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Original Article

Effects of Corm Size, Organic Fertilizers, Fe-EDTA and Zn-EDTA Foliar Application on Nitrogen and Phosphorus Uptake of Saffron (*Crocus sativus* L.) in a Calcareous Soil under Greenhouse Conditions

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Abstract

A greenhouse research experiment was conducted. The experiment was arranged in factorial layout based on a completely randomized design. The mother corm size (0.1-4 g, 4.1-8 g and 8-12 g), organic fertilizers (cattle manure 15 t ha^{-1}, vermicompost 10 t ha^{-1}, chamomile compost 10 t ha^{-1} and control) and micronutrients (Fe-EDTA and Zn-EDTA) in two levels (foliar application and no application) were assigned as the first, second and third experimental factors, respectively. Based on the results, with increasing mother corm size, formation of small corms (0.1-4 g) decreased, whereas the percentage of medium (4.1-8 g) or large size (more than 8 g) corms increased. The highest corm yield was observed when cattle manure was applied. Moreover, foliar application increased daughter corm yield in medium and larger size corms. Phosphorus and nitrogen concentration in daughter corms increased with increasing the size of mother corms. Organic fertilizers significantly increased phosphorus and nitrogen concentration in all size of corms: phosphorus content in large daughter corms increased five times on account of cattle manure application. Proper nutrient management during the first year of saffron propagation could improve corm number than rather corm weight.

Keywords: cattle manure, daughter corms, micronutrients, vermicompost

Introduction

Saffron (*Crocus sativus* L.) belongs to Iridaceae family and is a perennial and autumn-flowering geophyte plant, which produces 1 to 3 purple flowers from each corm (Molina *et al*., 2005; Kumar *et al*., 2009). Saffron is mostly distributed in regions with low annual rainfall, cold winters and hot summers (Sepaskhah and Kamgar-Haghighi, 2009; Koocheki and Seyyedi, 2016; Koocheki *et al*., 2016). However, being triploid with chromosome number $2n = 3x = 24$ and basic number of $x = 8$, saffron is unable to produce viable seeds and it is propagated only in a vegetative way by cultivation of corms (Kumar *et al*., 2009; Ali *et al*., 2013). During each season, saffron propagates by replacement or daughter corms produced from the mother plants (Kumar *et al*., 2009; Koocheki *et al*., 2014).

In agroecosystems, identifying the factors affecting quantity and quality of agricultural products is a crucial part of the field management, and should be taken into account in order to achieve optimum yield (Koocheki *et al*., 2006).

Saffron is cultivated mainly in Irano-Touranian region and west of Asia with low annual rainfall (Sepaskhah and Kamgar-Haghighi, 2009) and low availability of nutrients specially phosphorus, zinc and iron (Sameni and Kasraian, 2004; Adhami *et al*., 2006; Najafi-Ghiri *et al*., 2013). Determination of relationships between application of different sources of nutrients and saffron growth and development would be ultimately useful for fertilizer management.

Flower and corm yields of saffron depend substantially on soil related parameters (Husaini *et al*., 2010; Avarseji *et al*., 2013; Kirmani *et al*., 2014). Among these parameters, organic matter content and available phosphorus content are known as most important factors (Nehvi *et al*., 2010; Koocheki *et al*., 2012; Chaji *et al*., 2013). In this regard, Behdani *et al*. (2006) have reported that in the central and southern parts of Khorasan province in Iran, up to 67% of yield changes are due to organic fertilizer application and phosphorus availability in soil. The soil organic content is a crucial soil property for better growth of the plants and is historically increased via application of organic substances such as cattle manure, crop

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residue and other type of composts which includes the remains of microorganisms and soil micro fauna (Allievi *et al*., 1993; Damodar Reddy *et al*., 2000; Arancon *et al*., 2005). Organic matters affect plant growth directly by supplying nutrients and indirectly by modifying soil physical properties which in turn improves the root environment and stimulate plant growth (Bandyopadhyay *et al*., 2010; Koocheki and Seyyedi, 2015).

In alkaline or calcareous soils, the solubility of micronutrients (Seyyedi *et al*., 2015) such as iron and zinc (Fairbanks *et al*., 1987; Cakmak *et al*., 1997; Kalayci *et al*., 1999; Zuo *et al*., 2000) is particularly low, and plants grown on such soil often suffer from deficiencies. Soluble ferrous Fe tends to become oxidized to ferric oxide, which is insoluble, as well as the limitation of iron uptake by root cell cytosol (Nikolic and Kastori, 2000) and inhibit iron transport to shoots and its transfer from apoplasm to cytoplasm in shoot tissues (Nikolic and Romheld, 2002). Zinc is necessary for root cell membrane integrity, growth, differentiation and development of plants (Cakmak and Marschner, 1988; Cakmak, 2000). As suggested by Parker, Aguilera and Thomason (1992), root cell membrane permeability is increased under zinc deficiency, which might be related to the functions of zinc in cell membranes. From this point of view, external iron and zinc concentrations could mitigate the adverse effect of alkalinity.

Nitrogen is one of the main chemical elements required for plant growth and photosynthesis (Cechin and de Fatima Fumis, 2004; Salvagiotti *et al*., 2009), therefore plays a major role in achieving the maximum economic yield of saffron (Chaji *et al*., 2013; Kirmani *et al*., 2014; Koocheki and Seyyedi, 2015). In addition, by considering the particular role of phosphorous in cell division, ATP generation, chlorophyll synthesis (Schachtman *et al*., 1998) and plant reproductive growth (White and Veneklaas, 2012), as well as saffron yield improvement (Naghdi Badi *et al*., 2011), study on phosphorus and nitrogen uptake is essential to better understand the changes in corm yield of saffron.

In addition to nutrient management, corm size is a crucial factor for flowering potential in saffron (Kumar *et al*., 2009; Khan *et al*., 2011). It has been reported that there is a close relationship between corm size and the number of flowers in saffron (De Mastro and Ruta, 1993; Gresta *et al*., 2008; Koocheki and Seyyedi, 2015). Economically, corm weight is an important factor as small corms produce none or fewer flowers during the first year of saffron cultivation (Kafi *et al*., 2006). Furthermore, behaviour of mother corms for daughter corms production has been considered as a paramount importance for commercial scale of corm production (Khan *et al*., 2011). There is an evidence for enhancement of leaf area and dry matter as corm size increased, which resulted in more daughter corms in the end of season (Renau-Morata *et al*., 2012). Hence, it seems that the mother corm size is an important indicator of efficiency for other inputs such as organic or mineral fertilizers, which should be considered when selecting corms.

The aim of the hereby study was to investigate the role of corm size, organic fertilizers, iron and zinc foliar application on daughter corm number and yield. Moreover, phosphorous and nitrogen uptake in daughter corms were investigated.

Materials and Methods

In order to investigate the effects of mother corm size, organic fertilizers and micronutrient foliar application on corm yield and nitrogen, as well as phosphorus uptake of saffron, a greenhouse experiment (temperature of 16-22 °C and 65-70% relative humidity) was conducted at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The experiment was arranged in a factorial layout based on a completely randomized design with three replications. The mother corm size (0.1-4 g - small, 4.1-8 g - medium, and 8-12 g - large), organic fertilizers (cattle manure 15 t ha^{-1}, vermicompost 10 t ha^{-1}, chamomile compost 10 t ha^{-1} and control) and micronutrients (Fe-EDTA and Zn-EDTA) in two levels (foliar application and no application) were assigned as the first, second and the third experimental factors, respectively.

Before corm planting, chemical and physical attributes of the collected soil for the experiment were determined through soil sampling and analysis (Table 1). In July, saffron corms were planted in plastic boxes (30 × 50 × 50 cm) filled with mixture of soil and different levels of organic fertilizers (Table 2). There were 16 planted corms in each box and irrigation was performed immediately after planting. During growing season, plants were irrigated well so that no water stresses imposed. Since alkaline soil, with low iron and zinc solubility was used in this study, iron and zinc foliar application (0.4% Fe + 0.1% Zn from Fe-EDTA and Zn-EDTA) was done twice in the following months.

At the end of the growing season, in June, corms were pulled out from the soil and daughter corms were evaluated in terms of corm yield and size, separately as follows: 0.1-4 g, 4.1 to 8 g and more than 8 g.

Furthermore, in each group of corms, nitrogen (Kjeldahl-PECO, Model, psu 55) and phosphorus (Spectrophotometer, Jenway Model, 4510) were estimated by the Kjeldahl (AOAC, 2000) and Murphy and Riley (Murphy and Riley, 1962) methods, respectively.

For statistical analysis, analysis of variance (ANOVA) and Duncan's multiple range test were performed using SAS 9.3 software.

Table 2. Chemical properties of organic fertilizers used within the experiment

Sample	Organic carbon (%)	Total N (%)	Total P (%)	pH	EC (dS m^{-1})
Cattle manure	24.63	2.98	1.96	11.65	12.84
Vermicompost	33.11	2.07	1.86	7.81	11.87
Chamomile compost	31.91	1.98	1.45	7.14	9.84

Table 1. Chemical properties of the soil used within the experiment

pH	EC (d.S.m^{-1})	$CaCo_3$ (%)	Organic carbon (%)	Total nitrogen (%)	Olsen-P (mg kg^{-1})	K (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
8.43	1.30	19.98	0.36	0.05	6.50	145	2.01	0.33

Results and Discussion

The number and weight of daughter corms

The results revealed that the greatest number of daughter corms, as well as the highest corm yield, was obtained when large corms (8-12 g) were planted. In other words, smaller corms produced less and smaller new daughter corms. In addition, with increasing mother corm size, formation of small corms (0.1-4 g) decreased, whereas the percentage of medium (4.1-8 g) or large size (more than 8 g) corms increased (Tables 3 and 4).

More growth of daughter corms as the result of large mother corms might be due to more stored nutrients at early stages of growth and also more nutrient absorption during growing season. Since saffron growth in early stages depends highly on stored assimilates and nutrients, thus larger corms produce more roots and dry matter than smaller corms (Renau-Morata *et al*., 2012). Similar results were obtained by Sabet-Teimouri *et al*. (2013) who reported that larger corms (6-8 g) produce more dry matter as roots and corms, compared with smaller corms (2-4 or 4-6 g). They have also reported that an increase in corm size caused more water absorption and nutrient uptake, which leads to better transportation into leaves.

When large corms (8-12 g) were planted, the number of daughter corm increased five times and 302.7 corms were lifted from each m^2. After corm sorting, it was found that 77.8, 18.3 and 3.9% of corms belong to small, medium and large size groups, respectively. On the other hand, corm yield increased by 10% and reached to 727.2 g m^{-2}, when large mother corms were planted; however, similar results were obtained when small and medium size corms were planted (Tables 3 and 4). Based on average values, small corms (less than 4 g) were responsible for 80.8% of corms and 45.4% of yield (Tables 3 and 4). In general, daughter corm formation depends on mother corm size (Renau-Morata *et al*., 2012) and nutritional conditions during growth and development (Rezvani Moghaddam *et al*., 2013a).

According to the results, it seemed that saffron tends to produce more number of corms rather than large corms during the first year. In this regard, it has been reported that organic or biofertilizers increase corn number rather than corms weight in primary years (Rezvani Moghaddam *et al*., 2013b).

From data on Table 3 it was noted that though organic fertilizers had no significant effect on the number of small daughter corms, medium and large size corms were significantly affected by organic fertilizers, whether cattle manure, vermincompost or chamomile compost was used. In a similar study, Rezvani Moghaddam *et al*. (2013a) observed increased corm number and corm weight on account of mushroom compost application. They have stated that an increase in corm number and corm weight might be due to an easy availability of nutrients, especially nitrogen and phosphorous, from compost and thus improvement of physical and chemical properties of soil.

Among organic fertilizers, cattle manure had the highest effect on corm numbers from medium and large size mother corms. In addition, the highest corm yield was observed when cattle manure was applied (Tables 3 and 4). On the other hand, although micronutrient foliar application had no significant effect on small corm number, the effect

Table 3. Comparison of the number of saffron corm under different treatments

Fertilizer treatments	Number of daughter corms (m^2)			Total daughter corms (m^2)
	0.1 – 4 g	4.1 – 8 g	More than 8 g	
Corm size (C)				
0.1 – 4 g	96.33 (82.96%) c	18.33 (16.21 %) c	1.0 (0.84 %) c	115.67 c
4.1 – 8 g	197.17 (81.80 %) b	37.17 (16.31 %) b	4.0 (1.90 %) b	238.33 b
8.1 – 12 g	237.33 (77.82 %) a	53.83 (18.26 %) a	11.5 (3.92 %) a	302.67 a
Average	176.94	36.44	5.50	218.89
Foliar application (F)				
No application	187.44 (84.18 %) a	30.78 (14.33 %) b	4.22 (1.49 %) b	222.44 a
Application	166.44 (77.53 %) a	42.11 (19.52 %) a	6.78 (2.95 %) a	215.33 a
Average	176.94	36.44	5.50	218.89
Organic fertilizer (O)				
Cattle manure	193.56 (79.32 %) a	40.44 (17.42 %) a	8.89 (3.26 %) a	242.89 a
Vermicompost	171.56 (79.25 %) a	40.22 (18.38 %) a	5.56 (2.37 %) b	217.33 a
Chamomile compost	166.67 (78.03 %) a	41.11 (19.90 %) a	5.11 (2.07 %) b	212.89 a
Control	176.00 (86.84 %) a	24.00 (11.99 %) b	2.44 (1.17 %) c	202.44 a
Average	176.94	36.44	5.50	218.89
C	**	**	**	**
F	NS	**	**	NS
O	NS	**	**	NS
C × F	NS	NS	NS	NS
C × O	NS	NS	NS	NS
F × O	NS	NS	NS	NS
C × F × O	*	NS	NS	NS

Means in each column, followed by similar letter(s) were not significantly different at 5% probability level, using Duncan's Multiple Range Test.
**, * and NS were significant at the 0.01 and 0.05 levels of probability and no significant, respectively.

Table 4. Comparison of saffron corm yield under different treatments

Fertilizer treatments	Yield of daughter corm (g m^{-2})			Total yield of daughter corm (g m^{-2})
	0.1 – 4 g	4.1 – 8 g	More than 8 g	
Corm size (C)				
0.1 – 4 g	102.64 (51.15 %) c	92.78 (44.52 %) c	12.28 (4.33 %) c	207.69 c
4.1 – 8 g	247.48 (50.50 %) b	193.24 (39.15 %) b	50.30 (10.36 %) b	491.01 b
8.1 – 12 g	296.70 (41.91 %) a	302.46 (41.15 %) a	128.03 (16.93 %) a	727.19 a
Average	215.61	196.16	63.54	475.30
Foliar application (F)				
No application	220.48 (53.03 %) a	170.29 (38.46 %) b	52.25 (8.51 %) b	443.01 b
Application	210.73 (42.67 %) a	222.03 (44.75 %) a	74.82 (12.58 %) a	507.58 a
Average	215.61	196.16	63.54	475.30
Organic fertilizer (O)				
Cattle manure	225.34 (43.71 %) a	218.66 (41.51 %) a	99.57 (14.78 %) a	543.56 a
Vermicompost	182.83 (40.34 %) a	223.81 (47.46 %) a	71.59 (12.21 %) ab	478.23 a
Chamomile compost	223.71 (47.16 %) a	215.53 (44.09 %) a	53.77 (8.75 %) bc	493.01 a
Control	230.54 (60.20 %) a	126.64 (33.38 %) b	29.20 (6.43 %) c	386.39 b
Average	215.61	196.16	63.54	475.30
C	**	**	**	**
F	NS	**	*	*
O	NS	**	**	**
C × F	*	NS	NS	NS
C × O	NS	NS	NS	NS
F × O	NS	NS	NS	NS
C × F × O	NS	NS	NS	NS

Means in each column, followed by similar letter(s) were not significantly different at 5% probability level, using Duncan's Multiple Range Test.
**, * and NS were significant at the 0.01 and 0.05 levels of probability and no significant, respectively.

Table 5. Comparison of characteristics of phosphorus uptake of saffron corm affected by different treatments

Fertilizer treatments	Phosphorus concentration in daughter corms (g kg^{-1})			Phosphorus content in daughter corms (g m^{-2})			
	0.1 – 4 g	4.1 – 8 g	More than 8 g	0.1 – 4 g	4.1 – 8 g	More than 8 g	Total
Corm size (C)							
0.1 – 4 g	1.26 b	1.29 b	0.28 b	0.13 c	0.12 c	0.02 c	0.27 c
4.1 – 8 g	1.31 b	1.34 ab	1.09 a	0.32 b	0.27 b	0.07 b	0.66 b
8.1 – 12 g	1.40 a	1.41 a	1.32 a	0.42 a	0.44 a	0.18 a	1.03 a
Foliar application (F)							
No application	1.34 a	1.32 a	0.82 a	0.30 a	0.23 b	0.07 b	0.60 b
Application	1.32 a	1.37 a	0.97 a	0.28 a	0.32 a	0.11 a	0.71 a
Organic fertilizer (O)							
Cattle manure	1.42 a	1.44 a	1.16 a	0.32 a	0.32 a	0.15 a	0.80 a
Vermicompost	1.39 a	1.42 a	0.97 a	0.26 ab	0.32 a	0.10 b	0.68 a
Chamomile compost	1.41 a	1.40 a	0.92 a	0.31 ab	0.31 a	0.07 bc	0.70 a
Control	1.08 b	1.12 b	0.54 b	0.25 b	0.15 b	0.03 c	0.43 b
C	*	*	**	**	**	**	**
F	NS	NS	NS	NS	**	*	*
O	**	**	**	*	**	**	**
C × F	NS	NS	NS	NS	NS	NS	NS
C × O	NS	NS	NS	NS	NS	*	NS
F × O	NS	NS	NS	NS	NS	NS	NS
C × F × O	NS	NS	NS	NS	NS	NS	NS

Means in each column, followed by similar letter(s) were not significantly different at 5% probability level, using Duncan's Multiple Range Test.
**, * and NS were significant at the 0.01 and 0.05 levels of probability and no significant, respectively.

increased for the yield of medium or large size corm. Moreover, foliar application increased daughter corm yield in medium and larger size corms (Tables 3 and 4). Micronutrient foliar application, increased number of corm and corm yield of large corms 60.7 and 43.2%, respectively (Table 3 and 4).

In geophye plants, which have an underground storage organ, assimilates, nutrients and water are transferred into storage organs at the end of the growing season (Chaji *et al*., 2013). Because of the role of iron and zinc in reproductive growth and final yield (Cakmak and Marschner, 1988; Cakmak, 2000; Heitholt *et al*., 2003; Moosavi and Ronaghi,

Table 6. Comparison of characteristics of nitrogen uptake of saffron corm affected by different treatments

Fertilizer treatments	Nitrogen concentration in daughter corms (g kg^{-1})			Nitrogen content of daughter corms (g m^{-2})			
	0.1 – 4 g	4.1 – 8 g	More than 8 g	0.1 – 4 g	4.1 – 8 g	More than 8 g	Total
Corm size (C)							
0.1 – 4 g	9.80 c	10.25 c	2.22 c	1.01 c	0.97 c	0.13 c	2.10 c
4.1 – 8 g	11.41 b	11.03 b	9.02 b	2.81 b	2.15 b	0.58 b	5.54 b
8.1 – 12 g	11.85 a	11.82 a	11.92 a	3.51 a	3.61 a	1.55 a	8.68 a
Foliar application (F)							
No application	11.05 a	11.03 a	7.15 a	2.49 a	1.94 b	0.62 b	5.05 b
Application	10.99 a	11.02 a	8.28 a	2.40 a	2.54 a	0.89 a	5.83 a
Organic fertilizer (O)							
Cattle manure	11.48 a	11.49 a	9.239 a	2.69 a	2.59 a	1.21 a	6.50 a
Vermicompost	11.44 a	11.47 a	8.63 a	2.16 a	2.65 a	0.87 ab	5.68 b
Chamomile compost	11.12 a	11.04 b	7.71 a	2.54 a	2.42 a	0.63 bc	5.59 b
Control	10.04 b	10.13 c	5.28 b	2.37 a	1.31 b	0.31 c	3.99 c
C	**	**	**	**	**	**	**
F	NS	NS	NS	NS	**	*	*
O	**	**	**	NS	**	**	**
C × F	NS	NS	NS	NS	*	NS	NS
C × O	NS	NS	NS	NS	NS	NS	NS
F × O	NS	NS	NS	NS	NS	NS	NS
C × F × O	NS	NS	NS	NS	NS	NS	NS

Means in each column, followed by similar letter(s) were not significantly different at 5% probability level, using Duncan's Multiple Range Test.
**, * and NS were significant at the 0.01 and 0.05 levels of probability and no significant, respectively.

2010) it seemed that foliar application of these micronutrients could affect saffron corm growth and flowering in saffron grown in alkaline soils.

Phosphorous and nitrogen contents in daughter corms

There were significant differences between different mother corm size in terms of phosphorus and nitrogen concentration in daughter corms (Tables 5 and 6). Phosphorus and nitrogen concentration in daughter corms increased with increasing the size of mother corms (Tables 5 and 6). Similarly, the highest phosphorus and nitrogen contents were measured in the case of the largest corms (Tables 5 and 6).

As mentioned, an increase in phosphorus and nitrogen concentration of daughter corms, formed from large mother corms, might be due to more storage of assimilates and nutrients in mother corms, which cause more roots and dry matter production during different growth stages (Renau-Morata *et al*., 2012). Increase in phosphorus content of daughter corms guarantee more flower yield in the following year.

According to Tables 5 and 6, foliar application had no significant effect on phosphorus and nitrogen concentration in daughter corms. By contrast, organic fertilizers significantly increased phosphorus and nitrogen concentration in all size of corms (Tables 5 and 6). Since for the current experiment it was used alkaline soil (pH 8.4), with low available phosphorus (8.5 mg kg^{-1}), it was noted that saffron plant had high reaction to the phosphorus availability (due to organic fertilizers). In addition, organic fertilizers and foliar application led to an increase in phosphorus and nitrogen contents in all daughter corm sizes (Tables 5 and 6). For instance, phosphorus content in large daughter corms increased five and three times on account of cattle manure and vermicompost application.

Increase in leaf area, nutrient content in leaves and saffron flower yield have been reported also by Amiri (2008), who applied cattle manure for saffron cultivation and concluded that cattle manure improved physical and chemical properties of soil. Similar results were found by Rasouli *et al*. (2013), when vermicompost was applied and apparently an increase in chlorophyll, phosphorus and zinc content was observed in saffron leaves.

Conclusions

In general, the results indicated that cattle manure, vermicompost and foliar application of iron and zinc increased the number of daughter corm and its yield. In addition, using larger corms and application of organic fertilizer improved corm yield, as well as phosphorus and nitrogen concentration in new saffron plants. The results state that more phosphorus and nitrogen concentration by daughter corms causes more daughter corms as result of organic fertilizer application. Finally, the obtained results emphasize that proper nutrient management during the first year of saffron propagation could improve corm number than rather corm weight.

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