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The Influence of Spermidine and Biofertilizer Application on the Growth, Yield and Some Active Constituents of Saffron Plant (Crocus sativus L.)

Saad Ali Ihsan
Pharmacy College, Babylon University,Street No. (60), Hilla, Babylon, , Iraq
E-mail: saaddawood50@gmail.com

Maher H.S. Al-Mohammad and Sabah N.K. Al-Thamir
Pharmacy College, Babylon University,Street No. (60), Hilla, Babylon, , Iraq
E-mail: pharmacy@babylon.edu.iq

Abstract
A Filed experiment was conducted during the growing season of 2012-2013 to study the effect of spraying spermidine at concentrations (0, 50 and 100 mg.L^{-1}) and Biofertilizer applications at levels (0, 125 and 250 kg.ha^{-1}) and their interactions on (growth plant height, number of leaves , fresh and dry weight of herb, number of flowers and flowers fresh weight), yield (stigmas fresh and dry weight, stigmas yield, number of corms, corms weight and corms yield) and some active constituents (crocin, picrocrocin, and safranal) of saffron plant *Crocus sativus* L.. The treatments were distributed in a factorial experiment conducted in a Randomized Complete Block Design (RCBD) with three replicates. The means were compared by Duncan's Multiple Range Test ($P \leq 0.05$).Both the spermidine and the biofertilizer improved plant growth and yield parameters. Biofertilizer was more effective than spermidine. The concentrations of spermidine at 100 mg.l^{-1} and biofertilizer level at 250 kg.h^{-1} strongly affect growth and yield properties. Consequently, the active constituents of stigmas were increased ($P \leq 0.05$). Active constituent's content of stigmas was significantly improved by spermidine at 100 mg.l^{-1}×biofertilizer at 250 kg.h^{-1}. Biofertilizer was more effective than spermidine in increasing safranal content compared with crocin and picrocrocin.
Keywords: Saffron, Spermidine, Biofertilizer

INTRODUCTION

Saffron is a food spice obtained from the flower stigmas of *Crocus sativus* L. plant which is a member of the Iridaceae family. It is use as a food additive, since it possesses powerful coloring and flavoring agent (Abdullaev, 1993; Abdullaev and Frenkel, 1999), saffron is used to cure many human diseases such as asthma, arthritis, it is also useful in treating cold and coughs, some medicines containing saffron are used to treat acne and several skin diseases (Kirtikar and Basu, 1993 ; Akhondzadeh S. et al., 2005), saffron is beneficial in the treatment of several digestive disorders, especially valuable in flatulent colic, it also counteracts spasmodic disorders, as a sedative in addition it is used in case of fevers as antipyretic, melancholia and enlargement of the liver and spleen, also it is used in medicines that reduce inflammation and in the treatment of kidney disorders (Noorbala A.A. et al., 2005; Moshiri E. et al., 2006), its analgesic properties which due to its glycosidic constituents (Sampathu et al., 1984; Winterhalter and Straubinger, 2000),
The main active constituents of saffron are crocin, picrocrocin, and safranal (main component for characteristic aroma). However, recent studies have shown that saffron has Antitumor and Anticarcinogenic activities in biological systems both *in vivo* and *in vitro* (Nair et al., 1991; García-Olmo et al., 1999; Premkumar et al., 2001; Abdullaev et al., 2003).

Spermidine is one of the major polyamine forms in plants. They are able to bind with several negatively charged molecules, such as DNA (Basu et al., 1990, Pohjanpelta and Holtta, 1996), membrane phospholipids and proteins (Tassoni et al., 1996). Polyamines are involved in protein phosphorylation (Ye et al., 1994) and post transcriptional modification of DNA (Basu et al. 1990). Polyamines were localized in the vacuoles, mitochondria and chloroplasts (Slocum and Galston 1985), and detected in thylakoid membranes of spinach and photosystem II (Borrell et al*.,* 1995). Growth, fresh and dry weights and total terpenoid constituents of *Mentha piperita* L. were improved by the application of polyamines (Youssef et al., 2002), (Al-Mohammad and Al-Zorfy, 2012) found that spraying putrescine at concentration 100 mg.L^{-1} improved growth, flowers number, flower fresh and dry weights and active constituents of flowers for *Tagetes erecta* L..

On the other hand saffron has a very low harvested yield (stigmas). However yield is significantly influenced by nutrient management (McGimpsey et al., 1997; Fernández, 2004), especially N-fertilizers influence significantly on quantity and quality of yield (Amiri, 2008). Application of chemical fertilizers in infertile soil increased yield of corms and stigmas (Sampathu et al., 1984), (Dhar et al., 1988) found that applying 40 to 50 kg N ha^{-1} increase corms production and stigmas yield. Koocheki et al. (2006) found that 20 to

80% of saffron yield is attributed to soil fertility (available phosphorus, mineral nitrogen, and exchangeable potassium). Mollafilabi, (2003) reported that up to 100 kg ha^{-1} ammonium phosphate has been used in some saffron fields, which encouraged vegetative growth and decreased the yield (Housini, 2004).

The present aims of the study are to observe the influence of spermidine and biofertilizer on the stimulation of vegetative growth, yield of corms , stigmas and the active constituents of stigmas in saffron plants.

MATERIALS AND METHODS

The experiment was conducted in a private farm, in Babylon governorate (Iraq), during cropping season of (2012 – 2013), the soil texture at the experimental site was loam with approximately 1.61% organic matter, pH (8.1), EC (1.1) dSm^{-1}, total N (0.56%), available P (24.8) ppm and available K (510) ppm. The field was prepared conventionally and dividing into plots, the area of each experimental unit (plot) was 6 m^2 (3 × 2 m). The corms of saffron were obtained from a field belong to Mashhad Agricultural Company in Mashhad (Iran), it's weight about (8-10) g, corms were planted at 15 September, 2012 by hand, with 20 cm rows distances, 15 cm the distance between the corms in the lines, corms planted at about 10 cm deep.

The experimental treatments were consisted of two factors: The growth regulator Spermidine at concentrations of (0, 50 and 100 $mg.L^{-1}$), spraying each concentration for two times during the vegetative stage at 15 November and 1 December, and the Biofertilizer which consist of 17% phosphorus, 20% sulfur, 16% organic matter, 1% zinc and about 500 g of bacteria populations (*Thiobacillus inoculums*) for each 25 kg fertilizer, the role of sulfur is to lower soil pH when oxidize by the bacteria producing sulfuric acid which in turn solubilizes phosphorus making it available for absorption by plant roots. Biofertilizer was added at levels of (0, 125 and 250 $kg.ha^{-1}$) in two applications first 30 days after planting, and the second 30 days from the first. The treatments were distributed in Factorial experiment conducted in a Randomized Complete Block Design (RCBD) with three replicates. Collected data analyzed by using GenStat program and means were compared by Duncan's Multiple Range Test (DMRT) at probability level 0.05 according to Daniel (1999).

At final stage of vegetative growth the growth properties were measured: plant height (cm), leaves number, fresh and dry weight of herbs (g), while the flowers number and flowers fresh weight (plant/mg) were measured at flowering stage which was began at January. Harvesting of flowers were done at dawn during 2 weeks. Yield parameters were included immediately separation of stigmas from flowers by hand and weighted freshly (mg) then dried to determine dry weight (mg) and its dry yield (mg/m^2). Corms were harvested at April and the measurements included on: corms number, corm weight (g) and corms yield (kg/m^2). The extraction and assay method of the three active constituents of saffron stigmas (crocin, picrocrocin, and safranal) done according to the procedure of Souret and Weathers (2000).

RESULTS AND DISCUSSION

Results in Table (1) indicate that application of spermidine or biofertilizer gave significant ($P \leq 0.05$) effects on growth properties of saffron plants during vegetative and flowering stages compared to the controls. Treatment of spermidine at concentration 100 $mg.1^{-1}$ or biofertilizer at level 250 $kg.ha^{-1}$ resulted in highest values of the growth parameters during two physiological stages (vegetative and flowering). The increment percentage in plant height, number of leaves, fresh and dry weights of herbs, number of flowers and flowers fresh weight that resulted from the foliar applications of spermidine were evaluated to be 7.25%, 19.75%, 11.19%, 7.39%, 12.30% and 13.86% respectively, and for biofertilizer were 10.03%, 21.65%, 13.11%, 8.59%, 13.95% and 16.36% respectively. These results indicated that both factors had a stimulation effect on growth characteristics during the differential growth stages. The interaction treatment between 100 $mg.1^{-1}$ spermidine × 250 $kg.ha^{-1}$ biofertilizer showed superiority significant ($P \leq 0.05$) for all treatments on growth properties, and the increment percentage for properties above were evaluated to be 17.00%, 39.27%, 23.63%, 15.12%, 25.41% and 28.79% respectively.

Results in Table (2) showed that treatments with spermidine or biofertilizer gave more impact when the concentrations or levels were increased; moreover, they have significant ($P \leq 0.05$) effects on yield properties of saffron plants compared to the control. Also spermidine at concentration100 $mg.1^{-1}$ and biofertilizer at level 250 $kg.ha^{-1}$ resulted in highest values of the yield parameters. The increment percentage in stigmas fresh and dry weight (mg), stigmas yield (mg/m^2), corms number, corm weight (g) and corms yield (kg/m^2) that resulted from the treatments with spermidine were evaluated to be 13.01%, 16.28%, 14.85%, 14.16%, 14.31% and 30.84% respectively, and for biofertilizer were 15.86%, 19.32%, 16.63%, 17.45%, 22.32% and 43.28% respectively. These results were referred that both factors had stimulation effect on yield characteristics and biofertilizer had more effect than spermidine. Spermidine treatment enhanced phytohormones which could play an important role as signals and regulators of the growth and development of plant endogenous polyamine (Shunquan *et al*., 2001). Biofertilizer was required to provide the soil and the plant with macro and micro nutrients then stimulate all metabolism cycle. The interaction treatment between 100 $mg.1^{-1}$ spermidine × 250 $kg.ha^{-1}$ biofertilizer superiority significant ($P \leq 0.05$) for all treatments on growth properties, and the increment percentage for

properties above were evaluated to be 30.27%, 35.33%, 31.71%, 30.74%, 41.28% and 84.71% respectively.

Data presented in Table (3) show that spermidine and biofertilizer effects on some active constitutes (crocin, picrocrocin and safranal) on saffron stigmas, and these effects were significantly variable compared to the control. Furthermore, spermidine concentration at 100 $mg.1^{-1}$ and biofertilizer level at 250 $kg.ha^{-1}$ resulted in the highest values of pigments content in saffron stigmas, and more effect was done on safranal compared with crocin and picrocrocin. The increment percentage in safranal that resulted from the treatment with spermidine and biofertilizer were evaluated to be 9.61% and 11.86% respectively. The interaction treatment between 100 $mg.1^{-1}$ spermidine × 250 $kg.ha^{-1}$ biofertilizer showed a superiority significant ($P \leq 0.05$) for all treatments on pigments, and the increment percentage in crocin, picrocrocin and safranal were evaluated to be 3.12%, 3.96% and 20.56% respectively.

CONCLUSION

1- Biofertilizer was more effective than spermidine on the growth, yield and safranal of saffron plants .
2- The active constituents of Saffron stigmas (Safranal, Crocin and Picrocrocin) were significantly improved by Spermidine at (100 mg/L) X Biofertilizer at (250 kg/ha).
3- Increasing concentrations of Spermidine and Biofertilizer may gives significant results on the growth, yield and active constituents of Saffron plants, but it still needs further studies.

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Table (1): Effect of Spermidine, biofertilizer and their interactions on saffron growth properties

Treatments	Plant height (cm)	Leaves No.	FW (g)	DW (g)	Flowers No.	Flowers FW (mg)
Sp^{0}	19.85 c	8.24 c	14.71 c	2.98 c	7.61 c	117.56 c
Sp^{50}	20.64 b	9.05 b	15.48 b	3.08 b	8.01 b	124.52 b
Sp^{100}	21.29 a	9.86 a	16.36 a	3.20 a	8.55 a	133.86 a
Bi^{0}	19.42 c	8.08 c	14.47 c	2.94 b	7.45 c	114.63 c
Bi^{125}	21.00 b	9.23 b	15.71 b	3.12 a	8.23 b	127.94 b
Bi^{250}	21.36 a	9.83 a	16.37 a	3.19 a	8.49 a	133.38 a
$Sp^{0} \times Bi^{0}$	18.94 h	7.69 h	14.09 g	2.91 d	7.28 h	112.69 g
$Sp^{0} \times Bi^{125}$	20.12 f	8.14 g	14.71 f	2.97 cd	7.66 f	116.81 fg
$Sp^{0} \times Bi^{250}$	20.50 e	8.88 e	15.34 e	3.05 c	7.90 e	123.19 de
$Sp^{50} \times Bi^{0}$	19.46c	8.06 g	14.41f	2.95 d	7.42 g	114.61 fg
$Sp^{50} \times Bi^{125}$	21.02 d	9.18 d	15.68 d	3.11 bc	8.17 d	127.13 cd
$Sp^{50} \times Bi^{250}$	21.43 c	9.91 c	16.34 c	3.18 b	8.45 c	131.81 c
$Sp^{100} \times Bi^{0}$	19.85 g	8.50 f	14.91 f	2.96 d	7.66 f	116.58 ef
$Sp^{100} \times Bi^{125}$	21.87 b	10.38 b	16.75 b	3.28 a	8.86 b	139.88 b
$Sp^{100} \times Bi^{250}$	22.16 a	10.71 a	17.42 a	3.35 a	9.13 a	145.13 a

Means with similar letters in each column are not significantly different at the 5% level of probability according to DMRT.

Table (2): Effect of Spermidine, biofertilizer and their interactions on saffron yield properties

Treatments	Stigmas FW (mg)	Stigmas DW (mg)	Stigmas yield DW (mg/m^2)	Corms No.	Corm weight (g)	Corms yield (Kg/m^2)
Sp^0	5.46 c	3.33 c	201.37 c	6.24 c	2.00 c	0.753 c
Sp^{50}	5.82 b	3.56 b	215.42 b	6.63 b	2.17 b	0.870 b
Sp^{100}	6.17 a	3.90 a	231.28 a	7.12 a	2.29 a	0.985 a
Bi^0	5.36 c	3.25 c	197.28 c	6.06 c	1.90 c	0.693 c
Bi^{125}	5.86 b	3.70 b	220.71 b	6.82 b	2.25 b	0.923 b
Bi^{250}	6.21 a	3.87 a	230.09 a	7.11 a	2.32 a	0.993 a
$Sp^0 \times Bi^0$	5.19 g	3.17 g	190.09c	5.92c	1.72c	0.611c
$Sp^0 \times Bi^{125}$	5.44 fg	3.36 fg	201.52c	6.23c	2.08c	0.778c
$Sp^0 \times Bi^{250}$	5.74 de	3.54 de	212.51b	6.57b	2.21b	0.871b
$Sp^{50} \times Bi^0$	5.39 g	3.24 fg	199.58c	6.08c	1.91c	0.705c
$Sp^{50} \times Bi^{125}$	5.92 d	3.66 cd	219.31b	6.78b	2.28a	0.928b
$Sp^{50} \times Bi^{250}$	6.14 c	3.79 c	227.39a	7.03a	2.32a	0.979a
$Sp^{100} \times Bi^0$	5.51 ef	3.33 ef	202.18c	6.17c	2.06c	0.763c
$Sp^{100} \times Bi^{125}$	6.23 b	4.09 b	241.30a	7.46a	2.38a	1.065a
$Sp^{100} \times Bi^{250}$	6.76 a	4.29 a	250.36a	7.74a	2.43a	1.128a

Means with similar letters in each column are not significantly different at the 5% level of probability according to DMRT.

Table (3): Effect of Spermidine, biofertilizer and their interactions on saffron active constituents (mg/g DW)

Treatments	Crocin	Picrocrocin	Safranal
Sp^0	88.95 c	40.45 c	3.43 c
Sp^{50}	89.20 b	40.77 b	3.61 b
Sp^{100}	89.50 a	41.18 a	3.76 a
Bi^0	87.82 c	40.28 c	3.37 c
Bi^{125}	89.85 b	40.98 b	3.66 b
Bi^{250}	89.98 a	41.15 a	3.77 a
$Sp^0 \times Bi^0$	87.59 g	40.12 g	3.21 f
$Sp^0 \times Bi^{125}$	89.56 f	40.52 f	3.43 e
$Sp^0 \times Bi^{250}$	89.69 e	40.71 e	3.66 c
$Sp^{50} \times Bi^0$	87.86 fg	40.41 fg	3.33 f
$Sp^{50} \times Bi^{125}$	89.81 d	40.89 d	3.71 c
$Sp^{50} \times Bi^{250}$	89.92 c	41.02 c	3.79 b
$Sp^{100} \times Bi^0$	88.02 f	40.31 ef	3.58 d
$Sp^{100} \times Bi^{125}$	90.17 b	41.53 b	3.84 ab
$Sp^{100} \times Bi^{250}$	90.32 a	41.71 a	3.87 a

Means with similar letters in each column are not significantly different at the 5% level of probability according to DMRT.

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