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Research Article
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Investigating the Effect of Different Levels of Nitrogen Fertilizer on Quantitative and Qualitative Characteristics of *Chrysanthemum* (cv. Borna)

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Abstract

Chrysanthemum is one of the five main cut flowers in Iran. Knowledge about the optimal range of macro-nutrientsespecially nitrogen (N), for the best quantitative and qualitative characteristics of chrysanthemum is of great importance. Randomized complete blocks design in three replications was implemented in this research. Five levels of N included 0, 75, 150, 225 and 300 kg/ha (ammonium nitrate) has been applied to chrysanthemum "Borna" cultivar in Mahalat city of Iran. These traits were measured: Score of life after harvest, branch number, flower numbers, flower longevity, days to flowering, chlorophyll, dry weight, shoot fresh weight, crown diameter, stem diameter and flower diameter. The results showed that maximum crown diameter, flower longevity, chlorophyll index, fresh and dry weight of the plant were obtained at the level of 150 kg/ha, compared to the control. Also, the highest total absorption of macro-nutrients (nitrogen, phosphorus and potassium (NPK)), and micro-nutrients (iron, manganese, zinc and copper) was observed at the level of 150 kg/ha fertilization. According to the results, N application in the level 150 kg/ha can be recommended to have best growth condition for the "Borna" cultivar of chrysanthemum in Mahalat city.

Keywords: Chrysanthemum, Growing and flowering indices, Macro-and micro-nutrients, N fertilizer.

INTRODUCTION

Chrysanthemum is the second most popular flower in the world, and one of the five main cut flowers in Iran (Taghipour *et al*., 2019). Cultivation area of chrysanthemum in 2019 was 253 hectares in Iran, and 15 hectares of which are in Markazi province and mainly in Mahalat city (Taghipour *et al*., 2019). One of the factors affecting the quality of chrysanthemum cultivars is the management of plant nutrition, especially macro-nutrients such as nitrogen (N). N is one of the main macro-nutrients for plant growth, and it is a growth bottleneck in plant nutrition and a key element (Vojodi Mehrabani, 2017). Also, the excessive use of application of chemical fertilizers such as N fertilizers in order to increase the amount of horticultural and agricultural products, along with the industrialization of countries, put the world in the risk of environmental pollutions (Aldhous, 2000).

Chrysanthemum is one of the fast-growing plants with high nutritional requirements especially N, that the environmental conditions including balanced and optimal nutritional status could lead to increase in photosynthesis in this plant (Teja *et al*., 2017). The availability of N during the first seven weeks of growth is very vital for the plant, however for example N fertilizer should not be given to the plant when the diameter of the flower bud reaches 1-1.5 cm (Vojodi Mehrabani, 2017). Satar *et al*. (2016) recommended the application of N and P for chrysanthemum in 100 to 200 kg/ha concentration, respectively. Rajan *et al*. (2019) studied different amounts and sources of N in the form of nitrate, ammonia, and urea in chrysanthemum (cv. Thai Chen). They found that the best form of N was nitrate at a level of 200 kg/ha, which caused the highest plant height, stem diameter, dry and wet weight shoot of the plant, and the maximum number of flowers. Chopde *et al*. (2015) studied the growth, flowering and yield of chrysanthemum cultivars, under the influence of N fertilizers. They reported the application of 300 kg/ha of N caused the most appropriate growth and quantitative and qualitative performance of the product. Also, one of more recent researches showed that application of 280 mg/kg of N increased the number of flowers, diameter of flower stalk, diameter of stem compared to the control (Ali and Mjeed, 2017).

Most of the literature reported that application of N fertilizers had a significant positive effect on plant height, number of flowers, flower diameter, stem diameter, bud length and flower stalk diameter in different cultivars of chrysanthemum. However, lack of research on the effect of N fertilizer application on Borna cultivar of chrysanthemum in Iran was our reason to conduct this research. Moreover, the use of chemical fertilizers, especially N, in chrysanthemums in the Mahalat city has not been subjected to special recommendations, which causes no-application or in some cases excessive application of fertilizers in this region.

The objective of this research was to investigate the effect of N fertilizer (ammonium nitrate) in five levels (0, 75, 150, 225 and 300 kg/ha) on the quantitative and qualitative characteristic of Borna cultivar of chrysanthemum. Furthermore, ability of the plants to absorb macro-nutrients (NPK) and micro-nutrients (iron, manganese, zinc and copper) from the soil was monitored in this study.

MATERIALS AND METHODS

Study site and the experiment

The experiment was conducted on Borna cultivar of chrysanthemum, in a farm in the National Research School of Flowers and Ornamental Plants (1747 m above sea level, longitude E 30° 27′ 50′′ and latitude N 33° 54′ 30′′), Mahalat, Iran. According to table 1, the average temperature during the experiment were 22/10 ºC (day/night) and relative humidity of

43%. The soil texture was sandy loam and the soil classification was determined as Entisols. Characteristics of the soil are given in table 2, and also the result of irrigation water analysis was shown in table 3.

Table 1. Climatic conditions.

Average minimum temperature (ºC)	Average maximum temperature (ºC)	Average relative humidity (%)	Total hours of sunshine (h)	Total precipitation (mm)
10.2	22.1	43	3146.6	280.6

Table 2. Average results of soil analysis (depth of 0-30 cm) before planting in the experimental area.

Sand	Silt	Clay	EC	pH	OC	TN	P	K	Fe	Zn	Mn	Cu	B	CEC
	%		dS/m		%					mg/kg				$Cmol^+$/ kg
56	32	12	1.35	7.9	0.51	0.05	13	293	2.58	0.62	4.46	0.58	0.82	9.6

Table 3. Irrigation water properties.

EC	pH	SAR	Carbonate	Bicarbonate	Sulphate	Chlorine
(dS/m)				($Cmol^+$/kg)		
0.89	7.9	1.11	0	4.1	2.67	2.11

The experiment was implemented in a randomized complete block design in three replications. Nitrogen treatment was applied at five levels of 0, 75, 150, 225 and 300 kg/ha of N from the source of ammonium nitrate (NH_4^+) fertilizer (from N1 to N5, respectively).

Rooted chrysanthemum were planted in plots of 1×1 m, at distances of 20×25 cm (20 plants per m^2). Other fertilizers included triple superphosphate, potassium sulfate, magnesium sulfate, sequestrin138 (with 6% iron), manganese sulfate, zinc sulfate, copper sulfate, and boric acid have been applied in concentrations of 200, 100, 100, 40, 30, 40, 20, and 20 kg/ha, respectively, in all treatments. Irrigation was done according to the needs of the plant, once a week, using basin irrigation method.

N fertilizer was added into the soils in four stages; the first time a quarter of the fertilizer was applied just before planting (June 20th), second quarter 15 days after planting, third quarter 30 days after planting, and the last quarter 45 days after planting. The first fertilizer is mixed with the soil and the fertilizers after planting are spread on the soil.

Soil and plant properties

Soil properties included soil texture, pH, EC, CEC, organic carbon (OC), concentration of macro-nutrients (NPK) and micro-nutrients (Fe, Mn, Zn, Cu, B) were determined before plant cultivation in depth of 0-30 cm (Tekaya *et al.,* 2014; Ogunkunle *et al.,* 2020). The source of irrigation water supply of the National Flower and Ornamental Plant Research Institute was an aqueduct, and the water properties were analyzed included EC, pH, sodium adsorption ratio (SAR), carbonate, bicarbonate, sulphate, and chlorine.

Plant growth properties were determined in the pre-flowering stage, by taking newly matured leaves. Chlorophyll index was measured using a chlorophyll meter (Spad 502, Minolta, Japan) in five new mature leaves per plant (Lu *et al.,* 2022). Vegetative plant properties were determined included; plant height, stem diameter with digital caliper, branch number, and fresh

and dry weights by digital balance. The absorption of NPK and micro-nutrients (Fe, Mn, Zn, Cu) by leaves were also measured (Tekaya *et al.,* 2014; Ogunkunle *et al.,* 2020). The measurement of elements, including iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), was done by atomic absorption, based on previous studies (Tekaya *et al.,* 2014; Ogunkunle *et al.,* 2020). Nitrogen was also measured by the Kjeldahl method (Tekaya *et al.,* 2014; Roussos *et al.,* 2017).

During the flowering period, measurements were included; time to start flowering, flower longevity, number of flowers, flower diameter, crown diameter, flower quality and life after harvest score.

Statistical analysis

The collected data has been statistically analyzed using SAS software version 9.2. Mean comparison and analysis of variance of the data of soil and plant properties was performed using LSD test at level of 5%. Data was normalized by min and max normalization.

RESULTS AND DISCUSSION

Soil characteristics before planting and irrigation water properties

Based on table 2, the soil texture was sandy loam and light enough to cultivate ornamental flowers, and also good for root development. The nutritional status of the soil showed that N concentration was low, and therefore application of N fertilizer was necessary for the best plant growth. However, according to table 2, other nutritions concentration has also been considered to apply P, K and micro-nutrients fertilizers into the soil to have better nutritional balance for plant growth and flowering. Table 3 showed that the irrigation water was neither salty nor sodic, therefore it was suitable to cultivate ornamental flower of chrysanthemum.

Plant properties and nutrients absorption

Based on table 4, the effect of N levels on all of the plant properties was statistically significant according to the LSD test. table 5 showed the plant growth and flowering properties affected by different N levels. Some of the plant growth properties like crown diameter and dry weight shoot had higher amounts in N2 and N3 levels (75 and 150 kg/ha N fertilizers, respectively) compared to the control (N1=0 kg/ha N fertilizer). Plant height, flower diameter and numbers were highest in N2 level (75 kg/ha N fertilizer) compared to the control. Crown diameter, plant fresh and dry weights shoot, chlorophyll, flower longevity and score of life after harvest were highest in N3 level (150 kg/ha N fertilizer). Therefore, it can be concluded that N application at the level of ≤150 kg/ha increased the quantitative and qualitative properties of the plant compared to the control (N1).

Table 4. The results of analysis of variance of the plant properties.

S.o.V	df	Plant height	Flower diameter	Stem diameter	Crown diameter	Fresh weight shoot	Dry weight shoot
Replication	2	87.816*	0.147^{ns}	26.62*	1.517^{ns}	0.181^{ns}	844.7^{ns}
Nitrogen (N)	4	19.47*	0.647**	11.69*	1.500*	0.060*	474.8*
CV (%)		19.78	11.97	33.02	16.61	11.26	48.92

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test.

Continued Table 4. The results of analysis of variance of the plant properties.

S.o.V	df	SPAD	Days to flowering	Flower longevity	Flower numbers	Branch number	Score of life after harvest
Replication	2	1.641^{ns}	0.017^{ns}	0.355^{ns}	19.16^{ns}	135.27**	1.35^{ns}
Nitrogen (N)	4	17.637*	9.33*	0.031*	7.90*	34.608*	0.942*
CV (%)		8.25	2.86	27.55	25.76	46.85	26.40

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test.

Table 5. Comparison of mean values and the effect of different levels of nitrogen fertilizer on quantitative and qualitative characteristics of chrysanthemum.

N level	NH_4NO_3	Plant height	Flower diameter	Stem diameter	Crown diameter	Fresh weight shoot	Dry weight shoot
	kg/ha	cm	cm	cm	cm	g	g
N1	0	33.97 abc	2.68 b	1.73 a	16.17 b	104 d	24.81 d
N2	75	37.5 a	3.44 a	1.5 ab	15.83 b	164 b	38.38 b
N3	150	34.43 ab	3.17 ab	1.8 ab	17.33 a	223 a	50.78 a
N4	225	31.4 abc	3.11 ab	1.1 b	10.33 d	98 e	22.52 c
N5	300	28.23 c	2.70 b	1.57 ab	12.33 c	114 c	25.21 cd

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Continued Table 5. Comparison of mean values and the effect of different levels of nitrogen fertilizer on quantitative and qualitative characteristics of chrysanthemum.

N level	NH_4NO_3	SPAD	Days to flowering	Flower longevity	Flower numbers	Branch number	Score of life after harvest
	kg/ha	mg/100 g FW					
N1	0	57.13 a	134.7 b	8.66 a	6.66 b	7.33b	2.33 bc
N2	75	57.17 a	134 b	8 ab	7.33 a	8.6 ab	3 ab
N3	150	57.63 a	134.7 b	8.67 a	6.33 b	8.00 b	3.33 a
N4	225	54.83 b	137.3 a	7.66 b	4.66 c	9.33 a	2.00 c
N5	300	54.93 b	135 b	7.66 b	6.66 b	5.00 c	2.00 c

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

The increase in fresh and dry weight shoot of the plant may be due to increase in nutrient availability with enhancing N levels up to 150 kg/ha, this is in agreement with the results reported by Gangwar *et al.* (2012). The increase in fresh and dry weight shoot of the plant might also be due to an increase in plant spread, number of branches and leaf area, which in turn could increase number of flowers. It might be because of increased carbohydrate reserve for the development of floral primordia apart from the structural development of the plant (Teja *et al.,* 2017). It was also stated that increase in flower numbers and flower yield might be due to higher levels and balanced application of nitrogen and phosphorus (Satar *et al.,* 2016). Teja *et al.* (2017) found that plant height increased with application of nitrogen up to 200 kg/ha, and they considered it as essential role of nitrogen in the biosynthesis of nucleic acids, therefore it plays a vital role in promoting the plant growth. Further, nitrogen has been identified as an important constituent of chlorophyll, proteins and amino acids, thereby enhancing the rate of photosynthesis. The increase in plant height also might be due to greater uptake of nutrients into the plant system through soil application, which involved in the cell division, cell elongation

as well as protein synthesis, and finally enhanced the stem length and vegetative growth (Teja *et al.,* 2017). According to table 5, The application of N fertilizer up to 150 kg/ha increased the life of flower after harvest, which may cause the lack of N and therefore led to reproductive growth overcomes the vegetative growth and extended life after harvest (Grewal and Tanya, 2016). Photosynthetically produced carbohydrates are transported to the points of growth are used predominantly in the synthesis of nucleic acids and proteins, hence the application of nitrogenous fertilizers during the vegetative growth phase to the plants controls the growth of the plant to a larger extent (Teja *et al.,* 2017).

The application of all nitrogen levels had significant effect on the vegetative and floral parameters like plant height, number of primary branches, leaves, root suckers, flowers per plant, size of flower and delayed days taken to full bloom (Grewal and Tanya, 2016).

N application in the range of 75 to 225 kg/ha caused the highest branch numbers compared to the control (N1) (Table 5). Nitrogen in the range of 75 to 225 kg/ha (150 kg/ha in average) may increase the photosynthetic activity by increasing the size of the photosynthesizing resources (chlorophyll, number of branches and leaf surface). Teja *et al.* (2017) also reported that increase in the branch numbers could be due to the basic role of nitrogen in photosynthesis, thereby resulting in more number of leaves and branches per plant. Therefore, it facilitates the development of flowers with more photosynthesis, and improves conditions for increasing cell division and expanding the number of flower tissue cells and flower size.

However, at N4 and N5 levels (225 and 300 kg/ha N fertilizers, respectively) most of the plant properties had lowest amounts, which revealed that application of higher N fertilizers (> 150 kg/ha) in our work did not show positive effect on plant growth properties.

Plant nutrients in leaves were measured included NPK as macro-nutrients and Fe, Mn, Zn and Cu as micro-nutrients (Fig. 1). The results showed that N3 level (150 kg/ha N fertilizer) had maximum concentrations of all nutrients compared to the control (N1=0 kg/ha N fertilizer). It revealed that 150 kg/ha concentration of N fertilizer for chrysanthemum, Borna cultivar, led to the best nutrient absorption by plants and it was in accordance with the increase trend of most of the growing and flowering properites of chrysanthemum (Table 5). Moreover, according to fig.1, K among the macro-nutrients and Fe and Mn among the micro-nutrients had the highest concentrations. It was due to the initial concentration of the K, Fe and Mn in the soil before planting and fertilization (see table 2), which were maximum among the macro- and micro-nutrients, respectively. Therefore, absorption of K and Fe and Mn by plants increased in comparison to the other nutrients.

The increase of NPK, absorption by plants in application of N in level of 150 kg/ha compared to the control was in consistent with the results reported by Rajan *et al.* (2019). The demand for nutrients absorption by plant could be increased due to the increase in photosynthesis sources as mentioned earlier.

Cluster analysis of chrysantemum based on physical and chemical properties of the leaves was shown in fig. 2. Cluster I included nitrgent treatment at 0, 75 and 300, had medium values for days to flowering. Cluster II included nitrogen treatmet at 150 through 225, had lowest values for days to flowering.

To determine the dispersion of chrysantemum cultivars, principle components analysis (PCA) was used (Fig. 3). According to the fig. 3, the variances explained by the first two components, PC1 and PC2, were 49.2 and 30.7%, respectively. In addition, the strongest positive correlations with leaf N, P, K, Fe, Mn, Zn and Cu, plant height and numbers, stem and crown diameter,fresh and dry weight shoot belonged to PC1. It also showed the strongest positive correlations with flower longevity, flower diameter and chlorophyll and branch number. Days to flowering and were strongly and negatively correlated with PC1 and PC2 (Fig. 3).

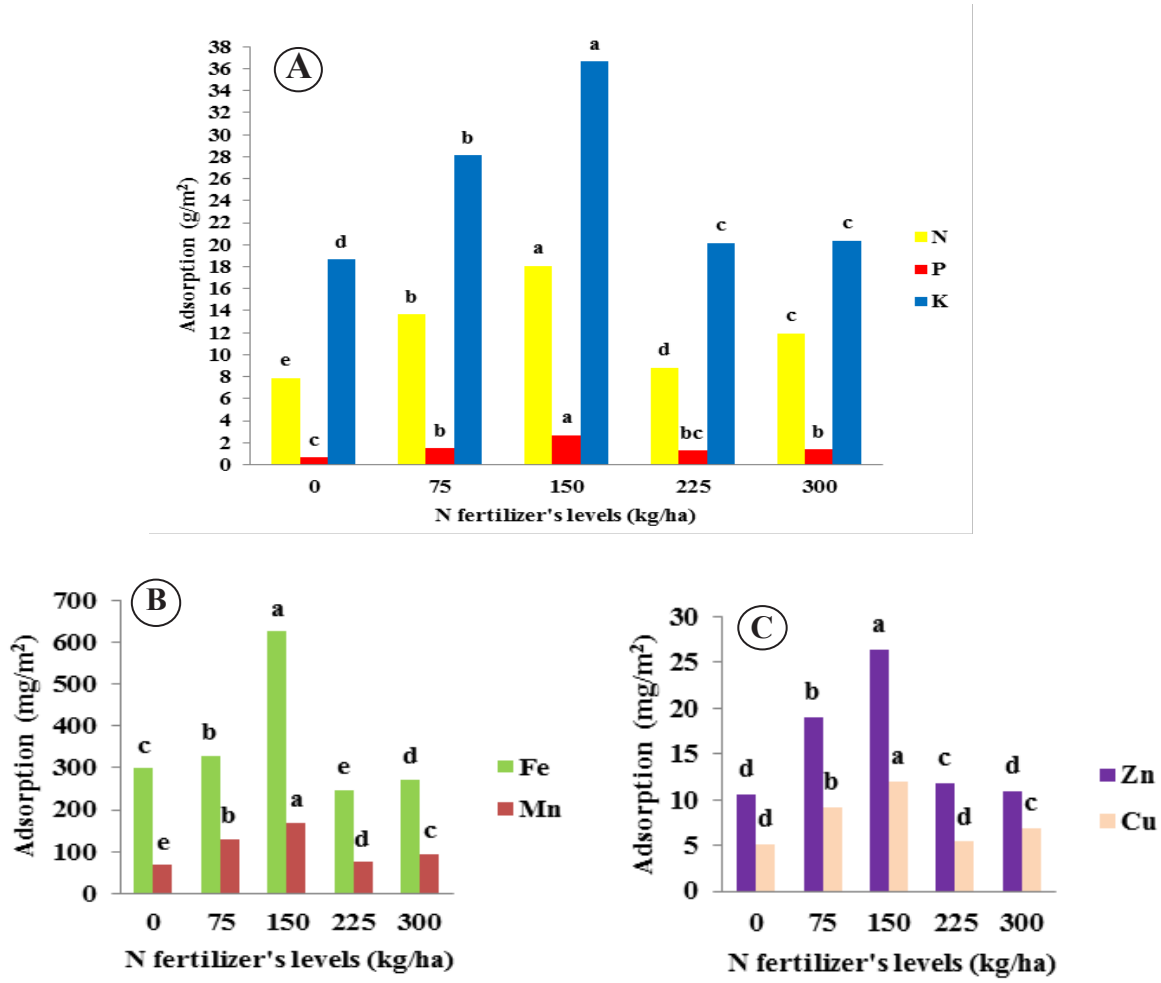

Fig. 1. The effect of different N levels on mean adsorption of macro-nutrient (a), micro-nutrients of Fe and Mn (b), and Zn and Cu (c) by plant leaves.

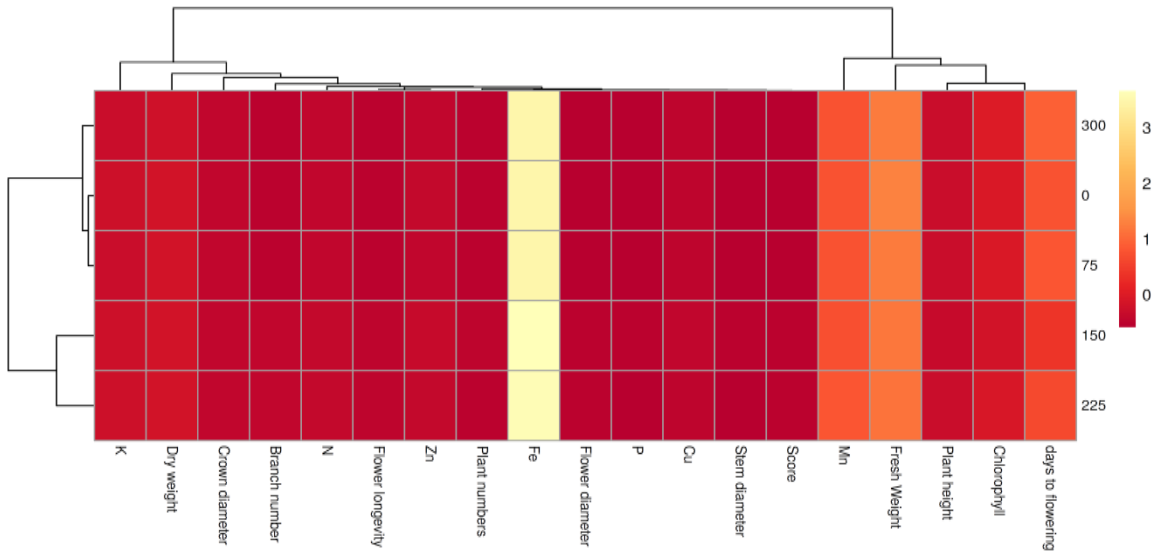

Fig. 2. Clustering heatmap analysis of chrysantemum based on physical and chemical properties of leaf (color gradient from low (dark red), medium (red) to high (yellow). Nitrogen levels: 0, 75, 150 and 225 kg/ha).

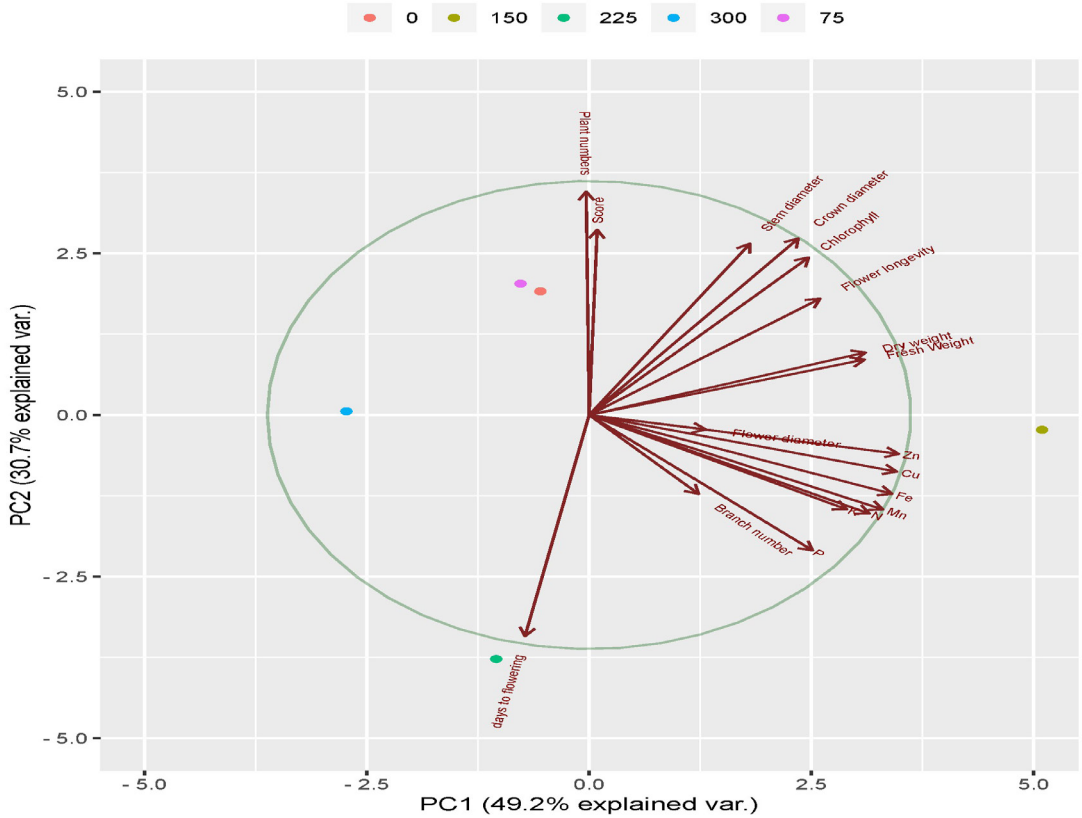

Fig. 3. The principle cluster analysis (PCA) of chrysantemun based on physical and chemical properties of leaf according to the first and the second principal components (PC1/PC2):7

Soil NPK concentrations after harvest

Based on table 6, the effect of N levels on all of Soil NPK concentrations after harvest was statistically significant according to the LSD test. The soil NPK after harvest was affected by N levels of fertilizers, and was higher in N1, N2 and N3 levels than N4 and N5 levels (Table 7). The amount of available P and K remaining in the soil after harvest decreased due to the increase in the levels of N application (N4 and N5, 225 and 300 kg/ha, respectively). The possible reason for this finding could be an increase in soil microbial activity due to high concentration of N, which led to increase in P and K absorption by the soil microorganism (Gu *et al*., 2021; Guo *et al*., 2022). Therefore, NPK at high levels of N application mostly consumed by soil microbes and the remaining amounts in the soil samples decreased.

Also, the soil NPK after harvest were lower in N3 (150 kg/ha) than N2 and N1 (75 and 0 kg/ha, respectively) (Table 7), which means the higher plant growing and flowering indices in N3 level (see table 7) led to the greater NPK adsorption by plants and low remaining amounts of NPK in the soil. Liu *et al*. (2022) showed that nitrogen application (between 100-200 kg/ha) improved plant growth, increased grain yield and regulated amino acid and mineral nutrient concentration delivery rates in plant roots, which also led to higher P and K uptake and translocation ability.

Table 6. The results of analysis of variance of Soil NPK concentrations after harvest.

S.o.V	df	N	P	K
Replication	2	0.002**	120.313*	39.699 ^{ns}
Nitrogen (N)	4	0.001*	112.98*	3828.86*
CV (%)		32.34	28.01	14.13

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test.

Table 7. The effect of different N levels on soil NPK concentration after harvest.

N levels	N	P	K
	%	mg/kg	
N1	0.068a	24.37a	261.6a
N2	0.064a	25.83a	244.6ab
N3	0.061a	20.36bc	244.5ab
N4	0.052b	19.82bc	223.9b
N5	0.048b	18.82c	217.2b

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

CONCLUSION

According to the increasing effects of different levels of N fertilizers on most of the observed quantitative and qualitative plant properties compared to the control (no N fertilizer application), it should be mentioned that N was an essential element for Borna cultivar of chrysanthemum growth. Normally, increase in amount of nitrogen fertilizers causes an increase in cell division, which leads to higher plant height, the number of branches in the plant, the leaf area, and the wet and dry weight of the plant. Among different applied N levels (0-300 kg/ha), N level of 75 and 150 kg/ha had the best effect on growing and flowering properties of chrysanthemum. Plant height, flower diameter and numbers were highest in N2 level (75 kg/ha N fertilizer) compared to the control. Crown diameter, plant fresh and dry weights shoot, chlorophyll, flower longevity and score of life after harvest were highest in N3 level (150 kg/ha N fertilizer). Increase in plant chlorophyll and fresh and dry weights shoot led to more photosynthesis by plants and therefore better plant growth in N3 level (150 kg/ha N fertilizer), which enhanced macro- and micro-nutrients absorption by plant. Also, higher N levels (225 and 300 kg/ha N fertilizers) had not positive effects on plant growth and could not be recommended. However, NPK concentrations in soil after harvest were lowest in the higher N levels in this study (225-300 kg/ha), which revealed that N application in high concentrations may increase soil microbial activity, and therefore the macro-nutrients have been consumed mostly by the soil microorganisms and remaining amount of the elements in the soil decreased.

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Enhancing Germination of *Habenaria janellehayneana* (Orchidaceae): Insight from Asymbiotic and Symbiotic Methods

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Abstract

Habenaria janellehayneana Choltco, Moloney, & Yong Gee (Orchidaceae) is a lithophytic orchid with striking pink flowers that is endemic to Phitsanulok Province, northern Thailand. Only a few populations of this species are found in Phu Hin Rong Kla National Park. To maintain rare plant species in *ex situ* collections thereby preventing extinctions, along with the aim of mass propagation for ornamental reasons, it is crucial that suitable propagation methods are developed. In this paper, we describe protocols for the asymbiotic and symbiotic germination of *H. janellehayneiana*. Of the four growing media tested, germination percentages were greatest on ½ VW (18.97%), followed by ½ MS (14.20%), MS (12.46 %), and VW (11.93 %) at 16 weeks, and protocorm development was most advanced (stage 4) within 10 weeks. Of the three plant growth regulators tested, including 6-benzylaminopurine (BAP), gibberellic acid (GA), and thidiazuron (TDZ), at 0, 1, 3, and 5 mg/L concentrations, 1 mg/L BAP significantly enhanced seed germination ($P < 0.05$) when compared to the control (8.47%). For symbiotic seed germination, two non-mycorrhizal endophytic fungi isolates of the genera *Aspergillus* and *Colletotrichum* increased seed germination by 14.03% and 11.00% respectively, when compared to the control (6.15 %). These findings demonstrate that it is possible to germinate the seeds of *H. janellehayneana* via both asymbiotic and symbiotic method, with a symbiotic approach providing the best outcomes, and this could assist in the conservation of this and other rare terrestrial orchids, as well as increase their value in the ornamental market.

Keywords: Micropropagation, Mycorrhiza, Ornamental plant, Terrestrial orchids.

INTRODUCTION

Although, orchids often constitute a significant proportion of regional floras in terms of species numbers (Christenhusz and Byng, 2016; Fay, 2018), they are characterized by low reproductive success (Neiland and Wilcock, 1998; Zhang and Gao, 2021) and are thus typically present at low abundance (Zhang and Gao, 2021). The reasons for this appear to be associated with ecological specialization at key life cycle stages, notably their requirement for myco-heterotrophic germination (Rasmussen *et al.*, 2015; Yeh *et al.*, 2019), as well as their subsequent transition to autotrophism for seedling establishment (Rasmussen *et al*., 2015) and dependence on specific vectors (mostly insects) for pollination (Swarts and Dixon, 2009; Ackerman *et al*., 2023). Not only does this restrict the geographic range and ecological amplitude of many species, but it also renders them highly sensitive to extraneous threats. As a result, orchids are regarded as facing a disproportionately high degree of extinction risk as compared with other taxa (Fay, 2018), with declining numbers and population fragmentation causing genetic erosion and a breakdown in key ecological processes (Gale *et al*., 2018).

In vitro propagation is frequently highlighted as a useful means of propagating rare and threatened orchids for ex-situ conservation (Stewart and Kane, 2007; Swarts and Dixon, 2009; Fay, 1992; Fay, 2018), and tissue culture technology has been widely applied to the mass propagation of various orchids of significant commercial value (Abebe *et al.*, 2009; Mohanty *et al*., 2012; Paek *et al.*, 2011; Zeng *et al.*, 2016; Zanello *et al.*, 2022). However, established *in vitro* protocols are limited to just a few high-profile genera or species and are not always transferable to less well studied taxa, particularly those, often rarer species with specific requirements for germination. Several approaches have been tested to overcome the difficulties of orchid seed germination, including both asymbiotic and symbiotic techniques, the use of mature/immature seeds, light/dark treatments, sterilization, scarification treatments, and modified culture systems (Arditti and Ghani, 2000; Rasmussen *et al.*, 2015; Setiaji *et al.*, 2021; Nongdam *et al*., 2023).

This genus *Habenaria* Willd. (Orchidaceae) contains about 928 terrestrial and lithophytic species and is characterized by the presence of a combination of derived floral traits with showy petals (Pridgeon *et al.*, 2001; Batista *et al*., 2013; Govaerts *et al.*, 2019). *Habenaria janellehayneana* Choltco. B. Moloney & Yong, a rare terrestrial species with pink flowers, was newly named in 2017 by Choltco *et al*. (2017) after concluding that it ought to be segregated from the widespread *H. rhodocheila* complex. Unlike *H. rhodocheila* Hance and *H. erichmichelii* Christenson, the stigmas of this species are basally parallel but convergent and touching (or nearly so) towards the apex. The species is native to Phitsanulok in northern Thailand and is regarded as a priority for conservation in the country (International Cooperation and Cooperation Group Wildlife and Wild Flora Protection Division, 2013; POWO, 2023). Because it has comparatively large, showy pink flowers, its population has declined due to poaching and the impacts of disturbance.

Habenaria species are notoriously difficult to propagate *in vitro* due to inherent barriers to seed germination and seedling establishment, with capsule maturity, medium nutrient content, culture method, growth factors and mycorrhizal fungi all being important factors (Stewart and Zettler, 2002; Keel *et al*., 2011). Several researchers have attempted symbiotic culture of *Habenaria* seeds, which has been shown to promote seed germination (Stewart and Kane, 2006a; Sangmanee *et al*., 2012). Further, Stewart and Kane (2006b) reported the method of asymbiotic seed germination of *H. macroceratitis*, but no leaf formation was observed. Sangmanee *et al*. (2012) examined the growth of *H. erichmichelii* in the presence of mycorrhizae and found that average plant height was increased when the culture medium was inoculated with fungal strains of *Humicola* sp. and *Oidiodendron* sp., but not with *Fusarium* sp., *Nodulisporium* sp. and *Trichoderma* sp. Symbiotic seed germination of *H. janellehayneiana,* on the other hand,

has never been reported. The aim of the present study was therefore to find the best conditions for both symbiotic and asymbiotic germination of this important species. Various media, plant growth regulators, and different fungal isolates, were examined.

MATERIALS AND METHODS

Plant material and seed storage

Habenaria janellehayneana is a terrestrial orchid that mostly grows on moist rocks besides streams and waterfalls in Phitsanulok Province, Thailand (Fig. 1). Mature undehisced plant capsules (7–8 weeks old) of *H. janellehayneana* (n=3) were collected with a permit from Phu Hin Rong Kla National Park in 2018. We used paper bags with silica gel for capsule storage until dehiscence, then stored the resulting brown seeds at 4°C in a sterile Eppendorf tube. Seed vigor was tested within 7 days after staining in a 1% triphenyl tetrazolium chloride (TTC) test at 30 ±2 °C for seven days (Lauzer *et al*., 1994), with embryos becoming orange or reddish in color considered viable.

Fig. 1. *Habenaria janellehayneana* at Phu Hin Rong Kla National Park, Thailand. A: Flower morphology; B: Habitat.

Fungal isolation and identification

Roots and rhizomes of plant specimens at vegetative and reproductive stages were collected in a sterile plastic bag, transported to the laboratory within 24–48 h, and refrigerated at 4°C before use. In the laboratory, the roots and rhizomes were then cleaned with tap water, trimmed into 1 cm sections and sterilized in a five min immersion in 0.5% NaOCl. Under a stereomicroscope, the segments were dissected transversely, and pelotons were taken from the cortical cells with a dissecting needle. The pelotons were washed with sterile distilled water five times, placed on a potato dextrose agar (PDA) plate adding both streptomycin and tetracycline at 100 mg/mL concentration, and incubated for 48–72 h at 30 ± 2 °C in the dark. Each fungal mycelia colony was sub-cultured on a fresh PDA media for purification.

The characterization and identification of fungi followed the methods by Zhu *et al*. (2008) for Rhizoctonia species. Genomic DNA of 14-day-old fresh fungal cultures was extracted using a universal and automated nucleic acid extraction system including MagLEAD 12gC machine (Hitachi Co., Ltd.) and a prefilled reagent cartridge for nucleic acid extraction MagDEA® Dx SV kit (Precision System Science Co., Ltd.). Five loci were amplified and

sequenced, including beta-tubulin (tub), chitin synthase 1 (chs-1), actin (act), glyceraldehyde-3-phosphate dehydrogenase (gadph), and the internal transcribed spacer regions (ITS). Genes were amplified and sequenced using the primer pairs ITS-1F + ITS4 (Gardes and Bruns, 2013; White *et al*., 1990), GDF1 + GDR1 (Guerber et al., 2003), CHS-354R + CHS-79F (Carbone and Kohn, 1999), ACT-512 F + ACT-783R (Carbone and Kohn, 1999), and Bt2a + Bt2b (Glass and Donaldson, 1995), respectively. The PCR mixture with a total volume of 25 µL contained 5 ng of genomic DNA, 1.25 unit of Taq DNA polymerase (GeneDireX, Inc.), and 0.2 µM of each primer. PCR amplifications were performed in T100 thermal cycler (Bio-Rad Laboratories Ltd., Thailand). The following thermocycling conditions were used: İnitial denaturation at 95 °C for 3 min, followed by 35 cycles of 40 s at 94 °C, 45 s at 54 °C (for ITS and tub2 gene) or 52 °C (for gadph, chs-1, and act genes), and 1 min at 72 °C, followed by a final step of extension at 72 °C for 7 min. Purified PCR amplicons were used to perform direct PCR sequencing of both DNA strands with Applied Biosystems™ 3500 Genetic Analyzer (Thermo Fisher Scientific (Thailand) Co. Ltd.). Using BioEdit (v.7.2.5; Hall, 1999), Forward and reverse primers were assembled to obtain consensus sequences that were subsequent deposited in GenBank. The resulting sequence data were edited and subsequently evaluated using BLAST-n (Altschul *et al*., 1997) to determine affiliation to other sequenced relatives.

For phylogenetic analysis, multiple DNA sequences of act, chs-1, chs-1, gadph, ITS, and tub2 were concatenated for isolate SUT-HJ-I04, and ITS and tub2 were concatenated for isolate SUT-HJ-I35. The DNA sequences were aligned using ClustalW multiple alignment (Thompson *et al*., 1994) and manually adjusted where necessary using BioEdit (v.7.2.5). For phylogenetic analysis, DNA sequences from the Colletotrichum boninense species complex and C. gloeosporioides were used as outgroups for isolate SUT-HJ-I04, while sequences from the Aspergillus species complex section Terrei and A. neoflavipes in section Flavipedes were used as an outgroup for isolate SUT-HJ-I35. Maximum Likelihood (ML) phylogenetic tree with bootstrap (1000 replicates) were constructed with RaxML v.8 (Stamatakis, 2014) and plotted with FigTree (v.1.4.4; http://tree.bio.ed.ac.uk/software/figtree/).

The following four different basal media modified with 2% sucrose, 15% coconut water and 0.8% agar were used to test their influence on seed germination and protocorm formation: (1) Vacin and Went (VW; Vacin and Went, 1949), (2) ½ VW, (3) Murashige and Skoog (MS; Murashige and Skoog, 1962), and (4) ½ MS. Separately, we also enriched the ½ VW medium with the following three plant growth regulators at 0, 1, 3, and 5 mg/L concentrations to assess their impact on germination and early growth: 6-benzylaminopurine (BAP), gibberellic acid (GA), and thidiazuron (TDZ).

Seeds were sterilized in 10% Clorox for 10 min, rinsed with distilled water, sterilized in 3% hydrogen peroxide for 10 min, and washed in sterilized water three times for five min before sowing on each medium. About 100 surface sterilized seeds were sprinkled in a Petri dish containing 20 mL of solidified media, sealed with parafilm, and kept at 25 ± 2 °C in darkness for four weeks and then transferred to a 16 h light/8 h dark cycle for 12 weeks. All treatments consisted of four independent replicates. A 1–5-point growth scale was used to evaluate germination and development, as described by Stewart and Zettler (2002): No germination (stage 0), embryo swollen with production of rhizoid (stage 1), enlarged embryo with testa ruptured (stage 2), protomeristem appearance (stage 3), first leaf emergence (stage 4), and first leaf elongation (stage 5). For the evaluation of seed germination, 100-150 seeds per plate and four replications were marked. The seed germination percentage formula shown below was then used to determine seed germination at each stage. The state of each seed was determined by stereomicroscope examination.

$$\text{Seed germination (\%)} = \frac{\text{number of seeds germinated in each stage}}{\text{number of total mature seeds}} \times 100$$

Symbiotic seed germination

We evaluated the efficacy of 35 fungal isolates (8 *Rhizoctonia*-like and 27 endophyte isolates) in facilitating *H. janellehayneana* symbiotic seed germination using the modified method of Stewart and Kane (2006a). The seed surface disinfection was the same as described above. About 100 viable seeds were sown on a nylon mesh and placed onto 110/ oatmeal agar (OMA) which had has its pH adjusted to 5.5. A 5 mm-diameter plug was then excised from the edge of 7-day old, actively growing mycelium of each fungal inoculum (Yam and Arditti, 2009), and this was inoculated onto the oatmeal agar medium with uninoculated plates serving as a control. Four replicates of each treatment were wrapped in parafilm and kept at 25 ± 2 °C in darkness for four weeks, followed by 12 weeks at 16 h light/8 h dark. The germination and developmental stages were graded in the same manner as described above.

Statistical analysis

A completely randomized design (CRD) was used to set up all of the studies. To normalize variability, the data were transformed to the square root of the arcsine before analysis. The statistical software package SPSS V16.0 (SPSS Inc., Chicago, USA) was used for ANOVA, and the means were compared using Duncan's Multiple Range Test (P=0.05).

RESULTS AND DISCUSSION

Asymbiotic seed germination

The TTC test of *H. janellehayneana* seeds revealed a mean stainability of 14.89 ± 1.77%, which was very low and could be caused by low pollination rates in nature. Within four weeks after sowing, seeds were swollen and were scored as stage 1 (embryo swollen; Fig. 2) in all tested media. At 16 weeks, the ½ VW media showed the highest germination (18.97%), followed by ½ MS (14.20%), MS (12.46%), and VW (11.93%).

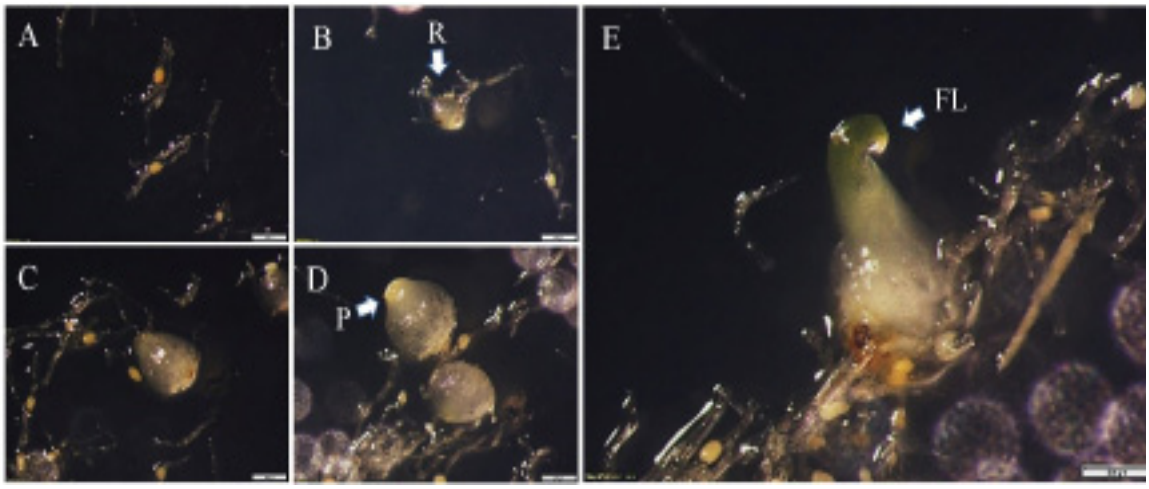

Fig. 2. Protocorm developmental stages of *H. janellehayneana* on ½ VW agar. A: Stage 0 (no germination); B: Stage 1 (embryo swollen with rhizoids present); C: Stage 2 (embryo enlargement with ruptured testa); D: Stage 3 (protomeristem appearance); E: Stage 4 (first leaf emergence); FL: First emerged leaf; P: Protomeristem; R: Rhizoids. bar = 500 µm.

All media supported advanced protocorm development up to stage 4 (leaf emergence) within 10 weeks, with no significant difference among them for stages 1–4; however, ½ VW had the highest frequencies for all stages (Table 1).

Table 1. Effect of basal media on seed germination and development of *H. janellehayneana* for 16 weeks.

Media	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Total
			(%)			
VW	6.70 ± 1.75	2.66 ± 1.50	1.64 ± 1.05	0.91 ± 1.06	0	11.93 ± 2.66^{b}
½VW	12.00 ± 3.00	2.75 ± 2.62	1.92 ± 1.41	2.28 ± 1.12	0	18.97 ± 2.47^{a}
MS	9.40 ± 4.32	1.39 ± 1.87	0.69 ± 0.46	0.97 ± 1.37	0	12.46 ± 2.49^{b}
½MS	9.01 ± 3.19	2.67 ± 2.48	1.33 ± 0.94	1.18 ± 0.49	0	14.20 ± 4.66^{ab}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's Multiple Range Test. Each mean value is determined by stereomicroscopic examination.

These results are similar to those previously reported by Thummavongsa *et al*. (2022), in which both half- and full-strength MS and VW media supported germination of *H. rhodocheila*, but ½ VW gave better performance overall. This might, perhaps, be due to its phosphate-rich regime, although different species in the same genus might be expected to have different media preferences. Stewarts and Kane (2006) showed that, among six tested media, percent seed germination of *H. macroceratitis* was greatest on both KC and LM (about 89%). On the other hand, *H. edgeworthii* Hook.f. ex. Collett exhibited the highest seed germination rates on a MS with 1.0 µM α-naphthalene acetic acid (NAA) (Giri *et al*., 2012).

Different types and concentrations of plant growth regulators had different effects on seed germination and growth of *H. janellehayneana* (Table 2). The addition of BAP, GA, and TDZ resulted in enhanced seed germination percentages, ranging from 8.33% to 13.16%, as compared with the control (8.47%). Media with 1 mg/L BA added gave the highest germination percentage (13.16%), which significantly differed from the control and 1 mg/L TDZ treatments. Seeds grown on media with 1, 3, 5 mg/L BAP and 3 mg/L GA proceeded to stage 4 (protocorm), as did those on the control, whereas seeds on media with 1, 3 mg/L GA and 3 mg/L TDZ stopped at stage 3. On the other hand, seeds on media with 5 mg/L TDZ added stopped at stage 1, indicating that a high TDZ concentration has an inhibitory effect on protocorm development (Table 2).

Table 2. Effect of plant growth regulators on seed germination and development of *H. janellehayneana* cultured on modified ½VW media for 16 weeks.

Treatment	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Total
			(%)			
control	6.98 ± 1.95	0.55 ± 1.11^{ab}	0.75 ± 0.58^{abc}	0.18 ± 0.37^{ab}	0	8.47 ± 1.87^{b}
1 mg/L BAP	10.01 ± 2.78	0.98 ± 0.89^{ab}	1.79 ± 0.98^{a}	0.37 ± 0.43^{ab}	0	13.16 ± 3.51^{a}
3 mg/L BAP	7.69 ± 3.15	0.76 ± 0.66^{ab}	0.89 ± 1.04^{abc}	0.71 ± 0.82^{ab}	0	10.06 ± 3.59^{ab}
5 mg/L BAP	9.64 ± 3.12	0.61 ± 0.42^{ab}	0.49 ± 0.62^{bc}	0.24 ± 0.49^{ab}	0	10.99 ± 3.39^{ab}
1 mg/L GA	7.80 ± 1.52	0.71 ± 0.12^{ab}	0.56 ± 0.38^{bc}	0^{b}	0	9.08 ± 1.04^{ab}
3 mg/L GA	8.29 ± 2.82	1.24 ± 0.88^{a}	0.18 ± 0.36^{bc}	0.24 ± 0.49^{ab}	0	9.95 ± 2.37^{ab}
5 mg/L GA	8.99 ± 3.27	0.60 ± 0.72^{ab}	0^{c}	0^{b}	0	9.59 ± 3.82^{ab}
1 mg/L TDZ	8.15 ± 1.69	0.18 ± 0.36^{ab}	0^{c}	0^{b}	0	8.33 ± 1.71^{b}
3 mg/L TDZ	8.69 ± 3.57	0.36 ± 0.42^{ab}	0.55 ± 1.11^{ab}	0^{b}	0	9.62 ± 3.41^{ab}
5 mg/L TDZ	8.97 ± 1.39	0^{b}	0^{c}	0^{b}	0	8.97 ± 1.39^{ab}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's Multiple Range Test. Each mean value is determined by stereomicroscopic examination.

Our findings agreed with those of several previous researchers who have described the asymbiotic seed germination of other terrestrial orchid species and found low germination and slow development. Stewart and Kane (2006b) reported that seeds of H. macroceratitis placed on ML and MM media supplemented with BAP only attained stage 4 protocorms within 16 weeks. Similarly, Piyatrakul (2014) observed that only 5.48% of H. rhodocheila seeds germinated on a modified VW medium (CMU1 with 0.1 mg/L NAA and 1 mg/L BAP added) after 20 weeks, and no stage 5 protocorms were observed. However, Thammavongsa *et al*. (2022) reported a seed germination range of 15.78–27.92% of the same orchid species on ½VW medium with the presence of stage 5 protocorms.

Symbiotic seed germination

We obtained thirty-five fungal isolates from the roots and rhizomes of H. janellehayneana at the vegetative and reproductive stages. The hyphae were noticed after seven days of culture. The morphological characteristics of these isolates on PDA were white, light purple to yellow in color, and some were identified as Rhizoctonia-like fungi according to Sneh *et al*. (1991). The results of co-culture of H. janellehayneana seeds with all 35 fungi isolates for 16 weeks are shown in table 3.

Table 3. Effect of fungal isolates on germination and development of *H. janellehayneana* seeds for 16 weeks.

Treatment	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Total
			(%)			
OMA	5.25 ± 0.86^{b}	0.67 ± 0.45	0.22 ± 0.44	0	0	6.15 ± 0.92^{c}
OMA + HJ-I04	10.10 ± 1.66^{a}	0.89 ± 0.72	0	0	0	11.00 ± 1.78^{b}
OMA + HJ-I35	10.87 ± 2.58^{a}	2.24 ± 1.94	0.91 ± 1.06	0	0	14.03 ± 1.03^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's Multiple Range Test. Each mean value is determined by stereomicroscopic examination.

Only seeds inoculated with one of two fungal isolates, namely SUT-HJ-I04 and SUT-HJ-I35, began to swell and germinate. Seeds treated with the SUT-HJ-I35 isolate exhibited the highest germination rate (14.03%), which was significantly higher compared to that for the other isolates, and they reached stage 3 protocorms, whereas the seeds treated with SUT-HJ-I04 stopped developing at stage 2, suggesting high mycorrhizal specificity or potentially a requirement for mycobiont switch (Umata *et al*., 2022). The results of BLAST searches using the ITS sequence data from these two fungal isolates are shown in table 4. The BLAST search identified SUT-HJ-I04 and SUT-HJ-I35 as Colletotrichum boninense and Aspergillus terreus, with 99.85 % and 100.00 % identity, respectively (Table 4).

Table 4. BLAST searches using the ITS sequence data of fungal isolates from *H. janellehayneiana*.

Isolate	Accession no.	Identity (%)	BLAST search result (Accession no./ taxonomic affiliation)
SUT-HJ-I4	OR074487	99.84	*Colletotrichum boninense* (MF076585.1)/Glomerellales
SUT-HJ-I35	OR074489	100.00	*Aspergillus terreus* DTO 403-C9 (MT316343.1)/Eurotiales

Further phylogenetic analysis based on multiple gene sequences indicated that SUT-HJ-I35 was grouped with *A. terreus* indeed (Fig. 3) but isolate SUT-HJ-I04 should be identified as *C. karstii* (Fig. 4). Our data suggest that these non-mycorrhizal fungi are more important for seed germination than previously thought.

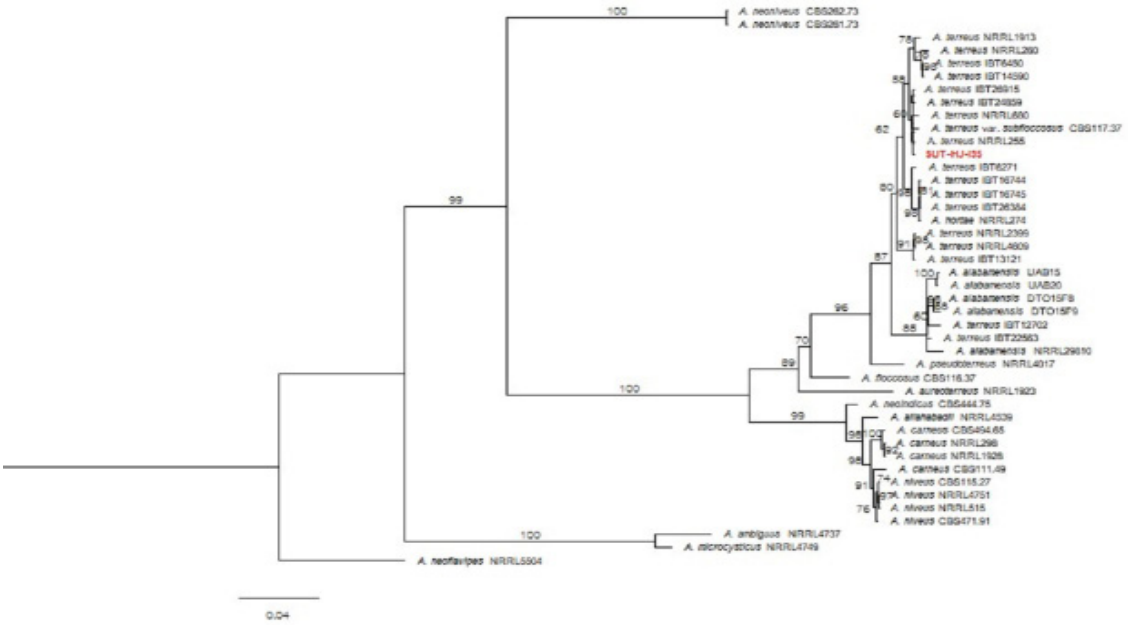

Fig. 3. Maximum Likelihood (ML) tree obtained based on phylogenetic analysis of ITS and tub2 sequence data of the isolate SUT-HJ-I35 and *Aspergillus* section Terrei. Numbers above branches are bootstrap values. Only values above 50% are indicated. The species *A. neoflavipes* NRRL5504 in section Flavipes was selected as an outgroup.

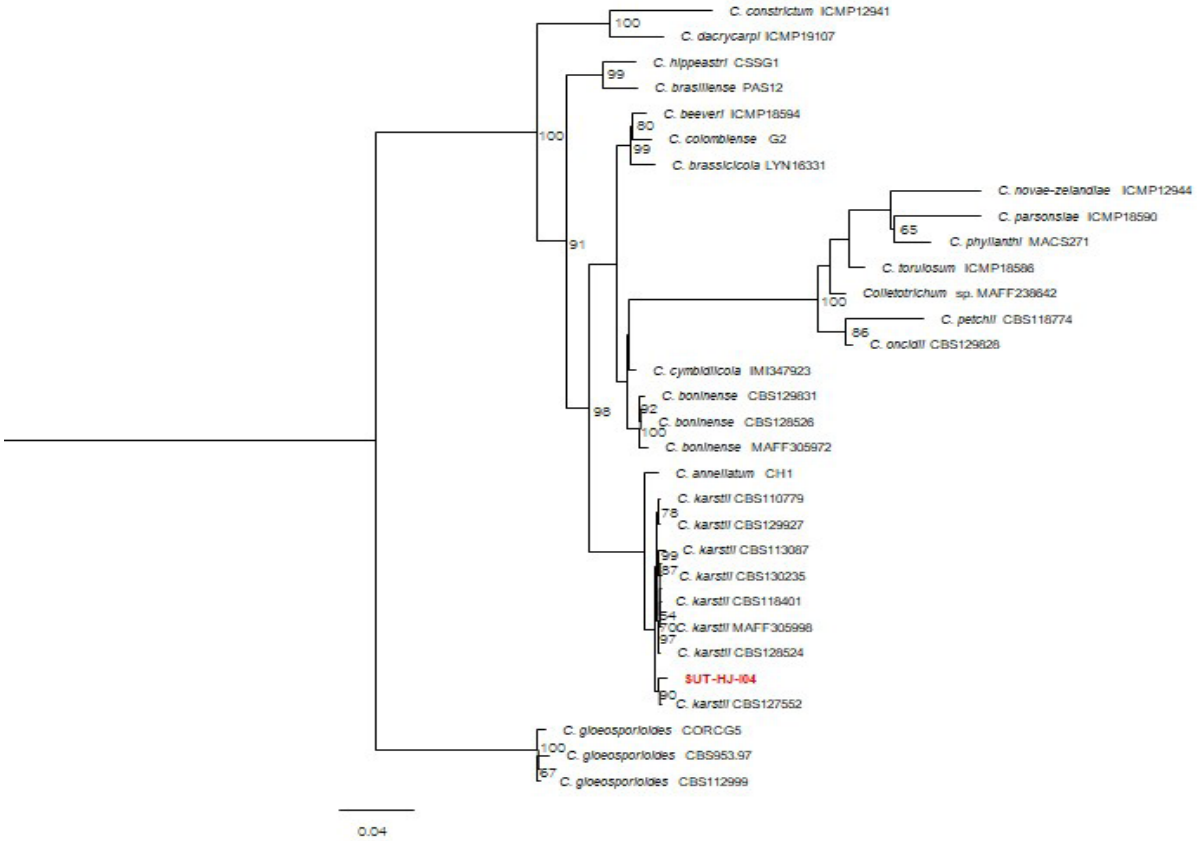

Fig. 4. Maximum Likelihood (ML) tree obtained based on phylogenetic analysis of concatenated sequences of the act, chs-1, gadph, ITS and tub2 genes of the isolate SUT-HJ-I04 and *Colletotrichum boninense* species complex. Numbers above branches are bootstrap values. Only values above 50% are indicated. The species *Colletotrichum gloeosporioides* species complex was selected as an outgroup.

The finding that *Colletotrichum* and *Aspergillus* have a role in seed germination of *H. janellehayneana* is consistent with prior work. Non-mycorrhizal fungi species, including *Colletotrichum, Aspergillus, Alternaria, Penicilli, Trichoderma* and *Fusarium* species, have been isolated from many orchid species (Cig *et al*., 2018; Alomía *et al*., 2022). Endophytic *Colletotrichum* fungi from *Bletilla ochracea* (Tao *et al*., 2013), *Dendrobium* spp. (Chen *et al*., 2010; Ma *et al*., 2018; Meng *et al*., 2019; Sarsaiya *et al*., 2020) and *Pogoniopsis schenckii* (Sisti *et al*., 2019) have been reported. Despite its high pathogenicity on seedlings, Shah *et al*. (2019) reported that *Colletotrichum* enhanced the growth of adult individuals of *Dendrobium* species. *Aspergillus* fungi, on the other hand, are not yet known to promote seed germination in orchids (Ma *et al*., 2015; Cig *et al*., 2018). Moreover, *A. fumigatus* was reported as an opportunistic orchid pathogen in *Laelia* orchids (Almanza-Álvarez *et al*., 2017).

More research is needed to assess the potential physiological and ecological benefits of non-mycorrhizal fungi commonly found in orchid roots. Some fungi produce active substances that may benefit orchids by increasing their tolerance to abiotic stress, allowing them to adapt to a variety of environmental circumstances or fighting to pathogens and insects (Ma *et al*., 2015). Some fungi may even breakdown local soils and offer nutrients for orchid growth and development (Li *et al*., 2021). Using the appropriate fungal strain may improve germination success.

Since all orchids rely on mycorrhizal partners to germinate naturally, symbiotic germination is now a widely employed technique and helpful strategy for terrestrial orchid conservation efforts. The symbiotic technique has been successfully applied to germinate three *Habenaria* species from Florida, USA, including *H. repens*, *H. quinquiseta*, and *H. macroceratitis*, with germination percentages ranging from 5.8–55.1%. The highest germination rates for all species were achieved using a *Ceratorhiza* isolate (Stewart and Zettler, 2002). Only *H. repens* seedlings developed stage 5, while none of *H. quinquiseta* or *H. macroceratitis* seeds developed beyond stage 2. Similar to their results, our study showed that none of the seedlings of *H. janellehayneana* inoculated with *Colletotrichum* or *Aspergillus* developed beyond the stage 2 or stage 3, respectively. Further investigations should explore how such seedlings can continue development thereafter. Studies on other orchid species have documented a need for multiple fungal species to achieve full development. For example, Chutima *et al*. (2011) showed that endophytic fungi isolated from *Pecteilis susannae* (L.) Rafin. enhanced seed germination up to 86.20% when the seeds were also grown with *Epulorhiza* sp. Similarly, a combination of *Ceratobasidium* sp., *Flavodon* sp., and *Tulasnella* sp. isolates induced significantly higher germination rates in *Paphiopedilum villosum* (Lindl.) Stein. as compared with uninoculated control treatments (Khamchatra *et al*., 2016). In addition, using *Ceratobasidium* strains achieved a high germination frequencies of up to 80% whereas *Tulasnella* strains supported a germination percentages close to 60% (Alomía *et al*., 2017).

CONCLUSION

For asymbiotic seed germination of *H. janellehayneana*, the highest germination percentages were obtained on ½ VW or with the addition of 1 mg/L BAP, and seeds on this medium developed to stage 4 protocorms within 10 weeks. In the case of symbiotic seed germination, however, two non-mycorrhizal endophyte fungi isolates obtained from the roots of wild-grown adult *H. janellehayneana* plants promoted seed germination via a symbiotic effect in co-culture. This research suggests that these fungal isolated may be effective for symbiotic early seed germination of this orchid species, but they are less effective for further growth. More specific mycorrhizal fungi might be needed for

seed germination enhancement and onward development of this (and other) terrestrial orchid species. Nevertheless, orchid growers may achieve more consistent results in the propagation of this terrestrial orchid using asymbiotic germination.

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Expectation of Chamomile Fundamental Oil Abdicate by Using the Artificial Neural Network System

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Abstract

The aim of this research was to forecast the proportion and production of chamomile essential oils by employing an artificial neural network system reliant on specific soil physicochemical characteristics. Various chamomile cultivation sites were explored, and 100 soil samples were transported to the greenhouse. The pH, EC, K, OM (organic matter), CCE (calcium carbonate equivalent), and clay content in the soils ranged from 8.75 to 7.94, 1.6 to 1.0, 381 to 135, 2.30 to 0.22, 69 to 16, and 55.6 to 32.0, respectively. Growth parameters, essential oil percentage, and yield were measured. The artificial neural network modeling aimed to predict essential oil concentration and yield using three sets of soil properties as predictors: 1- lime, clay, pH and EC; 2- nitrogen, phosphorus, potassium and clay; 3- lime, clay, silt, gravel, nitrogen, phosphorus, potassium, pH and EC. Consequently, three pedotransfer functions (PTFs) were formulated using the multi-layer perceptron (MLP) with the Levenberg-Marquardt training algorithm to estimate chamomile essential oil content. The evaluation of results indicated that the third PTF (PTF3), developed using all independent variables, exhibited the highest accuracy and reliability. Furthermore, the findings suggested the feasibility of predicting chamomile essential oil concentration and yield based on soil physicochemical properties. This has significant implications for land suitability assessments, identifying areas conducive to chamomile cultivation, and planning for essential oil yields.

Keywords: Artificial neural network (ANN), Calcium carbonate equivalent (CCE), Multilayer perceptron, Nitrogen.

INTRODUCTION

Ensuring optimal nutrition is a crucial factor in enhancing both the quality and quantity of crops, a condition profoundly impacted by the soil environment (Ajili Lahiji *et al*., 2018; Tofighi Alikhani, 2021). The role of soil characteristics in influencing crop yield has been emphasized by Peng *et al*. (2012), highlighting the indispensability of mineral elements in the process of photosynthesis for the production of organic materials in leaves. Each macronutrient plays a distinct role in the metabolic processes governing plant growth (Benier *et al*., 1985). The nutritional status, particularly the balance between elements extracted from air and soil, significantly influences plant flowering.

Given the significance of advancing the cultivation of medicinal plants and harnessing their products as natural, health-compatible ingredients, employing diverse cultivation and nutrition methods is essential to augment the essential oil and active compound content of medicinal plants (Ajili Lahiji *et al*., 2018; Savitikadi *et al*., 2020). Chamomile is an annual or perennial plant belonging to the Asteraceae family. The plant improves appetite and reduces swelling, pain and sweating (Ubessi *et al*., 2019). Chamomile are native to temperate regions of Asia and Europe and are grown around the world for their high medicinal, cosmetic, and dietary values (Wan *et al*., 2019). It has been used for thousands of years in ancient Greece, Rome and Egypt. In China, the detailed uses of this plant were first recorded in Uyghur medicine. Experienced TCM practitioners believe that preparations containing chamomile have a soothing effect. Additionally, the plant is used in traditional, homeopathic and Unani preparations (Zhao *et al*., 2015). There are two main varieties of chamomile: *Matricaria chamomilia* L. and *Anthemis nobilis* (L.). *Matricaria chamomilia* L. belongs to the genus Matricaria. This is an annual plant and the flowering period is from May to July in China. *Chamaemelum nobile* L. is a perennial plant of the genus Chamaemelum. The flowering period is from April to May in China (Dai *et al*., 2022). *Matricaria chamomilia* L. is relatively common and has been the subject of much research and use. Currently, 26 countries around the world have included this plant in their pharmacopoeia. Chamomile heads are often used as medicine (Petronilho *et al*., 2011). Chamomile contains flavonoids, coumarins, volatile oils, terpenes, sterols, organic acids and polysaccharides, among other compounds. Possessing a wide range of compounds, chamomile has various pharmacological activities such as anti-cancer, anti-infectious, anti-inflammatory, anti-oxidant, hypoglycemic, hypotensive, hypolipidemic, anti-allergic, anti-depressant and neuroprotective, among many other effects (Zhao, 2018). In general, this plant has special research value. However, there is little criticism on this topic in the literature. This article provides a comprehensive overview of plant properties and distribution, traditional uses, chemical composition, pharmacological effects, and quality control methods of chamomile (Dai *et al*., 2022).

Notably, the growth and essential oil yield of chamomile are influenced by factors such as calcium and magnesium, with magnesium exhibiting a more significant impact than calcium (Upadhyay and Patra, 2011). In the realm of agriculture, a key challenge lies in predicting crop yield based on readily accessible indicators. Various factors, including soil nutrition and physicochemical properties, affect plant yield and essential oil content (Belal *et al*., 2016; Radkowski and Radkowska, 2018; Mohammadi Torkashvand *et al*., 2020). Hypotheses suggest that predicting yield is feasible by assessing nutrient concentrations in leaves (Ajili Lahiji *et al*., 2018; Mohammadi Torkashvand *et al*., 2020), fruits (Mohammadi Torkashvand *et al*., 2019), or soil characteristics (Rahmani Khalili *et al*., 2020; Tashakori *et al*., 2020). Such predictions

enable effective planning for fertilization, soil selection, and future financial considerations for farmers.

In predicting natural variables, transfer functions, especially artificial neural networks (ANN), have proven more efficient in heterogeneous natural systems like soil and plants compared to traditional regression methods. Numerous studies have successfully employed ANN to estimate soil variables and predict crop yield based on factors such as weather, soil properties, and growth characteristics (Zhou *et al*., 2008; Bocco *et al*., 2010; Mokhtari Karchegani *et al*., 2011; Besalatpour *et al*., 2013; Dai *et al*., 2014; Moghimi *et al*., 2014; Marashi *et al*., 2017; Marashi *et al*., 2019). Tashakori *et al*. (2020) specifically highlighted the superior accuracy of ANN in estimating saffron yield compared to multiple linear regression and adaptive neuro-fuzzy inference system models.

A study by Poorghadir *et al*. (2021) underscored the influence of soil properties and nutrition on the yield and essence percentage of crops. This study aims to delve into the impact of soil characteristics on the concentration and quantity of chamomile essential oils, exploring the feasibility of using artificial neural networks to estimate these essential oil parameters based on critical physicochemical soil properties.

MATERIALS AND METHODS

Soil experiments

Multiple locations for chamomile cultivation in Kermanshah and Hamadan provinces, West of Iran, were investigated. From 20 areas, a total of 100 soil samples (five from each area) were collected at a depth of 0-30 cm and sent to IAU, Science and Research Branch, Tehran, Iran. The sampling areas shared similar topographic and climatic characteristics. Soil samples underwent air-drying, and clods were fragmented into small particles using a plastic hammer before passing through a 2 mm sieve (Klute, 1986). Laboratory analyses, focusing on phosphorus, nitrogen, potassium nutrients, pH, electrical conductivity (EC), texture, and organic matter, were conducted using 0.5 kg of each soil sample. The remaining soil was dedicated to greenhouse experiments. Soil pH and EC were measured in saturated soil extracts, soil texture was determined hydrometrically, and calcium carbonate equivalent (CCE) was assessed through titration (Paye *et al*., 1948). Nitrogen was measured via the Kjeldahl method (Goos, 1995), and available potassium and phosphorus concentrations were determined by flame emission and spectrophotometry methods after soil extraction (Soltanpour and Schwab, 1977; Emami, 1996). Organic matter content was determined by the Walkley and Black method (1934). Statistical data regarding soil properties are presented in table 1.

Greenhouse experiment

In a completely randomized design, 100 distinct soil samples were applied to plots with dimensions of 30 by 35 cm and a depth of 25 cm, with 20 seeds planted in each plot. Following germination and early growth, the number of plants per plot was reduced to 10 during the quadruple stage. Standard field operations, including irrigation, weed control, and pest management, were uniform across all plots. Upon full flowering, flowers were harvested at a maximum length of five centimeters, dried at 60 ºC, and the dry flower yield per plot (kg ha^{-1}) and essential oil concentration were determined. Essential oil content was measured using the Clevenger apparatus and expressed as g/100 g of dry flowers, while essential oil yield was reported as kg ha^{-1} based on dry flower yield.

Artificial neural network

The multilayer perceptron (MLP) learning rule, a feedforward network, was employed in this study. The Levenberg-Marquart back propagation algorithm facilitated training, utilizing the Tangent axon function as the activation function. The design of the artificial neural network was executed using NeuroSolutions 5.05 software. Data were divided into training (60%), validation (20%), and test (20%) sets. Three sub-series of variables were used as input variables for estimating essential oil percentage and yield. The efficiency of three pedotransfer functions (PTF1, PTF2, and PTF3) was compared, each developed using different sets of predictors. The evaluation criteria included the coefficient of determination (R^2) and root mean square error (RMSE). The number of neurons in the input layer corresponded to the input parameters, while the complexity of the network was determined by the number of hidden layers. The output layer neurons matched the number of output parameters. Model precision and accuracy were assessed using the test set data after training with the training and validation set data.

$$R^2 = 1 - \frac{\sum_1^N (y_i - \hat{y}_i)}{\sum_1^N (y_i - \bar{y}_i)^2}$$

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

In which: yi, $\bar{y}$,$\hat{y}$ respectively, the measured dependent variable, its mean and the estimated dependent variable, and N is the number of observations. Other criteria used to evaluate the precision of transition functions were the Geometric Mean Error Ratio (GMER) and Geometric Standard Deviation of error ratio (GSDER):

$$\text{GMER} = \exp\left(\frac{1}{N}\sum_1^N \ln\left(\frac{\hat{Y}_i}{Y_i}\right)\right)$$

$$\text{GSDER} = \exp\left(\frac{1}{N-1}\sum_{i=1}^{N}\left[\ln\left(\frac{\hat{Y}_i}{Y_i}\right) - \ln(\text{GMER})\right]^2\right)^{1/2}$$

The Geometric Mean of Error Ratio (GMER) serves as an indicator of the level of agreement between measured and estimated values. A GMER equal to one signifies a complete alignment between measured and predicted values. A GMER greater than one suggests that the predicted values surpass the measured values, while a GMER less than one indicates that the estimated values are lower than the measured values. On the other hand, the Geometric Standard Deviation of Error Ratio (GSDER) functions as a measure of data dispersion. A value close to one indicates minimal dispersion, and the deviation from one reflects the extent to which most estimates deviate from the measured data.

RESULTS AND DISCUSSION

Table 1 shows the correlation between the variables studied and the percentage and yield of the essential oils. The results presented in this table could be important to find input data series to the neural network. As seen, the percentage and yield of essential oil with organic matter showed a positive and significant correlation.

Table 1. Correlation between the input variables of the neural network and the amount of essential oil and essential oil yield.

Variable	pH	EC	N	P	K	OM	Lime	Sand	Silt	Clay	Essential oil	Essential oil yield
pH	1											
EC	0.511**	1										
N	-0.101	0.152	1									
P	0.806**	0.546**	-0.382*	1								
K	-0.390*	-0.419**	0.099	-0.323*	1							
OM	0.414**	0.675**	0.362*	0.443**	-0.046	1						
Lime	-0.267	0.015	0.001	-0.287	0.044	-0.544**	1					
Sand	-0.032	-0.339*	-0.439**	-0.029	0.268	-0.577**	0.648**	1				
Silt	-0.202	0.219	0.269	-0.069	-0.201	0.409**	-0.258	-0.408**	1			
Clay	0.188	0.178	0.243	0.084	-0.12	0.276	-.465**	-0.711**	-0.353*	1		
Essential oil	0.092	-0.024	0.391*	0.278	0.434**	0.355*	-0.181	0.155	0.153	-0.277	1	
Essential oil yield	0.235	0.163	0.401*	0.423**	0.382*	0.449**	-0.101	0.226	0.246	-0.421**	0.919**	1

*and **: Significant at $P < 0.05$ and $P < 0.01$, respectively.

Correlation among variables

The correlation between the variables examined and the percentage and yield of essential oils is depicted in table 2, offering valuable insights into potential input data for the neural network. Our findings reveal a positive and significant correlation between the percentage and yield of essential oil with organic matter, N, P, K contents, and clay. Consistent with our results, Jat and Ahaheat (2006) demonstrated that bio-fertilizers containing nitrogen, phosphorus, and potassium enhance the growth and essential oil production of fennel plants. Phosphorus plays a pivotal role in seed and flower germination, vegetative growth, fruit maturation, and metabolic processes (Bennett, 1993; Malakouti and Shahabi, 2000; Malakouti *et al*., 2008). Additionally, potassium influences herbal essential oil quantity and quality by affecting metabolic pathways and enzymatic activity (Pacheco *et al*., 2008). The correlation findings align with previous studies emphasizing the role of these elements in enhancing essential oil production (Nurzynska-Wierdak, 2013; Cecílio Filho *et al*., 2015; Chrysargyris *et al*., 2017).

Table 2. Input data for constructing a neural network in three different transfer functions and the characteristics of neural networks made.

Transition function	Model inputs	Number of hidden layers no.	Number of hidden layer nodes 1	Number of hidden layer nodes 2	Number of hidden layer nodes 3	Type of transfer function	Type of target function
PTF1	N, P, K and clay	3	4	2	1	Tangent axon	Levenberg-Marquardt algorithm
PTF2	pH, EC, Organic matter and clay	3	4	4	2	Tangent axon	Levenberg-Marquardt algorithm
PTF3	All variables	1	10	-	-	Tangent axon	Levenberg-Marquardt algorithm

Given the significant correlation with essential oils, three data series were designated as input for the artificial neural network (ANN), as outlined in table 3, considering the number of hidden layers and nodes.

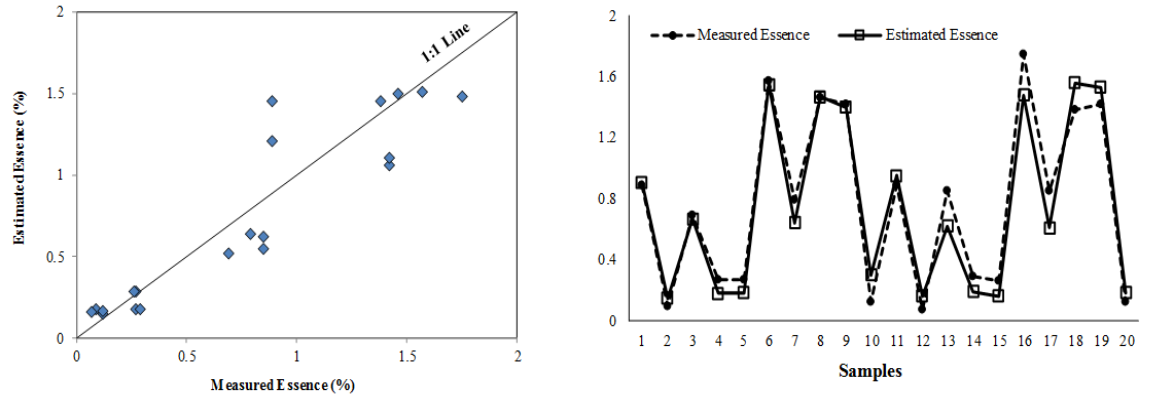
Table 3. Determination coefficient (R2), error (RMSE), GMER and GSDER in two sets of training and test data in predicting essential oil concentration (g/100 g).

Transition function	Data series	R^2	RMSE	GMER	GSDER
PTF1	Training	0.7856	0.226	1.27	2.37
	test	0.8271	0.201	1.04	1.24
PTF2	Training	0.9662	0.072	0.91	2.27
	test	0.8954	0.237	0.83	1.57
PTF3	Training	0.9562	0.023	0.98	1.22
	test	0.9478	0.086	1.02	1.32

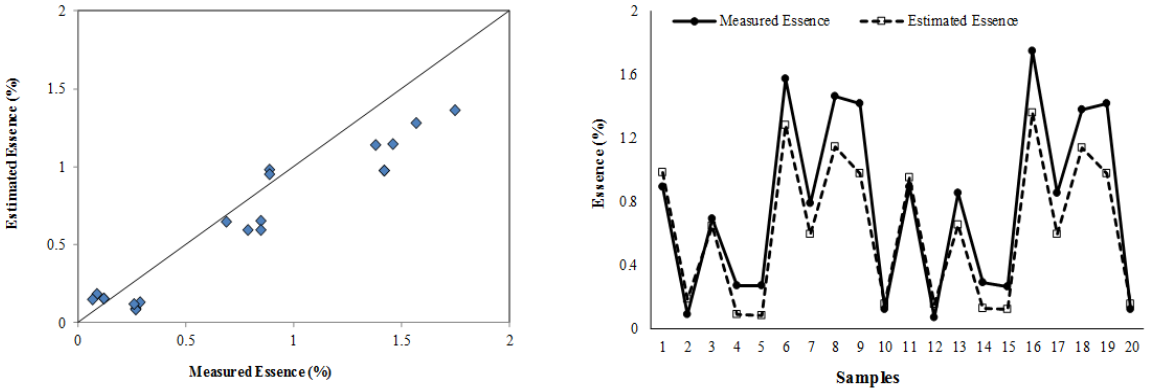
Estimation of essential oil

Fig. 1 illustrates the distribution and alignment of actual (measured) and estimated essential oil percentages for three input data series. R^2 values, as presented in table 4, indicate the accuracy of the ANN models in predicting essential oil percentages. Notably, the inclusion of all nine variables in the third transfer function (PTF3) resulted in a higher accuracy (R^2)

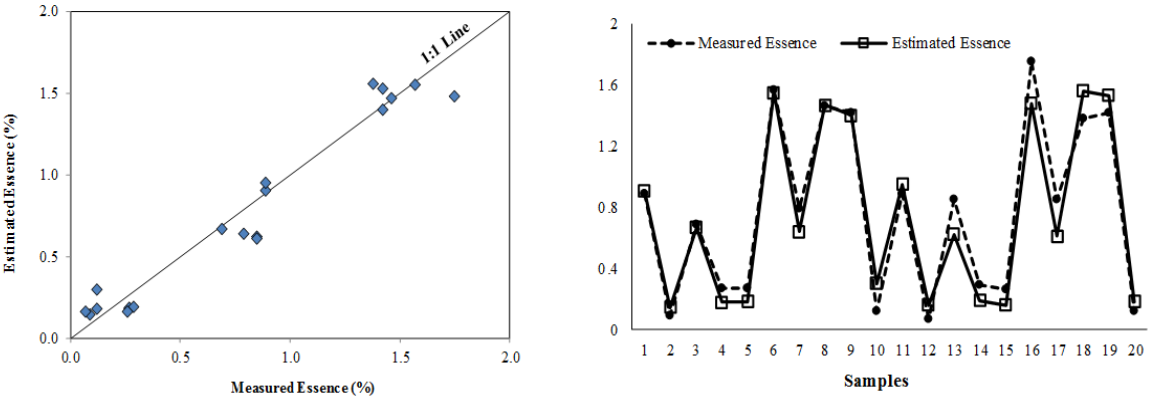
in both training (96.62%) and test (94.78%) data sets compared to PTF1 and PTF2. This increase in variables led to a reduction in error, as indicated by the GMER and GSDER values, demonstrating improved precision in the estimation of chamomile essential oil percentage.



A) First dataset



B) Second dataset



C) Third dataset

Fig. 1. Measured values (actual) and estimated concentration of essential oils in diagram 1: 1 test data and their conformance.

Table 4. Determination coefficients (R2), error (RMSE), GMER and GSDER in two sets of training and test data to predict the essential oil yield (kg / ha).

Transition function	Data series	R^2	RMSE	GMER	GSDER
PTF1	Training	0.7984	1.27	1.47	2.16
	test	0.9125	0.670	1.01	1.25
PTF2	Training	0.8646	0.536	1.19	1.38
	test	0.8323	0.998	1.26	1.45
PTF3	Training	0.9654	0.088	0.98	1.10
	test	0.9251	0.608	0.92	1.20

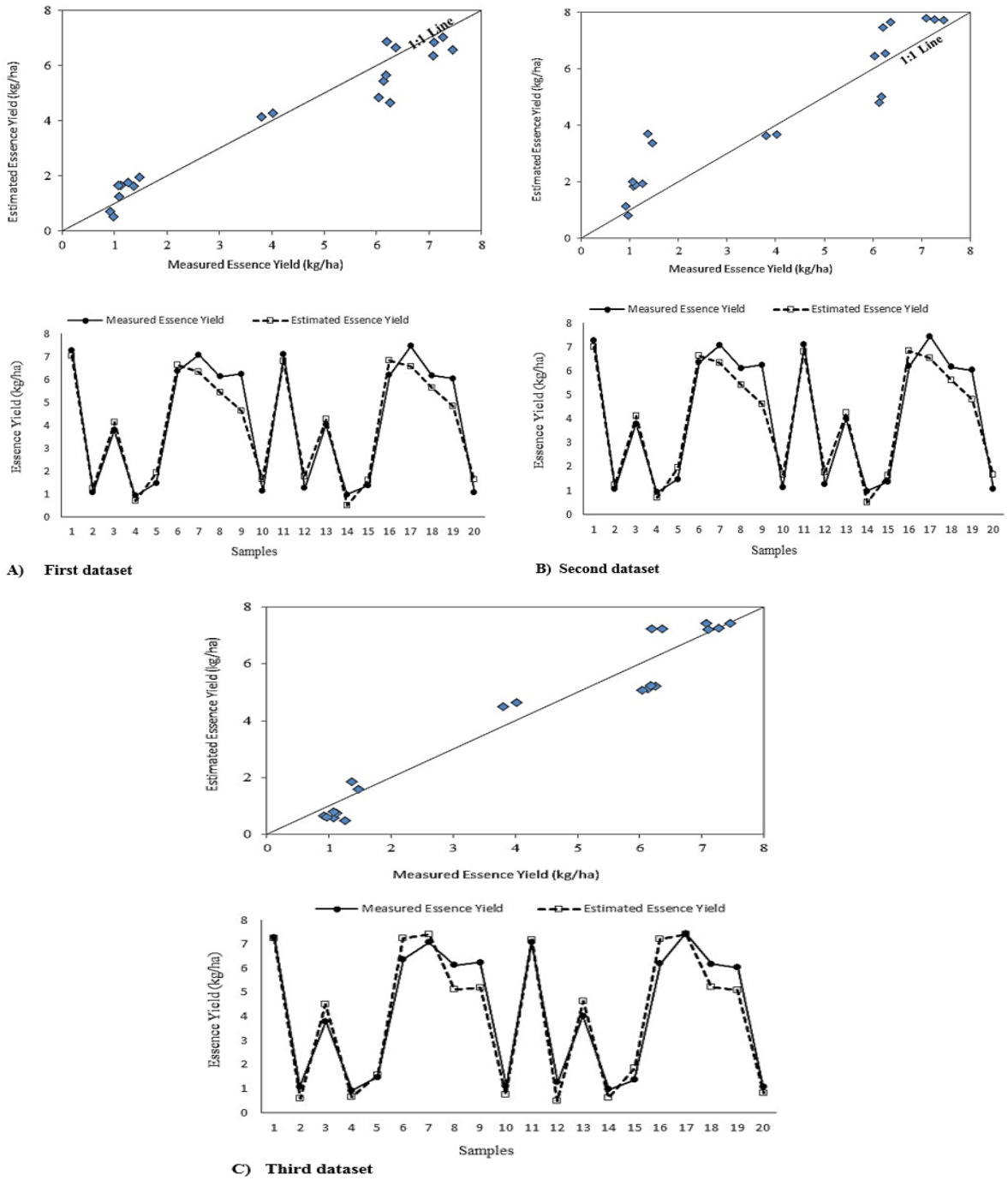


Fig. 2. Measured (actual) and estimated essence yield in diagram 1: 1 test data and their conformance.

Similarly, Fig. 2 displays the dispersion of measured yield values and the estimated essential oil values for the test series. The accuracy of the model improved in the third transfer function (PTF3), with an R^2 of 91.25% in the test data set. Despite a lower estimation in PTF3, the model exhibited the least error, as indicated by the GMER and GSDER values, demonstrating enhanced accuracy in predicting chamomile essential oil yield.

These results align with findings from studies employing ANN for crop yield prediction, emphasizing the importance of considering multiple variables for improved accuracy (Mohammadi Torkashvand *et al*., 2017; Akbar *et al*., 2018; Niazian *et al*., 2018). In summary, the comprehensive consideration of all nine soil variables led to a more accurate and reliable estimation of chamomile essential oil content and yield, showcasing the potential of ANN models in precision agriculture and decision-making systems.

CONCLUSION

In summary, the findings indicate that the third transfer function, incorporating nine variables as input for the neural network, proved to be the most precise in estimating both the concentration and yield of essential oil. This function exhibited the highest R^2 values and the lowest RMSE values, showcasing superior accuracy. Furthermore, the estimated values from this function demonstrated the closest alignment with observed values, displaying minimal deviation from the 1:1 line. The proposed model's evaluation metrics for estimating essential oil percentage in the test series data were as follows: $R^2 = 94.78$, RMSE = 0.86, GMER = 1.02, and GSDER = 1.32. Similarly, for essential oil yield in the test series data, the metrics were $R^2 = 91.51$, RMSE = 0.608, GMER = 0.92, and GSDER = 1.20. These results affirm the high accuracy and precision of predicting chamomile essential oil concentration and yield based on soil physicochemical properties. This predictive capability holds significance in determining land suitability, allowing for the identification of areas conducive to chamomile cultivation and facilitating strategic planning for essential oil yields. Future research avenues could explore additional soil characteristics or combinations thereof in conjunction with artificial neural networks for chamomile essential oil prediction. Additionally, the evaluation of alternative models, such as neuro-fuzzy, warrants consideration for estimating the concentration and essential oil yields not only for chamomile but also for other medicinal plant species.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Antioxidant Compounds, Minerals, and Nutrients of Different Chrysanthemum Genotypes

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Abstract

Chrysanthemum is a major ornamental flower in the world that is important medicinally and nutritionally. This research in 2022 investigated on 20 chrysanthemum genotypes produced and bred in the Ornamental Plants Research Center of Mahallat with regard to their nutritional, mineral, and antioxidant compounds in a completely randomized design. The results showed that the genotypes differed in all studied traits. The highest Ca (74.1 mg/kg FW), Fe (2.231 mg/kg FW), and Se (0.233 mg/kg FW) were obtained from codes 326, 110, and 562, respectively. The highest Zn content (0.315 mg/kg FW) was related to codes 562 and 134. Codes 540 and 603 were related to the highest vitamin A (0.086 mg/kg) and vitamin C (13.58 mg/100 g FW), respectively. Code 751 had the highest protein level of 1.483%. Codes 540 and 138 exhibited the lowest and highest fiber percentages of 13.06 and 22.34%, respectively. The best genotypes in petal anthocyanins and carotenoids were codes 674 and 108, respectively. codes 684 and 354 had the highest and code 751 had the lowest flavonoid content. The highest and lowest total phenols were observed in codes 326 and 462, respectively. Based on the results8, the 20 genotypes of chrysanthemum bred and produced in Iran, codes 110 and 326 are the most appropriate option in terms of nutritional and biological value, and these species can be used as a new and available food source to partially supply the nutrient requirement of the human body.

Keywords: Edible flower, Healthy eating, New food source, Plant protein, Vegetarianism.

INTRODUCTION

Our ancestors have proven that flowers are edible, but their culinary use has flourished in recent years. In the past, flowers were mostly consumed for their medicinal effects. However, they are used in modern nutrition and gastronomy as an ingredient or decorative material of various foods, and their effect on improving the appearance, taste, flavor, smell, and texture of foods, as well as their nutritional value, have increased their use in preparing various stews, sauces, soups, salads, desserts, and beverages. Presently, edible flowers are especially popular among the proponents of healthy lifestyles as a complete or part of the food (Nicolau and Gostin, 2015; Saurabh and Barman, 2020).

Marigold, primrose, hollyhock, chrysanthemum, carnation, rose, common sage, garden nasturtium, violet, and yucca are examples of famous edible flowers (Nicolau and Gostin, 2015; Guiné *et al*., 2019). Past studies have revealed that edible flowers are a rich source of proteins, carbohydrates, fatty acids, phenol compounds, sugars, vitamins, flavonoids, alkaloids, and antioxidants, that their application can significantly meet the food requirements of humans, and that they can even substitute main foods, especially during the shortage of seasonal or fresh foods (Fakhri *et al*., 2021; Netam, 2021).

Chrysanthemum (*Chrysanthemum morifolium* L.) from the family of Asteraceae is in the list of top ten cut flowers in the world. This species is medicinally, nutritionally, industrially, and environmentally important, in addition to its decorative value, so its consumption as a medicinal and edible plant in its origin, China, has a history of over 3000 years (Chen *et al*., 2021; Hadizadeh *et al*., 2022). Vitamins, flavonoids, sesquiterpenes, and unsaturated fatty acids are some biologically active compounds detected in chrysanthemums. Almost all parts of chrysanthemums (roots, stems, leaves, and petals) have medicinal applications (Skrajda-Brdak *et al*., 2020; Long *et al*., 2022). But, petals are their edible part, which is consumed in fresh, dried, steam-cooked, and cooked forms (Sugawara and Igarashi, 2009; Long *et al*., 2022).

Owing to the ornamental value of cut chrysanthemum flowers in the world, it has been subject to extensive breeding efforts, which have resulted in the production of many genotypes with significant ornamental traits, especially in Iran. New cultivars are often developed to improve the visual and qualitative features, such as flower color, size, form, aroma, quality, and longevity (Yan *et al*., 2019), which are some of the most important features considered by the consumers of edible flowers when deciding on flower selection and consumption. Many genotypes of chrysanthemum have been produced in Mahallat County, Iran, but no research has ever been conducted on their edibility. Thus, the present research aimed to determine the nutritional value and antioxidant compounds of 20 chrysanthemum genotypes produced in this county as edible flowers.

MATERIALS AND METHODS

To determine the minerals, vitamins, and antioxidant compounds of 20 chrysanthemum genotypes, an experiment was conducted based on a completely randomized design. The flowers were selected out of 1000 chrysanthemum genotypes produced and bred in the Ornamental Plants Research Center of Mahallat. The rooted cuts of the 20 target genotypes were planted in a controlled greenhouse in Mahallat in mid-September 2022. Fig. 1 show the characteristics of the genotypes used in this study. The flowers were harvested in late October 2022 when they were fully open. The harvested flowers were transferred to the experimental location (Islamic Azad University of Rasht) in proper packages and taking care of safe transportation principles.

To assess the traits, 10 flowers were selected from each genotype and were packed in lidded containers. The flowers were kept in a refrigerator (4 ºC) during the experiment.

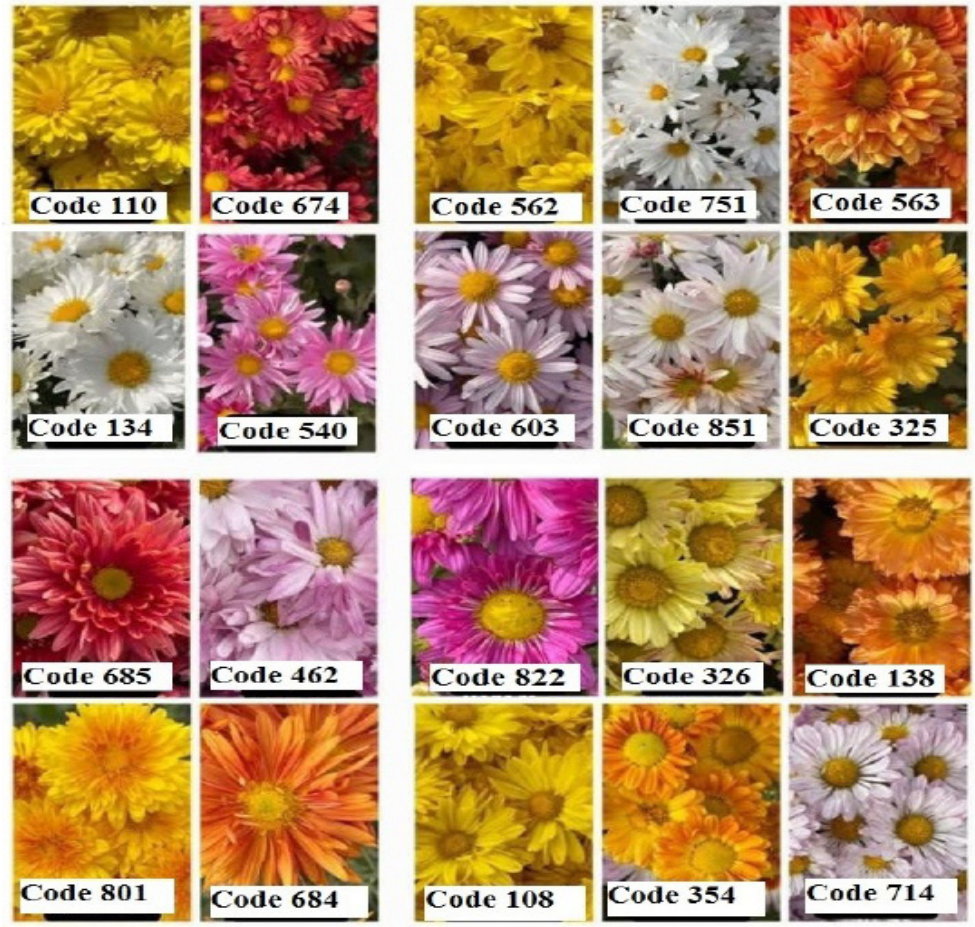

Fig. 1. Chrysanthemum genotypes used in the present study.

Assessment of traits

Minerals (Ca, Fe, Zn, and Se)

To measure the concentrations of minerals in chrysanthemum petals, their 1-g ash was extracted by Rengel and Romheld's (2000) method. Then, Ca, Fe, Zn, and Se concentrations were determined by the atomic absorption device.

Petal protein

The Kjeldahl method was employed to find out the petal protein content of the genotypes. In this method, the N percentage of the samples was determined, and the following formula was, then, used to estimate the petal protein percentage:

$$\text{Protein (\%)} = N \times 6.25$$

Vitamin C

The vitamin C content was determined by using the method of titration with 2,6-dichlorophenolindophenol. The vitamin C content of the petals was calculated by the following equation in mg/100 g fresh weight (FW) (Mazumdar and Majumdar, 2003):

$$\text{Vitamin C} = \frac{e \times d \times b}{c \times a} \times 100$$

in which a is the sample weight, b is the volume of metaphosphoric used in extraction, c is the volume of solution taken for titration, e is the volume of the dye solution consumed for each sample, and d is the dye factor, which is calculated by the following equation:

$$d = \frac{0.5}{\text{The amount of dye solution used for the titration of the standard sample}}$$

Petal anthocyanin and carotenoid

Mazumdar and Majumdar's (2003) method was used to measure the anthocyanin and carotenoid content of the chrysanthemum petals. So, 0.5 of the fresh petal was extracted by acid method to determine the anthocyanin content and by acetone 80% to determine the carotenoid content. Then, their absorbance was read with a PD-103 UV APEL spectrophotometer at 535 nm for anthocyanin and at 440, 645, and 663 nm for carotenoid. Finally, the anthocyanin and carotenoid contents were estimated by the following equations:

$$\text{Anthocyanin (mg/100g FW)} = \frac{e \times b \times c}{d \times a} \times 100$$

in which e is the sample weight, b is the volume of the sample used for measurement, c is the whole of the synthesized solution, d is the volume of the sample taken, and a is the reading of the spectrophotometer.

$$\text{Petal carotenoid (µg/g FW)} = 4.69 \times A_{440} - 0.268 \times (20.2)A_{645} + (8.02)A_{663}$$

Raw fiber

The percentage of raw fiber was calculated by the following equation as per the procedure described by Aryapak and Ziarati (2014):

$$\text{Raw fiber percentage} = \frac{\text{Raw fiber weight}}{\text{Initial sample weight}} \times 100$$

Tota phenol content

The total phenol content was determined by Singleton *et al.*'s (1999) method for which a reaction mixture was made of the plant extract, distilled water, diluted Folin, and sodium carbonate. Also, the standard solution was developed by gallic acid and pure methanol at the rates of 0, 6.25, 12.5, 25, 50, and 100 µL. Eventually, an APEL PD-303UV spectrophotometer and a standard curve were used to find out the total phenol content of the petals in mg gallic acid equivalent (GAE) per 100 g FW.

Total flavonoid content

The total flavonoid content was measured by Du *et al*.'s (2009) method. After the petal extract was prepared, the absorbance of the samples was read at 506 nm with a spectrophotometer, and the total flavonoid content was calculated in mg catechin equivalent (CE) per 100 g FW using a standard curve.

Data analysis

The data collected were analyzed by the SPSS 19 statistical software package. The means were compared by the LSD test at the $P < 0.05$ and $P < 0.01$ levels.

RESULTS

Minerals

According to the analysis of variance (ANOVA), the 20 studied genotypes differed in minerals (Fe, Zn, Ca, and Se) significantly (Table 1). The comparison of means showed that Codes 110, 326, and 603 had the highest (3.231, 2.403, and 2.368 mg/kg FW, respectively) and Codes 685, 801, and 138 had the lowest Fe content (1.143, 1.248, and 1.295 mg/kg FW, respectively). Regarding Zn, Codes 562 and 134 had the highest content (0.315 mg/kg FW) and did not differ from Code 354 (0.303 mg/kg FW) significantly. The lowest Zn content (0.175 mg/kg FW) was observed in Codes 674, 325, 326, and 684. The highest Ca content was 74.1 mg/kg FW exhibited by Code 326. Also, Codes 684, 138, and 801 had the lowest Ca content (45.46, 45.83, and 47.03 mg/kg FW, respectively), showing no significant differences from one another. The highest and lowest Se contents were observed in Codes 562 (0.233 mg/kg FW) and 603 (0.026 mg/kg FW), respectively (Table 2).

Table 1. Analysis of variance of the effect of different genotype on traits measured in chrysanthemum.

S.o.V	df	MS					
		Fe (ppm)	Zn	Ca	Se	Vitamin A	Vitamin C
Repetition	2	0.0085^{ns}	0.00653^{**}	7.15^{ns}	0.00058^{ns}	0.000011^{ns}	0.061^{ns}
Genotype	19	0.05467^{*}	0.000519^{*}	483.3^{**}	0.00593^{**}	0.00558^{**}	0.879^{**}
Error	38	0.01524	0.000137	64.04	0.0002	0.0000045	0.079
CV (%)	-	6.95	5.07	13.49	24.38	21.7	12.29

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test, respectively.

Vitamins A and C

The chrysanthemum genotypes differed in vitamins A and C significantly ($P < 0.01$) (Table 1). Code 540 was the best genotype in the vitamin A content (0.0868 mg/kg FW) followed by Codes 674 (0.028 mg/kg FW) and 138 (0.020 mg/kg FW) in the second and third ranks, respectively. The weakest genotypes in this trait were Codes 851 (0.0016 mg/kg FW) and 110 (0.0017 mg/kg FW). There was no significant difference between these two genotypes (Table 2).

Code 603 was the richest genotype in vitamin C (13.58 mg/100 g FW), but it did not show any significant differences from Codes 462, 562, 82, and 540 (13.39, 13.33, 13.30, and 13.10 mg/100 g FW, respectively). The lowest vitamin C contents were recorded by Codes 685 (11.71 mg/100 g FW), 354 (11.86 mg/100 g FW), and 751 (11.96 mg/100 g FW), respectively (Table 2).

Table 2. Mean comparison of the effect of different genotype on traits measured in chrysanthemum.

Genotype	Fe (mg/kg F.W.)	Zn (mg/kg F.W.)	Ca (mg/kg F.W.)	Se (mg/kg F.W.)	Vitamin A (mg/kg F.W.)	Vitamin C (mg/100 g F.W.)
Code 108	1.936^{cde}	0.187^{ih}	57.86^{e-h}	0.048 $^{b-f}$	0.0073^{de}	12.70^{cde}
Code 110	3.231^{a}	0.268^{b}	53.76^{jk}	0.060^{bcd}	0.0017^{h}	12.10^{f-i}
Code 134	1.866^{de}	0.315^{a}	61.00^{de}	0.052^{b-e}	0.0028^{fgh}	12.93^{bcd}
Code 138	1.295^{ghi}	0.245^{cd}	45.83^{l}	0.058^{b-e}	0.0200^{c}	12.23^{e-h}
Code 325	1.435^{fgh}	0.175^{i}	55.90^{g-j}	0.068^{bc}	0.0020^{gh}	12.26^{e-h}
Code 326	2.403^{b}	0.175^{i}	74.10^{a}	0.045^{c-f}	0.0025^{fgh}	12.46^{def}
Code 354	1.481^{fg}	0.303^{a}	62.66^{cd}	0.060^{bcd}	0.0023^{gh}	11.86^{hi}
Code 462	2.100^{c}	0.198^{gh}	59.96^{d-f}	0.052^{b-e}	0.0033^{fgh}	13.39^{ab}
Code 540	1.528^{f}	0.222^{ef}	70.60^{b}	0.035^{ef}	0.0868^{a}	13.10^{abc}
Code 562	1.90^{cde}	0.315^{a}	54.60^{i-k}	0.233^{a}	0.0098^{d}	13.33^{ab}
Code 563	1.750^{e}	0.268^{b}	52.86^{jk}	0.052 $^{b-e}$	0.0055^{efg}	12.36^{e-g}
Code 603	2.368^{b}	0.257^{cb}	57.5^{f-i}	0.026^{f}	0.0024^{gh}	13.58^{a}
Code 674	1.458^{fg}	0.175^{i}	69.30^{b}	0.035^{ef}	0.0280^{b}	12.53^{def}
Code 684	1.306^{ghi}	0.175^{i}	45.46^{l}	0.043^{def}	0.0022^{gh}	12.13^{f-i}
Code 685	1.143^{i}	0.210^{gf}	55.30^{h-k}	0.046^{c-f}	0.0025^{fgh}	11.71^{i}
Code 714	1.820^{de}	0.268^{b}	64.70^{c}	0.036^{ef}	0.0041^{e-h}	12.56^{def}
Code 751	1.365^{fgh}	0.222^{ef}	52.26^{k}	0.043 $^{d-f}$	0.0036^{fgh}	11.96^{g-i}
Code 801	1.248^{hi}	0.187^{ih}	47.03^{l}	0.069^{b}	0.0060^{ef}	12.36^{e-g}
Code 822	1.960^{cd}	0.233^{ed}	58.56^{efg}	0.046^{b-f}	0.00233^{gh}	13.30^{ab}
Code 851	1.878^{de}	0.222^{ef}	54.70^{h-k}	0.0603^{bcd}	0.0016^{h}	12.46^{d-f}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Total protein

Based on ANOVA, the studied 20 chrysanthemum genotypes differed in protein content significantly ($P < 0.01$) (Table 3). As is seen in fig. 2, Code 751 was the superior genotype (1.483%) followed by Codes 603, 562, and 108 in the next ranks, respectively. The lowest protein content was related to Code 801 (0.771%), but it had no statistically significant difference from Codes 563, 326, and 462 (Fig. 2).

Table 3. Analysis of variance of the effect of different genotype on traits measured in chrysanthemum.

		MS					
S.o.V	df	Total protein	Total fiber	Total flavonoids	Total phenol	Total anthocyanin	Petal carotenoid
Repetition	2	0.0015^{ns}	0.867^{ns}	2.683*	0.189^{ns}	14.7^{ns}	53903**
Genotype	19	0.1147**	21.406*	16.7**	16.07**	924.42*	44798**
Error	38	0.0063	7.38	0.7481	3.67	20.66	941.205
CV (%)	-	8.12	16.42	21.74	18.41	20.57	14.59

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test, respectively.

Total raw fiber

The raw fiber content was significantly ($P < 0.05$) influenced by the genotype (Table 3). The best genotype was Code 138, which contained 22.34% fiber. Codes 108, 326, 110, and 134 did not differ from one another significantly and were all the best treatments as a source of raw fiber. The lowest raw fiber content was observed in Codes 540 (13.06%) and 822 (13.78%), not differing from one another significantly (Fig. 3).

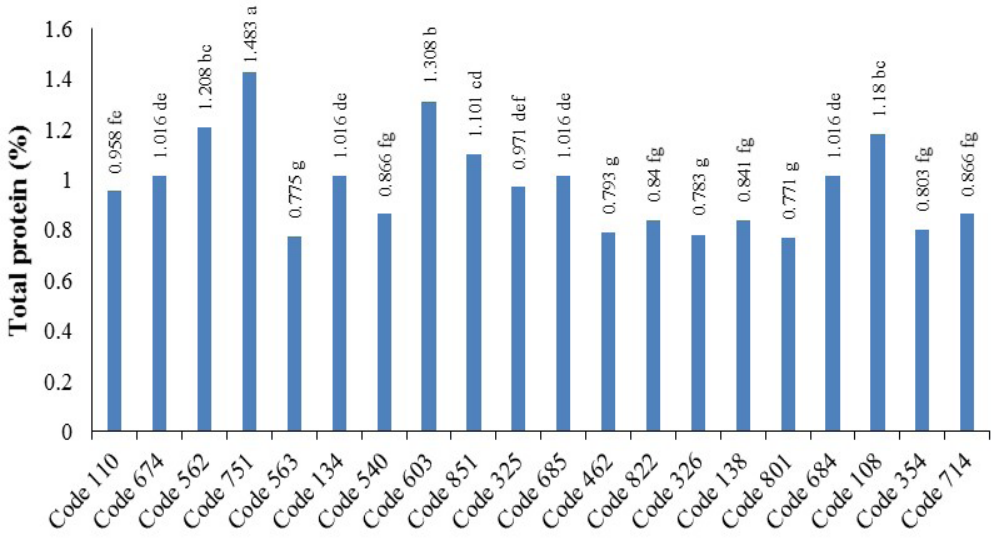

Fig. 2. Mean comparison of the effect of different genotype on total protein.

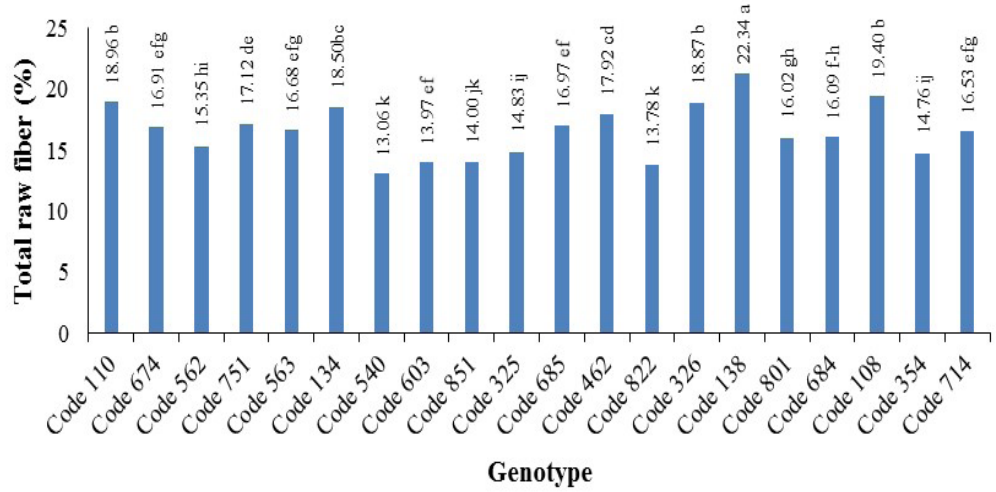

Fig. 3. Mean comparison of the effect of different genotype on total row fiber.

Total phenols

There were statistically significant ($P < 0.01$) differences among the genotypes in total phenols (Table 3). The total phenols content was the lowest in Code 462 (7.93 mg/g FW) and the highest in Code 326 (12.546 mg/g FW). However, the superior genotype did not differ from Codes 138 (11.566 mg/g FW) and 603 (11.536 mg/g FW) significantly. So, all these three genotypes were the best in total phenols (Fig. 4).

Total flavonoid

As revealed by ANOVA, the studied genotypes differed in total flavonoid significantly at the $P < 0.01$ level (Table 3). The best genotypes in this trait, which did not differ significantly from one another, were Codes 684 (5.81 mg/g FW) and 354 (5.39 mg/g FW). In contrast, Code 751 had the lowest total flavonoid content of 3.50 mg/g FW (Fig. 5).

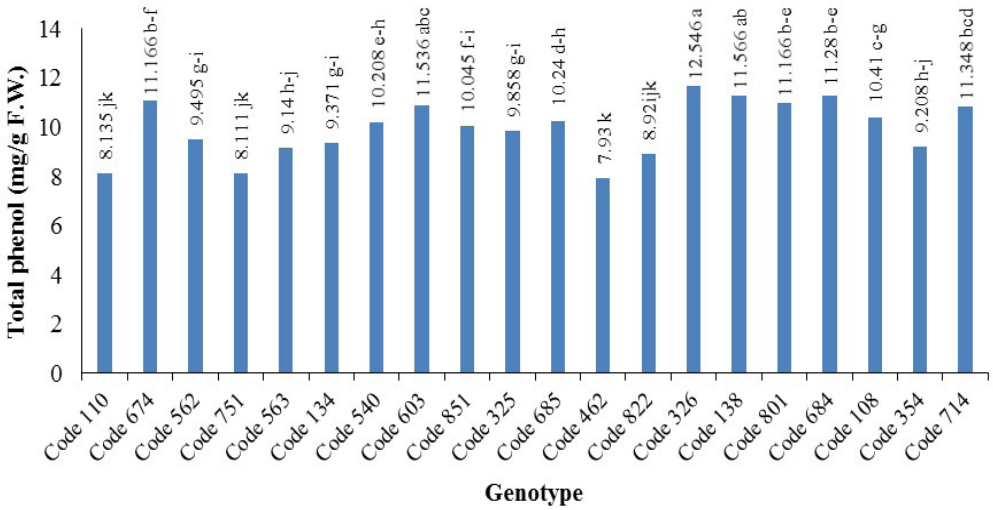

Fig. 4. Mean comparison of the effect of different genotype on total phenol.

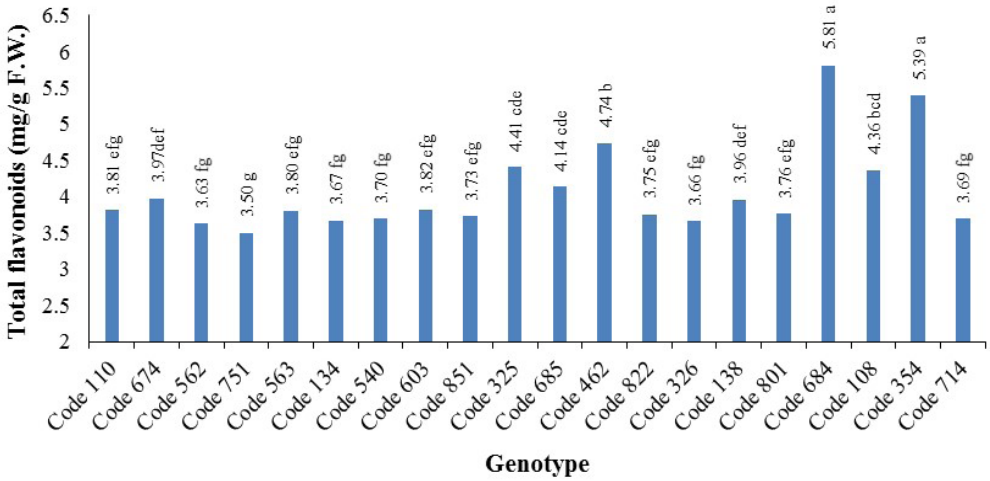

Fig. 5. Mean comparison of the effect of different genotype on total flavonoids.

Total anthocyanin in petals

The genotypes significantly ($P < 0.05$) differed in total anthocyanin content (Table 3). The highest anthocyanin content was 60.83 mg/100 g FW observed in Code 674 and the second-highest was 52.56 mg/100 g FW observed in Code 822. The lowest anthocyanin contents were observed in Codes 134 (5.60 mg/100 g FW), 751 (6.20 mg/100 g FW), and 108 (6.86 mg/100 g FW), respectively (Fig. 6).

Total carotenoid in petals

It was found from ANOVA that the carotenoid content of the petals differed among the genotypes significantly ($P < 0.01$; Table 3). Code 108 (482.3 µg/g FW) was the best. Codes 801, 563, 684, and 674 were also successful genotypes in this trait. But, the lowest petal carotenoid content (71.7 µg/g FW) was related to Code 603, which did not differ from Codes 134, 540, 751, 851, 462, 822, and 714 significantly (Fig. 7).

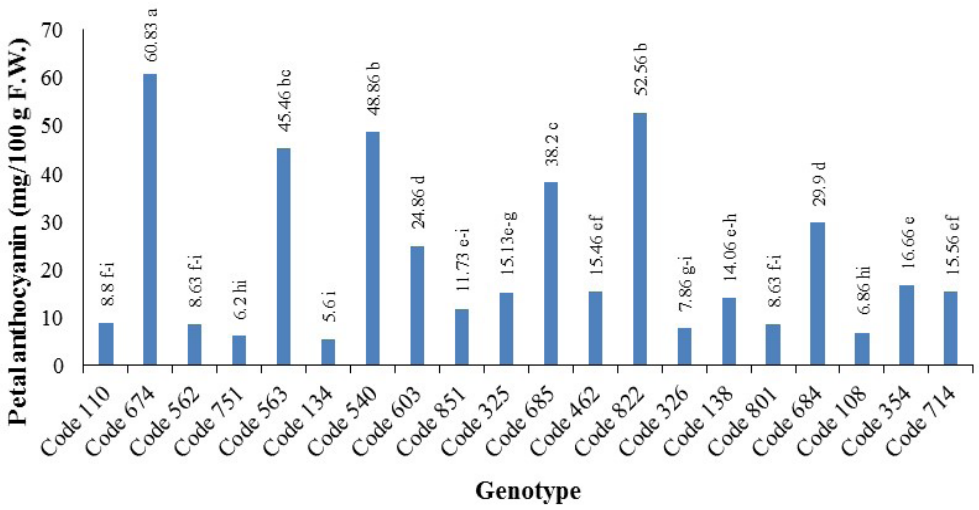

Fig. 6. Mean comparison of the effect of different genotype on total anthocyanin.

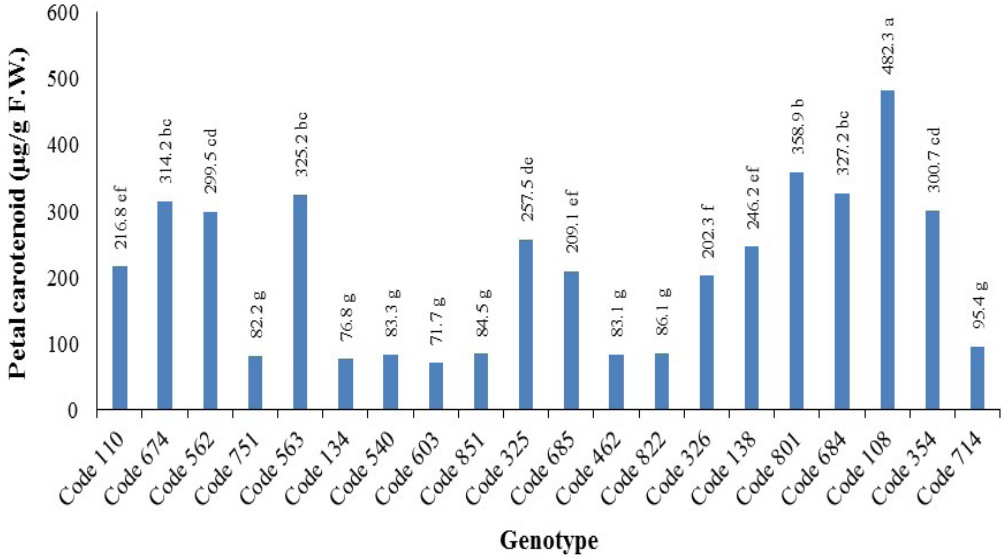

Fig. 7. Mean comparison of the effect of different genotype on petal carotenoid.

CORRELATION RESULTS

Table 4 shows the correlation between the measured traits. As can be seen, there was a positive and significant correlation between phenol, flavonoid and vitamin C, which can be mentioned as the most important characteristics of an edible flower. Also, there was a positive and significant correlation between the amount of carotenoid and vitamin C, phenol, flavonoid, fiber and element selenium. The amount of fiber in the studied edible flowers was positive and significant with all traits except anthocyanin, selenium and protein. The relationship between protein and vitamin C, phenol, flavonoid, iron, zinc, calcium and fiber was also positive and significant. The positive correlation between the investigated traits is a desirable feature for the consumption of edible flowers.

Table 4. Correlation between measured traits.

	Carotenoid	Anthocyanin	Vitamin C	Phenol	Flavonoid	Fe	Zn	Ca	Se	Fiber	Protein
Carotenoid	1.000										
Anthocyanin	0.054	1.000									
Vitamin c	0.254*	0.270*	1.000								
Phenol	0.384**	0.239	0.803**	1.000							
flavonoid	0.471**	0.216	0.744**	0.649**	1.000						
Fe	0.021	-0.053	0.632**	0.395**	0.343**	1.000					
Zn	0.075	0.044	0.713	0.452**	0.472**	0.557**	1.000				
Ca	0.130	0.343**	0.846**	0.737**	0.595**	0.599**	0.572**	1.000			
Se	0.262*	-0.249*	0.146	-0.012	0.014	0.089	0.352**	-0.026	1.000		
Fiber	0.422**	-0.006	0.772**	0.716**	0.646**	0.540**	0.563**	0.632**	0.047	1.000	
Protein	0.154	0.016	0.684**	0.538**	0.480**	0.417**	0.522**	0.503**	0.196	0.539**	1.000

*and **: Significant at $P < 0.05$ and $P < 0.01$, respectively.

DISCUSSION

The introduction of a new nutrient into the human food regime depends on various factors, such as its nutritional and mineral compounds, antioxidants, and effect on human health. Edible flowers, e.g., chrysanthemums, are an invaluable source of biologically active compounds that have drawn the attention of people, especially the proponents of a healthy lifestyle (Alves *et al*., 2021; Chen *et al*., 2021). Edible flowers have different concentrations of minerals, vitamins, proteins, sugars, fatty acids, and antioxidant compounds (Espejel *et al*., 2019). Minerals are important constituents in foods and play an essential role in preserving the natural functioning of the human body. For instance, Ca is a vital element in the structure of bones and teeth, and Fe, Zn, and Se are vital elements in the structure of enzymes (Charibzadeh and Jafari, 2017). The minerals required by the human body are received from the food regime directly or indirectly. So, an ideal food regime for human health must be rich in minerals (Huang *et al*., 2020).

Our results showed that the chrysanthemum genotypes had varying concentrations of Ca (45.46-74.10 mg/kg FW), Fe (1.143-3.231 mg/kg FW), Zn (0.175-0.315 mg/kg FW), and Se (0.026-0.233 mg/kg FW). Since the daily human demand for Ca, Fe, Zn, and Se has been estimated at 550-650, 5.6-6.5, 7-9, and 0.02-0.025 mg, respectively (Huang *et al*., 2020), it is evident that the chrysanthemum genotypes cannot supply these daily mineral demands by themselves, but they can only partially meet them as a new food ingredient.

In Mlcek *et al*.'s (2021) study, the Fe content was recorded at 3.12 and 4.02 mg/kg in edible begonia and rose flowers, respectively. So, the Fe content of chrysanthemum Code 110 (3.231 mg/kg FW) was higher than that of the begonia and lower than that of the rose. The begonia and rose flowers outperformed the studied genotypes of chrysanthemums in Zn and Ca. All flowers studied by Rop *et al*. (2012) (carnation, marigold, and violet) except for rose (3.55 mg/kg) had higher Fe, Zn, and Ca contents than our studied chrysanthemum genotypes.

Vitamin C is a strong antioxidant that plays a key role in the body's natural metabolism. The vitamin C content has been recorded at 2.6-44.9 mg/kg FW in many edible flowers (Demasi *et al*., 2021). It was 11.71-13.58 mg/kg FW in the studied genotypes of chrysanthemum, which

is acceptable among edible flowers. The human body's daily need for vitamin C is 95-100 mg. The vitamin C content of the apple, orange, and kiwifruit has been estimated at 5, 35, and 93 mg/100 g FW, respectively (Cruz-Rus *et al*., 2012; Demasi *et al*., 2021). So, all studied genotypes of chrysanthemum had higher vitamin C content than the apple.

Vitamin A was among the vitamins assessed in this research and its content in different genotypes was estimated at 0.0016-0.0868 mg/kg FW. Since carotenoids are the precursor of vitamin A (Skrajda-Brdak *et al*., 2020), the genotypes were expected to have higher carotenoids than vitamin A, but it was the opposite.

Proteins and fibers are other invaluable compounds in edible flowers so that plant fibers are regarded as an essential component of the food regime that can reduce the risk of cardiovascular diseases, diabetes, hypertension, and so on. The human body daily needs 25-35 g of fiber, which should be supplied from food sources (Jakubczyk *et al*., 2022). Previous research has reported the raw fiber percentage at 1.468 and 0.491% of FW in carnation and orange day-lily (Stefaniak and Crzeszczuk, 2019) and 12.7, 13.89, and 28 g/kg in agave, aloe vera, and broccoli, respectively (Fernandes *et al*., 2017) whereas it was in the range of 13.06-22.34% of FW for chrysanthemum genotypes in our study. Code 138 had the highest raw fiber content (22.34%), so it was the most appropriate source of raw fiber among the studied genotypes. Also, the chrysanthemum genotypes had 0.771-1.483% of proteins, which was lower than that of carnation (5.61%) and orange day-lily (3.346%) (Stefaniak and Crzeszczuk, 2019). Mlcek *et al*. (2021) reported the protein content of begonia, pot marigold, and marigold at 4.51, 8.98, and 9.34 mg/kg FW, respectively. Thus, the chrysanthemum genotypes studied in this research were richer in protein than these three edible flowers.

The health benefits of edible flowers largely depend on their antioxidants (phenols, flavonoids, anthocyanins, and carotenoids). So, it is important to identify and quantify the antioxidants of edible flowers as a natural food source (Zheng *et al*., 2019). We found that the total phenols, total flavonoids, and anthocyanins were 7.93-12.546, 3.50-5.81, and 5.6-60.83 mg/g FW in different genotypes of chrysanthemum, respectively. It has been reported that the total flavonoids, phenols, and anthocyanins in the apple flesh were 0.501-1.41, 0.379-3.77, and 0.65-5.66 mg/g FW, respectively (Rabiei *et al*., 2019), so the chrysanthemum genotypes had higher total phenols, flavonoids, and anthocyanins than the apple cultivars studied by Rabiei *et al*. (2019).

Mlcek *et al*. (2021) found that the total phenols in begonia, pod marigold, rose, and marigold were 4.82, 3.65, 4.45, and 4.78 g/kg FW, respectively. Rop *et al*. (2012) reported the total flavonoids in the edible flowers of carnation, rose, marigold, and violet at 2.27, 2.04, 1.90, and 1.99 g/kg FW, respectively. So, the chrysanthemum genotypes were superior to these flowers in total phenols. The total phenols and flavonoids of nine rose cultivars were in the ranges of 798.67-2978.89 and 78.64-531.54 mg/100 g FW, which are much greater than that in chrysanthemums. Espejel *et al*. (2019) state that purple, orange, red, and violet flowers have higher anthocyanin content than yellow and white flowers. In our research too, the chrysanthemum genotypes whose flowers were white (Codes 134 and 751) had lower anthocyanin. The highest anthocyanin content was related to Code 674 whose petals were red. However, in addition to flower color, anthocyanin content is influenced by environmental conditions, genotype, and flowering steps and may even be lower in some flowers with higher antioxidant capacity (Espejel *et al*., 2019). Compared to Stefaniak and Crzeszczuk (2019), the anthocyanin content of the chrysanthemum genotypes was lower and higher than that of

carnation (443.47 mg/100 g FW) and daylilies (2.77 mg/100 g FW), respectively. Yang and Shin (2017) showed that the anthocyanin content was 0.61-502.64 mg/100 g FW in nine rose cultivars and that the cultivars with dark (red) color had higher anthocyanins. In the present work, the highest anthocyanin content was observed in the genotype with red flowers.

CONCLUSION

Based on the results, the chrysanthemum genotypes that were investigated here had nutritional and antioxidant values and differed in nutrients, minerals, and antioxidants significantly. The genotypes that had the highest Fe, Ca, Se, total phenols, and total carotenoids were Codes 110, 326, 562, 326, and 108, respectively. It should be noted that the flowers of these genotypes were yellow. Code 751, which had white flowers, had the highest protein content. The highest raw fiber and total flavonoid were recorded by Codes 138 and 684 whose flowers were orange. Finally, the genotypes whose flowers were purple produced the highest anthocyanins (Code 674), vitamin A (Code 540), and vitamin C (Code 603). Therefore, the studied genotypes of chrysanthemum can be used as a nutritious source in the food regime to enjoy their high food and antioxidant values.

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Physiological Responses and Nutritional Implications of *Physalis alkekengi* L. Under Varied Salinity Stress and Si and Se Nanoparticle Treatments

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Abstract

This study systematically investigates the physiological responses of *Physalis alkekengi* to diverse conditions of salinity stress (0, 50, 100, and 200 mM NaCl), coupled with the application of selenium (Se) nanoparticles at concentrations of 25 and 50 mgl^{-1}, as well as silicon (Si) nanoparticles at concentrations of 100 and 200 mg l^{-1}. The experiment involved a thorough examination of many characteristics connected to biomass, such as antioxidant enzyme activity, fatty acid composition, and elemental content. This analysis was conducted at varying levels of salinity and with the addition of nanoparticles. The findings revealed that exposure to salt stress has a detrimental effect on both plant development and fruit output, leading to changes in vegetative and morphological characteristics. The utilization of Se and Si nanoparticles had a significant alleviating impact on stress caused by salinity. The correlation matrix analysis revealed complex correlations among the examined parameters, emphasizing the interrelated responses of *P. alkekengi* to environmental stressors and nanoparticle interventions. Principal Component Analysis (PCA) revealed the hidden patterns and connections between variables, highlighting the significant influence of biomass-related features, antioxidant enzymes, and fatty acid content on the observed variability. The results of this study enhance our knowledge of the physiological processes that regulate *P. alkekengi*'s reaction to high salt levels. Additionally, it offers valuable information on the possible beneficial impacts of Se and Si nanoparticles in reducing the negative consequences of salinity stress. The study's comprehensive breadth increases its relevance to future research focused on optimizing growth circumstances and strengthening the resistance of *P. alkekengi* in demanding situations.

Keywords: Antioxidant defense system, Fatty acid composition, Nanoparticle, Salinity stress.

INTRODUCTION

Physalis alkekengi L., a member of the solanaceae family, is notable in both ornamental and therapeutic applications. The plant's fruit, referred to as the bladder cherry, Chinese lantern, Japanese lantern, strawberry groundcherry, or winter cherry is known in different regions has a diverse range of uses including food, medicine, and industry (He *et al.*, 2023). This plant species is well-known in different regions, such as China, Japan, Korea, and portions of Europe. Fossils of its seeds have been found dating back to the miocene and pliocene geological periods (23 million to 5.3 million years ago) in Europe (Liang *et al.*, 2024). In addition to its visual attractiveness, *Physalis alkekengi* L. has been of great significance in traditional medicine worldwide (Bahmani *et al.*, 2016).

Physalis alkekengi L. is abundant in biologically active substances such as steroids, flavonoids, phenylpropanoids, alkaloids, nucleosides, terpenoids, megastigmane, aliphatic derivatives, organic acids, coumarins, and sucrose esters (Liang *et al*., 2024). Throughout history, it has been widely used for its various therapeutic benefits. The applications of this substance include reducing fever, producing anti-inflammatory reactions, providing analgesic effects, possessing antibacterial capabilities, and exhibiting antiviral activity (Popova *et al.*, 2022). The field of Chinese medicine has effectively utilized the medicinal properties of the plant by harnessing the therapeutic potential of its fruit juice. *Physalis alkekengi* L. remedies have been used to treat a variety of ailments including abscesses, coughs, fevers, sore throats, acidic excretions, gout, arthritis, hemorrhoids, hepatitis, bronchitis, and to strengthen the liver. The plant's historical acknowledgment as a medicinal substance with the ability to induce fetal abortion and prevent pregnancy enhances its traditional importance (Bahmani *et al*., 2016, He *et al*., 2023). Moreover, the plant's chemical substances, such as physoaline and solenoid alkaloids, have shown effectiveness in inhibiting mycobacterium TB, highlighting its promise in fighting tuberculosis (Liang *et al*., 2024).

Although, there is a significant amount of information available on the therapeutic benefits of *Physalis alkekengi* L., there is a lack of research on how it responds to environmental stresses, specifically salt (Bahmani *et al*., 2016; Bosch *et al.*, 2016; Helvacı *et al.*, 2010; Li *et al.*, 2018). This study seeks to fill this void by investigating the influence of Si and Se nanoparticles on the physiological reactions of *Physalis alkekengi* L. in the presence of high salt levels. Despite its abundance in the Earth's crust, Si has not traditionally been considered necessary for plant growth. Recent findings demonstrate its importance in strengthening cell walls, increasing plant resistance to diseases, and raising overall plant quality (Abdoli *et al.*, 2020; Monroy-Velandia and Coy-Barrera, 2021). The presence of Si in plants has a dual purpose: It enhances the strength of cell walls and also functions as a mobile element inside the plant. This makes Si an important supplement for many plant species, especially when they are experiencing stressful conditions. The crucial importance of its potential lies in its ability to stimulate growth, increase photosynthesis, reduce evaporation and transpiration, strengthen leaves, and improve overall plant quality. This is particularly significant when considering environmental challenges like salinity (Al-aghabary *et al.*, 2005; Banerjee *et al.*, 2021).

Si has a complex impact on the ability of plants to withstand stress. Under unfavorable circumstances, it provides defense against harm caused by the sun, minimizes the loss of water through a dual cellulose layer in the leaf's outer covering, and improves the process of photosynthesis. In addition, Si enhances the ability to withstand biological and physical pressures, such as high salinity, heavy metals, and drought. The application of this technology

improves the efficiency of water consumption, stimulates the production of dry matter, and increases the water potential in leaves during periods of drought stress (Guerriero *et al.*, 2016; Karimi *et al.*, 2020; Khan *et al.*, 2021). The deposition of Si on the cell walls of xylem provides protection against water scarcity, particularly in dry environments. This process plays a crucial role in the metabolic, physiological, and structural functions of plants (Moradi *et al.*, 2022; Mushtaq *et al.*, 2020).

Se, at low concentrations, enhances plant growth and alleviates the adverse impacts of environmental stressors such as low temperature, water scarcity, high salinity, and toxicity caused by heavy metals. Se acts as a vital antioxidant, boosting the body's ability to withstand oxidative stress and maintaining the integrity and functionality of cells. Se's application mitigates heavy metal toxicity, enhances chloroplast enzymes, and promotes plant growth in stressful conditions. The recent application of nano-Se as a fertilizer in agriculture, due to its exceptional purity and absorption efficiency, demonstrates its potential to serve as a more efficient stress-reducing agent. The increased effectiveness of nano-Se is due to its greater specific surface area compared to conventional particles, which presents a promising opportunity for implementing environmentally sustainable stress management approaches (Badawy *et al.*, 2021; Golubkina *et al.*, 2022; Hajiboland and Keivanfar, 2012).

Se, although not directly engaged in the fundamental metabolic processes of plants, has shown a significant function in improving both the growth of plant structures and the production of offspring, particularly when plants are subjected to challenging environmental or biological circumstances (Bisht *et al.*, 2022; Ghasemian *et al.*, 2021). Se's positive effects are diverse and involve enhanced activity of antioxidant enzymes and increasing levels of antioxidant molecules. Research has indicated that Se is effective in improving growth under drought stress conditions and delaying aging caused by flowering in annual plants (Hawrylak-Nowak, 2022; Jiang *et al.*, 2017; Karimi *et al*., 2020). However, further in-depth studies are necessary to fully understand the overall mechanisms by which Se helps to reduce environmental stress (Bisht *et al*., 2022).

Nanotechnology is revolutionizing plant science in the modern era. Nanoparticles, with a size smaller than 50 nm, possess distinct characteristics that allow them to enter plant cell walls and impact physiological responses. The utilization of metal nanoparticles, specifically, has demonstrated potential in enhancing both the quantitative and qualitative attributes of plants, while also providing a method to counteract biological stressors (Sanzari *et al.*, 2019). This paper addresses the lack of research on the effects of Si and Se nanoparticles on *Physalis alkekengi* L. in saline environments.

The main goal of this work is to examine the previously unexamined impacts of nano-sized Si and Se particles on the physiological responses of *Physalis alkekengi* L. plants under saline circumstances, considering the gaps in knowledge mentioned earlier. Our objective is to clarify the mechanisms by which these nanoparticles can improve a plant's ability to withstand salt stress. This research will not only deepen our understanding of how plants respond to stress, but also potentially provide new approaches to alleviating environmental stress in agriculture. By examining the complex interaction of Si, Se, and *Physalis alkekengi* L. in the presence of saline circumstances.

MATERIALS AND METHODS

Plant materials

The seeds of *Physalis alkekengi* L. were obtained from Pakan Bazr Company, located

in Isfahan, Iran. The study employed Se nanoparticles (NPs) in the form of Se dioxide acquired from Sigma-Aldrich, USA. Described as having a spherical shape, with a CAS number of 7446-08-4, a size that falls between 10-40 nm, a purity level of 99.9%, a specific surface area of 30-50 $m^2g^{-1,}$ and a true density of 3.89 g cm^{-3}. The Si dioxide used in this study was obtained from Sigma-Aldrich, USA. It consisted of Si nanoparticles (Si NPs) with a CAS number of 7631-86-9. The quality of the Si nanoparticles was 99.5%, and their particle size ranged from 10 to 20 nm. The active surface area of the nanoparticles was measured to be 450 g m^{-2}.

Conditions for growth and experimental design

The experimental design was organized in a factorial configuration, utilizing a randomized complete design (CRD) with three replicates in the year 2022. The seeds were planted in 4-L pots filled with a substrate consisting of a 2:1 ratio of cocoa to perlite. The greenhouse maintained a photoperiod of 16 hours of light and 8 hours of darkness, together with a relative humidity ranging from 65% to 80%. The experimental setting was the greenhouse of the University of Zanjan, Zanjan, Iran, located at an elevation of 1661 (36° 40' 25" N, 48° 29' 04" E). During the growth period, plants were nourished with a Hoagland solution and irrigated with distilled water until they reached the 4-leaf stage. Following that, the plants were subjected to saline conditions and exposed to varying concentrations of NaCl (50, 100, and 200 mM) every three days for a total of 50 days. In addition, the plants were treated with Si nanoparticles (Si NPs) at concentrations of 100 and 200 mg l^{-1} and Se nanoparticles (Se NPs) at concentrations of 25 and 50 mg l^{-1}. These treatments were applied three times, with a 15-day gap between each application, starting from the stage when the plants had four leaves. The sampling was performed when the fruits reached maturity, and their physiological and biochemical features were assessed.

Shoot dry weight

After subjecting the plant aerial parts to dehydration in an oven set at 72 °C for a period of 24 hours, their weight in the dry state was measured using a digital scale with a precision of 0.01 g.

Fruit weight and yield

The individual weight of each fruit was measured using a digital scale, and the total fruit yield was calculated by adding all the weights of all the fruits on each plant.

Calyx size

The fruit calyx's dimensions, including its length and width, were precisely measured using a caliper.

Catalase enzyme activity measurement

The enzyme extraction process involved pulverizing plant leaf samples in amortar using liquid nitrogen, resulting in a powdered form. Afterward, 0.5 ml of sodium phosphate buffer with a pH of 6 was introduced, and then the mixture was spun at a speed of 13,000 revolutions per minute. The supernatant obtained was used to measure the total protein content and catalase enzyme activity. The spectrophotometric determination of catalase activity was conducted at a wavelength of 240 nm for a duration of 30 seconds. This was achieved by utilizing a 20 mM sodium phosphate buffer with a pH of 7, along with 20 µL of 30% hydrogen peroxide (H_2O_2) as

the electron acceptors. The quantification of catalase activity was determined by measuring the enzyme units (mg^{-1} of protein), following the established procedure described by (Anderson, 2002).

Superoxide dismutase (SOD) enzyme activity measurement

The activity of the SOD enzyme was assessed in leaf samples by measuring its ability to inhibit the photochemical reduction process of nitrobuterazolium (NBT). The reaction mixture comprised 50 mM phosphate buffer (pH 7), 13 mM methionine, 0.1 mM sodium ethylenediaminetetraacetate (Na-EDTA), 75 µM nitrobuterazolium (NBT), 75 µM riboflavin, and 100 µL of the extract. Spectrophotometric measurements were conducted at a wavelength of 560 nm at two-minute intervals to determine absorbance. The SOD activity was quantified in enzyme units mg^{-1} of protein using the methodology developed by (Giannopolitis and Ries. 1977).

Anthocyanin measurement

The anthocyanin content was quantified using (Wagner, 1979) methodology. 1 g sample of fruit was combined with 10 mL of acidic methanol. The resulting mixture was then stored in darkness at a temperature of 4 ºC for a duration of 24 hours. The absorbance of the supernatant was measured at a wavelength of 520 nm using a spectrophotometer after centrifugation. The anthocyanin content was determined by applying the formula $A = Ɛbc$, where Ɛ represents the extinction coefficient (3300 mM cm^{-1}), A is the absorption value, b is the width of the cuvette (1 cm), and c is the quantity of anthocyanin in milligrams of cyanidin-3-glucoside per 100 g.

Analysis of sodium and potassium content in leaves

The leaf samples were pulverized using a mortar after being completely dried in the air. Subjecting the sample to a temperature of 500 ºC for a duration of 6 hours and subsequently dissolving it in a solution of 2 M nitric acid facilitated the measurement of sodium and potassium levels. The solution volume was ultimately modified to 25 ml by adding double-distilled water. Subsequently, measurements were carried out using a film-photometry apparatus (model PFP7, JENWAY, England) in accordance with the approach described by (Chapman and Pratt, 1962).

Leaf chlorine measurement

In order to determine the amount of chlorine present, a sample of 100 mg of powdered plant tissue was treated with 10 ml of 0.5 M nitric acid to extract the chlorine content. Following an hour of drying the extract at a temperature of 80 °C, a volume of one mL of the extract was utilized to measure the chlorine content utilizing a colorimetric method at a wavelength of 480 nm using the Epoch 2 instrument, in accordance with the procedure outlined by (Munns and Tester, 2008).

Quantification of phosphorus content in leaves

The phosphorus content was measured using a Unico spectrophotometer. The plant samples were incinerated in a furnace at a temperature of 550 °C. Subsequently, Barton's reagent and 70% perchloric acid were added. The spectrophotometric measurement of absorbance was conducted at a wavelength of 450 nm, following the methodology described by (Ryan *et al.*, 2009).

Fatty acid analysis

The determination of the fatty acid composition in the fruit involved the extraction of oil via the Soxhlet method, followed by gas chromatography (GC) for profiling. The GC analysis was conducted using a Thermo-UFM (Ultera Fast Model) gas chromatograph equipped with a Ph-5 capillary column measuring 10 meters in length, 0.1 mm inner diameter, and 0.4 µm thickness. The column featured a dimethyl stationary phase siloxane on its inner surface, with a 5% phenyl coating. The thermal program of the column ranged from 60 to 280 °C, with a temperature increase rate of 80 °C min^{-1}. Detection was carried out using a Flame Ionization Detector (FID), and helium served as the carrier gas with an inlet pressure to the column set at 0.5 kg cm^{-2}. The detector chamber maintained a temperature of 290°C, while the injection chamber was set at 280 °C (Sowmiya *et al.*, 2021).

Statistical analysis

The data obtained from measuring various variables in this study were initially recorded in Excel and then analyzed using SAS statistical software version 9.4. In order to evaluate the importance of average data, comparisons were carried out using Duncan's test at either the 1% or 5% level of significance. The graphs and figs were produced using Excel software. PCA and hierarchical clustering were performed using XLSTAT software version 2022-4 the clustering procedure utilized Ward's algorithm and Euclidean distance as the fundamental principles for grouping.

RESULT

Shoot dry weight

Salinity stress significantly reduced shoot dry weight ($P<0.01$), with the highest impact observed at 200 mM salinity, causing a 29% decrease compared to the control. Se and Si nanoparticles had a significant positive effect on shoot dry weight ($P<0.01$), increasing it by 8% and 10%, respectively, in 25 and 200 mg l^{-1} treatments compared to the control. The interaction between salinity stress and nanoparticles also significantly influenced shoot dry weight ($P<0.01$), indicating a mitigating effect of Se and Si on salinity-induced reduction. In the 200 mM salinity treatment, Se at 25 and 100 mg l^{-1} increased shoot dry weight by 34% and 22%, respectively, showcasing the potential of these nanoparticles in enhancing plant resilience to salinity stress (Fig. 1-a).

Fruit morphology

Calyx length: Salinity stress significantly impacted calyx length ($P<0.01$), causing a notable decrease at higher salinity levels, with the lowest value of 38.4 mm observed at 200 mM. Se and Si nanoparticles treatments significantly increased calyx length compared to the control, reaching the maximum at 50 and 200 mg l^{-1} Se treatments (Fig. 1-d).

Calyx width: Salinity stress ($P<0.01$) led to a substantial decrease in calyx width, with the lowest value of 31.6 mm at 200 mM. Se and Si nanoparticles treatments significantly increased calyx width compared to the control, with the highest values observed at 25 and 200 mg l^{-1} Se treatments (Fig. 1-e).

Fruit fresh weight: Salinity stress significantly reduced fruit fresh weight ($P<0.01$), showing a decreasing trend with increasing salinity levels. Se and Si nanoparticles treatments significantly increased fruit fresh weight compared to the control, with the highest fresh weight observed at 200 mg l^{-1} Se treatment (Fig. 1-b,c).

Fruit yield: Salinity stress significantly reduced fruit yield (P<0.01), exhibiting a marked decline with increasing salinity levels, reaching the lowest at 200 mM. Se and Si nanoparticle treatments significantly increased fruit yield compared to the control, with the highest yield observed at 50 and 200 mg l^{-1} Se treatments (Fig. 1-f,g).

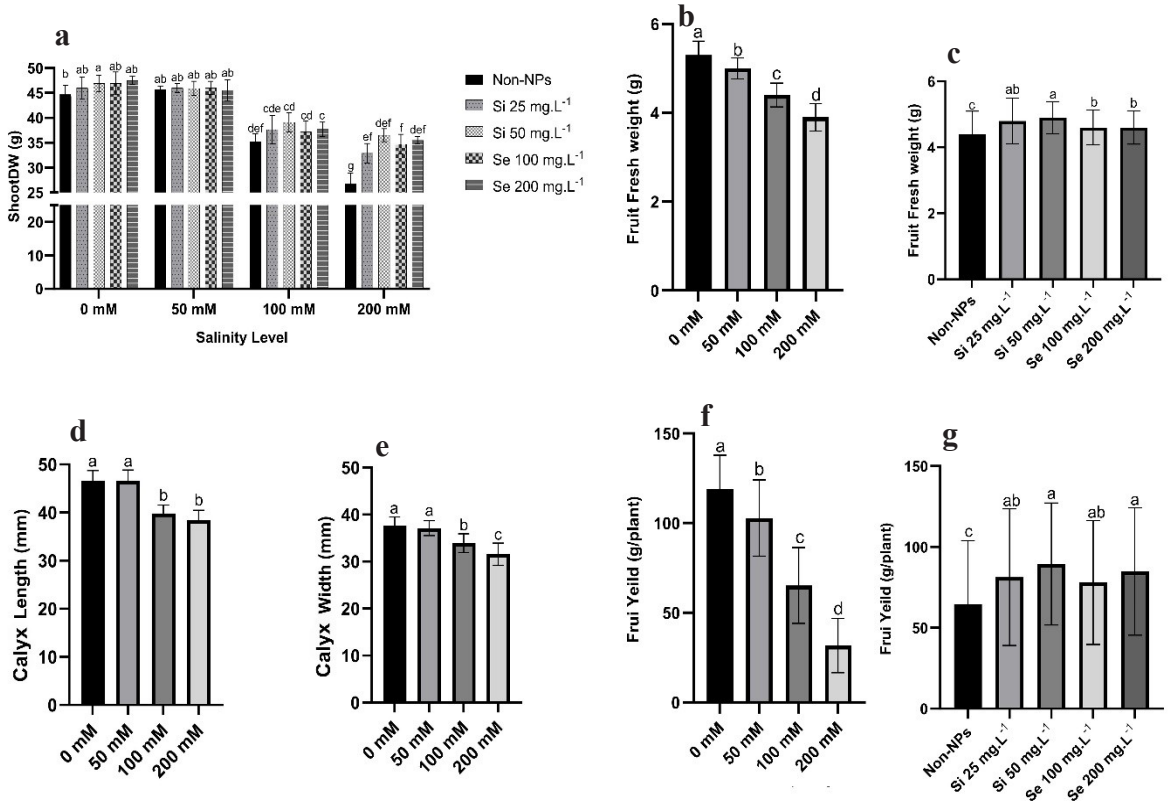

Fig. 1. Presents a comprehensive overview of key physiological parameters in *Physalis alkekengi* L. under varying salinity concentrations and nanoparticle treatments. The subplots include (a) Shoot dry weight, illustrating the impact of different treatments on plant biomass; (b) Fruit fresh weight under distinct salt concentrations, elucidating the correlation between salinity stress and fruit weight; (c) Fruit fresh weight under diverse nanoparticle concentrations, highlighting the effect of nanoparticles on fruit development; (d) Calyx length in response to varying salt concentrations, indicating the influence of salinity on fruit calyx morphology; (e) Calyx length under different nanoparticle treatments, emphasizing the role of nanoparticles in modulating calyx length; (f) Fruit yield in distinct salt concentrations, providing insights into the relationship between salinity stress and overall fruit productivity; and (g) Fruit yield under different nanoparticle concentrations, revealing the impact of nanoparticles on enhancing or mitigating fruit yield.

Biochemical traits

Catalase enzyme activity

The impact of salinity stress on catalase enzyme activity was statistically significant at the 1% level (P<0.01). Salinity-induced stress led to a marked increase in catalase enzyme activity, with the highest observed activity at 200 mM salinity (0.532 enzyme units). Notably, treatments involving Se and Si nanoparticles resulted in a considerable reduction in catalase enzyme activity compared to the control treatment, where the highest activity was recorded in the control treatment (0.481). Specifically, Se treatments at 25 and 100 mg l^{-1} exhibited an 11% and 12% decrease, respectively, relative to the control condition (Fig. 2-b,c).

Superoxide dismutase (SOD) enzyme activity

Salinity stress significantly heightened SOD enzyme activity ($P<0.01$), peaking at 200 mM salinity (5.82 enzyme units). In contrast, treatments involving Se and Si nanoparticles demonstrated a significant reduction in SOD enzyme activity compared to the control expect in Se 100 mg l^{-1}, with the highest activity observed in the control treatment (4.64 enzyme units). Se treatments at 50 and 200 mg l^{-1} resulted in a notable 7% and 10% reduction, respectively, in SOD enzyme activity relative to the control (Fig. 2-d,e).

Anthocyanin content

Salinity stress up to 50 mM elicited a significant increase in anthocyanin content, followed by a subsequent decline at higher stress levels ($P<0.01$). Se and Si nanoparticle treatments induced a notable augmentation in anthocyanin content compared to the control condition, where the lowest amount was recorded (2.29 mg). Specifically, Se treatments at 25 and 200 mg l^{-1} demonstrated a substantial 21% and 27% increase, respectively, in anthocyanin content relative to the control (Fig. 3-a).

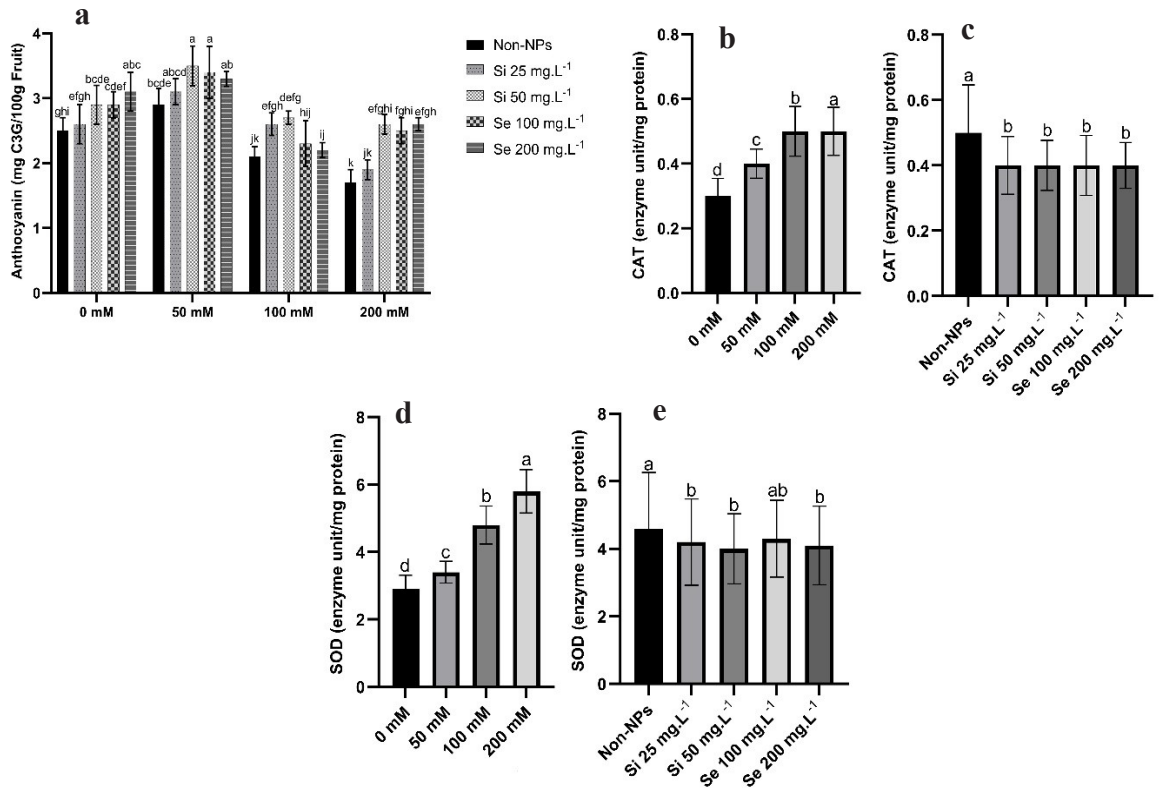

Fig. 2. Provides a detailed depiction of crucial biochemical parameters in *Physalis alkekengi* L. subjected to varying salinity concentrations and nanoparticle treatments. The subplots encompass (a) Anthocyanin levels, illustrating the influence of different treatments on the plant's anthocyanin content; (b) Catalase (CAT) activity under distinct salt concentrations, elucidating the correlation between salinity stress and CAT enzyme response; (c) CAT activity under diverse nanoparticle concentrations, highlighting the effect of nanoparticles on modulating CAT enzyme activity; (d) Superoxide dismutase (SOD) activity in response to varying salt concentrations, indicating the impact of salinity on the plant's antioxidant defense mechanism; and (e) SOD activity under different nanoparticle treatments, emphasizing the role of nanoparticles in regulating SOD enzyme activity.

Leaf elemental composition

Sodium (Na)

The impact of salinity stress on leaf sodium content was statistically significant at the 1% level ($P<0.01$). Increasing salinity stress levels led to a substantial rise in leaf sodium content, reaching its peak at 200 mM salinity (7.54 mg g^{-1}), while the control treatment exhibited the lowest sodium content (1.86 mg g^{-1}). Se and Si nanoparticle treatments induced a significant reduction in leaf sodium content compared to the control. Specifically, Se treatments at 50 and 200 mg l^{-1} resulted in a noteworthy 27% and 32% decrease, respectively, in leaf sodium content relative to the control. The interaction effect of salinity stress and Se and Si nanoparticles on leaf sodium was also significant ($P<0.01$), showcasing a notable reduction in leaf sodium levels in the presence of Se and Si (Fig. 3-a).

Chlorine (Cl)

Salinity stress significantly increased leaf chlorine content ($P<0.01$), with the highest observed value at 200 mM salinity (50.33 mg g^{-1}). Conversely, Se and Si nanoparticle treatments caused a substantial reduction in leaf chlorine content compared to the control, demonstrating a pronounced decrease in chlorine levels. Se treatments at 25 and 200 mg l^{-1} resulted in a considerable 21% and 27% reduction, respectively, in leaf chlorine content relative to the control. The interaction effect of salinity stress and Se and Si nanoparticles on leaf chlorine was also significant ($P<0.01$), revealing a mitigating influence on chlorine levels by Se and Si treatments (Fig. 3-b).

Potassium (K)

Salinity stress significantly decreased leaf potassium content ($P<0.01$), reaching its lowest at 200 mM salt stress treatment (26.13 mg g^{-1}), while the control treatment exhibited the highest potassium content (35.73 mg g^{-1}). In contrast, Se and Si nanoparticle treatments induced a significant increase in leaf potassium content compared to the control. Se treatments at 200 mg l^{-1} demonstrated a notable 15% increase in leaf potassium content relative to the control (Fig. 3-c,d).

Phosphorus (P)

Salinity stress led to a significant decrease in leaf phosphorus content ($P<0.01$), with the lowest observed value at 200 mM salinity (2.86 mg g^{-1}) and the highest in the control treatment (6.26 mg g^{-1}). Se and Si nanoparticle treatments induced a significant increase in leaf phosphorus content compared to the control. Se treatments at 25 and 100 mg l^{-1} resulted in a considerable 12% and 14% increase, respectively, in leaf phosphorus content relative to the control. Notably, no significant difference in leaf phosphorus was observed between different levels of Se and Si nanoparticles (Fig. 3-e,f).

Total essential fatty acid (TEFA)

The analysis of total essential fatty acids (TEFA) across different treatments revealed notable variations in fatty acid composition. Under salinity stress at varying levels (50, 100, and 200 mM), the TEFA content exhibited a discernible trend. Specifically, in the absence of nanoparticles (Without NP), TEFA values remained relatively stable, indicating a limited impact of salinity on the overall essential fatty acid profile. However, the introduction of Si nanoparticles (Si 25 and Si 50 mg l^{-1}) resulted in marginal decreases in TEFA, suggesting a potential modulating effect of Si on essential fatty acid composition. Se treatments (Se 100 and Se 200 mg l^{-1}) showed a tendency to maintain TEFA levels comparable to the control, hinting at a protective role against salinity-induced alterations in essential fatty acids. Further investigations may elucidate the specific mechanisms by which Si and Se nanoparticles influence TEFA synthesis and accumulation under salinity stress (Fig. 4-a,b).

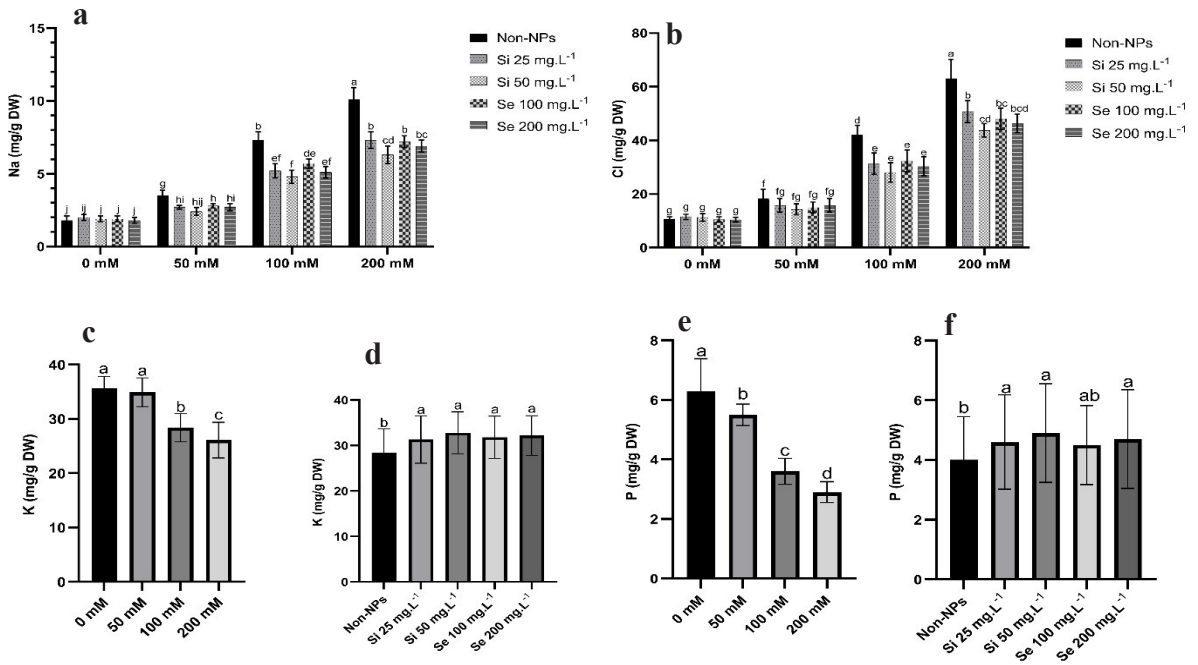

Fig. 3. Provides a comprehensive insight into the ionomic profile of *Physalis alkekengi* L. under varying salinity conditions and nanoparticle treatments. The subplots encompass (a) Sodium (Na) levels, illustrating the plant's response to different salt concentrations; (b) Chloride (Cl) levels, depicting the variation in Cl content under different salt treatments; (c) Potassium (K) concentrations in response to diverse salinity levels, delineating the plant's K dynamics under salt stress; (d) K levels under various nanoparticle treatments, demonstrating the influence of nanoparticles on K uptake; (e) Phosphorus (P) content under different salinity conditions, providing insights into the impact of salt stress on P accumulation; and (f) P concentrations in response to distinct nanoparticle treatments, highlighting the role of nanoparticles in modulating P levels.

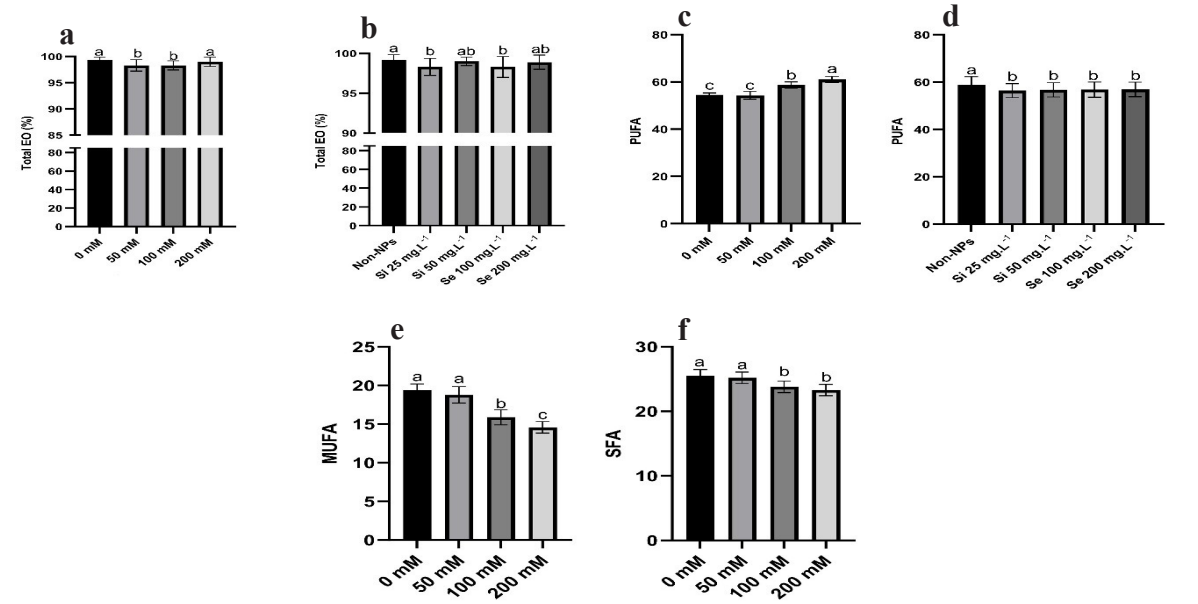

Fig. 4. Provides a detailed overview of the fatty acid composition in *Physalis alkekengi* L. under distinct salinity conditions and nanoparticle treatments. The subplots present (a) Total fatty acid levels, elucidating variations in the overall fatty acid content under different salt concentrations; (b) Total fatty acid concentrations in response to various nanoparticle treatments, illustrating the impact of nanoparticles on the total fatty acid profile; (c) Polyunsaturated fatty acids (PUFA) levels under different salt conditions, showcasing the plant's response to salt-induced stress; (d) PUFA concentrations under diverse nanoparticle treatments, indicating the role of nanoparticles in influencing PUFA content; (e) Monounsaturated fatty acids (MUFA) variations in different salt concentrations, shedding light on the plant's MUFA dynamics under salt stress; and (f) Saturated fatty acids (SFA) alterations under different salt conditions, providing insights into how salt stress affects SFA composition.

Saturated fatty acids (SFAs)

The analysis of saturated fatty acids (SFAs) demonstrated nuanced responses to salinity stress and nanoparticle treatments. Across different salinity levels, SFAs exhibited fluctuations with variations in the type and concentration of nanoparticles. In the absence of nanoparticles (Without NP), SFAs experienced modest changes under increasing salinity. However, Si nanoparticle treatments (Si 25 and Si 50 mg l^{-1}) displayed a trend of maintaining SFAs at levels comparable to or slightly below the control, suggesting a potential mitigating effect on salinity-induced SFA alterations. Se treatments (Se100 and Se 200 mg l^{-1}) demonstrated a similar trend, highlighting the capacity of Se to regulate SFA content under salt stress conditions. Further investigations into the molecular pathways involved in SFA metabolism and regulation could enhance our understanding of the observed trends (Fig. 4-f).

Monounsaturated fatty acids (MUFAs)

The examination of monounsaturated fatty acids (MUFAs) revealed distinct patterns influenced by salinity stress and nanoparticle treatments. Salinity stress at varying levels prompted changes in MUFA content, with a general trend of decrease observed. In the absence of nanoparticles (without NP), the decline in MUFAs under salinity stress was notable. However, Si nanoparticle treatments (Si 25 and Si 50 mg l^{-1}) demonstrated a potential mitigating effect, with MUFA levels exhibiting moderation compared to the control. Se treatments (Se 100 and Se 200 mg l^{-1}) also showed a trend of preserving MUFA levels, suggesting a protective role against salinity-induced reductions in monounsaturated fatty acids. Exploring the underlying molecular mechanisms governing MUFA metabolism and regulation in response to nanoparticle treatments could provide valuable insights (Fig. 4-e).

Polyunsaturated fatty acids (PUFA)

Polyunsaturated fatty acids (PUFAs) exhibited dynamic responses to salinity stress and nanoparticle treatments. Salinity stress at varying levels led to fluctuations in PUFA content, indicating the sensitivity of these fatty acids to environmental stressors. In the absence of nanoparticles (without NP), PUFA levels displayed a general decreasing trend under salinity stress. However, Si nanoparticle treatments (Si 25 and Si 50 mg l^{-1}) showcased a potential mitigating effect, with PUFA content showing moderation compared to the control. Se treatments (Se 100 and Se 200 mg l^{-1}) also demonstrated a trend of maintaining PUFA levels, suggesting a protective role against salinity-induced alterations in polyunsaturated fatty acids. Exploring the molecular mechanisms involved in the synthesis and regulation of PUFAs under nanoparticle treatments could contribute to a comprehensive understanding of their role in plant responses to salinity stress (Fig. 4-c,d).

Correlation matrix

The Pearson correlation matrix analysis revealed intricate interrelationships among diverse physiological parameters in the experimental context. Strong positive correlations were observed between biomass-related traits (DW shoot, calyx dimensions, FW fruit, and frui Y), indicating coordinated responses. Concurrently, a negative correlation between these traits and antioxidant enzymes (CAT and SOD) suggests a potential trade-off between growth and antioxidant activity. Sodium (Na) and chloride (Cl) exhibited a consistent co-occurrence, negatively correlating with antioxidant enzymes, implicating their role in ionic stress response. Potassium (K) and phosphorus (P) displayed positive correlations with biomass, emphasizing their significance in plant growth. Fatty acid composition (Total essential fatty acid, SFA, MUFA,

PUFA) exhibited weak correlations with other parameters, suggesting relative independence. Treatment effects, particularly Se, and Si nanoparticles, hinted at nuanced shifts in correlations, underlining their potential in modulating plant responses (Fig. 5-a).

Principal component analysis (PCA)

Principal component analysis (PCA) was conducted to elucidate intricate patterns within the extensive dataset derived from the experimental conditions. The eigenvalue analysis revealed that the first five principal components (F1-F5) collectively accounted for a substantial 97.44% of the total variability, highlighting their significance in capturing the underlying structure of the data. The eigenvectors associated with each parameter provided insights into their contributions to the respective principal components. Biomass-related traits, antioxidant enzymes, and fatty acid composition exhibited noteworthy contributions to specific principal components, emphasizing their distinctive roles in the observed variance. The cumulative percentage of variability further underscored the efficacy of the selected principal components in collectively representing the majority of the dataset. The loadings and squared cosines of the variables provided a nuanced understanding of the strength and reliability of the associations, guiding the nuanced interpretation of the PCA results (Fig. 5-b).

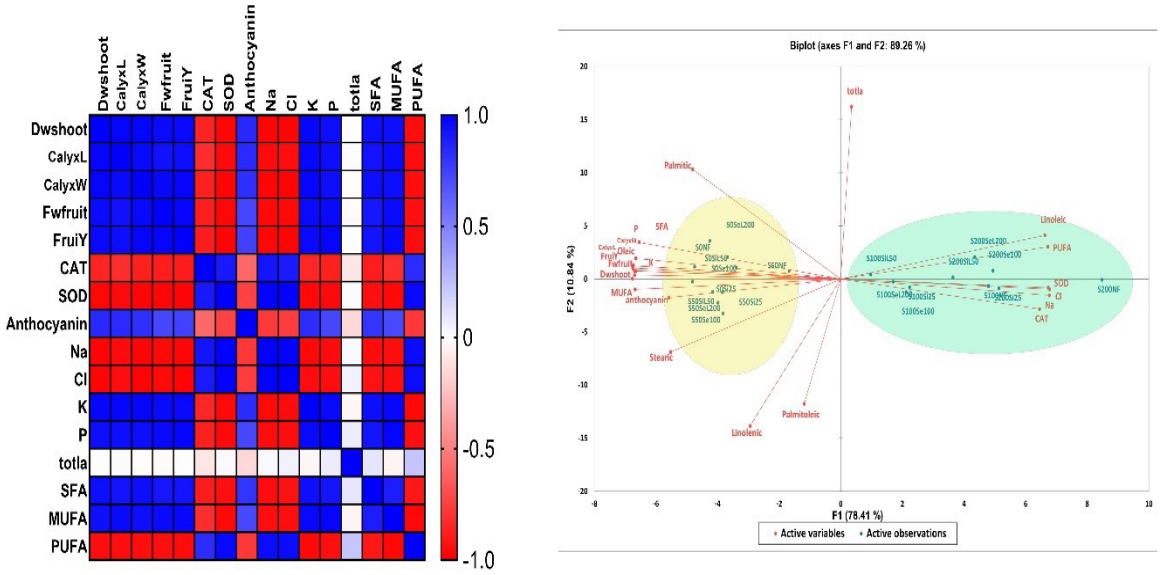

Fig. 5. Encapsulates two crucial analytical components: (a) a Correlation matrix and (b) PCA, both contributing to a comprehensive understanding of the intricate relationships and multivariate patterns within the dataset. Subplot (a) displays the correlation matrix, employing Pearson correlation coefficients to elucidate the interdependencies among diverse parameters, such as shoot dry weight, fruit yield, enzymatic activities (CAT, SOD), anthocyanin content, and elemental concentrations (Na, Cl, K, P). The correlation matrix aids in identifying potential associations or trends between these variables. Subplot (b) features principal component analysis. The eigenvalues, eigenvectors, and variable contributions are presented, offering insights into the major determinants shaping the observed patterns.

DISCUSSION

This experiment rigorously investigated the effects of salinity stress on the physiological and biochemical aspects of plant growth in *P. alkekengi* L. The findings demonstrate substantial changes in shoot dry weight, fruit morphology, and multiple biochemical characteristics, indicating the adverse impact of elevated salinity levels on plant well-being. Furthermore, the utilization of Se and Si nanoparticles exhibited encouraging remedial effects, alleviating the

detrimental outcomes of salinity stress (Afshari *et al.*, 2021; Ali *et al.*, 2021; Ghasemian *et al*., 2021).

The augmentation of soil salinity levels instigates a cascade of physiological responses in plants, primarily through the elevation of external osmotic pressure. This results in a diminished capacity for water absorption, thereby compromising plant turgor and reducing femininity (Shomali *et al.*, 2021). Concurrently, salt stress disrupts water secretion processes, leading to the accumulation of salts within plant tissues, ultimately resulting in a decline in overall plant vigor. The accompanying perturbations in enzymatic activities and metabolic processes further contribute to the detrimental effects of salinity stress (Gaafar *et al.*, 2020; Hawrylak-Nowak, 2022; Monroy-Velandia and Coy-Barrera, 2021). However, the introduction of Se and Si nanoparticles serves as a strategic intervention, manifesting positive effects on plant aerial parts under salt stress conditions.

The incorporation of nanoparticles demonstrates notable enhancements in plant growth dynamics, water absorption, and nutrient assimilation, culminating in an overall increase in plant weight. Se nanoparticles, acknowledged for their essential role in plant physiology, exhibit a capacity to elevate enzyme activity and metabolic processes, thereby mitigating the adverse impacts of salinity stress on plants (Alsaeedi *et al.*, 2019; Alsamadany *et al.*, 2022; Gaafar *et al*., 2020). The current findings are consistent with previous studies highlighting the beneficial effects of Si and Se nanoparticles, underscoring their role in augmenting plant biomass under salinity stress conditions.

Salt stress-induced alterations in fruit quality, encompassing changes in color, texture, and phenolic content, pose considerable challenges to the commercial value of crops (Monroy-Velandia and Coy-Barrera, 2021; Mushtaq *et al*., 2020). However, the application of Se and Si nanoparticles emerges as an effective strategy to counteract these adverse effects, contributing to increased plant weight and size (Afshari *et al*., 2021; Alsamadany *et al*., 2022; Kiumarzi *et al.*, 2022). Moreover, the discernible reductions in fruit yield attributed to diminished water and nutrient absorption, increased vulnerability to diseases, and compromised resistance under salt stress conditions can be effectively mitigated through nanoparticle interventions (Ghasemi-Soloklui *et al.*, 2023).

The multifaceted contributions of Se nanoparticles, characterized by their involvement in plant growth, development, and antioxidant activity, offer significant improvements in fruit yield (Abdoli *et al*., 2020; Garza-García *et al.*, 2021; Ghasemian *et al*., 2021; González-García *et al.*, 2021). The present study aligns with prior research reports, indicating increased fruit yield in strawberries with the application of Se nanoparticles. Notably, the concentration of nanoparticles proves to be a critical factor, with the 200 mg l^{-1} level demonstrating superior efficacy in influencing plant performance. This underscores the importance of nanoparticle dosage in tailoring interventions for optimal plant responses under salinity stress conditions.

The discernible impact of salt, even in low concentrations within the roots and leaves, manifests in the heightened activity of the catalase enzyme. This catalytic response exhibits organ-specific variations, influenced by the diverse sodium concentrations present in different plant organs. A pivotal biochemical consequence of salinity stress in plants is the accumulation of reactive oxygen species (ROS), leading to perturbations in cellular redox balance and the onset of oxidative stress, as elucidated by previous research (Mushtaq *et al*., 2020). In response to stressful environmental conditions, plants mobilize antioxidant compounds, with varying quantities among different plant species, to counteract the deleterious effects of activated oxygen

species. The delicate equilibrium between ROS generation and neutralization by antioxidants becomes disrupted under stress, resulting in oxidative damage at the cellular level (Alam *et al.*, 2022; Moradi *et al*., 2022). Reactive oxygen species, including hydrogen peroxide, are formed during metabolic processes and environmental stress, underscoring the continuous exposure of photosynthetic aerobic organisms to these species. The catalase enzyme plays a pivotal role in eliminating hydrogen peroxide from plant cells, leading to a concurrent decrease in its activity. Consequently, the heightened activity of antioxidant enzymes, such as catalase, constitutes an essential adaptive mechanism employed by plants to combat salinity-induced stress (Ali *et al*., 2021; Kiumarzi *et al*., 2022). Also, the stability of SOD and CAT levels in nanoparticle treatments, despite harsh salt stress conditions, underscores the efficacy of nanoparticle interventions in mitigating cellular oxidative stress. By reducing ROS concentrations within the cells, nanoparticle treatments effectively dampen the need for significant alterations in SOD and CAT activity. This resilience highlights the potential of nanoparticle-based approaches in buffering against environmental stressors, thereby preserving cellular homeostasis even under challenging conditions (Zia-ur-Rehman *et al.*, 2023).

The adaptive response to salinity stress encompasses an augmentation in chlorophyll content and the enhanced activity of antioxidant enzymes like catalase, peroxidase, and polyphenol oxidase. The elevation in these antioxidant defenses serves as a strategic mechanism for plants to contend with the surge in reactive oxygen species triggered by salt stress (Zahedi *et al.*, 2019). Notably, the application of nanoparticles has garnered attention for its reported efficacy in increasing the content of antioxidant enzymes, as evidenced by various studies (Ghasemi-Soloklui *et al*., 2023; Ghasemian *et al*., 2021; Golubkina *et al*., 2022; Hawrylak-Nowak, 2022; Moradi *et al*., 2022). Researchers postulate that certain nanoparticles possess distinctive antioxidant enzyme-like properties, thereby aiding plants in mitigating oxidative conditions induced by environmental stressors (Zia-ur-Rehman *et al*., 2023).

Salt stress induces the production of superoxide radical anions ($O_2^{\bullet-}$) within plant cells, primarily attributed to stomatal closure and diminished carbon dioxide fixation, leading to reduced growth. Concurrently, heightened respiration in these conditions contributes to the generation of detrimental ions within the cell's mitochondria. The pivotal response involves the elevation in superoxide dismutase enzyme activity, crucial for the detoxification of superoxide ions and consequent reduction in plant damage. Under stress conditions, superoxide dismutase efficiently reacts with superoxide anion radicals, producing water and oxygen as byproducts (Alam *et al*., 2022; Moradi *et al*., 2022). Si and Se nanoparticles exhibit the potential to mitigate oxidative damage by modulating antioxidant defense systems, encompassing both enzymatic and non-enzymatic components (Patel *et al.*, 2023; Sanjay and Shukla, 2021). Notably, the application of nanoparticles and Se under stress conditions led to a decrease in enzyme activity, indicative of their stress-moderating and ROS-reducing capabilities. This aligns with findings reporting reduced antioxidant enzyme activity with nanoparticle application under stress conditions in beans (Zadegan *et al.*, 2023). Although, antioxidant activity can enhance plant tolerance to harsh conditions, the presented data from this study suggest that nanoparticle treatments may not consistently influence this parameter across all treatments. The lack of significant differences in plant antioxidant concentrations between nanoparticle treatments compared to the control group underscores the variability in the effectiveness of nanoparticle strategies in modulating plant stress responses (Morales-Espinoza *et al.*, 2019).

The impact of salinity stress on fruit anthocyanins, crucial phenolic compounds contributing to red, blue, and purple hues in fruits and vegetables, is multifaceted. Salinity-

induced alterations in enzyme activity and plant metabolism can diminish anthocyanin production and concentration, especially under extreme stress levels like 200 mM. Elevated salinity conditions may heighten the oxidation potential of the environment, potentially oxidizing anthocyanins and reducing their content and activity in the fruit (Denaxa *et al.*, 2022). The observed nuanced response in the current study, where mild salinity levels increased anthocyanin content while severe levels decreased it, echoes findings reported by Denaxa *et al*. (2022) and Yaghubi *et al.* (2019) in strawberries. Encouragingly, the application of nanoparticles and Se emerges as a positive influence on fruit anthocyanins. Nanoparticles exhibit the capacity to augment anthocyanin production and concentration, while Se nanoparticles not only boost anthocyanin levels but also enhance their antioxidant activity (Banerjee *et al*., 2021). Consistent with previous research, an augmentation in anthocyanin content with Se application has been reported (Fatemi *et al.*, 2021; Sheikhalipour *et al.*, 2021).

Plants respond to high levels of sodium ions in their surroundings by activating mechanisms that regulate the balance of sodium ion concentrations inside and outside of cells. Unlike potassium ions, which are maintained at high concentrations within cells, plants actively reduce sodium ion levels inside cells in order to achieve equilibrium (Muhammad *et al.*, 2022). The increase in salt concentration in the growth medium hinders the plant's ability to maintain a balance of sodium ions, leading to a higher accumulation of sodium ions in the leaves. The current findings are consistent with previous research conducted by (Alam *et al*., 2022; Moradi *et al*., 2022), which have demonstrated a decrease in sodium ion levels in leaves by using nanoparticles and Se.

Under conditions of elevated sodium chloride concentration in the root environment, there is an increase in the accumulation of chloride ions in plant organs. Nanoparticles and Se act as chlorine ion adsorbents, specifically attracting and absorbing these ions, including those that enter the plant through the soil. Nanoparticles and Se function as inhibitory agents on chlorine ion permeation channels in plant cells. This leads to a decrease in the penetration of chloride ions and subsequently reduces their accumulation in leaves (Kiumarzi *et al*., 2022). In addition, these substances strengthen the plant's defense mechanism by promoting the production of antioxidants and improving its ability to withstand salinity stress. As a result, the accumulation of chlorine ions in the leaves is reduced (Hawrylak-Nowak, 2022; Kiumarzi *et al*., 2022; Muhammad *et al*., 2022).

Salinity stress, marked by an elevation in soil salt concentration, hampers the uptake of potassium by plant roots. The disparity in osmotic conditions between the soil and roots, coupled with the hindrance of potassium transportation within the plant, leads to a reduction in the transfer of potassium from the roots to the leaves. In addition, exposure to high levels of salt can cause cellular harm, leading to a decrease in the ability of damaged cells to transport potassium (Habibi and Aleyasin, 2020; Hawrylak-Nowak, 2022; Muhammad *et al*., 2022). As a result, there is a decrease in the potassium levels in the leaves (Sardar *et al.*, 2023). Se nanoparticles enhance the antioxidant defense system, reducing oxidative damage, protecting cells and cell membranes from salt-induced stress, and promoting potassium uptake and transport in plants. Increased potassium uptake and transport contribute to heightened potassium concentrations in leaves during periods of salt stress. Nanoparticles have been shown to enhance plant tolerance to saline conditions by improving the activity of potassium transfer pumps in roots, leading to increased potassium absorption and transfer in plants (Wu *et al.*, 2018).

The presence of high levels of salt in the soil has a detrimental effect on the ability of plant roots to absorb phosphorus. This is caused by the disruption of the root's water balance

and an increase in the deposition of phosphate in the soil. Salinity stress causes changes in the tissue structure and function of roots, leading to a decrease in the movement of phosphorus within the plant (Okon, 2019; Yadav *et al.*, 2011). This limits the transport of phosphorus from the roots to the leaves and reduces the phosphorus content in the leaves. Moreover, alterations in enzyme activity caused by salinity, specifically phosphatase, result in reduced absorption and utilization of phosphorus by plants (Bouras *et al.*, 2022; Sardar *et al*., 2023). Prior studies, exemplified by Golubkina *et al*. (2022), have demonstrated the effectiveness of nanoparticles and Se in enhancing the phosphorus content in leaves. This indicates their potential use in reducing the negative impact of salinity stress on the absorption and utilization of phosphorus by plants.

Beyond its gustatory appeal, the *P. alkekengi* fruit stands out for its nutritional richness, contributing to potential human health benefits. The discerned decline in fatty acids can be ascribed to the impeding influence on both unsaturated and saturated fatty acid synthesis, culminating in diminished oil content and consequential shifts in the fatty acid composition. The plant's adaptive responses to environmental and growth nuances extend beyond impacting performance metrics, influencing the intricate synthesis of secondary metabolites and bioactive compounds (Terletskaya *et al.*, 2021). In concordance with previous study on wheat (Weinstock *et al.*, 2006), our investigation illuminates a discernible inverse correlation between linoleic acid and oleic acid. Furthermore, our study reveals an augmentation in polyunsaturated fatty acids and a concurrent reduction in non-monounsaturated fatty acids under stress conditions, mirroring the trends documented by He and Ding (2020) and Zadegan *et al*. (2023). These consistent patterns underscore the universality of the observed alterations in fatty acid composition in response to environmental stressors and treatment modalities.

The correlation matrix analysis offers a thorough comprehension of the complex physiological relationships within the experimental framework, revealing insights into the dynamic reactions of *P. alkekengi* to different situations and treatments. The significant positive relationships reported across biomass-related parameters, such as shoot biomass, calyx dimensions, fruit fresh weight, and fruit yield, highlight a synchronized response to environmental stressors. The inverse relationship between these growth-related characteristics and antioxidant enzymes (CAT and SOD) implies a possible trade-off, indicating the allocation of resources between growth and stress response mechanisms. The elements sodium (Na) and chloride (Cl) consistently showed a negative connection with antioxidant enzymes, suggesting their participation in the plant's response to ionic stress. The discovered positive associations between potassium (K) and phosphorus (P) with biomass-related variables highlight the crucial roles of these nutrients in promoting plant growth. The fatty acid composition, including the total essential fatty acid, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), showed relatively modest associations with other measures. This suggests that they exhibit a certain level of independence in their reactions. The introduction of Se and Si nanoparticles in treatment had subtle impacts on correlations, emphasizing their potential in influencing the complex physiological dynamics of *P. alkekengi*.

The PCA conducted on the broad range of parameters in this experiment reveals the complex interrelationships and underlying patterns among the variables. The primary eigenvalues corresponding to the initial five main components (F1-F5), which together account for 97.44% of the overall variability, highlight the crucial significance of these components in capturing the fundamental characteristics of the dataset. The specific principal components were primarily influenced by biomass-related features, antioxidant enzymes, and fatty acid

content, emphasizing their unique contributions to the observed variability. The loadings and squared cosines of the variables highlighted the robustness and validity of these correlations. The distinct responses to the treatment were evident by closely analyzing the eigenvectors. Parameters such as DW shoot, calyx L, and calyx W showed significant correlations with individual main components. The significant impact of fatty acids, such as palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic, was demonstrated in determining the observed differences in various treatments. The cumulative proportion of variability demonstrates that the chosen principal components jointly captured most of the dataset's complexity. The effectiveness of PCA in identifying treatment-specific patterns improves our comprehension of *P. alkekengi*'s response to different conditions, offering a comprehensive perspective on the relationship between biomass-related characteristics, antioxidant enzymes, and fatty acid composition.

CONCLUSION

In conclusion, this study highlights the significant impact of salinity stress on *Physalis alkekengi* L., resulting in decreased growth and fruit yield alongside heightened activities of antioxidant enzymes as a response to oxidative stress induced by salt exposure. Application of Se and Si nanoparticles via foliar spraying demonstrated promising efficacy in mitigating salinity stress, particularly within the concentration range of 25 to 100 mg l^{-1} respectively, indicating a potential avenue for enhancing plant tolerance to adverse conditions. The superior performance of Se and Si nanoparticles compared to the control underscores the potential of nanotechnology in optimizing plant responses to environmental stressors, with their surfaces exhibiting notable superiority in promoting overall plant health and performance. The ability of nanoparticles to modulate salinity stress and enhance antioxidant enzyme activities and boosting plant tolerance to abiotic stress such as alkaline or salt stress presents novel opportunities for sustainable agricultural practices aimed at improving crop yields and quality. Moving forward, further research should explore the molecular mechanisms underlying the positive effects of Se and Si nanoparticles on plant responses to salinity stress, paving the way for tailored interventions and nano-based solutions to bolster crop resilience in the face of environmental challenges. This study thus contributes valuable insights into the potential applications of nanotechnology in agriculture, offering a pathway towards sustainable and resilient crop production amidst changing environmental conditions.

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Soft Modeling of Factors Affecting Export Development of Ornamental Plants and Flowers Industry in Mazandaran Based on Interpretive Structural Modeling (ISM)

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Abstract

Mazandaran's share of ornamental plants and flowers industry's exports has been decreasing over the past ten years, despite its proximity to CIS countries. The aim of research was to achieve a stratified model of effective factors in the export development of ornamental plants and flowers in Mazandaran in the form of a comprehensive stratified operational model that has a practical nature. By reviewing the research literature and attracting the opinions of experts, the subject was identified in the form of 22 factors and 98 indicators. The basis of the research was the judgments of a group of experts and export experts and export producers from Mazandaran, who were selected based on a purposeful judgmental sampling. The validity of the researcher-made questionnaire was confirmed by experts. The data obtained from the questionnaires were analyzed using interpretative structural modeling, and were drawn in terms of influence or impact on 6 levels in an interactive network. The findings showed that factors "International political and economic characteristic" and "Recreation of government in development of export" have the greatest impact on the export development of the flowers and ornamental plants industry and are considered the most basic factors and the factors "Improvement of commercial diplomacy", "Sustainable growth of economic" were also at the lowest level of effectiveness. Also, the driving force and dependence of other factors were also identifyed, in order to explain the effectiveness of the factors in the export development of this industry.

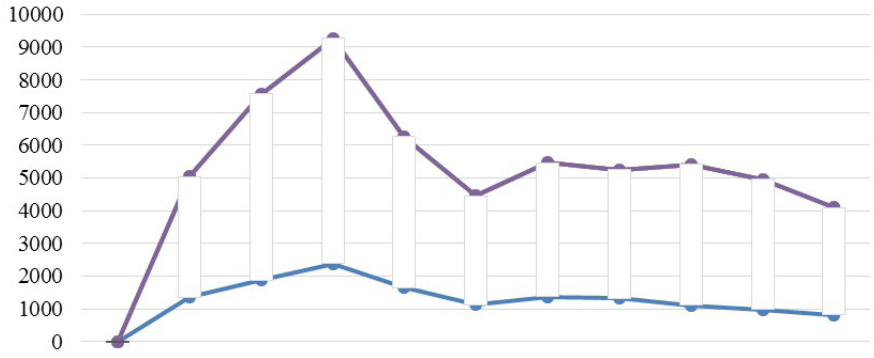
Keywords: Export, Flowers industry, ISM, Mazandaran, Ornamental plants, Soft modeling.

INTRODUCTION

The export of agricultural pruducts plays a significant role in non-oil exports and is more stable than other industries. Now days, production/growing of flowers and ornamental plants, as one of the important activities in the agricultural sector, play an essential role in aesthetic gratification, general decoration, beautification, employment opportunity, export growth, livelihood, trade and commerce, improving the environment etc. According to the geographical and agricultural conditions, production of flowers and ornamental plants have a suitable situation in Iran. Development of agricultural extension and education services, agricultural cooperatives and unions, development of infrastructure and facilities, export facilitation, marketing, practical research, etc., can contribute to the sustainability of this industry (Hajimirrahimi and Ghasemi, 2023).

Export of flower and ornamental plant is one the most commercial transactions in some countries in such a manner that a country like Netherlands gain a high amount of its income from export of flower and plant. This country is so similar to Mazandaran province in terms of climate and weather (Azarkish *et al*., 2015). According to the high quality of the produced flowers, export of different types of flower and plant is so low. Potential ability of Mazandaran province for producing ornamental plant and flower and excellence of this product in terms of quantity, quality, diversity of color, appropriate size, etc. makes Mazandaran province as the first rank in producing apartment flowers by producing 27.5 million vase per year and the second rank by producing 527 cutting branch per year (Agriculture Jihad, 2016).

Most of the transit of ornamental plants and flowers from Iran are to other countries. The flowers entered into those countries are packed again in new packages and new names and exported to other countries by their own name. Export flowers are sent to UAE, Saudi Arabia, Kuwait, Bahrain, Iraq, China, Japan, Vietnam, Taiwan, and a small amount to Germany and France (Khosh-Khoui *et al*., 2021). Despite the fact the Mazandaran is one of the important hubs of producing ornamental plants and flowers and has a high potential in practicing, they have no remarkable rile in the field of export and in the global markets (Zamanian, 2009). Most of the customers of Iran for purchasing flower are Iraq, Azerbaijan, Ukraine, Moldavia, Belarus, Georgia, Armenia, Tajikistan, Kyrgyzstan, Uzbekistan, Turkmenistan, Kazakhstan, and Russia that are mainly northern neighbors of Iran and Mazandaran. With this potential, almost 98% of the cutting flowers are used for domestic markets (Khosh-Khui *et al*., 2021). Also Mazandaran's share in the export of flowers and ornamental plants has been decreasing in the last ten years.



Top line: VALUE (1000dollars)
Bottom line: WEIGHT (tons)

Fig. 1. The amount of ornamental plants and flowers export in Mazandaran province during last 10 years (Source: Agricultural Jihad of Mazandaran province).

Surveys show that the flower and ornamental plants industry in Iran and many different countries of the world is facing various problems and challenges (Darras, 2021). For example, research has shown that the scientific level of agricultural operators is very low and there is no optimal awareness and knowledge in the field of production, which emphasizes the need for training and the presence of specialist forces (Namvar and Ommani, 2022). Classified monitoring variables affecting the export development of ornamental plants and flowers industry in Mazandaran province can be a good action for achieving a reasonable export performance and cause access to the non-oil incomes. Since there is very few researches about the issue of export of ornamental plants and flowers especially in province, the mentioned study are studies that have the closes relationship with this field.

Spier's research results showed that the most important challenges facing ornamental plants and flowers industry are the low income of producers compared to production costs, transportation problems, compliance with quality standards and proper packaging (Spier *et al*., 2020). In another research, issues such as land ownership, pest and plant diseases management, lack of access to sufficient water sources and lack of sufficient support from the government had been identified as the most important challenges for flowers and ornamental plants industry in the Darossalaam from Tanzania (Pastory *et al*., 2020). By examining the sustainability challenges of the value chain of potted plants in Germany, the most important challenges of industry are explained as follows: The environmental aspect: Lack of water, the use of pesticides and carbon footprint, from the social aspect: low wages and difficult working conditions, and from the economic aspect: challenges related to profitability and compliance with standards (Havardi-Burger *et al*., 2020). In the review of orchid plants development strategies in Indonesia, the lack of technical recommendations for producers and lack of access to capital, were the most important internal factors and also lack of labor recruitment, lack of use of network marketing and online media for advertising, and lack of creativity in production through exhibitions had been the most important external factors (Sri *et al*., 2021).

Khosh-Khui *et al*. (2021) said that due to the high price of plants and flowers in Iran and lack of high amount of export, this industry has not important role in profitability and gaining foreign currency for Iran and according to the high quality of the produced plants and flowers in Iran, export of different types of plants and flowers is so low and we don't look at the production of ornamental plan as an strategic industry. Khalilabadi *et al*. (2016), concluded that economic factors and social factors have a positive effect on flower and ornamental plants export of Thran province. Azarkish *et al.* (2015), showed that there is a direct relation between the level of infrastructures and improvement of macroeconomic factors for both Iran and Kenya and infrastructures are effective in removing of limitations of small farmers, credits, warehousing, market distribution of product, insurance, access to new technology, services of promotion, roads and ports, telephone communications, irrigation, and rules. Amiri *et al*. (2014) categorized the impediments and problems of producers in sectors of producing, harvesting, packaging, processing and exporting and also marketing ornamental plant. Mostashar Nezami *et al.* (2013) classified the factors effective in export of Iranian plant and flower into two internal and external class. Dana *et al.* (2012) expressed that most of the persons active in this field have a look at the common allowances of the government and pay less attention to the commercial nature and content and responsible attention to the demand of customers and conditions of the target market. Estelaji and Pazoki (2012), show that the first priority in the global markting pattern is the product. Nikooie *et al.* (2010) said that share of producer from the price sold to the consumer and efficiency of marketing is so low. Zamanian (2009) reached that in more than

70 of the producers, the manner of production is traditional and the industrialized degree is so weak. This research aims to identify the driving and dependent factors of export development of ornamental plants and flowers industry in Mazandaran province and provide a classified structured model in order to determine that each of the factors are placed in what cluster of independent, bonder and autonomous factors.

MATERIALS AND METHODS

The current exploratory research is applied in terms of goal. Data of this study were collected via documenting and surveying methods. Questionnaires for the analysis of two-way relationships of the components, which included 18 effective components in the export development of this industry, were distributed among 14 expert participants. With targeted sampling, participants who had helped to identify the components in qualitative section of the study, evaluate the bilateral relationship among the factors and the analytical matrixes are designed. Participants in this research were exporters and traders of ornamental plants and flowers industry in the Mazandaran province, managers and experts of different levels of Industry, Mine and Trade Organization, Agriculture Jihad, export chamber of commerce, flower and plant union, transportation management in Amirabad port and airports of Mazandaran Province that have been collaborated with researchers in the qualitative part of the research. The participants cooperated in the best condition for collecting the research information and in evaluation of the questionnaire. At the rating section, the ISM pairwise comparison questionnaire. Interpretive Structural Modeling (ISM), which is an interactive learning process where a set of elements are structured into a comprehensive system model, is used as the approach. ISM helps in determining the sequence and purpose of complex relationships between elements in the system. According to the complexity of policy and foreign commerce and also number of factors effective in export production, ISM method is used for identification of relation between factors and diagnosis of impact and effect of each of them. The characteristic investigated in the ISM approach is the relationship between the elements. Since all possible relationships are examined in the form of a matrix, therefore, the structural-interpretive questionnaire in itself has validity. The characteristic investigated in the ism approach is the relationship between the elements. Since all possible relationships are examined in the form of a matrix, therefore, the structural-interpretive questionnaire in itself has validity (Habibi and Afridi, 2022). In order to implement the ISM modeling approach and determine the relationship between the factors as well as to create order in the elements of the problem, it is necessary to go through the following 7 steps:

1. **Identifying elements:** Identifying elements related to the topic. This step can be done by reviewing the theoretical literature or using any group problem solving technique.

2. **Field relations of SSIM:** Establishing contextual relationships between elements, considering which pairs of elements will be tested.

3. **Initial access matrix:** Creating a structural self-interaction matrix that shows how the elements of a system pairwise have relationships with each other.

4. **Final access matrix:** Production of SSIM matrix.

5. **Determination of level of variables:** Matrix commutability states that if element A is related to B and element B is related to C, then A is also related to C.

6. **Drawing ISM:** Matrix alignment to reach different levels. Draw a diagram of relationships and remove transitional links.

7. **Review on ISM:** Reviewing the ISM model, to discover conceptual mismatches and also to make necessary changes (Raut *et al*., 2018).

RESULTS

First stage: **"Identifying elements"**

In the research conducted by Mahdiee and Fani (2022) all the factors affecting export development of ornamental plants and flowers industry in Mazandaran province were identified.

Second stage**: "Field relations of SSIM"**

Experts examined the relationship between the factors. Evaluation of experts is shown in table 1.

Table 1. SSIM for factors affecting the export development of ornamental plants and flowers industry in Mazandaran province.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	Global red ocean	❁	V	V	X	A	A	V	A	A	A	V	V	V	V	A	V	V	V
2	Global evaluation		❁	V	A	A	A	A	X	X	V	A	A	A	A	V	V	V	V
3	Development of hardware infrastructure for export			❁	V	A	A	V	A	V	A	V	A	V	A	A	V	V	V
4	Development of software infrastructure for export				❁	A	X	V	V	X	V	V	V	V	V	V	V	V	V
5	International political & economic characteristics					❁	V	V	V	V	V	V	V	V	V	V	V	V	V
6	Recreation of government in development of export						❁	V	V	V	O	V	O	V	V	V	V	V	V
7	Export terminal							❁	X	A	X	A	X	X	A	V	V	V	V
8	Managerial capabilities								❁	V	V	A	X	V	O	V	V	V	V
9	Competitive strategies									❁	V	A	A	A	A	A	V	V	V
10	Marketing and export branding										❁	A	X	X	V	V	V	V	V
11	Technology											❁	V	V	X	V	V	V	V
12	Making official and network for export												❁	X	V	V	V	V	V
13	Experience and Commitment in export													❁	A	V	V	V	V
14	Processing exported products														❁	V	V	V	V
15	Removing export Hydrocephalus & Export Barriers															❁	V	V	V
16	Targeted modeling from pioneer countries																❁	X	V
17	Sustainable growth of economic																	❁	X
18	Improvement of commercial diplomacy																		❁

Third stage: Initial access matrix

Access matrix or initial access is obtained from conversion of SSIM to zero and one matrix.

Fourth stage: Final access matrix

The secondary relation between elements should be analyzed so that the initial access matrix be combatable.

Fifth stage: Determination of level of variables

In this stage, the classification of element that is determination of early and late sets is performed that formation of the conical matrix is created. After determination of the output and input set for each of the elements by obtaining common area of two sets, their classification is done. For example, result of determination of input and output set, common in the first level of factors of export development of ornamental plant and flowers in Mazandaran, the factors No. 17 and 18 are recognized as the first level. By determination of the first level element, this element will be separated from other elements, then another level of element is formed vial a similar process. These determined levels are used in formation of diagram (Fig. 3) and final

model. Commonly, factors that have equal output set and bilateral relations set which means that the common set of them be the same with their output set; the first level or upper level creates a hierarchy. Therefore, on this basis, the upper level is not the source of other elements. Therefore, in determination of the second level factors, through the analyzed commons, the 16th factor as the second level and 1st, 2nd, 3rd, 7th, 9th, 10th, 12th, 13th, 14th, and 15th as the third level and 4th and 8th and 11th factors as the fourth level and 6th factor as the fifth level and finally, the 5th level as the 6th level.

Sixth and seventh stage: Drawing ISM and a review on ISM

In this step, according to the levels of factors and by considering the final access matrix, the ISM is drawn (Fig. 2). The final model is drawn in 6 levels that show that the factors of lower levels have more impact on other factors in form of a hierarchy.

Fig 2. ISM for determination of factors affecting the export development of ornamental plants and flowers industry in Mazandaran province.

At the end, the variables affecting the export development of ornamental plants and flowers industry in Mazandaran provinceare analyzed in terms of permeability and correlation. Purpose of this analysis is to determine the power of permeability and amount of correlation of variables. The 18 variables of the present study are classified in 4 clusters based on the driving number.

►Cluster 1 including autonomous factors that have weak driving and relation power and are separated from the system and their bond with the system is so weak and low.

►The dependent factors compromise the second cluster that have low driving power and high relation power.

►The third cluster includes bonders that have driving power and high relation power. This factors are not fixed since any change happened in the impact other variables and the feedback of these changes may be felt.

►The fourth cluster includes dependent factors that have high driving power but weak relation power.

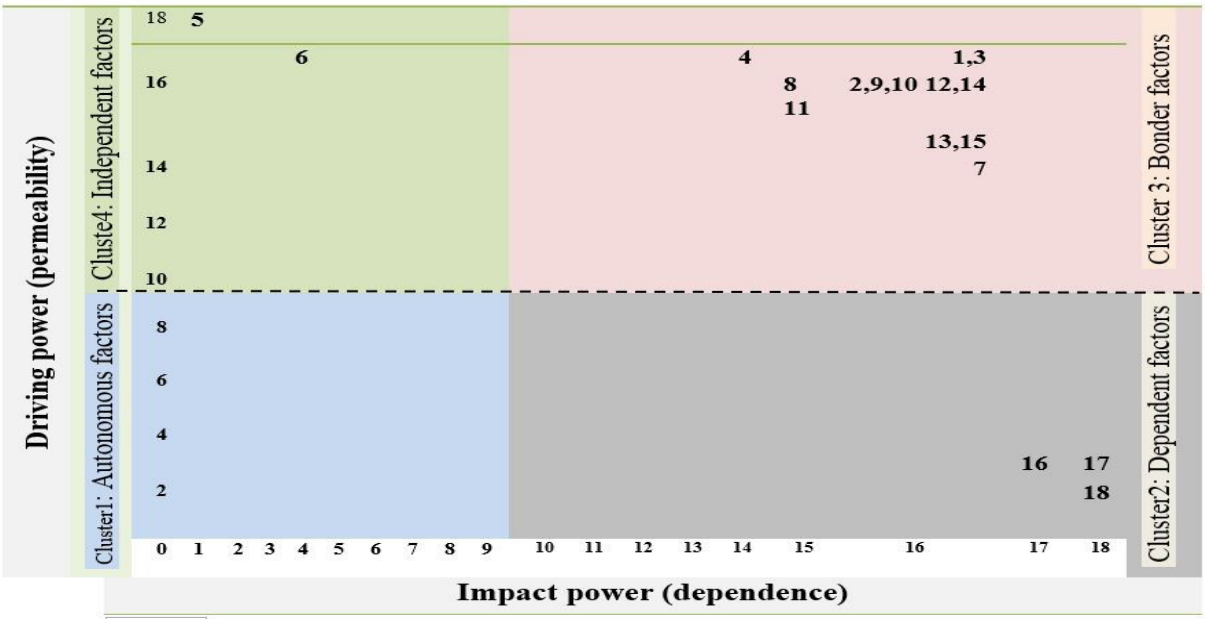


Fig. 3. Clustering factors affecting the export development of ornamental plants and flowers industry in Mazandaran province.

About the amount of driving of each of the factors: The result of the research show that the factors ″Recreation of government in development of export″ and ″International political and economic characteristics″ have the highest amount of driving power and are considered as the most fundamental items. Factors ″Sustainable growth of economic″ and ″Improvement of commercial diplomacy″ due to having high relation power and low driving power are affected by other factors. Other factors have equal driving and relation power.

About the relation of the driving factor with dependent factors that as per fig. 3: There is a unilateral relation between some of the factors like the ″Relation of recreation of government in development of export″ that is placed in dependent factors cluster with intermediate factors like ″Development of software and hardware infrastructure of export″. Relation of some of the factors was a bilateral relation like relation of factor ″ Making official and network for export ″ with factor ″Processing export products″.

The structure of the factors in 6 levels of importance in Fig. 2 and the diagram in the Fig. 3 clustered the variables affecting the export development of ornamental plants and flowers industry in Mazandaran province in 4 categories. The clusters expresses that the factors ″International political and economic characteristics″ and ″Recreation of government in development of export″ have high impact on other factors and other factors affect them a bit are place in cluster of independent factors and in terms of logic of relations of cause and effect, it is considered as cause and fundamental basis of other factors.

On the other hand, the factors of ″Sustainable growth of economic″ and ″Improvement of commercial diplomacy″ have low impact and affect high from other factors are placed in cluster of dependent factors and in logic relation of cause and effect, it is considered as effect of other factors. The factors ″Removing export hydrocephalus and export barriers″, ″Managerial capabilities″, ″Experience and commitment in export″, ″Export terminal″, ″Processing exported products″, ″Global evaluation″, ″Marketing and export branding″, ″Global red ocean″, ″Making official and network for export″, ″Competitive strategies″, ″Development of

hardware infrastructure for export", "Development of hardware infrastructure for export" and "Technology" are considered as intermediate variables and placed in cluster of bonder factors; therefore, these factors as the intermediate factors are non-sustainable factors that have high driving power and high relation power. But none of the factors of the present study are placed in cluster of autonomous factors which means that there is no autonomous variable in the research and all variables can be put in the cause and effect relations.

DISCUSSION

Mazandaran province is considered as one of the largest producers in the country's flower and ornamental plants industry. Unfortunately, despite the emphasis on export in sixth development plan (of Iran), amount of export of industry of ornamental flower and plant in Mazandaran was reduced remarkably. In this research, first, the bivariate relationships between 18 effective factors in the development of flower and ornamental plants export in Mazandaran province were examined by experts in this field, and the final matrix showed that the basic and principle of export of this industry in Mazandaran province is in "Improvement of International political and economic space" which confirms the findings of Mahdiee and Fani (2022), Mirrahimi *et al.* (2022), and Namvar and Ommani (2022). In other word, the major problems in the field of export is sanctions, fluctuation of foreign currency and other international factors that cast a shado on the export of ornamental plant and flowers industry. In general, in the planning and policies of this sector, the government should improve the political and economic environment of flower and ornamental plant industry, like other agricultural products, by thinking of solutions for sanctions and also reducing the production costs of this industry as Khosh-Khui *et al*. (2021), Pastory *et al.* (2020) and Khalilabadi *et al*. (2016) mentioned in their articles. The adoption of supportive policies by the government can be a good action for achieving a reasonable export performance and cause access to the non-oil incomes as Azarkish *et al.* (2015) mentioned in their article. Khosh-Khui *et al.* (2021) also considered development of foreign investment in Iranian flower industry, removing sanctions and joining the global flower industry as the most effective ways for realizing export goals and said that in the field of non-petroleum export, conditioned to preparing in frastructures, it will gain good foreign currency and has a good profitability.

As mentioned in the ISM model of this research, after the factors "International political and economic characteristics" and "Recreation of government", factors "Development of hardware, software and technological infrastructure for export" have had the greatest effect on the export of this industry. The meaning of infrastructures is the necessary facilities for reduce product waste, improving quality, volume of export, packaging and sorting industry, distribution and supply logistics chain, financial, insurance and Banking infrastructure for export. Azarkish *et al*. (2015), showed that for both Iran and Kenya, infrastructures are effective in removing of limitations of small farmers, credits, warehousing, market distribution of product, insurance, access to new technology, services of promotion, roads and ports, telephone communications, irrigation, and rules. The policies should be made in direction of removing infrastructure shortage relevant to production and export of ornamental plants like producing in traditional greenhouses, construct cold storage room, using mechanized system of standard packaging and fast, safe and appropriate transport. Estelaji and Pazoki (2012), showed that the first priority in the global markting pattern is the product. The elements of place, commendatory and persuasive activities and price are the next priorities. The factors of product: Producing

with a modern and industrial method, using pioneer countries, packaging, appearance, quality and diversity in production, the factors of place and distribution canals: Export terminal in the region, recognition of structure of foreign markets, existence of equipment and facilities of warehousing, sales agency in target market and finally activity of specialists of marketing. The factors of persuasive policies including experience of pioneer countries, advertising, international exhibitions, and persuasive policies of the government and finally for the price, and the factors of cost price in farm, financial support of family, foreign currency policies of government, impact of inflation in Iran and foreign investment. Nikooie *et al.* (2010) showed that due to existence of high waste, the technical efficiency is low but the price efficient is high due to high price of retailing than the sale at farm; therefore, the results of total efficiency is high. In the next degrees of importance, with less influence, factors ″Export barriers″, ″Managerial capabilities″, ″Experience and commitment″, ″Export terminal″, ″Processing exported products″, ″Global evaluation″, ″Global red ocean″, ″Networking″ and ″Competitive strategies″ were introduced in the ISM model. These results were consistent with the findings of Ghasemi and Hajimirrahimi (2023), Darras (2021), Sri *et al*. (2021), Khosh-Khui *et al.* (2021), Pastory *et al*. (2020), Havardi-Burger *et al*. (2020), Spier *et al*. (2020) and Amiri *et al*. (2014).

So, with pay more attention to the aspects related to the improvement of international political and economic space in the field of export, more than ever, to make Iran benefit from the abundant profits of this income-generating industry.

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مدل‌سازی نرم عوامل مؤثر بر توسعه صادرات صنایع گل و گیاهان زینتی مازندران بر اساس مدل‌سازی ساختاری تفسیری (MSI)

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چکیده

از ایران به‌عنوان یکی از ده کشور برتر، در تولید گل و گیاهان زینتی در جهان، یاد می‌شود. استان مازندران، یکی از بزرگترین تولیدکنندگان گل و گیاهان زینتی کشور محسوب می‌شود. با این وجود، علیرغم هم‌جواری با کشورهای مصرف کننده‌ی گل و گیاهان زینتی (کشورهای CIS)، سهم مازندران از صادرات این صنعت در ده سال گذشته رو به کاهش بوده است. هدف پژوهش حاضر دستیابی به مدل سطح‌بندی شده از مولفه‌های موثر در توسعه صادرات گل و گیاهان زینتی مازندران است که در قالب یک مدل عملیاتی با رویکردی جامع و ماهیتی کاربردی تبیین گردد. با بررسی ادبیات تحقیق و بررسی نظر کارشناسان، مولفه‌ها در قالب ۲۲ عامل و ۹۸ شاخص شناسایی شد. مبنای تحقیق، قضاوت گروهی از کارشناسان و خبرگان صادرات و تولیدکنندگان صادراتی مازندران بوده است که بر اساس نمونه‌گیری قضاوتی هدفمند انتخاب شدند. روایی پرسشنامه محقق ساخته توسط متخصصان تایید شد. داده‌های به دست آمده از پرسشنامه‌ها با استفاده از مدلسازی ساختاری تفسیری مورد تجزیه و تحلیل قرار گرفت و بر اساس میزان تاثیرگزاری و تاثیرپذیری، در یک شبکه تعاملی ۶ سطحی ترسیم شد. یافته‌های تحقیق نشان داد که عوامل «مولفه‌های سیاسی و اقتصادی بین‌المللی» و «بازآفرینی دولت در توسعه صادرات» بیشترین تأثیر را بر توسعه صادرات صنعت گل و گیاهان زینتی داشته و از اساسی‌ترین عوامل موثر بر آن محسوب می‌شوند. «بهبود دیپلماسی تجاری»، «رشد پایدار اقتصادی» نیز در پایین‌ترین سطح اثربخشی قرار داشتند. همچنین نیروی محرکه و وابستگی سایر عوامل بررسی شد تا اثرگزاری مولفه‌ها در توسعه صادرات این صنعت تبیین گردد.

کلید واژه‌ها: صادرات، صنعت گل، ISM، مازندران، گیاهان زینتی، مدل‌سازی نرم.

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پاسخ‌های فیزیولوژیکی و تاثیر تغذیه‌ای تیمار نانوذرات Si و Se در گیاه عروسک پشت‌پرده (*Physalis alkekengi* L) تحت تنش شوری

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چکیده

این مطالعه به‌طور سیستماتیک پاسخ‌های فیزیولوژیکی گیاه عروسک پشت‌پرده به سطوح مختلف تنش شوری (۰، ۵۰، ۱۰۰ و ۲۰۰ میلی‌مولار NaCl)، همراه با کاربرد نانوذرات سلنیوم (۲۵ و ۵۰ میلی‌گرم در لیتر) و نانوذرات سیلیکون (۱۰۰ و ۲۰۰ میلی‌گرم در لیتر) را بررسی می‌کند. این آزمایش شامل بررسی کامل بسیاری از ویژگی‌های مرتبط با زیست توده، فعالیت آنزیم آنتی‌اکسیدانی، ترکیب اسیدهای چرب و محتوای عناصر بود. نتایج نشان داد که قرار گرفتن در معرض تنش شوری اثر مضری بر رشد گیاه و تولید میوه دارد که منجر به تغییرات معنی‌داری در ویژگی‌های رویشی و مورفولوژیکی می‌شود. استفاده از نانوذرات سلنیوم و سیلیکون تأثیر قابل‌توجهی بر کاهش تنش ناشی از شوری داشت. تجزیه و تحلیل ماتریس همبستگی، همبستگی‌های پیچیده‌ای را در بین پارامترهای مورد بررسی نشان داد، که بر پاسخ‌های گیاه عروسک پشت‌پرده به عوامل تنش‌زای محیطی و مداخلات نانوذره تاکید کرد. تجزیه و تحلیل مؤلفه اصلی (PCA) الگوهای پنهان و ارتباطات بین متغیرها را نشان داد، که تأثیر قابل توجه بر ویژگی‌های مرتبط با زیست توده، آنزیم‌های آنتی‌اکسیدانی و محتوای اسیدهای چرب را بر تغییرپذیری مشاهده شده برجسته کرد. نتایج این تحقیق دانش ما را در مورد فرآیندهای فیزیولوژیکی که واکنش گیاه عروسک پشت‌پرده به سطوح بالای نمک را تنظیم می‌کنند، افزایش می‌دهد. علاوه بر این، اطلاعات ارزشمندی در مورد اثرات مفید احتمالی نانوذرات سلنیوم و سیلیکون در کاهش پیامدهای منفی تنش شوری ارائه می‌دهد. نتایج جامع این مطالعه به تحقیقات آینده مرتبط با بهینه‌سازی شرایط رشد و تقویت مقاومت عروسک پشت‌پرده در شرایط تنش‌های محیطی افزایش می‌دهد.

کلید واژه‌ها: سیستم دفاعی آنتی‌اکسیدانی، اسیدهای چرب، نانوذرات، تنش شوری.

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بررسی ژنوتیپ‌های مختلف گل داودی از نظر ترکیبات آنتی‌اکسیدانی، عناصر معدنی و مغذی

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چکیده

داودی یکی از مهمترین گل‌های زینتی در جهان است که از نظر دارویی و خوراکی نیز حائز اهمیت است. در این پژوهش ۲۰ ژنوتیپ گل داودی تولید و اصلاح شده در پژوهشکده گل و گیاهان زینتی محلات از نظر ترکیبات مغذی، معدنی و آنتی‌اکسیدانی در قالب طرح کاملا تصادفی بررسی شدند. نتایج نشان داد که در بین ژنوتیپ‌های منتخب گل داودی اختلاف معناداری در همه صفات ارزیابی شده وجود دارد، بطوری‌که بیشترین مقدار کلسیم (۷۴/۱ میلی‌گرم در کیلوگرم وزن تر)، آهن (۲/۲۳۱ میلی گرم در کیلوگرم وزن تر) و سلنیوم (۰/۲۳۳ میلی‌گرم در کیلوگرم وزن تر) به‌ترتیب برای کدهای ۳۲۶، ۱۱۰ و ۵۶۲ ثبت شد. بیشترین مقدار روی (۰/۳۱۵ میلی‌گرم در کیلوگرم وزن تر) نیز به کدهای ۵۶۲ و ۱۳۴ تعلق داشت. بیشترین ویتامین آ (۰/۰۸۶ میلی‌گرم در کیلوگرم) و ویتامین ث (۱۳/۵۸ میلی گرم در ۱۰۰ گرم وزن تر) به‌ترتیب برای ژنوتیپ‌های کد ۵۴۰ و ۶۰۳ ثبت شد. کد ۷۵۱ (۱/۴۸۳ درصد) بالاترین سطح پروتئین و کدهای ۵۴۰ و ۱۳۸ نیز به‌ترتیب با ۱۳/۰۶ و ۲۲/۳۴ درصد کمترین و بیشترین درصد فیبر را داشتند. کدهای ۶۷۴ و ۱۰۸ نیز به‌ترتیب ژنوتیپ های برتر در صفات آنتوسیانین و کاروتنوئید گلبرگ بودند. کدهای ۶۸۴ و ۳۵۴ بیشترین و کد ۷۵۱ کمترین فلاونوئید کل را داشتند در حالیکه بیشترین و کمترین مقدار فنل کل به‌ترتیب به کدهای ۳۲۶ و ۴۶۲ تعلق داشت. با توجه به نتایج حاصل، از ۲۰ ژنوتیپ معرفی شده گل داودی که در داخل کشور اصلاح و تولید می‌شوند کدهای ۱۱۰ و ۳۲۶ از نظر ارزش غذایی و بیولوژیکی مناسب‌ترین گزینه هستند و می‌توان از این گونه‌ها به‌عنوان یک منبع غذایی جدید و در دسترس جهت تامین بخشی از مواد مغذی مورد نیاز بدن استفاده نمود.

کلید واژه‌ها: منبع غذایی جدید، گل خوراکی، پروتئین گیاهی، تغذیه سالم، گیاهخواری.

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پیش‌بینی عملکرد اسانس بابونه با استفاده از سیستم شبکه عصبی مصنوعی

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چکیده

هدف از این تحقیق پیش‌بینی نسبت و تولید اسانس بابونه با استفاده از سیستم شبکه عصبی مصنوعی متکی بر ویژگی‌های فیزیکوشیمیایی خاص خاک بود. مزارع مختلف کشت بابونه مورد بررسی قرار گرفت و ۱۰۰ نمونه خاک به گلخانه منتقل شد. OM، K، EC، pH (ماده آلی)، CCE (کربنات کلسیم معادل) و میزان رس در خاک‌ها از ۸/۷۵ تا ۷/۹۴، ۱/۶ تا ۱/۰، ۳۸۱ تا ۱۳۵، ۲/۳۰ تا ۰/۲۲، ۶۹ تا ۱۶ و ۵۵/۶ به ۳۲/۰ رسید. پارامترهای رشد، درصد اسانس و عملکرد اندازه‌گیری شد. مدل‌سازی شبکه عصبی مصنوعی با هدف پیش‌بینی غلظت و عملکرد اسانس با استفاده از سه مجموعه از ویژگی‌های خاک به‌عنوان پیش‌بینی‌کننده انجام شد: ۱) نیتروژن (N)، فسفر (P)، پتاسیم (K)، رس ۲) رس، سیلت ۳، EC، pH) رس، سیلت، شن، سنگریزه، فسفر، پتاسیم، نیتروژن، pH و EC. در نتیجه، سه تابع انتقال (PTF) با استفاده از پرسپترون چند لایه (MLP) با الگوریتم آموزشی Levenberg–Marquardt برای تخمین محتوای اسانس بابونه فرموله شد. ارزیابی نتایج نشان داد که سومین PTF (PTF3) که با استفاده از همه متغیرهای مستقل توسعه یافته است، بالاترین دقت و پایایی را از خود نشان می‌دهد. علاوه بر این، یافته‌ها امکان پیش‌بینی غلظت و عملکرد اسانس بابونه را بر اساس ویژگی‌های فیزیکوشیمیایی خاک پیشنهاد کرد. این امر پیامدهای قابل‌توجهی برای ارزیابی تناسب زمین، شناسایی مناطق مساعد برای کشت بابونه و برنامه‌ریزی برای بازده اسانس دارد.

کلید واژه‌ها: شبکه عصبی مصنوعی (ANN)، کربنات کلسیم معادل (CCE)، پرسپترون چند لایه، نیتروژن.

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افزایش جوانه‌زنی *Habenaria janellehayneana* (Orchidaceae): بررسی روش‌های غیرهمزیستی و همزیستی

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چکیده

گل *Habenaria janellehayneana* Choltco, Moloney, & Yong Gee (ارکیداسه) یک ارکید لیتوفیت با گل‌های صورتی است که بومی استان فیتسانولوک در شمال تایلند است. فقط تعداد کمی توده‌های این گونه در پارک ملی فوهین رانگ کلا مشاهده شده است. برای حفظ گونه‌های گیاهی نادر در کلکسیون‌های "در محل" می‌بایست از انقراض ممانعت کنیم و با هدف تولید (تکثیر) انبوه برای اهداف زینتی، مهم است که روش‌های مناسب را برای رشد و نمو آن ها فراهم کنیم. در این مقاله ما دستورالعمل هایی را برای جوانه‌زنی همزیستی و غیر همزیستی این گیاه ارائه می‌کنیم. از ۴ محیط کشت آزمایش شده در محیط کشت ۱/۲ واسین و ونت (۱۸/۹۷ درصد) بیشترین جوانه‌زنی طی ۱۶ هفته حاصل شد و بعد از آن محیط ۱/۲ موراشیگ و اسکوگ (۱۴/۲ درصد)، موراشیگ و اسکوگ (۱۲/۴۶ درصد) و واسین و ونت (۱۱/۹۳ درصد) و رشد و نمو شبه پیازها بالاترین میزان (مرحله ۴) طی ۱۰ هفته مشاهده شد. از بین سه تنظیم‌کننده رشد مورد آزمایش، شامل ۶ -بنزیل آمینوپورین، جیبرلیک اسید و تیدیازرون در غلظت‌های صفر، ۱، ۳ و ۵ میلی‌گرم در لیتر، غلظت یک میلی‌گرم در لیتر ۶ -بنزیل آمینوپورین جوانه‌زنی را در سطح آماری ۵ درصد در مقایسه با شاهد (۸/۴۷ درصد) بهبود بخشید. در جوانه‌زنی بذرهای همزیست، دو ایزوله قارچ اندوفیت غیر مایکوریزا از جنس‌های آسپرژیلوس و کولتوتریکوم جوانه‌زنی را به‌ترتیب تا ۱۴/۰۳ و ۱۱ درصد و در مقایسه با شاهد ۶/۱۵ درصد افزایش دادند. این یافته‌ها اثبات می‌کند که امکان جوانه‌زنی بذر این گیاه از هر دو روش همزیستی و غیر همزیستی وجود دارد ولی روش همزیستی نتایج بهتری را به همراه داشت و می‌توان به این گیاه نادر کمک کرد که ذخایرشان حفظ شود و نیز ارزش (بهای) آن‌ها در بازار گیاهان زینتی بالاتر رود.

کلید واژه‌ها: ریزازدیادی، میکوریزا، گیاهان زینتی، ارکیده خاکی.

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بررسی تأثیر سطوح مختلف کود نیتروژن بر خصوصیات کمی و کیفی گل داوودی (رقم. برنا)

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چکیده

گل داوودی یکی از پنج گل اصلی شاخه بریده در ایران است. دانش در مورد محدوده بهینه مواد غذایی پرمصرف به ویژه نیتروژن (N) برای بهترین ویژگی‌های کمی و کیفی ارقام اصلاح شده گل داوودی از اهمیت بالایی برخوردار است. در این تحقیق طرح بلوک‌های کامل تصادفی در سه تکرار اجرا شد. پنج سطح نیتروژن شامل ۰، ۷۵، ۱۵۰، ۲۲۵ و ۳۰۰ کیلوگرم در هکتار (نیترات آمونیوم) بر روی گل داوودی، رقم برنا، در شهرستان محلات اعمال شد. ویژگی‌های اندازه‌گیری شده در این پژوهش حداکثر ارتفاع گیاه، قطر گل، قطر تاج، قطر ساقه، طول عمر گل، نمره کیفی، شاخص کلروفیل، وزن تر و خشک اندام هوایی گیاه، تعداد شاخه در بوته و تعداد روز تا گلدهی بود. نتایج نشان داد که حداکثر ارتفاع گیاه، قطر گل، قطر تاج، قطر ساقه، طول عمر گل، نمره کیفی، شاخص کلروفیل، وزن تر و خشک اندام هوایی گیاه، تعداد شاخه در بوته و تعداد روز تا گلدهی در سطح ۱۵۰ کیلوگرم در هکتار نسبت به شاهد به‌دست آمد. همچنین بیشترین جذب کل عناصر پرمصرف (نیتروژن، فسفر و پتاسیم) و کم مصرف (آهن، منگنز، روی و مس) در سطح کود ۱۵۰ کیلوگرم در هکتار مشاهده شد. بر اساس نتایج، کاربرد نیتروژن در سطح ۱۵۰ کیلوگرم در هکتار برای داشتن بهترین شرایط رشد برای گل داوودی رقم برنا توصیه می‌شود.

کلید واژه‌ها: داوودی، کودنیتروژن، شاخص‌های رشدی و گلدهی، عناصر غذایی کم و پرمصرف.

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