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Investigation of Tuberose's Quantitative Characteristics and Identification of Its Associated Pathogens under Different Levels of Nitrogen and Potassium in Khuzestan Province

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This study aimed to assess the effect of different levels of nitrogen and potassium on tuberose's quantitative characteristics and identification of its associated pathogens in the Khuzestan province of Iran. The experiment was carried out at Safiabad Agricultural Research and Education and Natural Resources Center as a completely randomized factorial design with 3 replications. Factors included four levels of nitrogen (0, 100, 200 and 300 kg N ha⁻¹) as Urea, three levels of potassium (0, 150 and 300 kg K₂O ha⁻¹) as potassium sulfate and a constant level of phosphorous $(100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1})$ as super phosphate. In current study, leaf nitrogen, spike length, leaf length, SPAD readings, leaf area index (LAI), optical depreciation coefficient, disease percentage and absorbed photosynthetically active radiation parameters were measured. The results showed that the effect of nitrogen on leaf length, SPAD readings, optical depreciation coefficient, absorbed photosynthetically active radiation, and disease percentage was significant at 0.05 probability level while on leaf nitrogen, spike length, and LAI was significant at 0.01 probability level. Potassium had no any significant effect on corresponding parameters in this experiment. In addition, the causal agents of necrotic spots on the tips and edges of the leaves and petals were identified as Botrytis sp., Curvularia sp., and Fusarium sp. while the main cause of necrotic spots on the petals was a thrips, which is probably either F.intonsa or F.schultzei species. Overall, the application of 100 kg ha-1 of nitrogen and no application of K2O are the best treatments and recommended for florists (in planting large bulbs).

Keywords: Macro elements, Plant pest and disease, Polianthes tuberosa, Quantitative parameters.

Abstract

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is a perennial herbaceous plant belongs to the family Agavaceae, and native to tropical and sub- tropical regions. It is one of the flowering bulbs which is mainly used as cut flowers (Bahadoran and Salehi, 2015). Tuberose is widely cultivated in Iran due to the presence of optimal climatic condition and high demand for it in internal and international markets (Shour *et al.*, 2010). As reported officially, tuberose is ranked fourth among the cut flowers in Iran, with an approximately total area harvested of 288 ha and around 29 million cut flowers (Anonymous, 2020). Iran benefits unique advantages over the world's major producers of flowers because of weather and lighting conditions. However, the flowers produced in Iran come with limited export value due to non-compliance with nutritional principles (Enfejari and Bayat, 2018). Adequate and proper nutrition of cut flowers (e.g. tuberose) is greatly important and, indeed, is essential to meet quantitative and qualitative standards in the production of these flowers (Shour, 2003). Nitrogen plays a vital role in the growth and physiology of plants and is a main component involved in the formation of amino acids, proteins, and nucleic acids. It also contributes in shaping structural parts of chlorophyll and ATP and is used by plants more than other elements (Tabatabaei, 2009).

It has been previously shown that the application of 200 kg ha⁻¹ nitrogen can improve yield and growth indicators (e.g. the height of the flowering stem, spike length, stem diameter and nitrogen content) in tuberose (Khalaj and Edrisi, 2013). Application of nitrogen increases plant height, number of leaves, florets and bulblets in tuberose (Taher et al., 2013). Flowering period mainly depends on the amount of nitrogen applied. It has also been demonstrated that the content of nitrogen, phosphorus and potassium is significantly increased in the leaves after application of nitrogen, phosphorus, and potassium as fertilizers, however the content of nitrogen, phosphorus and potassium in the leaves is generally higher than in bulb (Amarjet et al., 2001). Increase in the number of florets is due to the synthesis of amino acids, the formation of chlorophyll, and the better transfer of carbohydrates, which ultimately leads to better spike growth and an increase in the number of florets (Parmer, 2007). High levels of nitrogen enhance the synthesis of amino acids and stimulate the accumulation of protein in the leaves (Akbari et al., 2011). Furthermore, it has been proved that light absorption by vegetation is increased concomitantly with an increase in leaf area index (LAI) (Koocheki and Khajeh Hosseini, 2008). In fact, there is a direct relationship between LAI and leaf nitrogen content in most crops (Koochaki and Soltani, 1996). An increase in the absorption of radiation due to the application of nitrogen as a fertilizer has been shown in different studies (e.g. Al-Badawy et al., 1995; Latiri-Souki et al., 1998; Gohari, 1994). LAI in plants is varied depending on environmental conditions and genotype of the plant (Fageria, 1992). The optical depreciation coefficient factor indicates the rate of light reduction in the plant community. Light depreciation coefficient less than one means vertical leaves with a compact distribution whilst values higher than one indicates horizontal leaves in the canopy (Jones, 1992). Reduced optical depreciation coefficient (more vertical leaves) allows light to penetrate the canopy, influences more leaves in low amounts of radiation and raises the rate of carbon exchange. This elevates the efficiency of radiation absorption in plants growing under limited light sources (Kiniry et al., 2005). The optical depreciation coefficient depends on the angle of radiation and the angle and position of the leaves on the plant, playing a crucial role in the optimal use of light (Dewitt, 1965).

Fungal diseases (e.g. *Botrytis* blight) are one of the main limiting factors of tuberose production industry particularly in cold and wet seasons (Sharga, 1982). Moreover, worms and thrips are main insects affecting tuberose plants as pest. Although, aphids and spiders are also able to make damage on the tuberose (Roy and Kenneth, 1989). Generally, tuberose pest and diseases can negatively have a major impact on its quantitative and qualitative parameters, thus necessitating their accurate identification in order to adopt proper management strategies. Moreover, it is well known that macro-elements particularly N and K play a major role in management of the plant

diseases considering the foregoing topics, this work mainly attempts to assess the quantitative characteristics of tuberose treated with different levels of nitrogen and potassium fertilizers and identify its associated pathogens.

MATERIALS AND METHODS

This study was carried out at the Safiabad Agricultural Research and Education and Natural Resources Center (SARENRC) during the year of 2019 to study physiological and biochemical characteristics of tuberose under different levels of nitrogen and potassium. A plot of land was selected and land preparation operations (e.g. plowing, disking and leveling) were done in early May. Then plots, intervals between plots and replications were designed. The rate of urea fertilizer needed was calculated and weighed based on four levels of 0, 100, 200 and 300 kg ha⁻¹ of nitrogen, and then fertilizer was applied ($^{1}/_{3}$ before planting and the remaining 45 and 60 days after planting). Potassium levels were considered as 0, 150 and 300 kg ha⁻¹ K₂O based on potassium sulfate, which was applied wholly before planting. A constant level of phosphorous (100 kg P₂O₅ ha⁻¹) as superphosphate was applied across all treatments before. Chemical and physical properties of soil show in table 1 (Table 1).

Table 1. Chemical and physical properties of the soil in the depth of 0-30 cm.

Texture	Mn (ppm)	Zn (ppm)	Fe (ppm)	Cu (ppm)	K (ppm)	P (ppm)	OC (%)	рН	EC (dS m ⁻¹)	Depth
Silty Loam	4.66	0.9	7.6	1.6	159	11.8	0.92	7.72	1.8	0-30 cm

Leaves were analyzed by the Kjeldahl method using the AutoAnalyzer-Kjeltec-1030-Sweden machine to investigate leaf N status (Jafari Haghighi, 2003). Three leaves from three distinct plants in each plot were selected as the index and the leaves length was measured weekly. The length of spike was measured by a ruler from the junction point where the first floret emerges from the spike to the tip of the spike. Measurements were done in each plot at least on 12 randomly picked flower spikes, each with at least two opened florets. Then the mean of spike length was included in the calculations (Shour *et al.*, 2010).

Chlorophyll assay was performed at the peak of flowering using full leaves and at least ten readings per plot using chlorophyll meter SPAD (The Soil Plant Analysis Development) -502 (Minolta). Four plants whose flowers have been picked previously were randomly selected before flowering and their LAI values were calculated using leaf area meter (ADC Bio Scientific Ltd AM-300-002). The coefficient of light depreciation was measured using a tube PAR meter based on the Beer-Lambert law (or Beer's law).

$$I_i/I_o = e^{-KL}$$

I_i: Photosynthetically active radiation (PAR) under the canopy;

 I_0 : Photosynthetically active radiation (PAR) above the canopy;

L: Leaf area index (LAI);

K: Coefficient of light depreciation.

For this purpose, photosynthetically active radiation (PAR) above and under the canopy

was measured using Sunscan Δt (Devices LTD, England) and LAI was measured using leaf area meter (ADC Bio Scientific Ltd AM-300-002). The light depreciation coefficient (K) was then calculated using the foregoing formula. The percentage of radiation interception was measured by calculating photosynthetically active radiation (PAR) using the following equation:

$$PAR_1 = PAR_0 - PAR_1$$

Where PAR_1 is the amount of photosynthetically active radiation absorbed by the canopy, PAR_0 is the amount of photosynthetically active radiation above the canopy, and PAR_t is the amount of photosynthetically active radiation under canopy (transmitted radiation) (Akmal and Janssens, 2004).

The percentage of radiation interception was calculated using the following equation:

$$[(PAR_1/PAR_0) \times 100]$$

A tube PAR meter (Sunscan Δt Device LTD, England) was used to measure the rate of light penetration into the canopy (Rosati *et al.*, 2001). PAR assays were performed at the peak of flowering, at least for four points, and in the middle of the day (11 a.m. to 1 p.m.), when canopy receives maximum light.

Toobtain pathogenic isolates, symptomatic tissues including flowers and leaves were collected and transferred to the phytopathology laboratory of SARENRC. Infected segments were disinfected superficially using 1% sodium hypochlorite followed by rinsing with sterile distilled water three times. Subsequently, they were kept into humid chamber at 25 °C \pm 2 with a photoperiod of 12h for six days (Poletto *et al.*, 2020). After growing fungal structures, microscopic slides were then prepared and examined for visualization of fungal under a light microscope (Nikon ECLIPSE 80i, Japan). For disease incidence measurement, 20 flowers were randomly picked from each plot and the disease status was examined on infected plants to measure the disease incidence percentage per treatment. Infected spikes were counted and the disease percentage was calculated using the following equation (Kiani *et al.*, 2008):

Disease incidence (%) = (Number of infected flowers / Total number of flowers picked from each plot) × 100

Statistical analysis

In this study, the experiment conducted in a completely randomized factorial design with three replications. Data were analyzed with MSTAT-C (version: 6.1) using two-way ANOVA. Duncan multiple- ranges test (DMRT) was used to compare the means. Excel software (2013) was used to draw the graphs.

RESULTS AND DISCUSSION

The effect of nitrogen on leaf length, SPAD readings, optical depreciation coefficient, absorbed photosynthetically active radiation, and disease percentage was significant at 0.05 probability level while on leaf nitrogen, spike length, and leaf area index (LAI) were significant at 0.01 probability level. On the contrary, potassium and N×K had no significant effect on the measured parameters (Table 2).

Percentage of leaf nitrogen

ANOVA results showed that the effect of nitrogen fertilizer on the percentage of leaf nitrogen before flowering was significant at 0.01 probability level, but no significant effect was observed for potassium and N×K treatments (Table 2). An increase in the application of nitrogen fertilizer was concomitant with an increase in the percentage of leaf nitrogen, so that the highest percentage of leaf nitrogen (2.53 %) was observed after applying 300 kg ha⁻¹ nitrogen fertilizer (Table 3).

Spike length and leaf length

The effect of nitrogen fertilizer on spike length (at an alpha level of 0.01) and leaf length (at an alpha level of 0.05) was significant (Table 2 and 3). Increasing the rate of nitrogen applied up to 100 kg ha⁻¹ extends the spike length but at higher rates (i.e. over 100 kg) the length of the spike is reduced. Application of 100 kg ha⁻¹ nitrogen is the best treatment of applying nitrogen to achieve the maximum length of the spike, which is 12.4% higher than that of the control treatment. However, the highest leaf length was achieved after applying 300 kg ha⁻¹ of nitrogen, which is 12.35 % higher than the control treatment. Potassium application had no any significant effect on the length of the spike and leaves during the experiment.

As showed in fig. 1, the average weekly increase in leaf length in the control treatment indicates an approximately linear increase from the start of the growing season to the fifth week. Comparison between weeks, reveals a very small increase in leaf length because of the plant entering the flowering stage.

According to the above results, it can be concluded that with an increase in the rate of nitrogen fertilizer, the concentration of leaf nitrogen is increased; this consequently leads to increase leaf length due to the effect of nitrogen on vegetative growth. These findings are consistent with the results reported on tuberose by Kishore and Singh (2006) and Kadu *et al.* (2009). The number and length of leaves in tuberose is increased concomitant with an increase in the rate of nitrogen fertilizer (Mohana Sundaram *et al.*, 2003). The profitable effect of nitrogen on developing leaf length is related to the effect of nitrogen on the growth of metabolic carriers (Marchner, 1983). The optimal effect of nitrogen on leaf length might be due to its effect on components involved in protein synthesis as well as protoplasm components, which leads to increase chlorophyll content in the leaves. These factors contribute to cell division, enlargement, and differentiation, resulting in increased leaf length (Parmer, 2007). Kishore and Singh (2006) showed that the application of potassium does not significantly affect the number of leaves in tuberose.



Fig. 1. The average weekly increase in the leaf length in the control treatment.

SPAD readings and leaf area index (LAI)

Table (2) indicates that the effect of nitrogen on chlorophyll meter readings and LAI before flowering was significant at levels of 0.05 and 0.01, respectively. Potassium and its interaction with nitrogen had no significant effect on these parameters. With increasing nitrogen content, SPAD readings and the value of LAI increased, and the highest readings were observed in the application treatment of 300 kg ha⁻¹ of nitrogen, which are 31.5 % and 66.5 %, higher than that of the control treatment, respectively.

As illustrated in fig. (2), the content of nitrogen in leaves in the control treatment increased from 1.94 % to 2.53 % after applying 300 kg ha⁻¹ nitrogen. Subsequently, this increased the SPAD readings from 39.29 in the control treatment to 46.43 in the treatment of applying maximum nitrogen, indicating an increase in chlorophyll content per leaf with an increase in the application of nitrogen.

As stated, an increase in the rate of leaf nitrogen due to increasing the application of nitrogen fertilizer has led to an improvement in chlorophyll and photosynthetic activity, and ultimately the growth of the plant and LAI value (Parmer, 2007). As illustrated in fig. 3, LAI has been increased from 1.49 in control treatment to 2.49 in the treatment of applying 300 kg ha⁻¹ nitrogen (i.e. 67.1 % increase than the control treatment).



Fig. 2. The leaf nitrogen content and SPAD (The Soil Plant Analysis Development) readings in different treatments of nitrogen fertilizer.



Fig. 3. Leaf area index and leaf nitrogen content in different treatments of nitrogen fertilizer.

During a two-year trial, increased application of nitrogen raised LAI value in sugar beet, but without any effect on the number of the leaves (Gohari, 1994). Nitrogen enhances LAI by affecting the absorption of sunlight (Latiri- Souki *et al.*, 1998). The mixed application of nitrogen and nitroxin leads to an increase in photosynthetic pigments (chlorophylls a and b) in leaves and carotenoids in chrysanthemums (Nasri and Ghaderi, 2014).

Optical depreciation coefficient (K) and the percentage of absorbed photosynthesis active radiation (PAR)

According to tables 2 and 3, the effect of nitrogen on optical depreciation coefficient and percentage of absorbed photosynthetically active radiation (PAR) before flowering was significant at an alpha level of 0.05. However, potassium and its interaction with nitrogen had no significant effect on these parameters. By increasing the rate of nitrogen applied, the optical depreciation coefficient decreased whilst the percentage of absorbed PAR increased, with the minimum optical depreciation coefficient (0.419) and the maximum absorbed PAR (63.55 %) that were found after applying 300 kg ha⁻¹ of nitrogen. According to the table 3 and fig. 4, the maximum difference in the absorbed radiation was observed between the control treatment and applying 100 kg ha⁻¹ of nitrogen, and there was not much difference between the other nitrogen treatments. There were no significant differences between applying 100, 200, and 300 kg ha⁻¹ of nitrogen, but the application of 100 kg ha⁻¹ nitrogen seems to be the best treatment to achieve a high percentage of absorbed PAR.

According to table 3, as SPAD readings, LAI, and percentage of absorbed PAR are increased, the optical depreciation coefficient drops indicating a lower angle and more optimal arrangement of leaves, more efficient light penetration into canopy, and increased efficiency of light absorption. This coefficient has not been measured for tuberose so far and, hence, one of the objectives of this study was to determine the coefficient K and its application in different growth models.

Generally, the rate of plant photosynthesis is indicated by the leaf area index, and it can be claimed that the higher leaf area index indicates the higher photosynthetic level. That increases the light absorption by the vegetative parts and ultimately increases the photosynthetic efficiency. Light absorption by vegetative parts increases with an increase in LAI value (Koocheki and Khajeh Hosseini, 2008), in full agreement with the results of this study (Fig. 4). Nitrogen significantly affects the rate of light absorbed and improves the color of the leaves from yellowish green to dark green, which is a sign of increased chlorophyll. Also, a direct relationship is observed between the sugar beet LAI and leaf nitrogen content (Kouchaki and Soltani., 1996).



Fig. 4. LAI (Leaf area index) and absorbed radiation (%) in different treatments of nitrogen fertilizer.

iscussed earlier (Figs. 3 and 4), an increase in LAI and SPAD readings led to an increase in the absorbed radiation from 53.22 % in control treatment to 63.55 % in the treatment of applying maximum nitrogen, which is not unexpected (Fig. 4). The increase in the percentage of absorbed radiation after applying nitrogen fertilizer has been established (e.g. Al-Badawy *et al.*, 1995; Latiri-Souki *et al.*, 1998; Gohari, 1994). In brief, according to the table 2 and figs. 3, 4, and 5, increasing the rate of soil nitrogen can increase leaf nitrogen content, chlorophyll readings and content, the percentage of absorbed PAR and consequently photosynthesis. Elevated photosynthesis increases vegetative growth, LAI, and ultimately the percentage of absorbed PAR. The above tips show that the proper supply of nitrogen fertilizer is effective in the percentage of absorbed PAR and the optimal use of light by the plant.

Percentage of disease in tuberose petals

The effect of nitrogen on the disease incidence (%) was significant at an alpha level of 0.05, but the effect of other factors on this parameter was not significant (Table 2). By increasing the rate of nitrogen applied, the disease incidence (%) was increased particularly in the plots treated with 300 kg ha⁻¹ (Table 3). Leaf nitrogen content in the control treatment was 1.94 % which reached to the maximum value of 2.53 % after applying 300 kg ha⁻¹ of nitrogen fertilizer. This led to an increase in the disease incidence (%) from 72.4 % in control treatment to 93.41 % in the treatment of applying 300 kg ha⁻¹ of nitrogen (Table 2). An increase in leaf nitrogen content, unexpectedly leads to an increase in the disease incidence (%) due to the effect of nitrogen on plant fragility and reduction of plant resistance to parasitic and non-parasitic diseases (Younesi *et al.*, 2014). On the contrary, the rate of disease in plants can be diminished with proper management of applying nitrogen fertilizer (Younesi *et al.*, 2014). The minimum rate of the disease occurred in the treatment of applying no nitrogen fertilizer, where the minimum content of leaf nitrogen was also observed (Table 2).

Identification of pest and diseases in leaves and petals of tuberose

Our examinations on tuberose leaves in this study revealed that there are several spots on the tips of the leaves appeared as burned and necrotic symptoms (Fig. 5). Based on micro morphological characterizations, the identity of the samples were determined as *Fusarium* sp., *Botrytis* sp., and *Curvularia* sp.

At flowering stage, signs of burning and then light brown to gray necrosis spots were appeared on some parts of the petals, mostly in the middle petals of the spikes. These symptoms were also in association with the same fungal agents and a species of *Thysanoptera* (i.e. thrips) that seems to be very important in creating foregoing symptoms. Based on the results, the genus observed on the tuberose fields is presumably *Frankliniella*. The species of this genus are largely active on the flowers of plants, which cause burnt spots on the petals, and in high populations, they even lead to a complete flower- burning and diminish the vase life of the flowers (Bagheri, 2007). The species of this genus likely is one of the two species *F. intonsa* or *F. schultzei* (Bagheri, 2007). The accurate identification of this species is in progress at entomology laboratory of SARENRC.

CONCLUSION

The quality of flowers is the most important parameter in the production of tuberose that the farmers try to improve it with the least fertilizers. The spike length and the percentage of petal diseases are fundamental in determining the quality of flowers. These parameters come with a more balanced status presumably due to a more optimal balance of nutrients in the leaf in the treatment of applying 100 kg ha⁻¹ nitrogen fertilizer. The results of this study showed that if tuberose

		nitrogen	length	length	readings		coefficient	active radiation
Replication	2	0.058 ns	24.6 ns	3.8 ns	58.33 ^{ns}	0.236 ^{ns}	0.047**	314.04**
Nitrogen(N)	3	0.68**	77.3**	32.44*	220.68*	1.49**	0.015^{*}	211.95*
Potassium(P)	2	0.049ns	33.5 ^{ns}	1.38 ^{ns}	63.93 ns	0.083 ns	0.001^{ns}	13.47ns
$P \times N$	6	0.039ns	24.8^{ns}	16.65^{ns}	22.67 ^{ns}	0.113^{ns}	0.004ns	17.42ns
Error	22	0.051	14.9	6.56	33.50	0.101	0.005	28.39
CV (%)	I	9.78	6.11	7.16	13.66	15.72	14.45	8.83
*, ** and ^{ns} : \$	Significant at	P < 0.05, P < 0	0.01 and insig	nificant, resp	ectively.			
	_	able 3. Mean	comparison of	the effect of	nitrogen on	luantitative (haracteristics	ıf tuberose.
Nitrogen I (Kg ha ⁻¹)	Leaf nitroger	able 3. Mean h Spike lengtl (cm)	comparison of Leaf lengt	the effect of	nitrogen on gs L/	luantitative o	haracteristics Dptical oreciation	of tuberose. Absorbed photosynthetically ctive radiation (%)
Nitrogen I (Kg ha ⁻¹)	Leaf nitroger (%)	able 3. Mean Spike lengt (cm) 49.8 ^b	1 Leaf lengt (cm) 34.2 ^{ab}	the effect of h SPAI readin	nitrogen on gs L/	luantitative (I de 17°	haracteristics Dptical oreciation efficient a	f tuberose. Absorbed shotosynthetically ctive radiation (%) 53.22 ^b
Nitrogen I (Kg ha ⁻¹) 0	Leaf nitroger (%) 1.94° 2.21 ^{be}	able 3. Mean Spike lengt 49.8 ^b 55. 8 ^a	n Leaf lengt 34.2 ^{ab} 34.68 ^b	the effect of h SPAI 35.29 42.74	initrogen on L_{ℓ}	luantitative (I de de 17°	haracteristics Dptical oreciation efficient a 0.517 ^a	f tuberose. Absorbed photosynthetically ctive radiation (%) 53.22 ^b 61.52 ^a
Nitrogen I (Kg ha ⁻¹) 0 100	1 (%) 1.94 ^c 2.51 ^{ab}	Table 3. Mean 1 Spike length 49.8 ^b 55. 8 ^a 53.02 ^{ab}	n Leaf lengt (cm) 34.2 ^{ab} 34.68 ^b 35.76 ^{ab}	h SPAI readin 42.74	initrogen on \mathbf{g}^{s} \mathbf{L}^{j}	1 $\frac{1}{1}$	haracteristics Dptical preciation efficient a 0.517 ^a 0.479 ^{ab}	f tuberose. Absorbed photosynthetically ctive radiation (%) 53.22 ^b 61.52 ^a 63.22 ^a

nitrogen Leaf

length Spike

length Leaf

readings SPAD

Optical

Absorbed

incidence Disease Table 2. Analysis of variance of the effect of different levels of nitrogen and potassium on quantitative characteristics of tuberose.



Fig. 5. Contaminated tuberose leaves and petals.

fed with a balanced combination of nitrogen and potassium, its vegetative and reproductive activities would coordinate and balance. Some scientists have reported that potassium is the most demanded fertilizer by tuberose, whilst in this study, we found that potassium does not significantly affect the parameters studied, probably due to differences in the size of bulbs grown, potassium status in the soil and the rate of potassium release into the soil.

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