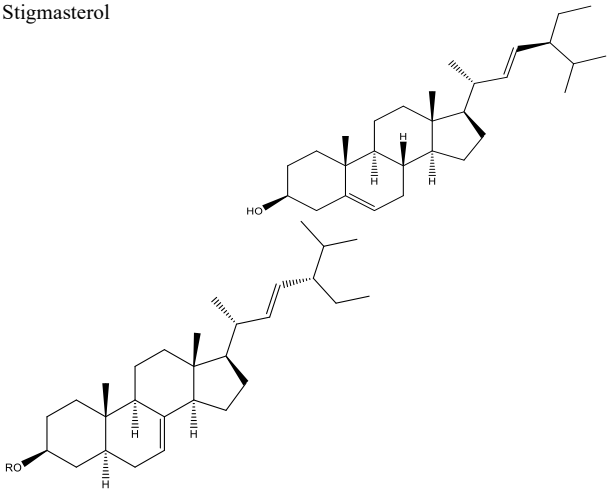
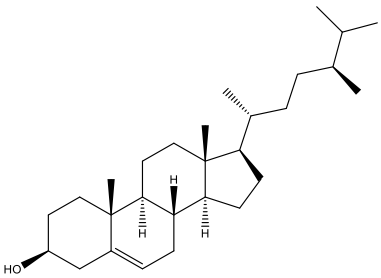
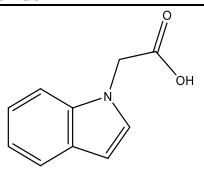
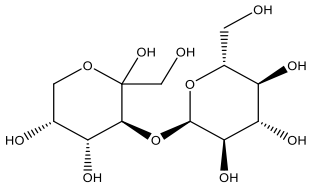
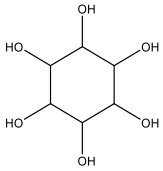
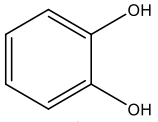
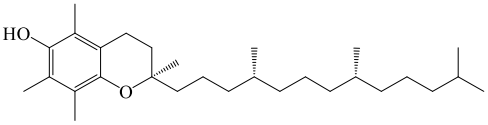
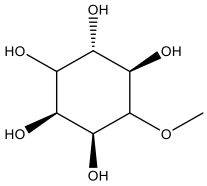
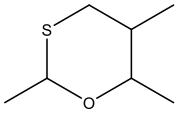
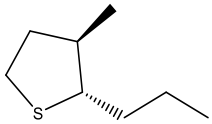
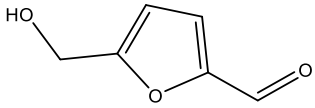
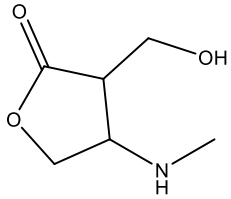
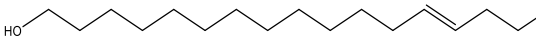
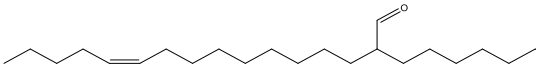
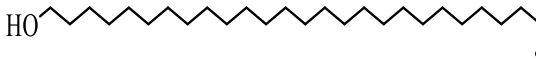
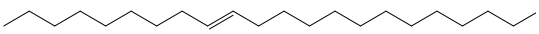
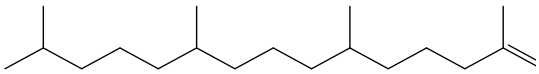
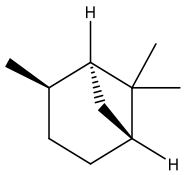
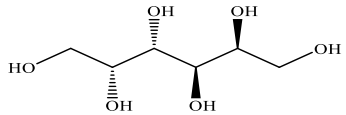
No.	Compound	Structure	Plant Organ	References
	5. Sterols			
78	Stigmasterol		Stem Bark Seeds Fruit Peel	(Kulkarni and Jamakhandi, 2018)
79	ά-spinasterol	R=H.	Stem Bark Leaf	
80	β-Glucoside–*α* spinasterol	R=Glu.	Leaf	
81	Campesterol		Wood Stem Bark	(Katekhaye and Laddha, 2015)
82	Pithogenin	C28H44O4	Seed	(Murugesan et al., 2019)
	6. Miscellaneous Compounds			
83	Indole-1-acetic acid			
84	D-Turanose		Seed	(Aldarhami et al., 2023)
85	Inositol			
86	Catechol		Bark	(Murugesan et al., 2019)
87	Tocopherol		Fruit	(Vargas et al., 2020)

Table 2. Different classes of chemical constituents of *P. dulce* (continued)

No.	Compound	Structure	Plant Organ	References
	6. Miscellaneous Compounds			
88	D-Pinitol		Fruit Peel	
89	2, 5, 6-trimethyl 1, 3-oxathiane			(Vargas et al., 2020)
90	Trans-3-methyl-2-N-propylthiophane		Fruit	
91	2-furan carboxaldehyde-5 (hydroxymethyl)			
92	3-(hydroxymethyl)-4-(methylamino)-dihydrofuran-2(3H)-one		leaf	(Wichaidit and Thongyoo, 2021)
93	13 octadecenol			(Vanitha and Manikandan, 2016)
94	2-octyl-cis-11-hexdecenal		Leaf	
95	Octacosanol			(Murugesan et al., 2019)
96	13-docosenamide			
97	2-hexadecene,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]		Leaf	(Vanitha and Manikandan, 2016)
98	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-, (1alpha,2beta,5alpha			
99	Dulcitol		Leaf	(Srinivas et al., 2018)

6. Nutritional Value

P. dulce fruits provide important vitamins like thiamine, ascorbic acid, riboflavin (Figure 2A), and several necessary amino acids like phenylalanine, valine, tryptophan, and lysine (Figure 2B). They also contain a small number of critical minerals including K, P, Na, Ca, and F (Figure 2C) (Dhanisha et al., 2022b). *P. dulce* fruit has the potential to stop oxidative damage and to scavenge free radicals due to the phenols, flavonoids, and saponins content (Katekhaye and Kale, 2012). According to reports, 100 g of seeds contain the following: ash (2.8%), carbohydrate (41.4%), fiber (7.8%), protein (17.7%), and water (13.5%) (Figure 2D). The fat is composed of many fatty acids as described in (Figure 2E) (Murugesan et al., 2019).

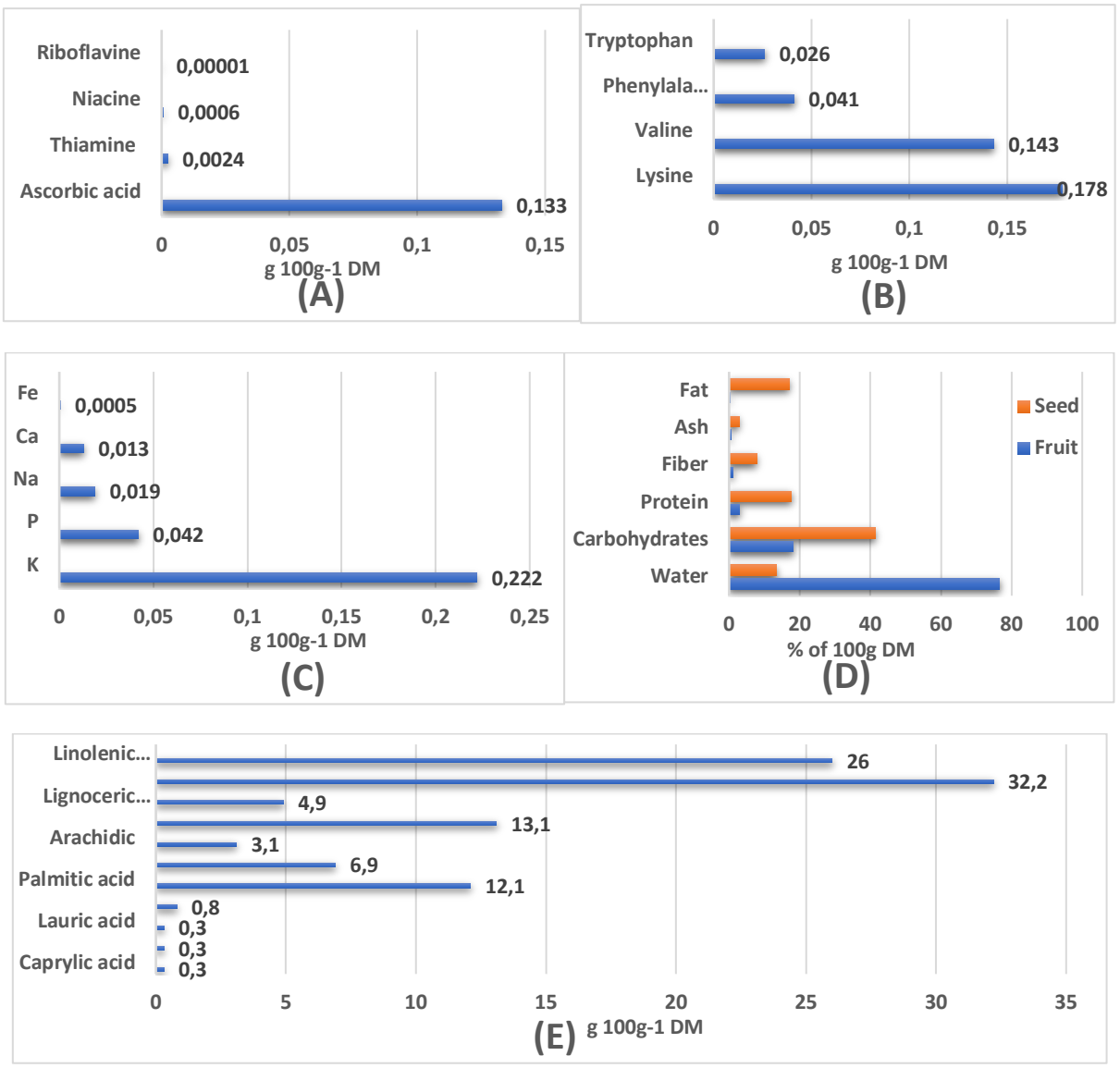


Figure 2. Nutritional value of different parts of *Pithecellobium dulce* (Roxib.) Benth. A. Fruit vital vitamin content. B. Fruit amino acids content. C. Essential minerals in fruit arils. D. Composition of dried fruit and seed. E. Seed Fat content).

7. Biological Activities

The different parts of *P.dulce* were used traditionally for many biological activities in which many of these activities have been proved scientifically by different studies. The presence of many biologically active phytoconstituents in the different parts of *P. dulce* may contribute to the anti-inflammatory, anti-diabetic, anti-diarrheal, anti-microbial, anti-convulsant, anti-ulcer, antioxidant, anti-cancer, hepatoprotective, cardioprotective and nephroprotective activities summarized in (Figure 3).

7.1. Analgesic /anti-inflammatory activity

The anti-inflammatory bisdesmodic triterpenoidal saponin (Dulcin) was identified from the seeds of *P. dulce* (Sahu and Mahato, 1994).

Leaves methanolic, ethanolic, and aqueous extract showed significant results when evaluated using the hot plate assay and acetic acid-induced writhing assay in mice for the analgesic activity and rat paw edema test for the anti-inflammatory activity (Sugumaran et al., 2009; Selvan and Muthukumaran, 2011). Another study in which the leaf's ethanolic extract was tested using the membrane stabilization of the HRBC (human red blood cell) assay and the albumin denaturation

inhibition assay compared to aspirin. The percentage of HRBC membrane stabilization was found to be 59.25% and the inhibition of albumin denaturation was 62.80% (Kalavani et al., 2016).

The anti-inflammatory properties of the aqueous extract of the bark, leaf, and fruit were investigated utilizing the inhibition of albumin denaturation method against diclofenac sodium as a standard medication. The three extracts inhibited albumin denaturation effectively. The maximum inhibition percentage for the bark extract was 52.73% (Nagendra et al., 2019).

The radiographic and histopathological examination of the joints revealed the antiarthritic activity of *P. dulce* leaf ethanolic extract at a dose of 250 mg kg^{-1}. Indomethacin was utilized as a reference drug and formaldehyde was used for the induction of arthritis (Mishra et al., 2021).

7.2. Anti-diabetic activity

The bark hydro-alcoholic extract was screened for antidiabetic activity using oral doses of 200 mg kg^{-1} and 400 mg kg^{-1} in alloxan-induced diabetic rats. The 400 mg kg^{-1} concentration showed significant antidiabetic activity and reduced cholesterol and triglyceride levels. glibenclamide was used as a reference drug (Praveen et al., 2010).

In vitro, α-amylase and α-glucosidase activity were evaluated using methanolic and 70% acetone leaves and bark extracts against acarbose. The extracts inhibited the sucrase enzyme more effectively than the maltase enzyme. (Katekhaye and Nagmoti, 2013).

The fruit ethanolic extract was tested for antidiabetic activity against gliclazide utilizing a daily 300 mg kg^{-1} oral dose administered to streptozotocin (STZ) induced diabetic rats. The extract showed significant inhibition in the blood glucose, glycosylated hemoglobin (HBA1C), urea, and creatinine levels. Aminotransferases, alkaline phosphatase (ALP), plasma protein, plasma insulin, and hemoglobin levels were all normalized. (Pradeepa et al., 2013).

The seed methanolic extract was studied for the inhibitory activity of α-glucosidase and α-amylase enzymes. The results demonstrated considerable efficacy against pancreatic-amylase and superior activity against maltase over sucrase enzyme (Nagmoti and Juvekar, 2013). Also, the oral administration of different doses of the methanolic extract resulted in a significant drop in HbA1C and fasting blood glucose while increasing serum insulin, total protein, liver glycogen levels, and body weight. Metformin was used as a reference drug (Nagmoti et al., 2015).

Two isolated compounds from the fruit peel methanolic extract which tested positive for the Molisch test were tested using non-enzymatic glycosylation of hemoglobin assay and enzymatic α-amylase assay. Compound 1 was more potent than compound 2 at concentrations of 0.2 mg dl^{-1} to 1.0 mg dl^{-1} (Praylin et al., 2015).

The anti-diabetic effect of the fruit peel aqueous extract was examined in STZ-induced diabetic rats by administering 200 mg kg^{-1} of the extract orally. Glibenclamide is used as a standard drug. The levels of urine sugar, blood glucose, HBA1C, ALP, glucose-6-phosphatase, fructose-1,6-bisphosphatase, aspartate transaminase (AST), total cholesterol, alanine transaminase (ALT) and triglycerides were reduced while the levels of liver glycogen, insulin, hexokinase, protein, superoxide dismutase, glutathione peroxidase, and catalase were decreased (Sukantha et al., 2016).

In dexamethasone-induced diabetic rats, aqueous and ethanolic leaf extracts were evaluated against pioglitazone via oral administration of 200 mg kg^{-1} and 400 mg kg^{-1}. They demonstrated considerable anti-diabetic and anti-hyperlipidemic efficacy. (Mule et al., 2016).

The saponin-enriched fraction from the seed extract was investigated for possible antihyperglycemic activity using the *in vitro* α-glucosidase and α-amylase inhibitory assay and *in vivo* sucrose tolerance test against standard drug acarbose. The extract inhibited both glucosidase and amylase enzymes more effectively than the conventional medication employed. It may be linked to limiting sucrose hydrolysis (Kumar et al., 2017).

7.3. Anti-diarrheal activity

Castor oil-induced diarrhea in rats was used to test the aqueous and ethanolic extracts of the leaves. The aqueous extract was more powerful. Diphenoxylate HCl was utilized as a control medication(Sugumaran et al., 2008a). Another study only employed the ethanolic extract of the leaf and loperamide as a control medication. The results demonstrated considerable antidiarrheal activity by prolonging the latent period and decreasing defecation frequency (Venu et al., 2016).

7.4. Anti-hyperlipidemic activity

In triton-induced hyperlipidemic rats, an oral dosage of 200 g kg^{-1} of *P. dulce* leaves aqueous extract was employed. The extract significantly reduced serum total cholesterol, phospholipids, triglyceride, LDL, and very low-density lipoproteins (VLDL) levels while increasing serum HDL levels. Fenofibrate was used as a standard treatment (Rajan and Kumar, 2010).

The crude methanolic extract of the seeds resulted in significant inhibition of the LDL, VLDL, triglycerides, and total cholesterol (Nagmoti et al., 2015).

7.5. Anti-microbial activity

Agar well diffusion assay was utilized for evaluating the anti-microbial effect of *P. dulce* leaf extract in aqueous and different organic solvents against *Enterococcus faecalis, Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus*, and *Staphylococcus epidermidis* Gram-positive bacteria and *Alcaligenes faecalis, Aeromonas hydrophila*, *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* gram-negative bacteria and eight fungi *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae*, *Alternaria alternata*, *Alternaria solani, Alternaria vitis*, and *Alternaria alternata.* The aqueous leaf extracts demonstrated no antimicrobial effect against all the examined microbes. While the different organic solvent extracts demonstrated outstanding activity against most of the examined bacteria (Kumar et al., 2013).

The antimicrobial activity of leaf methanolic extract against, *S. aureus*, and *S typhimurium*, as well as two fungal strains, *A. niger,* and *Candida albicans,* was studied using chloramphenicol as a standard drug. The extract demonstrated antibacterial activity against *S. aureus* greater than against *S. typhimurium*. Conversely, it had greater antifungal activity against *A. niger* than against *C. albicans* (Idris et al., 2020).

Different organic solvents and alkaloidal extracts of *P. dulce* leaves were evaluated for anti-microbial activity against *Mycobacterium tuberculosis, C. albicans, and A. niger* using rifamycin, fluconazole, and nystatin as standard drugs. All the extracts were inactive against *A. niger*. while the alcoholic and total alkaloidal extracts were active against *M. tuberculosis* and *C. albicans* (Shanmugakumar et al., 2006).

Bark methanolic extract was tested for anti-microbial activity against *A. fumigatus, C. albicans, S. aureus, E. coli, B. subtilis,* and *Proteus vulgari.* Gentamycin and ketoconazole were used as standard drugs. The extract was only active against *E. coli* and *P. vulgari* (Kotb et al., 2022).

7.5.1. Anti-bacterial activity

Using streptomycin as the reference medication, the ethyl acetate floral *P. dulce* fraction containing the flavonoid glycoside quercetin was shown to have antibacterial activity against *S. typhi* and *E. coli* gram-negative and *S. aureus* gram-positive (Chandran and Balaji, 2008).

The antibacterial activity of *P. dulce* fruit peel aqueous and several organic solvent extracts against various organisms was investigated. According to (Sukantha et al., 2011), the ethyl acetate fraction was effective against *S. aureus, E. coli, S. epidermis, K. pneumonia, E. faecalis, P. putida,* and *P. aeruginosa*. Whereas the methanolic extract was effective against *P. putida, S. aureus,* and *K. pneumonia*, the aqueous extract was active against *S. aureus* and *K. pneumonia* only, while the petroleum ether extract was effective only against *P. putida***.** On the other hand (Sukantha et al., 2014) reported that all the extracts displayed antibacterial properties, although the methanol extract had superior antimicrobial properties compared to the aqueous and ethyl acetate extracts. *S. aureus* and *K. pneumonia* were the organisms that were most sensitive to all the extracts, whilst *P. mirabilis* and *P. vulgaris* were the most resistant organisms. Polymyxin and rifampicin are used as standard drugs.

P. dulce biologically generated silver nanoparticles exhibited satisfactory antibacterial activity against *S. aureus*, *E. coli, P. aeruginosa,* and *C. albicans*. The higher the concentration of crude extract, the higher the diameter of the inhibition zone (Lakshmi et al., 2014).

The disc diffusion assay was utilized to test the leaves methanolic extract, n-hexane, ethyl acetate, chloroform, and aqueous fractions against two gram-positive bacteria (*B. cereus* and *S. aureus*) and four gram-negative bacteria (*Proteus, Shigella boydii, Pseudomonas specious* and *E. coli*). Kanamycin was used as a conventional medication. The growth of the gram-negative bacteria was

inhibited effectively by the ethyl acetate fraction, while the methanolic fraction was most active against gram-positive bacteria (Akter et al., 2020).

Ethyl acetate aril parts extract was evaluated against some gram-negative strains using the disc diffusion and the agar well diffusion assays and chloramphenicol as a standard drug. The treated sample showed activity against *Shigella flexneri, Salmonella enteric*, and *K. pneumoniae* (Hepzibah et al., 2017).

The leaf ethanolic extract was screened using three assays against seven gram-negative and gram-positive bacteria including *S. boydii, S. typhi, Pseudomonas, Shigella dyst-1, S. sonnie, S. fleas, Plesiomonas*, *Staphylococcus saprophyticus, S. aureus, and S. epidermidis*. Only *S. dyst-1* was susceptible to the antimicrobial effects of the leaf extract (Kulkarni and Jamakhandi, 2018).

P. dulce root extracts in hexane, benzene, ethyl acetate, and ethanol were evaluated using the disc diffusion assay against three gram-negative *(Acetobacter aceti, Acetobacter aceti,* and *K. pneumoniae)* and one gram-positive bacteria *(S. aureus).* The results demonstrated that polar extracts have more antibacterial activity than non-polar extracts (Bhat et al., 2018).

The methanolic extract of the seed demonstrated significant activity against clinically relevant multidrug-resistant bacteria in which *Acinetobacter baumannii* had a MIC of 233 mg ml^{-1}, while *E. coli* and *S. aureus* had MIC of 300 mg ml^{-1}. A molecular docking study was conducted to identify the best compounds with high affinity for two *S. aureus* receptors and low binding energy. Turanose had energy values of (-6.6 and -7.4) kcal mol^{-1}, whereas inositol had (-5.4 and -7.2) kcal mol^{-1} for 2XCT and 1JIJ receptors, respectively (Aldarhami et al., 2023).

7.5.2. Anti-fungal activity

Fungistatic and fungicidal activities of *P. dulce* seeds on plant pathogens such as *Penicillium digitatum, Botrytis cinerea, Rhizopus stolonifer,* and *Fusarium oxysporum.* The anti-fungal activity of the aqueous extract against *P. digitatum, B. cinerea,* and *R. stolonifera* is mostly due to the presence of kaempferol and a few other compounds (Bautista-Baños et al., 2003). In addition, the triterpenoidal saponins Pithedulosides A, B, E, F, and I inhibited the growth of *Colletotrichum gloeosporioides* mycelium and *R. stolonifer in vitro* (Shweta, 2013).

7.6. Anti-obesity activity

P. dulce fruit peel different organic solvent extracts at two different doses (100 and 200 mg kg^{-1}) were used for the assessment of anti-obesity compared to orlistat. The findings indicated that the petroleum ether, ethyl acetate, and methanolic extracts had potential anti-obesity activity (Jagadeeshwar et al., 2021).

7.7. Antioxidant activity

Six different methods were utilized to evaluate the antioxidant activity of the fruit pericarp methanolic and acidified methanol extracts and the anthocyanin extracted from them. According to the findings, acidified methanol extract has a higher vitamin C concentration and antioxidant scavenging activity than methanolic extract (Ponmozhi et al., 2011).

Leaves and wood bark methanolic and 70% acetone extracts were studied using DPPH, hydroxyl radical, superoxide radical, hydrogen peroxide (H_2O_2), nitric oxide (NO), hypochlorous acid, and singlet oxygen scavenging activity assays. The results revealed that the leaves and wood bark have significant antioxidant activity with good content of total phenolic and flavonoid and have good iron chelating activity (Katekhaye and Kale, 2012). *In-vitro* antioxidant activity assessment of different fractions of the crude methanolic leaf extract using DPPH, reducing power, hydroxyl radical, and H_2O_2 scavenging assays, revealed a significant antioxidant property with higher activity of ethyl acetate fraction (Akter et al., 2020).

Water-soluble polysaccharides isolated from the seeds were tested using scavenging of DPPH radicals, H_2O_2, and reducing power assays. The polysaccharides fraction showed a strong dose-dependent free radical scavenging activity compared to the standard ascorbic acid (Bagchi and Kumar, 2016).

In-vitro ferric-reducing antioxidant power (FRAP), DPPH, and NO assays were performed on aqueous, methanolic, and acetone *P. dulce* leaf extracts. The FRAP assay demonstrated that the water

extract had the most scavenging activity, while the DPPH assay revealed that the acetone extract had the highest activity and the NO assay revealed that the methanolic extract had the highest activity (Kumari, 2017).

A flavanol glycoside kaempferol- 3-O-α-rhamnoside isolated from the leaf ethyl acetate fraction demonstrated strong activity in the DPPH assay (IC_{50} 14.6 µg ml^{-1}). It effectively inhibited the oxidative damage of erythrocytes induced by AAPH and protected the plasmid DNA from oxidative degradation (Akter et al., 2022).

7.8. Antiparasitic activity

P. dulce fruit methanolic extract, the ethyl acetate fraction, and the identified compound N-malonyl-(þ)-tryptophan which was isolated from the methanolic extract were found to possess *in vitro* activity against *Hymenolepis nana* the most common intestinal tapeworm in humans globally (López-Angulo et al., 2019).

7.9. Anti-ulcerogenic activity

The hydroalcoholic fruit extract was tested for the anti- gastric (Megala and Geetha, 2010 and 2012b) and anti-duodenal ulcer (Megala and Geetha, 2015) activity, and the extract was administered pre- and post-ulcer induction. The gastric ulcer was induced by chemicals and stress and omeprazole was used as a reference drug. On the other hand, the duodenal ulcer was induced by cysteamine, and ranitidine was used as a reference drug. Both studies demonstrated significant anti-ulcerogenic activity.

When compared to ranitidine, the *P. dulce* alcoholic and aqueous extract of seeds were effective in preventing ulcers in pyloric-ligated rats and significantly lowered stomach volume, total acidity, free acidity, and ulcer index (Palanivel et al., 2014).

7.10. Anti-venom activity

P. dulce water bark extract was able to reduce the venom's capacity to necrotize tissue and hindered its lethality. Due to the extract's high tannin concentration, it successfully inhibited 90% of acetylcholine esterase activity. Using Autodock 3, the binding energies of tannic acid (14.7 kcal mol^{-1}), di-gallic acid (10.38 kcal mol^{-1}), and four other tannin compounds were examined. The extract non-selectively precipitates the venom protein while blocking the nicotinic acetylcholine receptor (Pithayanukul et al., 2005).

7.11. Cardio-protective activity

The cardioprotective activity of the aqueous and ethanolic fruit peel extract was assessed using the marker enzymes lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGPT), serum glutamate pyruvate transaminase (SGOT), and creatine phosphokinase (CPK) all of which were considerably increased by isoproterenol. The cardiac damage was greatly reversed by extract coadministration. Verapamil was used as a reference medication (Thangarajan et al., 2015).

Isoproterenol-induced heart injury is reversed by *P. dulce* fruit and floral extracts. The effects of plant extracts against myocardial infarction were substantially identical to those of the common cardioprotective drug verapamil (Srinivas et al., 2018).

7.12. Cytotoxicity activity

P. dulce leaf extract demonstrated a significant effect on breast cancer cells. The methanolic extract was used by (Poongodi and Hemalatha, 2015) and The IC_{50} value was found to be 112 µg $ml^{-1,}$ and 100% cell inhibition was achieved at 300 µg ml^{-1}. On the other hand, (Sharma, 2016) used the crude aqueous extract and the cytotoxicity was time and dose-dependent because 300 mg ml^{-1} of the extract reduced cell viability to 50% (IC_{50}) in 48 hours.

The bark and leaf extracts were studied against cervical cancer cells (HeLa) (López et al., 2013), human colorectal adenocarcinoma cell line (Caco-2) (Knauth et al., 2018), and hepatocellular (HepG-2) and colon (HCT-116) cell lines (Kotb et al., 2020). The first demonstrated that the aqueous bark extract was more cytotoxic than the leaf aqueous and ethanolic extracts. The second showed that the

methanolic leaf extract was more cytotoxic than the bark extract. While the third reported that the lipophilic fractions had no significant cytotoxic effect against HCT-116 and HepG-2.

P. dulce aqueous and ethanolic bark extracts' cytotoxic effects were assessed against three cell lines MCF-7, HCT-116, and HepG2 using the conventional MTT colorimetric technique at various doses. With a 1.71% cell viability, the plant's aqueous extract showed the highest level of toxicity against HepG2. On the other hand, with a viability of 6.05%, the ethanolic extracts had the highest toxicity against HCT-116. The plant's bark can be used to make anticancer medications using the right standardized techniques (Jalique et al., 2017).

The identified component Kaempferol-3-O-α-L-rhamnoside from the ethyl acetate fraction of the leaves methanolic extract demonstrated an anti-tumor effect on Ehrlich ascites carcinoma cells (EAC). The standard anticancer medicine vincristine demonstrated growth inhibition of 77.84±6.69% while the extract demonstrated 70.89 ± 6.62% EAC cell growth inhibition (Akter et al., 2022).

A significant activity of hydroalcoholic fruit extract against murine melanoma (B16F10) and lung adenocarcinoma in humans (A549) by MTT assay was reported with IC_{50} =119 and 114 g ml^{-1} respectively (Dhanisha et al., 2022a).

P. dulce seeds crude methanolic extract at different concentrations was utilized to evaluate the cell viability of colorectal (LoVo), human umbilical vein endothelial cells (HUVECs), MCF-7, and A-549 cell lines using MTT assay and doxorubicin as positive control. The LoVo cell line viability % was inhibited in a concentration-dependent manner by increasing the cell apoptosis rate, the number of cells at the sub-G1 phase of the cell cycle, and decreasing the rate of migration of LoVo cells in the scratch assay. When compared to LoVo cells IC_{50} 3.03 ± 0.1, the extract had a reduced cytotoxic effect on HUVEC with an IC_{50} of 6.24 ± 0.25 µg/ml. Octadecanedioic acid, hydroxystearic acid, linoelaidic acid, soya-saponin III, and kaempferol 7-O-beta-D-glucopyranoside were isolated in the same study and correlated to the anticancer activity (Alhamed et al., 2023).

7.13. Hepato-protective activity

Two studies were made to prove the hepatoprotective effect of *P. dulce* fruit (Manna et al., 2011) used the aqueous extract against carbon tetrachloride (CCl_4)-induced hepatic injury, while (Raju and Jagadeeshwar, 2014) used the ethanolic and aqueous extracts against alcohol and paracetamol-induced hepatic injury and silymarin as a standard drug. The first study showed that both pre and post-treatment with the extract protected against hepatic damage induced by CCl_4.The second study showed a significant hepatoprotective effect of the extracts compared to the toxic control.

Under *in vivo* conditions, *P. dulce* bark extract exhibited hepatoprotective activity at concentrations of 100 and 200 mg kg^{-1}. The extract resulted in a significant reduction in hepatic enzymes when compared with acetaminophen (Singh and Shukla, 2013).

In paracetamol-induced hepatotoxicity, an ethanolic extract of *P. dulce* leaves significantly reduced SGOT, SGPT, alkaline phosphatase (ALP), triglyceride, and bilirubin levels (Sul et al., 2021).

7.14. Mosquito repellent activity

Mosquito repellents obtained from natural sources can be used instead of chemically based repellents, which are usually poisonous to other creatures and may cause respiratory defects in humans. The aqueous leaf extract used for the synthesis of silver nanoparticles showed significant larvicidal activity against *C. quinquefasciatus* (Raman et al., 2012).

Various *P. dulce* leaf and seed extracts were tested for larvicidal and ovicidal effectiveness against mosquito vectors *Anopheles stephens*i and *Aedes aegypti*. The methanolic leaf extract demonstrated the most activity(Govindarajan et al., 2013).

Govindarajan and Rajeswaryn (2014) tested the adulticidal activity of *P. dulce* leaf and seed various solvent extracts against the filariasis vector mosquito *Culex quinquefasciatus*. Methanol extract had the strongest larvicidal and ovicidal action, followed by ethyl acetate, chloroform, benzene, and hexane extracts. The highest death rate (100%) was recorded at 500 mg L^{-1} and 750 mg L^{-1} of the leaf and seed extracts, respectively. Furthermore, crude extracts of the plant's leaf and seed protected against mosquito bites in a concentration-dependent way while causing no adverse reactions (Govindarajan and Rajeswary, 2015).

P. dulce leaf hydroalcoholic extract and its fractions were reported to possess an ovicidal activity against *Haemonchus contortus.* The isolated compounds coumaric acid, ferulic acid, quercetin, luteolin 7-O-rhamnoside and may be responsible for the activity (Olmedo-Juárez et al., 2022).

7.15. Nephroprotective activity

P. dulce fruit aqueous extract was given orally before and after the CCl4-producing toxin. Because of *P. dulce's* antioxidant activity, the crude extract reduced lipid peroxidation and protein carboxylation following CCl4 toxication. When compared to untreated rats given CCl4, the reactive oxygen species (ROS) were lower in the extract-treated animals, while antioxidant enzymes were higher. The aqueous extracts also inhibited and protected against renal DNA damage and cell death, hence preserving the kidneys from CCl4-induced oxidative damage (Pal et al., 2012).

A study was made to investigate the antioxidant benefits of *P. dulce* fruit methanolic extract against methotrexate (MTX)-induced hepatic and renal toxicities. Following oral administration of the extract at 40 mg kg^{-1} body weight for 10 days straight, the serum markers of the hepatic and renal toxicity and the levels of the pro-inflammatory cytokines, such as tumor necrosis factor-alpha (*TNF-α),* interleukin 6 (*IL-6)* and interleukin 1-beta *(IL-1β)* were reduced. When compared to the MTX alone group, the extract reduced the levels of tissue oxidative stress markers and improved the antioxidant status in the liver, kidneys, and lungs of mice (Dhanisha et al., 2021).

7.16. Neuropharmacological activity

Leaf aqueous and alcoholic extracts showed a clear reduction in locomotor activity, motor coordination, and hypnosis production but were unable to reduce the convulsions or mortality in mice. The alcoholic extract significantly reduced CNS activity better than the aqueous extract (Sugumaran et al., 2008b; Mule et al. 2011).

The aqueous, ethanolic extracts and the crude flavonoid fraction of the leaf were assessed for anticonvulsant activity using the maximal electroshock-induced seizure test and pentylenetetrazol (PTZ) assay. Phenytoin sodium was used as a standard drug. The results showed significant anticonvulsant activity (Sugumaran et al., 2008; Dhivya and Niranjan, 2013).

7.17. Spermicidal activity

Given the significance of saponins as potential spermicidal agents, tests for spermicidal properties were conducted on the saponins of *P. dulce*. Sapogenin demonstrated activity against human semen in a dilution of 0.03% (Shweta, 2013).

7.18. Acute and sub-acute toxicity

The hydroalcoholic fruit extract did not cause any hematological or biochemical abnormal changes. The LD_{50} was reported to be 3916 mg kg^{-1} and the potential minimum and maximum effective doses were found to be 100 and 300 mg kg^{-1} respectively (Megala and Geetha, 2012).

The up-down regulation approach was used to investigate the acute oral toxicity of the leaf ethanolic extract and the crude flavonoid fraction. They were safe at doses of up to 2000 mg kg^{-1} (Dhivya and Niranjan, 2013), whereas the saponin-enriched fraction of the seed extract was safe in mice at doses of up to 2000 mg kg^{-1} (Kumar et al., 2017).

The oral administration of 5 g kg^{-1} body weight as a single dose of the bark hydroalcoholic extract to Wistar rats resulted in no deaths or hazardous signs for 4 hours post-dose administration and 14 consecutive days. The LD_{50} of stem bark extract is higher than 5 g/kg (Toudji et al., 2017).

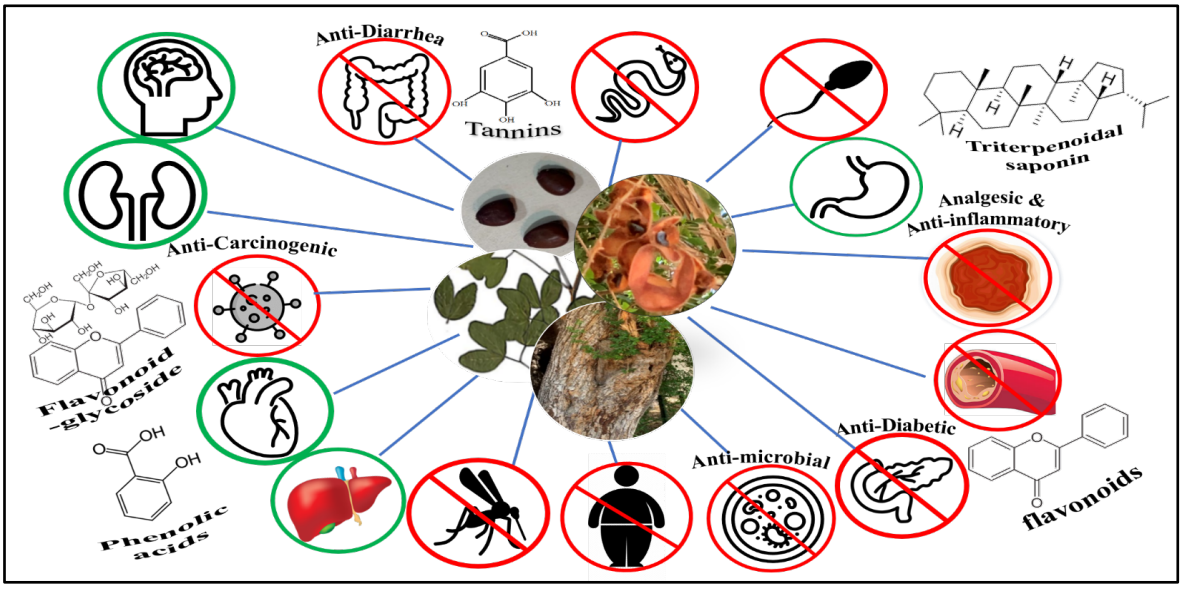


Figure 3. Different pharmacological activities of *Pithecellobium dulce* (Roxib.) Benth.

8. Conclusion

This review covers information regarding traditional uses, nutritional values, phytochemicals, and pharmacological activities of the different extracts as well as the pure compounds and the analysis of active compounds related to *P. dulce.* The various parts of the plant were utilized traditionally in treating many disorders in many countries. *P. dulce* provides important vitamins, amino acids, critical minerals, and many fatty acids which contribute to its nutritive value. The plant contains many chemical constituents such as triterpenoids, flavonoids, saponins, steroids, and glycosides which are responsible for many pharmacological activities. There is evidence from different *in-vivo* and *in-vitro* studies that the extracts and pure compounds found within *P. dulce* have antimicrobial, hepatoprotective, nephroprotective, cardioprotective, anticancer, antidiabetic, and antiulcerogenic activities via multiple pathways. Even though the chemical structure and pharmacological potential of a few of the constituents are known, the mechanisms of action must be studied further before they can be developed into therapeutics.

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Conflict of Interest

The authors have confirmed that there are no conflicting interests.

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