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Combinations of Iron and Manganese Have Variable Effects on the Quantitative and Qualitative Traits of Tuberose (*Polianthes tuberosa* L.)

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ABSTRACT

Understanding interactions and a balance between iron (Fe) and manganese (Mn) as fertilizers is crucial for optimizing tuberose growth. Here, a field experiment considered the effects of various Fe and Mn concentrations on quantitative features, qualitative traits, microelement absorption, and microelement distribution in tuberose plants. The treatments included Fe concentrations of 0, 15, 30, and 45 kg ha^{-1} along with Mn concentrations of 0, 10, and 20 kg ha^{-1} from $FeSO_4$ and $MnSO_4$ sources, respectively. According to the results, high Fe concentrations (30 and 45 kg ha^{-1}) negatively affected the uptake of Mn, Zn, and Cu in aerial parts and bulbs. However, an apt combination of Mn and Fe significantly improved several traits. In the first year, Fe (30 kg ha^{-1}) and Mn (20 kg ha^{-1}) significantly increased spike length (27.19%), floret length (46.22%), bulb count (43.60%), vase life (96.08%), and the percentage of opened florets (26.65%). In the second year, Fe (30 kg ha^{-1}) and Mn (20 kg ha^{-1}) resulted in even more improvements, including flower stalk count (78.48%), floret length (47.44%), vase life (32.02%), percentage of opened florets (41.31%), fresh (41.73%) and dry (54.40%) weights of the aerial parts, and bulb fresh (48.98%) and dry (61.54%) weights. The findings highlighted that the combined application of $FeSO_4$ (30 kg ha^{-1}) and $MnSO_4$ (20 kg ha^{-1}) had significantly positive effects on tuberose growth and relevant parameters. Tuberose development improved significantly in response to this combination treatment over the two years, which provides a valuable guideline for farmers and researchers when aiming to enhance tuberose yield.

Introduction

Tuberose (*Polianthes tuberosa* L.) belongs to the Agavaceae family and is a significant cut flower in tropical and subtropical regions, including Iran (Afifipour and Khosh-Khui, 2015; Bahadoran et al., 2016). Micronutrients are indispensable components of plant nutrition that stimulate and catalyze metabolic processes (Sharma et al.,

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2014; Patel et al., 2017), resulting in improved plant growth, flowering, and yield. These nutrients are vital for the growth and development of plants, ultimately leading to higher flower yield and quality.

Iron is necessary for many plant processes, including photosynthesis, nitrate and sulfate reduction, nitrogen assimilation, and the redox system. Moreover, plants require Fe for the synthesis of heme-containing proteins, such as cytochromes, catalase, and peroxidases, which are involved in electron transport and other physiological processes. Iron also plays a crucial role in the synthesis of chlorophyll and nucleic acid metabolism within the chloroplast (Malvi, 2011). On the other hand, Mn is involved in the redox reaction with the photosynthetic electron transport system. Mn deficiency can significantly impact the chloroplast, leading to interveinal chlorosis and growth retardation. Mn also helps prevent lodging and contributes to disease tolerance (Weisany et al., 2013). In developing countries, micronutrient deficiencies, specifically Fe and Mn, are prevalent issues that require treatments. The absorption of micronutrients is constrained by high pH levels in calcareous and alkaline soils (Ghasemi-Fasaei and Ronaghi, 2008; Sharma et al., 2014). Flowers and ornamental plants, including tuberose, are particularly prone to Fe deficiency, which can adversely affect vegetative and reproductive growth.

Previous studies have shown that nutrient elements improve qualitative and quantitative characteristics in cut flowers, such as tuberose. Shoor et al. (2010) studied the effects of various concentrations of $ZnSO_4$, $CuSO_4$, $MnSO_4$, and $FeSO_4$ fertilizers (0, 2, 4, and 6 mg L^{-1}) on *P. tuberosa* 'Double'. It was reported that 6 mg L^{-1} of $FeSO_4$ fertilizer significantly increased the inflorescence length and floret count. In addition, 6 mg L^{-1} of $CuSO_4$ increased floret diameter and the percentage of opened florets. They also found that applying $MnSO_4$ at 6 mg L^{-1} increased multiple measurable traits in tuberose, such as vase life, inflorescence, floret diameters, inflorescence and spike lengths, and floret count. Kumar and Chattopadhyay (2001) studied the effects of $ZnSO_4$, $CuSO_4$, and $FeSO_4$ fertilizers on the growth and yield of tuberose (*P. tuberosa* cv. Single) at four levels (0, 5, 10, and 15 kg ha^{-1}). It was reported that all treatments significantly increased the yield characteristic of tuberose compared to the control.

According to previous research, the most essential trace elements for tuberose are Fe, Zn, and Mn. As a matter of fact, Fe is the most important after sulfur, followed by Zn and Mn (De and Dhiman, 2001; Yadav et al., 2002). Yadav et al. (2002) evaluated the amounts of macro and micronutrient elements absorbed by tuberose. The findings revealed that tuberose plants absorbed 148 kg of nitrogen, 33 kg of phosphorus, 128 kg of potassium, 227 kg of calcium, 17 kg of magnesium, 407 g of Zn, 4587 g of Fe, 128 g of Cu, and 300 g of Mn per hectare. Khalaj et al. (2012) reported that nitrogen levels affected nitrogen uptake, spike length, and flower stalk diameter. Applying 200 kg ha^{-1} of nitrogen improved the growth and yield of tuberose, such as stalk height, flower stalk diameter, and bulb weight. Nitrogen, phosphorus, and potassium enrichments significantly improved spike production and floret quality.

In a study by Jain (2014), a treatment containing 0.8% Zn, 0.8% Fe, 0.2% B, and 0.4% Cu resulted in several improvements in plant characteristics in tuberose. The treatment maximized plant height (64.12 cm), leaf count per plant (25.37), leaf area (1414.23 cm^2), number of spikes per plant (3.07), spike length (82.73 cm), floret diameter (3.38 cm), number of florets per spike (34.33), floret length (7.11 cm), and flower duration (23.11 d). Also, the mentioned treatment significantly reduced the minimum days to first spike emergence (73.97 d). Tayade et al. (2018) demonstrated that applying 0.4% FeSO4 with 0.4% $ZnSO_4$ to tuberose foliage significantly increased the flower quality of tuberose, in multiple ways particularly spike length, spike diameter, rachis length, floret length, floret diameter, and vase life.

Iran has significant potential for exporting valuable cut tuberose flowers that can benefit the perfume industry. Therefore, priority is given to research methods that improve tuberose production and quality while recommending proper amounts of fertilizers to achieve optimal yields. The current study aimed to find proper Fe and Mn application levels to maximize tuberose quality and yield, thereby improving its important marketability traits. Also, the absorption of micronutrients, including Fe, Mn, Zn, and Cu, was evaluated in aerial parts and bulbs of tuberose over a two-year study period.

Materials and Methods

Plant materials and experimental conditions

This study was conducted in a factorial arrangement and as a randomized complete block design in a research farm at the Flower and Ornamental Plant Research Center, Ahvaz University, Iran. The experimental plot consisted of 12 treatments and 3 replications in plots. Each plot had an area of 1.5 m^2. Treatments included four levels of Fe (0, 15, 45, and 30 kg Fe ha^{-1}) sourced from $FeSO_4$ fertilizer (24% Fe) and three levels of Mn (0, 10, and 20 kg ha^{-1}) from $MnSO_4$ fertilizer (30% Mn). Prior to the experiment, we analyzed the water and soil properties (Table 1).

Table 1. Soil analysis of the experiment place in two years, and chemical analysis of irrigation water of the National Flower and Plant Research Station of Mahallat.

Soil analysis										
2018										
Soil texture		Sand		Silt		Clay		pH	EC (dS m^{-1})	
Silty- Loam		55		32.5		12.5		7.92	1.28	
T.N.V (%)	O.C (%)	N (%)	P	K	Fe	Zn	Mn	Cu	B	
34	0.5	0.05	10.32	200	1.8	0.74	1.2	0.68	0.74	
2019										
Soil texture		Sand		Silt		Clay		pH	EC (dS m^{-1})	
Silty- Loam		56		32		12		7.95	1.35	
T.N.V (%)	O.C (%)	N (%)	P	K	Fe	Zn	Mn	Cu	B	
	0.51	0.05	13.4	193	1.36	0.6	1.84	0.16	0.82	
Water analysis										
SAR	Sum Cation	Na^+	$Ca^{2+}+Mg^{2+}$	Sum anions (meq L^{-1})	$SO4^{2-}$	Cl^-	HCO_3^-	$CO3^{2-}$	pH	EC (dS m^{-1})
0.79	8.86	1.5	7.18	8.75	1.12	1.5	6.13	0	7.38	0.88

To prepare the plots for the experiment, the bulbs of double-flowered tuberose were planted at a spacing of 15 × 15 cm in the plots. In the first stage, the fertilizer was spread on the soil and then compost was added in the mixture. In the other two stages, ammonium nitrate fertilizer was spread on the soil and then the plots were irrigated. All chemical fertilizers except NH_4NO_3 were applied in two stages, i.e., at planting time and one month after the planting. Supplementary fertilizers, including KSO_4 (360 kg), NH_4NO_3 (500 kg), triple super phosphate (200 kg), $MgSO_4$ (100 kg), $CuSO_4$ (40 kg), and H3BO3 (20 kg), were also provided. To increase the efficiency of nitrogen consumption, ammonium nitrate fertilizer was given to the plots in 3 stages (before cultivation, 30 days, and 60 days after cultivation). According to weather statistics, the average rainfall at the site was 0.40 mm in 2018 and 0.73 mm in 2019 from June to mid-September. The mean maximum temperature during the experiment was 35.8 °C and 35.3 °C in 2018 and 2019, respectively, while the mean minimum temperature was 22.27 °C and 21.43 °C. The mean relative humidity during the experiment was 18.7% and 22.7% in 2018 and 2019, respectively. Planting operations were carried out in June 2018 and 2019 at the research farm of the Flower and Ornamental Plant Research Center. Operations including irrigation, weeding, and countermeasures against pests and diseases were carried out uniformly in all plots. The plants were harvested in the mid-September of the same year. Walnut-sized flowering bulbs were purchased from a specific commercial producer and planted at a depth of 10 cm beneath the surface.

Growth, flower traits, and vase life

At the time of harvest, plant height was measured with a ruler from the soil surface to the highest point of the flower cluster. The spike length was measured from the lowest to the highest end of the flower cluster with a ruler, floret length and flower stalk diameter were measured 20 cm from the soil surface with a caliper. Floret diameter, i.e., the diameter of the third floret from the bottom of the cluster, was measured with a caliper. To calculate the vase life, the flowers were moved to glass containers (vases) that contained 300 mL of distilled water. The water in the vases was replaced every 3 days, and almost 1 cm of each stem was cut at the same time. The vases were placed at 15 to 20 °C to resemble room temperature. The vase life of the cut inflorescences was considered when the number of senesced florets exceeded the number of open ones. Also, the loss or wilting of 50% of florets marked the end of vase life (Ezz et al., 2018). To determine the percentage of opened florets, the opened florets in each cluster per glass container were counted and calculated for each treatment group.

Aerial part and bulb micronutrient analysis

The aerial parts and bulb fresh weights were recorded after harvesting the plants. The samples were subsequently dried in an oven at 70 °C for 48 h to obtain their dry weights. To determine the Mn, Fe, Zn, and Cu contents of the aerial parts and bulbs, 0.5 g of the dry bulbs and the aerial parts were ground and dry-ashed at 500 °C for 5 h. The ashed samples were then dissolved in 5 mL of 2 N HCl and digested by heating at 80 °C for 30 min in a water bath. Then, the prepared extracts were used for reading Mn, Fe, Zn, and Cu concentrations via an atomic absorption device (Unicam Solar atomic absorption device and Genesys Spectronic model 20 spectrophotometer) (Barton, 1948).

Statistical analysis

Data were analyzed in SAS9.2 software, and the means were compared using Duncan's Multiple Range test ($P \leq 0.05$). Furthermore, the R software (version 4.3.2) was employed to create a clustering heatmap in multivariate analysis and correlation graphs. The correlation plot was drawn using a paired linear correlation method based on Pearson's method in the R 4.3.2 software while employing the ggcorrplot package. The clustering heatmap was also generated using the Gplot package.

Results

Effects of year on quality and quantity traits

According to ANOVA and its combined analysis, all measured traits showed significant differences ($P \leq 0.05$) in both years, except for spike length, floret diameter, bulb fresh weight, and Mn content of aerial parts (Table 2). Data collected in the two years were significantly different regarding most traits, and therefore the results for each year were reported separately. Plants in the first year showed maximum values of plant height (60.78 cm), spike length (25.21 cm), floret diameter (46.06 mm), floret number (26.88), aerial parts fresh and dry weights (116.35 and 13.84 g plant^{-1}), bulb fresh weight (37.93 g), vase life (12.09 d), Mn of aerial parts (40.48 mg kg^{-1} DW), bulb Fe (106.13 mg kg^{-1} DW), bulb Mn (20.12 mg kg^{-1} DW), Cu of the aerial parts (6.94 mg kg^{-1} DW), and bulb Cu (8.39 mg kg^{-1} DW). Plants in the second year showed maximum values of flower stalk diameter (7.01 cm), floret length (9.41 cm), bulb number (19.16 plant^{-1}), bulb dry weight (14.24 g), percentage of opened florets (69.02%), Fe content of aerial parts (336.82 mg kg^{-1} DW), Zn content of aerial parts (26.15 mg kg^{-1} DW), and bulb Zn (15.93 82 mg kg^{-1} DW) (SI1).

Effect of Fe on quality and quantity traits of tuberose

The analysis of variance in the first year showed that the main effect of Fe was significant ($P \leq 0.05$) on all traits, except for floret count and diameter and the Mn content of the aerial parts (data not shown). As shown in Table 3, the application of FeSO4 fertilizer (45 kg ha^{-1}) significantly increased most of the traits, i.e., plant height, spike length, flower stalk diameter, floret diameter and length, floret count, fresh and dry weights of the aerial parts, bulb count, bulb fresh and dry weights, vase life, the percentage of opened florets, and the Fe content in the aerial parts and bulbs, compared to the control plants in the first year. However, the Zn, Cu, and Mn contents in the aerial parts and bulbs decreased compared to the control plants. Applying 30 kg ha^{-1} of $FeSO_4$ fertilizer resulted in notable increases of 11.89%, 19.90%, 23.01%, and 39.11% in spike length, floret length, bulb count, and bulb dry weight, respectively, compared to the control plants (Table 3).

The analysis of variance for the second year showed that the main effect of Fe was significant ($P \leq 0.05$) on all traits, except for the Zn content of the aerial parts (data not shown). In the second year, the number and diameter of florets showed a significant increase by adding $FeSO_4$ fertilizer to the soil compared to the control treatment (Table 4). However, there was no significant difference among the Fe treatment levels on these traits. Flower stalk diameter increased significantly with increasing the levels of applied Fe in two years compared to the control treatment. An increase in flower stalk diameter was obtained by adding 30 kg ha^{-1} $FeSO_4$ fertilizer by about 11.27% and 39.21% compared to the control treatment in both years, respectively (Tables 3 and 4).

Table 2. Combined analysis of variance for different levels of Fe and Mn on some quantitative and qualitative traits of tuberose.

Source of Variation	df	Plant height	Spike length	Flower Stalk diameter	Floret diameter	Floret length	Floret number	Fresh weight of aerial part	Dry weight of aerial part	Bulb number	Bulb fresh weight	Bulb dry weight
Year	1	84.28**	7.67^{ns}	7.31**	7.61^{ns}	1.60**	77.69**	5152.50**	3.35**	93.39**	17.31^{ns}	43.40**
Rep (Year)	4	16.48	12.59	0.69	4.68	0.30	2.97	4.25	2.57	1.72	5.39	4.94
Fe	3	78.08**	46.42**	1.78**	8.79*	1.37**	42.32*	1138.69**	6.13**	59.48**	85.85**	44.12**
Mn	2	87.59**	35.47**	1.48**	5.94^{ns}	4.83**	31.56^{ns}	467.10**	2.02**	47.86**	70.76**	30.12**
Year×Fe	3	2.00^{ns}	1.34^{ns}	2.66**	4.26^{ns}	0.01^{ns}	1.78^{ns}	338.37**	1.87^{ns}	2.86^{ns}	22.60^{ns}	7.35*
Year×Mn	2	1.35^{ns}	0.96^{ns}	0.34^{ns}	2.48^{ns}	0.62^{ns}	4.99^{ns}	18.10^{ns}	1.16^{ns}	5.62^{ns}	1.22^{ns}	0.53^{ns}
Fe×Mn	6	11.47^{ns}	11.14^{ns}	0.41^{ns}	5.84^{ns}	0.22^{ns}	5.78^{ns}	242.00**	1.62^{ns}	4.57^{ns}	95.64**	9.58**
Year×Fe×Mn	6	5.97^{ns}	6.90^{ns}	0.24^{ns}	4.05^{ns}	0.10^{ns}	11.49^{ns}	133.12**	1.11^{ns}	7.28*	79.20**	12.74**
Error	44	11.05	6.55^{ns}	0.30	6.01	0.37	13.14	32.36	1.18	2.73	9.43	2.36
CV (%)		5.57	10.29	8.42	5.36	7.26	14.55	5.59	8.12	9.43	8.20	11.41

**, *, and ^{ns}: significant at α= 0.01, and α= 0.05, and non-significant, respectively.

In continued

Source of Variation	df	Vase life	Opened floret	Mn of aerial part	Fe of aerial part	Zn of aerial part	Cu of aerial part	Mn bulb	Fe bulb	Zn bulb	Cu bulb
Year	1	0.22**	829.32**	21.45^{ns}	11181.61**	74.87**	7.56**	515.25**	992.35**	21.67^{ns}	33.65**
Rep (Year)	4	0.56	31.36	9.26	246.77	4.484	0.45	4.27	90.50	13.49	0.48
Fe	3	7.373**	216.22**	42.72**	15694.33**	77.43**	8.13**	135.90**	5388.57**	1.60**	51.90**
Mn	2	1.57**	311.01**	32.83**	3491.90**	40.57**	9.38**	1.72^{ns}	2321.69**	39.94^{ns}	7.07**
Year×Fe	3	3.00^{ns}	4.15^{ns}	1.76^{ns}	520.93^{ns}	0.98^{ns}	0.18^{ns}	65.20**	440.82**	64.21*	30.73**
Year×Mn	2	0.92**	31.55^{ns}	2.16^{ns}	576.36^{ns}	3.74^{ns}	2.55*	29.07**	736.85**	91.23**	0.49^{ns}
Fe×Mn	6	5.93**	224.70**	6.73^{ns}	4535.38**	12.52*	0.99^{ns}	49.002**	621.56**	67.28**	7.49**
Year×Fe×Mn	6	4.18*	77.83**	8.25^{ns}	3829.11**	20.95**	1.26^{ns}	77.16**	1157.39**	18.66^{ns}	6.46**
Error	44	1.46	20.05	8.304	526.78	4.83	0.69	5.33	53.43	15.29	0.61
CV (%)		10.95	7.00	7.22	8.47	9.61	14.41	14.87	7.14	25.4	13.18

**, *, and ^{ns}: significant at α= 0.01, and α= 0.05, and non-significant, respectively.

Table 3. Mean comparison of different level of Fe on some quantitative and qualitative traits of tuberose in year 1.

Treatment	Plant height (cm)	Spike length (cm)	Flower stalk diameter (cm)	Floret length (cm)	Fresh weight of aerial part (g)	Dry weight of aerial part (g)	Bulb number	Bulb fresh weight (g)	Bulb dry weight (g)
Fe0	57.74^{b}	22.90^{b}	5.52^{b}	6.48^{c}	111.63^{c}	12.24^{c}	13.81^{b}	33.68^{b}	9.94^{c}
Fe15	59.98^{ab}	24.90^{ab}	5.87^{ab}	7.09^{bc}	115.20^{bc}	13.46^{b}	14.34^{b}	35.10^{b}	12.02^{b}
Fe30	62.63^{a}	25.62^{ab}	6.14^{ab}	7.77^{ab}	117.90^{ab}	14.46^{ba}	16.99^{a}	40.01^{a}	13.83^{a}
Fe45	62.76^{a}	27.40^{a}	6.58^{a}	8.34^{a}	120.66^{a}	15.19^{a}	18.37^{a}	42.92^{a}	14.97^{a}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

In continued

Treatment	Vase life (d)	Opened floret (%)	Fe of aerial part (g kg^{-1} DW)	Zn of aerial part (g kg^{-1} DW)	Cu of aerial part (g kg^{-1} DW)	Mn bulb (g kg^{-1} DW)	Fe bulb (g kg^{-1} DW)	Zn bulb (g kg^{-1} DW)	Cu bulb (g kg^{-1} DW)
Fe0	10.08^{c}	55.36^{b}	157.39^{c}	22.09^{a}	7.22^{a}	26.26^{a}	90.611^{c}	20.22^{a}	10.86^{a}
Fe15	11.61^{b}	57.57^{b}	205.34^{b}	20.67a	6.61^{a}	21.11^{b}	103.43^{b}	14.89^{b}	10.83^{a}
Fe30	12.83^{ab}	61.16^{a}	220.61^{ab}	18.39^{b}	6.56^{a}	17.06^{c}	109.39^{b}	12.44^{c}	7.11^{b}
Fe45	13.86^{a}	61.69^{a}	238.00^{a}	17.22^{b}	5.56^{b}	16.06^{c}	121.10^{a}	11.78^{c}	4.78^{c}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

Table 4. Mean comparison of different level of Fe on some quantitative and qualitative traits of tuberose in year 2.

Treatment	Plant height (cm)	Spike length (cm)	Flower Stalk diameter (cm)	Floret diameter (mm)	Floret length (cm)	Floret number	Fresh weight of aerial part (g)	Dry weight of aerial part (g)	Bulb number	Bulb fresh weight (g)
Fe0	56.31^{b}	22.79^{b}	5.61^{c}	43.40^{b}	8.53^{d}	20.97^{b}	74.22^{b}	11.16^{b}	17.64^{c}	34.30^{c}
Fe15	58.16^{ab}	24.33^{ab}	6.46^{b}	45.22^{a}	9.08^{c}	22.61^{ab}	79.66^{b}	13.44^{a}	18.42^{bc}	36.22^{bc}
Fe30	59.70^{a}	25.10^{a}	7.81^{a}	46.18^{a}	9.71^{b}	23.44^{a}	97.06^{a}	13.66^{a}	19.44^{b}	37.96^{ab}
Fe45	60.29^{a}	25.99^{a}	8.16^{a}	46.82^{a}	10.33^{a}	24.78^{a}	98.40^{a}	13.64^{a}	21.11^{a}	39.31^{a}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

In continued

Treatment	Bulb dry weight (g)	Vase life (d)	Opened floret (%)	Mn of aerial part (mg kg^{-1} DW)	Fe of aerial part (mg kg^{-1} DW)	Zn of aerial part (mg kg^{-1} DW)	Cu of aerial part (mg kg^{-1} DW)	Mn bulb (mg kg^{-1} DW)	Fe bulb (mg kg^{-1} DW)	Cu bulb (mg kg^{-1} DW)
Fe0	13.22^{b}	8.86^{c}	64.31^{b}	41.22^{a}	304.44^{c}	28.72^{a}	5.78^{a}	12.06^{a}	74.50^{d}	4.22^{a}
Fe15	13.74^{b}	9.91^{b}	67.33^{ab}	39.72^{a}	332.28^{b}	26.56^{b}	5.33^{ab}	11.06^{b}	88.06^{c}	3.39^{b}
Fe30	14.67^{ab}	10.19^{b}	71.56^{a}	39.44^{a}	350.00^{a}	25.17^{bc}	4.83^{b}	10.83^{b}	107.39^{b}	3.33^{b}
Fe45	15.34^{a}	10.91^{a}	72.89^{a}	37.17^{b}	360.56^{a}	24.17^{c}	4.22^{c}	9.83^{c}	124.89^{a}	3.00^{b}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

According to Tables 3 and 4, fresh and dry weights of aerial parts increased significantly with Fe levels in both years. However, with the addition of 30 kg ha^{-1} $FeSO_4$ fertilizer to the soil, these traits significantly increased by about 5.61% and 18.10% in the first year and by about 30.76% and 22.41% in the second year, respectively.

Vase life and opened florets percentage significantly increased by rising Fe levels in the soil in both years. As shown in Tables 3 and 4, plants treated with 45 kg ha^{-1} were able to significantly increase the longevity of tuberose cut flowers compared to the control plants of about 37.49% and 23.21% in the first and second years, respectively. There was no significant difference between 30 and 45 kg ha^{-1} of $FeSO_4$ fertilizer in all traits, except the Cu content of the aerial parts and bulbs and the Fe content of the bulb in the first year (Table 3). A notable increase of about 40.17% and 20.72% was found in the Fe content of the aerial part and bulb by adding 30 kg ha^{-1} $FeSO_4$ fertilizer compared to the non-treated plants (Table 3). By increasing the Fe level in two years, the amount of Zn and Cu of the aerial parts significantly declined. However, no significant decrease occurred in the Mn content of the aerial parts by adding Fe compared to the control plants in the first year (Table 3). In addition, a considerable reduction was found in Cu, Zn, and Mn of bulbs by about 55.99%, 41.76%, and 38.85%, respectively, by adding 45 kg ha^{-1} $FeSO_4$ fertilizer compared to the control plants in the first year. However, the decline of Cu (23.08%) and Zn (22.03%) contents in the aerial parts were lower than in the bulbs (Table 3).

Results in the second year showed a significant decrease in Cu, Zn, and Mn contents in the aerial parts of about 15.86%, 26.92%, and 9.84%, respectively, compared to the control plants. However, the Cu and Mn contents in bulbs decreased by about 28.95% and 18.43%, respectively (Table 4).

Effect of Mn on quality and quantity traits

The analysis of variance showed that the main effects of Mn were significant ($P \leq 0.05$) on plant height, spike length, flower stalk diameter, floret length, fresh and dry weights of the aerial parts, bulb count, bulb dry weight, the percentage of opened floret, vase life, Mn content of the aerial parts, and Fe, Zn, and Cu contents in the bulbs (data not shown).

As shown in Tables 5 and 6, with increasing the amount of Mn fertilizer, significant increases were observed in plant height, spike length, flower stalk diameter, floret diameter and length, floret count, fresh and dry weights of the aerial parts, bulb count, bulb fresh and dry weights, the percentage of opened florets, vase life, Mn concentration in the aerial parts and bulbs, compared to the control treatment in both years. However, the amount of Fe, Zn, and Cu in the aerial parts and bulbs declined. In the first year, there was no significant difference between 10 and 20 kg ha^{-1} of $MnSO_4$ fertilizer regarding most measured traits. However, a significant difference was found between the effects of these two concentration levels on flower stalk diameter, fresh weight of the aerial parts, bulb count, vase life, Cu content of the aerial parts, and bulb Fe and Zn contents (Table 5).

A significant increase of about 7.22%, 21.04%, and 18.64% was found in plant height, flower stalk diameter, and floret length, respectively, by adding 20 kg ha^{-1} of $MnSO_4$ fertilizer compared to the control treatment.

Applying 20 kg ha^{-1} $MnSO_4$ fertilizer increased the fresh and dry weights of the aerial parts significantly by about 8.38% and 18.13%, respectively. Also, it increased the bulb dry weight by about 20.67% compared to the control plants in the first year (Table 5). The bulb count increased markedly by about 26.37%, with increasing the Mn application to 20 kg ha^{-1} compared to the control treatment in the first year. Maximum bulb count per plant (17.69) was obtained by 20 kg ha^{-1} $MnSO_4$ fertilizer in the second year.

A notable rise (26.06% and 15.54%) was found in the aerial parts and bulb dry weight, respectively, when the plants were treated with 20 kg ha^{-1} $MnSO_4$ fertilizer compared to the control group in the second year (Table 6).

Vase life and the percentage of opened florets significantly increased by higher Mn levels in the soil in both years. However, the vase life of cut flowers in the first year (10 d) was higher than in the second year (9.34 d) in non-Fe-treated plants. According to results in Tables 5 and 6, plants treated with 20 kg ha^{-1} $MnSO_4$ fertilizer had a notably higher percentage of vase life than control plants by about 39.58% and 13.83% in the first and second years, respectively.

Plants exposed to 20 kg ha^{-1} $MnSO_4$ fertilizer had a higher percentage of opened florets by about 8.76% and 14.70% in the first and second years, respectively, compared to the control plants (Tables 5 and 6).

Table 5. Mean comparison of Mn effect on quality and quantity traits of tuberosa in year 1.

Treatment	Plant height (cm)	Flower stalk diameter (cm)	Floret length (cm)	Fresh weight of aerial part (g)	Dry weight of aerial part (g)	Bulb number	Bulb dry weight (g)	Vase life (d)
Mn0	58.53^{b}	5.47^{b}	6.75^{b}	111.92^{c}	12.78^{a}	14.00^{c}	11.45^{b}	10.00^{c}
Mn10	61.06^{ab}	6.00^{b}	7.50^{a}	115.83^{b}	13.64^{b}	15.94^{b}	12.81^{ab}	12.33^{b}
Mn20	62.75^{a}	6.62^{a}	8.01^{a}	121.29^{a}	15.09^{b}	17.69^{a}	13.82^{a}	13.96^{a}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

In continued

Treatment	Opened floret (%)	Mn of aerial part (mg kg^{-1}DW)	Fe of aerial part (mg kg^{-1} DW)	Cu of aerial part (mg kg^{-1} DW)	Fe bulb (mg kg^{-1} DW)	Zn bulb (mg kg^{-1} DW)	Cu bulb (mg kg^{-1} DW)
Mn0	56.40^{b}	38.33^{b}	220.63^{a}	7.08^{a}	122.78^{a}	17.63^{a}	8.96^{a}
Mn10	59.08^{ab}	40.67^{ab}	193.63^{b}	6.92^{a}	93.33^{c}	15.46^{b}	8.46^{ab}
Mn20	61.34^{a}	42.44^{a}	201.75^{ab}	5.46^{b}	102.29^{b}	11.42^{c}	7.77^{b}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

Table 6. Mean comparison of Mn effect on some quality and quantity traits of tuberosa in year 2.

Treatment	Plant height (cm)	Spike length (cm)	Flower stalk diameter (cm)	Floret diameter (cm)	Floret length (cm)	Fresh weight of aerial part (g)	Dry weight of aerial part (g)	Bulb number	Bulb fresh weight (g)	Bulb dry weight (g)
Mn0	56.91^{b}	23.28^{b}	6.18^{c}	44.13^{b}	8.48^{c}	82.54^{b}	11.42^{c}	17.98^{b}	34.75^{b}	13.03^{b}
Mn10	58.63^{ab}	25.04^{a}	7.04^{b}	45.80^{a}	9.41^{b}	88.71^{a}	13.11^{b}	19.62^{a}	37.70^{a}	14.64^{a}
Mn20	60.31^{a}	25.33^{a}	7.80^{a}	46.29^{a}	10.36^{a}	90.75^{a}	14.39^{a}	19.87^{a}	38.39^{a}	15.06^{a}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

In continued

Treatment	Vase life (d)	Opened floret (%)	Mn of aerial part (mg kg^{-1} DW)	Fe of aerial part (mg kg^{-1} DW)	Zn of aerial part (mg kg^{-1} DW)	Cu of aerial part (mg kg^{-1} DW)	Mn bulb (mg kg^{-1} DW)	Fe bulb (mg kg^{-1} DW)	Cu bulb (mg kg^{-1} DW)
Mn0	9.34^{c}	63.97^{b}	36.71^{c}	349.33^{a}	27.96^{a}	5.54^{a}	9.83^{a}	104.50^{a}	4.08^{a}
Mn10	9.98^{b}	69.73^{a}	39.46^{b}	336.00^{ab}	25.88^{b}	4.88^{b}	10.75^{b}	97.21^{b}	3.25^{b}
Mn20	10.63^{a}	73.37^{a}	42.00^{a}	325.13^{b}	24.63^{b}	4.71^{b}	12.25^{c}	94.42^{b}	3.13^{b}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

Applying 20 kg ha^{-1} $MnSO_4$ fertilizer significantly increased the Mn content of the aerial parts by about 10.72% and 14.42% in the first and second years, respectively, compared to the control treatment (Tables 5 and 6). However, a higher Mn accumulation of about 24.58% was found in bulbs of plants treated with a high level of Mn in the second year. Also, Cu, Zn, and Fe levels in the aerial parts declined in response to rising Mn levels in the soil (20 kg ha^{-1}) compared to the control treatment in both years. In the first year, adding 20 kg ha^{-1} $MnSO_4$ fertilizer caused a remarkable decrease in the Cu, Fe, and Zn contents of the bulbs by 13.31%, 16.68%, and 35.22% compared to the control treatment, respectively.

According to Table 6, in the second year, there were no significant differences between the effects of applying 10 and 20 kg ha^{-1} MnSO4 fertilizer regarding most of the measured traits. However, notable differences were found in flower stalk diameter, floret length, the dry weight of aerial parts, vase life, and Mn contents of the aerial parts and bulbs.

Interaction effects of Mn and Fe on quality and quantity traits

According to the analysis of variance in the first year, the interaction effect of Fe and Mn on most traits was not significant ($P \leq 0.05$), except for the fresh weight of the aerial parts, bulb fresh and dry weights, vase life, the percentage of opened florets, the Fe, Mn, Fe, and Zn contents of the aerial parts, and the Cu content of the bulbs (data not shown).

The analysis of variance in the second year showed that the interaction effect of Fe and Mn on most traits was not significant ($P \leq 0.05$), except for spike length, fresh weight of the aerial parts, bulb count, bulb fresh and dry weights, the percentage of opened florets, Fe and Zn contents of aerial parts, and Fe and Cu contents of the bulbs (data not shown).

As shown in Tables 7 and 8, the highest fresh weight of the aerial parts (125.86 g per plant) was obtained in response to 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn in the first year and 30 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (111.26 g per plant) in the second year. Compared to the control plants, a significant increase (14.67%) was found in the fresh weight of plants exposed to 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn. However, no significant difference occurred between the treatments with increasing concentrations of Mn and Fe in the soil.

Bulb fresh and dry weights increased significantly ($P \leq 0.05$) with the addition of Fe and Mn fertilizers in both years. The highest bulb fresh weights (46.07 g and 44.57 g) were obtained in response to 45 kg ha^{-1} Fe with 10 kg ha^{-1} Mn in both years (Tables 7 and 8). Adding 45 kg ha^{-1} Fe and 10 kg ha^{-1} Mn caused a notable increase (64.92% and 51.24%) in bulb fresh weight compared to the control in the first and second years, respectively.

The maximum bulb dry weight was observed by applying 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (17.03 g per plant) treatment in the first year and 30 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (16.8 g per plant) in the second year (Tables 7 and 8). Compared to the control plants, adding 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn remarkably enhanced bulb dry weight by about 148.04% in the first year, and also plants exposed to 30 kg ha^{-1} $FeSO_4$ with 20 kg ha^{-1} Mn showed a notable increase (61.54%) in bulb dry weight in the second year.

As shown in Table 8, maximum spike length (29.50 cm) was observed in response to 45 kg ha^{-1} Fe and 10 kg ha^{-1} Mn in the second year. A significant increase (29.57%) occurred in spike length by applying 45 kg ha^{-1} Fe and 10 kg ha^{-1} Mn.

The highest bulb count per plant (21.27) was obtained by applying 45 kg ha^{-1} Fe alone in the second year. The bulb count significantly increased (38.00%) in plots treated with 45 kg ha^{-1} Fe compared to the control plants (Table 8).

As shown in Tables 7 and 8, the longest vase life was obtained by using Fe 30 kg ha^{-1} with 20 kg ha^{-1} Mn (16.67 d) in the first year, and 45 kg ha^{-1} Fe with 20 kg ha-1 Mn (11.43 d) in the second year.

The percentage of opened florets reached a maximum value when applying 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (65.92%) in the first year. Also, it reached a maximum value (78.67%) by applying 45 kg ha^{-1} Fe with 10 kg ha^{-1} Mn in the second year (Tables 7 and 8).

The highest Fe level in the aerial parts was obtained by applying 15 kg ha^{-1} Fe alone (273.70 mg kg^{-1} DW) in the first year, and 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (380.33 mg kg^{-1} DW) in the second year (Tables 7 and 8).

The lowest and the highest Zn content in the aerial parts were found in the control treatment (without Mn and Fe treatment) (30.50 mg kg^{-1} DW) and in the 10 kg ha^{-1} Mn treatment alone in the second year (Table 7).

The maximum Mn of the bulb (31.93 mg kg^{-1} DW) was obtained in the treatment of 20 kg of Mn alone in the first year. However, the minimum value (8.67 mg kg^{-1} DW) was obtained by applying 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (Table 7).

Table 7. Interaction effects of Fe and Mn on some quantitative and qualitative traits of tuberose in year 1.

Treatments	Fresh weight of aerial part (g)	Bulb fresh weight (g)	Bulb dry weight (g)	Vase life (d)	Opened floret (%)	Fe of aerial part (mg kg $^{-1}$ DW)	Mn bulb (mg kg $^{-1}$ DW)	Fe bulb (mg kg^{-1} DW)	Zn bulb (mg kg^{-1} DW)	Cu bulb (mg kg^{-1} DW)
Fe0Mn0	109.76^{bcd}	27.93^{c}	6.87^{d}	8.50^{d}	48.26^{d}	174.00^{bcd}	29.33^{ab}	100.63^{cd}	19.50^{b}	9.50^{a}
Fe0Mn10	105.10^{cd}	33.13^{bc}	10.20^{cd}	9.90^{d}	62.61^{ab}	141.66^{d}	17.50^{a-e}	72.06^{e}	27.83^{a}	12.00^{a}
Fe0Mn20	120.03^{ab}	39.97^{ab}	12.77^{a-c}	11.83b^{cd}	55.24^{bcd}	156.50^{cd}	31.93^{a}	99.13^{cd}	13.33^{cde}	11.07^{a}
Fe15Mn0	104.13^{d}	34.37^{bc}	11.83^{b-d}	10.17^{d}	59.79^{abc}	273.70^{a}	21.50^{bcd}	120.30^{b}	18.17^{bc}	10.67^{a}
Fe15Mn10	123.26^{a}	37.47^{a-c}	12.43^{a-c}	12.33^{a-d}	49.82^{cd}	166.00^{cd}	18.50^{cd}	92.83^{d}	12.50^{cde}	10.50^{a}
Fe15Mn20	118.20^{ab}	33.47^{bc}	11.80^{b-d}	12.33^{a-d}	63.06^{ab}	176.33^{bcd}	23.33^{abc}	97.16^{cd}	14.00^{b-e}	11.33^{a}
Fe30Mn0	117.43^{ab}	45.87^{a}	15.20^{a-c}	11.00^{cd}	62.04^{ab}	204.33^{a-d}	17.83^{cde}	114.70^{bc}	17.00^{bc}	11.17^{a}
Fe30Mn10	115.20^{a-d}	36.87^{a-c}	12.63^{a-c}	10.83^{cd}	60.27^{ab}	239.16^{abc}	20.00^{cd}	103.30^{bcd}	10.50^{de}	5.67^{b}
Fe30Mn20	121.06^{ab}	37.30^{a-c}	13.67^{a-c}	16.67^{a}	61.12^{ab}	218.33^{a-d}	13.33^{de}	110.16^{bcd}	9.83^{e}	4.50^{b}
Fe45Mn0	116.33^{abc}	36.83^{a-c}	11.90^{b-d}	10.33^{d}	55.55^{bcd}	230.50^{abc}	16.50^{cde}	155.46^{a}	15.83^{bcd}	4.50^{b}
Fe45Mn10	119.76^{ab}	46.07^{a}	15.97^{ab}	16.23^{ab}	63.59^{a}	227.66^{abc}	23.00^{abc}	105.13^{bcd}	11.00^{de}	5.67^{b}
Fe45Mn20	125.86^{a}	45.87^{a}	17.03^{a}	15.00^{abc}	65.92^{a}	255.83^{ab}	8.67^{e}	102.70^{bcd}	8.50^{e}	4.17^{b}

In each column, means with similar letter(s) are not significantly different using the Duncan test at the 5% level.

Table 8. Interaction effects of Fe and Mn on some quantitative and qualitative traits of tuberose in year 2.

Treatments	Spike length (cm)	Fresh weight of aerial part (g)	Bulb number	Bulb fresh weight (g)	Bulb dry weight (g)	Opened floret (%)	Fe of aerial part (mg kg^{-1} DW)	Zn of aerial part (mg kg^{-1} DW)	Fe bulb (mg kg^{-1} DW)	Cu bulb (mg kg^{-1} DW)
Fe0Mn0	22.77^{b}	78.50^{cd}	15.33^{c}	29.47^{d}	10.40^{c}	50.67^{c}	370.66^{ab}	32.5^{a}	83.50^{d}	4.33^{a}
Fe0Mn10	21.87^{b}	75.06^{cd}	19.53^{ab}	41.13^{ab}	16.20^{ab}	65.73^{a-c}	267.00^{f}	29.17^{ab}	95.33^{c-e}	4.17^{a}
Fe0Mn20	23.73^{b}	69.10^{d}	18.07^{a-c}	32.30^{cd}	13.07^{a-c}	76.53^{a}	275.66^{ef}	24.50^{b-d}	44.66^{f}	4.17^{a}
Fe15Mn0	24.40^{b}	70.46^{a-c}	16.33^{bc}	37.00^{a-d}	12.93^{a-c}	69.73^{ab}	321.16^{c-e}	26.50^{a-d}	108.83^{a-c}	4.17^{a}
Fe15Mn10	22.83^{b}	91.93^{cd}	19.40^{ab}	32.80^{cd}	12.73^{bc}	58.13^{bc}	336.33^{b-d}	25.17^{b-d}	80.83d^{e}	3.17^{a-d}
Fe15Mn20	25.77^{ab}	76.56^{d}	19.53^{ab}	38.87^{a-c}	15.57^{ab}	74.13^{a}	339.33^{a-d}	28.00^{a-c}	74.50^{e}	2.83^{bcd}
Fe30Mn0	23.77^{b}	87.26^{a}	19.00^{ab}	37.67^{a-c}	13.43^{a-c}	66.67^{ab}	360.33^{a-c}	27.33^{a-d}	101.33^{b-d}	4.00^{ab}
Fe30Mn10	25.97^{ab}	92.63^{a-c}	18.33^{a-c}	32.30^{cd}	13.77^{a-c}	76.40^{a}	384.50^{a}	27.00^{a-d}	92.83^{cd}	3.17^{acd}
Fe30Mn20	25.57^{ab}	111.26^{b-d}	21.00^{a}	43.90^{a}	16.80^{a}	71.60^{ab}	305.16^{d-f}	21.17^{d}	128.00^{a}	2.83^{bcd}
Fe45Mn0	22.20^{b}	93.93^{ab}	21.27^{a}	34.87^{b-d}	15.37^{ab}	68.80^{ab}	345.16^{a-d}	25.50^{b-d}	124.33^{ab}	3.83^{abc}
Fe45Mn10	29.50^{a}	95.20^{a-c}	21.20^{a}	44.57^{a}	15.87^{ab}	78.67^{a}	356.16^{a-c}	22.17^{cd}	119.83^{ab}	2.67^{cd}
Fe45Mn20	26.27^{ab}	106.06^{a-c}	20.87^{a}	38.50^{a-c}	14.80^{ab}	71.20^{ab}	380.33^{ab}	24.83^{b-d}	130.50^{a}	2.50^{d}

In each column, means with similar letter (s) are not significantly different using the Duncan test at the 5% level.

The highest bulb Fe was obtained in the 15 kg ha^{-1} Fe alone (155.46 mg kg^{-1} DW) in the first year, and 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn treatment (130.50 mg kg^{-1} DW) in the second year (Tables 7 and 8).
The lowest and highest values of bulb weight were obtained in 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (4.17 mg kg^{-1} DW) and also by applying 10 kg ha^{-1} Mn alone (12 mg kg^{-1} DW), respectively, in the first year (Table 7).
The lowest and highest values of bulb weight were obtained by applying 45 kg ha^{-1} Fe with 20 kg ha^{-1} Mn (2.50 mg kg^{-1} DW) and the control treatment (4.33 mg kg^{-1} DW), respectively, in the second year (Table 8). Bulb Zn significantly decreased, down to 56.41% in response to high levels of Fe and Mn in the first year, compared to the control plants (Table 7).

Multivariate analysis of the interaction effect of Mn and Fe on quality and quantity traits

In the present study, plant height (PH) had positive correlation with spike length (SL) (r= 0.85), flower stalk diameter (FSD) (r= 0.94), floret diameter (FD) (r= 0.73), floret length (FL) (r= 0.92), fresh weight of aerial parts (FW) (r= 0.85), dry weight of aerial parts (DW) (r= 0.94), bulb count (BC) (r= 0.81) and vase life (VL (r= 0.90). All of the measured nutrient elements in aerial parts and bulbs had negative correlation with each other and also with other morphological traits in tuberose, except for Fe content in the bulbs (Fig. 1). Cluster analysis of tuberose was based on morphological and chemical properties of the leaves (Fig. 2). Cluster I, which included Fe30Mn20 and $Fe_{45}Mn_0$, detected low to medium values of FW, FD, PH, Mn of aerial parts (MnAP), bulb fresh weight (BFW), percentage of opened florets (OPP), and Fe in the bulb (FeB). However, it caused a high Fe value of aerial parts (FeAP). Cluster II included Fe15Mn10 and Fe30Mn10, which detected medium values of FW, FeB, and low values of MnAP, BFW, and FD. Cluster III included Fe0Mn0 and Fe15Mn0, characterized by plants with very low DW, FL, FSD, SL, BDW, BN, VL, Zn in the bulb (ZnB), Zn in aerial parts (ZnAP), Cu in the bulb (CuB), Cu in aerial parts (CuAP), and MnB. In cluster IV, Fe0Mn10 and Fe15Mn20 caused plants with very low CuAP, CuB, FL, and FSD. Within this cluster, these two treatments showed medium values of FD, MnAP, and BFW.

Discussion

The proper and balanced use of fertilizers can significantly enhance the quantity and quality of plants, particularly flowers and ornamental plants (Khalaj and Edrisi, 2012). The current study indicated that applying 45 kg ha^{-1} of $FeSO_4$ caused the highest values in all measured traits and caused a significant difference compared to the control treatment. Cut flowers, particularly tuberose plants, have essential features that enhance marketability, such as the length of the flowering stem. The findings demonstrated that Fe supplementation improved the length of flowering stems and spikes, likely due to the role of Fe in chlorophyll formation, photosynthesis performance, carbohydrate production, and nucleic acid metabolism. Additionally, Fe fertilizers enhanced Fe absorption in the leaves, resulting in increased activity of catalase and peroxidase enzymes, ultimately leading to higher Fe assimilation by the leaves (Marschner, 2012). The findings of this experiment are consistent with previous studies conducted by Kumar and Chattopadby (2001) on tuberose plants, Singh et al. (2015) on Lilium ‘Tresor’, and Fakhraei Lahiji (2012) on gladiolus 'Oscar'. Similar results by Barman and Pal (1993) supported the current findings. Shoor et al. (2010) reported that micronutrients such as Zn, Cu, and Fe increased the length of the flower spike, flowering stems, and the percentage of opened florets in tuberose plants. Additionally, a more efficient absorption of Fe in the plant increases the activity of peroxidase and catalase enzymes, resulting in a higher number of florets. Fe plays a crucial role in the biosynthesis of photosynthetic assimilates, which leads to increased vegetative growth of the plant and potentially improves flower quality by increasing the number of florets (Fakhraei Lahiji, 2012; Saeed et al., 2013; Kumar et al., 2022).
Tayade et al. (2018) reported that using 0.4% FeSO4 in foliar spraying resulted in significant improvements in various flowering parameters of tuberose, such as flower spike length (11.24%), spike diameter (15.66%), rachis length (20.23%), floret length (22.40), floret diameter (11.65%), and vase life (34.45%), compared to the control. This positive effect could be explained by a higher deposition of carbohydrates in the vegetative cells, leading to thicker cells and a potential increase in the flower vase life. The observed increase in the flower vase life may have resulted from the contribution of micronutrients, specifically Fe, in synthesizing plant hormones. The percentage of opened florets is desirable for cut tuberose flowers as it enhances their beauty.

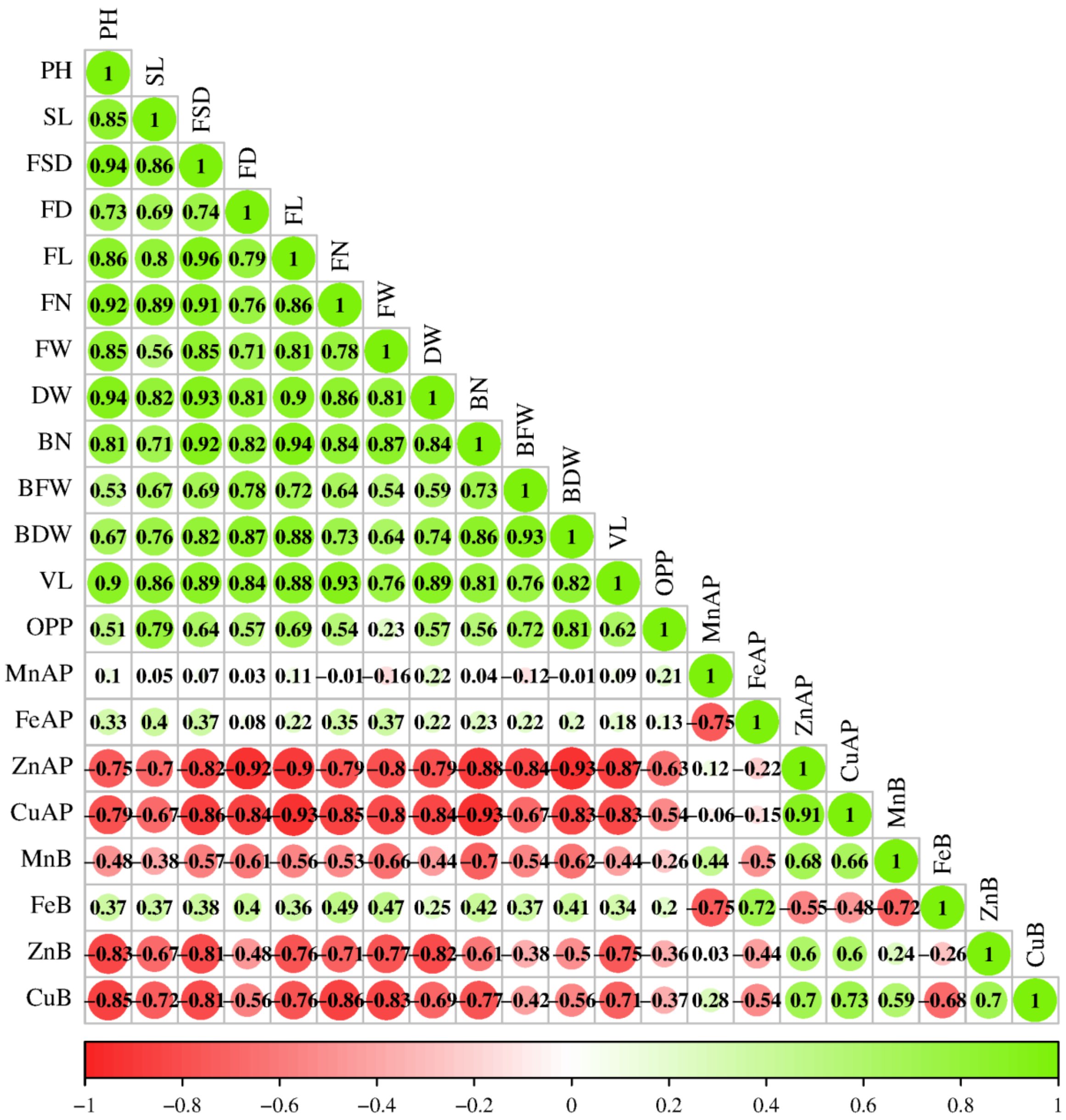

Fig. 1. Correlation heat map indicating correlations among micronutrients, quantitative and qualitative traits of tuberose from two years. Positive relationships are presented in green color and negative relationships are in red color. Abbreviations: plant height (PH), spike length (SL), flower stalk diameter (FSD), floret diameter (FD), floret length (FL), floret number (FN), fresh weight of aerial part (FW), dry weight of aerial part (DW), bulb number (BN), bulb fresh weight (BFW), vase life (VL), opened florets percentage (OPP), bulb (B), aerial parts (AP).

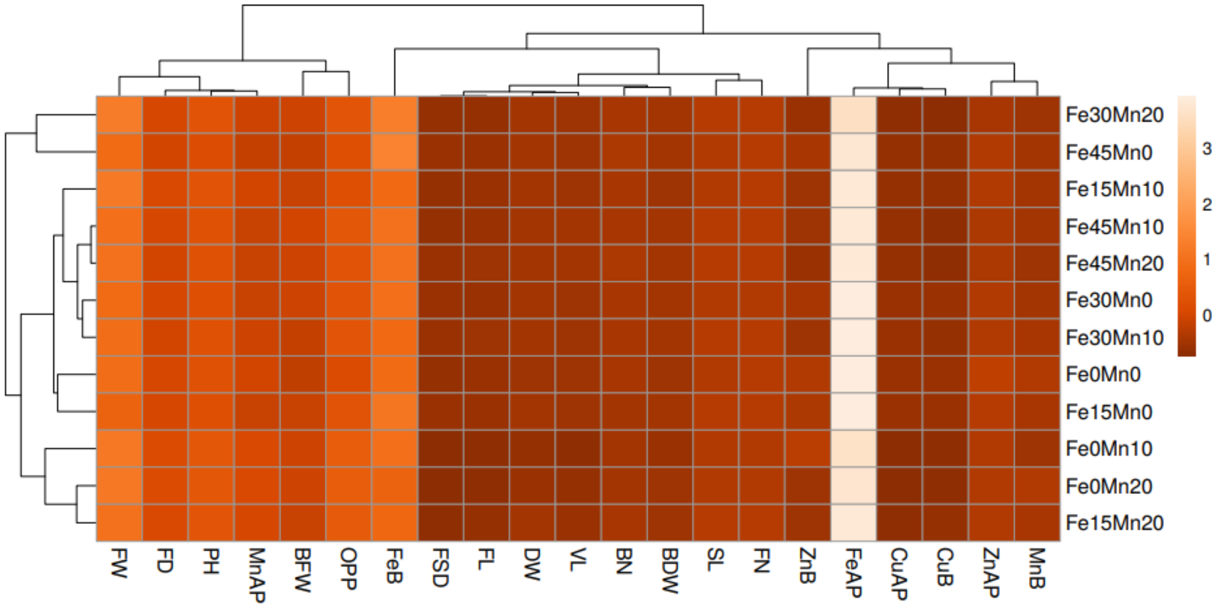

Fig. 2. Cluster analysis of treatments on tuberose based on physical and chemical properties of the leaf (gradient from low (dark brown), medium (orange) to high (pink). Abbreviations: plant height (PH), spike length (SL), flower stalk diameter (FSD), floret diameter (FD), floret length (FL), floret number (FN), fresh weight (FW) of aerial part, dry weight (DW) of aerial part, bulb number (BN), bulb fresh weight (BFW), vase life (VL), opened florets percentage (OPP), bulb (B), aerial part (AP).

Tables 3, 4, 7, and 8 demonstrate that adding $FeSO_4$ fertilizer affected the percentage of opened florets and the vase life. Similar to previous research, these findings suggest that $FeSO_4$ fertilizer can enhance carbohydrate metabolism and activate enzymes, thus increasing the carbohydrate content in the flowering stem. This increase in carbohydrates may strengthen the ability to open florets and extend the vase life of cut tuberose flowers (Shoor et al., 2010). In another study, foliar spraying of $FeSO_4$ effectively increased overall iron content in the plant tissues, thus extending the vase life of the flowers. Plants that received a combination of N (150 mg L^{-1}), $FeSO_4$ (2%), and citric acid (0.1% w/v) had the longest vase life after harvesting (Eidyan et al., 2014).

The appropriate application of Fe fertilizer can enhance the growth and performance of the aerial parts and roots by promoting better Fe absorption (Marschner, 2012). Furthermore, carbohydrate and Fe accumulation in the aerial parts, followed by their transfer to the roots, can improve root growth and development. The results showed that root fresh and dry weights, bulb count, and bulb Fe content increased by applying more Fe in both years. These findings are consistent with a previous study by Barman and Pal (1993). This effect is likely related to the role in activating several crucial enzymes, such as catalase, peroxidase, alcohol dehydrogenase, carbonic dehydrogenize, and tryptophan synthesis, etc., while assisting chlorophyll synthesis and various physiological processes that encourage plant growth and development (Saini et al., 2015; Haydar et al., 2022). Additionally, ascorbate peroxidase and catalase activities increased in soybean plants treated with $FeSO_4$ fertilizer (Dhaliwal et al., 2022). The beneficial effects of Fe application on the quantitative and qualitative traits of tuberose were also confirmed in previous research conducted by Kumar and Chattopadby (2001), who reported a significant difference in the growth and yield of tuberose in all treatments compared to the control due to Fe fertilizer application.

Tables 3 and 4 indicate that an increase in $FeSO_4$ fertilizer in the soil decreased the Mn uptake by the plants. In Figure 1, the heat map shows a negative correlation between Fe contents in the aerial parts and bulbs and reduced Mn content in the aerial part (r= -0.75) of tuberose. This result is attributable to the negative interaction between Fe and Mn, which is consistent with previous findings by Ghasemi Fasaei and Ronaghi (2008) that Fe fertilizer enhanced Fe uptake but reduced Mn, Zn, and Cu uptake in wheat plants. Alam et al. (2000) reported that Fe competes with other micronutrients, such as Mn, for the same non-specific sites. Similar to previous findings, the addition of Fe resulted in a reduction in the uptake of Mn, Zn, and Cu, but the rise in Fe uptake rate was considerably higher than the decline in

Mn, Zn, and Cu uptake rates. This suggests that Fe may have specific absorption and transportation sites in addition to non-specific ones, as reported by Ghasemi Fasaei and Ronaghi (2008). In contrast, other studies have shown that the application of Mn increased the Mn uptake only and had no significant effect on the uptake of other cationic micronutrients (Ghasemi-Fasaei and Ronaghi, 2008).

Furthermore, Moraghan et al. (2002) found that an increase in Fe consumption increased the yield and concentration of Fe in bean grains but decreased the concentration of Mn in the beans. Findings by Moosavi and Ronaghi (2011) also confirmed the current results. However, some reports have indicated that different sources of Fe fertilizer can increase the Zn and Mn concentrations of broad beans (Mahmoud et al., 2022), which disagrees with the present results. Similar to the present findings, Basar and Ozgumus (1999) discovered a negative correlation between the total Fe concentration and the concentrations of Zn and Mn in peach trees (Fig. 1).

Tables 5 and 6 demonstrated that applying 20 kg ha^{-1} of pure Mn resulted in the highest measured parameters compared to the control. Applying 20 g ha^{-1} $MnSO_4$ fertilizer likely increased nitrogen absorption, leading to an increase in plant height, flower spike length, and floret number per spike. These findings may be attributed to the role of Mn in chloroplast synthesis, photosynthesis, respiration, and nitrogen metabolism. Mn deficiency can also affect the photosynthetic efficiency of plants (Yano and Yachandra, 2014). Therefore, adding an appropriate concentration of $MnSO_4$ could improve the quality and quantity traits of tuberose. These results are consistent with previous research on bulbous plants, including tuberose (Shoor et al., 2010) and Gladiolus (Singh et al., 2012; Memon et al., 2013; Singh et al., 2015).

The longevity of flowers is an important trait in marketability. Higher longevity is a desirable quality trait in cut flowers. Nowadays, nutrient management, especially in microelements, before harvesting and using protective solutions after harvesting can become increasingly important in improving the vase life of cut flowers. In tuberose cut flowers, the end of their vase life is usually reached when wilted florets are more than healthy flowers. Mn is expected to play a crucial role in the vase life of tuberose cut flowers due to its involvement in carbohydrate metabolism, phosphorylation reactions, and the citric acid cycle (Shoor et al., 2010). Mn plays a significant role in superoxide enzyme activity, which protects tissues from oxidative damage and can contribute to longer vase life (Candas and Li, 2014). The increased presence of carbohydrates and Mn in the aerial parts, followed by their transfer to the roots, improves root growth and development, leading to an increase in bulb fresh weight, dry weight, number of bulbs, and bulb Mn content. Similar findings were obtained in a study by Barman and Pal (1993) on tuberose.

Micronutrients are known to improve the presence of chlorophyll pigments, proteins, and nucleic acid synthesis, thus enhancing growth and flowering in flower species such as gladiolus (Sharma and Khare, 2014), chrysanthemum (Vanlalruati et al., 2019), and marigold (Yadegari, 2013). Patel et al. (2017) also found that micronutrient application plays a significant role in the successful production of high-quality tuberose cut flowers and in improving the vase life of flowers. Fe and Zn are involved in photosynthesis and enhanced carbohydrate assimilation, resulting in luxurious vegetative and floral growth. Fe reportedly induced the production of healthy green leaves, leading to a better distribution of assimilates to the floral part. Hembrom and Singh (2015) also found that foliar spraying of 0.4% $FeSO_4$ on Lilium 'Tresor' resulted in significant improvements in assimilate distribution, with the treated plants having maximum bulb weight (12.61 g).

Applying Fe at any level by both application methods led to a significant increase in the chlorophyll index of Rosa hybrida 'Dallas' leaves compared to the control treatment. In particular, plants sprayed with 60 mg L^{-1} of Fe had the highest chlorophyll index according to the mean values of the two seasons (Salem et al., 2019).

Memon et al. (2013) concluded that gladiolus flowers treated with a combined application of 40 g $ZnSO_4$ and 20 g $FeSO_4$ had remarkable growth and flower yields. Similarly, Fe enhanced the flowering characteristics of tuberose by alleviating chlorosis and promoting the healthy green growth of leaves. This increased the production of assimilates and partitioning of flower growth, which likely contributed to higher flower production and increased yield (Patel et al., 2017).

Mudassir et al. (2021) applied micronutrients (specifically Fe, Zn, and B) either alone or in combinations on tuberose plants at different time intervals after planting (60, 90, and 120 days). The mixture of all three micronutrients resulted in significant improvements in various aspects of plant growth and development, such as increased chlorophyll index (60.28%), plant height (59.62%), stalk length (105.07%), fresh (153.96%) and dry (109.60%) plant weights, spike length (73.64%), number of florets

(163.05%), and vase life (55.56%) compared to plants that did not receive the micronutrient spray. Furthermore, they discovered that applying Fe alone resulted in a substantial increase of 31.28%, 91.46%, and 51.02% in plant height, floret count, and floret length, respectively, in tuberose plants.

Tables 5 and 6 indicate a contrasting relationship between Mn and Fe absorption in the aerial parts and bulbs, similar to the results obtained from the effect of high levels of Fe on the Mn content of aerial parts and bulbs.

Based on the results, an increase in Mn absorption by the plant led to a decrease in Fe absorption. This result may be attributed to the negative interaction between Fe and Mn (Gasemi Fasai et al., 2002). A negative correlation was also found between a high level of Mn in the soil and the absorption of other micronutrient elements in tuberose. Additionally, an inverse relationship was observed in the absorption of micro-nutrients, especially Cu and Zn elements, between the aerial parts and tuberose bulbs, although the decrease in micronutrients in the aerial parts and bulbs was not lower than the critical concentration.

Optimum consumption of Fe and Mn resulted in a significant increase in quantitative and qualitative characteristics of tuberose compared to control plants, likely due to the roles of these two elements in plants. Similar results were obtained in marigold (Balakrishnan et al., 2007; Yadegari, 2013), tuberose (Kalra et al., 1998; Halver et al., 2007; Shoor et al., 2010; Khalaj and Edrisi, 2012), chrysanthemum (Ganga et al., 2008), orchids (Ganga et al., 2009), and gerbera (Sahu et al., 2016). These findings are consistent with previous studies (Kumar and Chattopadhyay, 2001; Ghasemi-Fasaei and Ronaghi, 2008; Marschner, 2012).

Conclusion

To summarize, the current study indicated that the optimal combination of Mn and Fe application can significantly enhance the quantitative traits of tuberose, thus improving marketability. Particular focus was given to key marketability traits, such as flowering stem height, flower stalk diameter, fresh weight of aerial parts, and vase life. The Fe30Mn20 (30 kg ha^{-1} Fe and 20 kg ha^{-1} Mn), $Fe_{45}Mn_{10}$ (45 kg ha^{-1} Fe and 10 kg ha^{-1} Mn), and $Fe_{45}Mn_{20}$ (45 kg ha^{-1} Fe and 20 kg ha Mn) treatments led to superior results compared to the other treatments. The Fe30Mn20 treatment in particular was identified as the most effective. These findings highlight the positive effects of the mentioned treatments on various growth and flowering parameters in tuberose.

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Author contributions
MAK was the project administrator, SE performed data curation, writing, review, and editing. PSA conducted data curation and analysis using software applications.

Conflict of Interest
The authors indicate no conflict of interest in this work.

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