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The Effect of Exogenously Applied Plant Growth Regulators on Plant Development of Saffron (*Crocus sativus* L.)

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ABSTRACT: Plant growth regulators have important roles in plant growth and development. The externally applicable chemicals belong to different action classes and each one has a crucial and effective role at different plant growing stages. Saffron (*Crocus sativus* L.) is one of the most important and valuable medicinal and aromatic plant, belongs to Iridaceae family. Because of impossibility of generative propagation in nature, growing can be maintened asexually with daughter corms occured each year. In this experiment, the effects of exogenously applied paclobutrazol, indole-3-butyric acid, zeatin and picloram on saffron developmental stages were studied. Data were focused on aerial part and corm related characters which is important in developmental findings in this plant. In the treatments, paclobutrazol (10 or 20 mg L^{-1}), indole-3-butyric acid (1 or 3 mg L^{-1}), zeatin (1 or 3 mg L^{-1}) and picloram (5 or 10 mg L^{-1}) were applied alone besides control to saffron 45 days after planting to the soil under greenhouse condition. According to the data picloram concentrations have a retardant role in nodium activation, leaf number and corm developmental characters (number, weight, diameter and yield of daughter corm) but cause the highest plant height (60.98 cm in 10 mg L^{-1} and 57.37 cm in 5 mg L^{-1}). In 10 mg L^{-1} paclobutrazol treatments resulted as the best corm production (469 kg da^{-1}) than the other treatments and control. Zeatin at 3 mg L^{-1} concentration was found effective on the best nodium activation (2.81 active nodes/corm). In conclusion, saffron development is affected significantly by externally applied plant growth regulators under soil condition and the application methods can be progressed in studies on aimed parts of the plant.

Keywords: *Crocus sativus*, indole-3-butyric acid, paclobutrazol, picloram, zeatin

Safran (*Crocus sativus* L.)'da Bitki Gelişimi Üzerine Dışarıdan Uygulanan Bitki Gelişim Düzenleyicilerinin Etkisi

ÖZET: Dışsal uygulanabilen kimyasallar olan ve farklı etki sınıfları içinde bulunan bitki gelişim düzenleyicilerinin her biri bitki büyüme ve gelişiminin farklı aşamaları üzerinde hayati ve önemli işlevlere sahiptir. Tıbbi ve aromatik bitkiler içinde en önemli ve değerli bitkilerden biri olan safran (*Crocus sativus* L.) Iridaceae familyasına ait olup, doğal olarak generatif yolla üretilmesi mümkün olmadığından bitki gelişimi aseksüel olarak her yıl meydana gelen yavru kormlardan sağlanmaktadır. Bu araştırmada dışsal olarak uygulanan paclobutrazol, indole-3-butyric acid, zeatin and picloram maddelerinin safran gelişimi üzerine etkileri çalışılmıştır. Bitki gelişimi için önemli parametreler olan korm ve toprak üstü aksamı ile ilişkili karakterlerle ilgili veriler toplanmıştır. Çalışmada kontrol parsellerinin yanında paclobutrazol, indole-3-butyric acid, zeatin ve picloram bitki dikiminden 45 gün sonra cam serada toprağa uygulanmıştır. Elde edilen verilere göre picloram konsantrasyonları nodyum aktivasyonu, yaprak sayısı ve korm gelişim özellikleri (korm sayısı, ağırlığı, çapı ve yavru korm verimi) için geciktirici bir rol oynarken, en yüksek bitki boyu (10 mg L^{-1} de 60.98 cm ve 5 mg L^{-1} de 57.37 cm) elde edilmiştir. 10 mg L^{-1} paclobutrazol uygulamalarında, diğer uygulamalara ve kontrole nazaran en iyi korm verimine (469 kg da^{-1}) ulaşılmıştır. Zeatin 3 mg L^{-1} konsantrasyonda en iyi nodyum aktivasyonu göstermiştir (2.81 aktif nodyum/korm). Sonuç olarak safran gelişimi önemli derecede dışarıdan toprağa uygulanan bitki gelişim düzenleyicilerinden etkilenmekte olup uygulama metodu gelişimi hedeflenen bitki kısmı göz önüne alınarak geliştirilebilir.

Anahtar kelimeler: *Crocus sativus*, indole-3-butyric acid, paclobutrazol, picloram, zeatin

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INTRODUCTION

Saffron (*Crocus sativus* L.) is an autumn flowering, cormous perennial, Iridaceae family member plant. Although saffron plants have hermaphrodite flowers, there are some explanations about abnormalities at steps related with sexual propagation (Sampathu et al., 1984, Negbi et al., 1989, Renau-Morata et al., 2013) so cultivation of the plants is only possible via corm under field condition. Saffron stigmata is mainly used as medicinal and aromatic purposes. Demand of the stigmata is increasing as new applications in pharmacology emerge (Ríos et al., 1996). For this reason the studies on corm formation have increasing importance to obtain healthy and productive cultivation materials during the last decade. Therefore for this purpose some of the studies (Turhan et al., 2007, Amiri, 2008; Khan et al., 2011, Çavuşoğlu and Sülüşoğlu, 2012) have focused on effect of natural and chemical fertilization by applying to the soil in saffron. On the other hand there are a few studies (Aytekin and Acıkgoz, 2008) related with usage of plant growth regulators for saffron corm production under the field condition. Mostly the studies (Plessner et al., 1990, Sharma et al. 2008, Zeybek et al., 2012, Devi et al., 2011, 2014, Cavusoglu et al., 2013) on the effects of plant growth regulators have been done in *in vitro*.

In some cases results of field experiments with cormous or bulbous plants can be more productive, relaible, efficient and applicable. Based on this idea in the study, the effects of external usage of plant growth regulators directly to the soil, as promoter or retardant under greenhouse condition, on growth characteristics as aerial parts and aboveground parts of saffron were evaluated.

MATERIALS AND METHODS

The greenhouse trial were conducted in two growing season in 2011-2012 and 2012-2013 independently under Kocaeli city in Turkey. Before planting of saffron corms the soil was analysed. For analysis the soil samples taken from in a depth of 0-20 cm. Results indicated that pH: 7.18, E.C: 846 µS/cm, $CaCO_3$: 2.9% and organic material was 3.22%. Saffron corms were provided from any-treated growing areas. 10-24 mm in diameter corms (varied between 0.78 g - 6.44 g) were choosen and placed 10x10 cm distance between and within rows in 5 cm depth in to the soil in 15th of September 2011 and 15th of September 2012. Each sub-parcel was 0.18 m^2 consisted 18 corms in equal size (equal to 299.8 kg corm da^{-1}).

Experiments were conducted in a randomised block design (Figure 1.a) with three replication using 8 different applications (10 or 20 mg L^{-1} paclobutrazol, 1 or 3 mg L^{-1} indole-butyric acid, 1 or 3 mg L^{-1} zeatin and 5 or 10 mg L^{-1} picloram) addition to control. The plant growth regulators [Paclobutrazol ($C_{15}H_{20}ClN_{30}$) MW=293.79 g mol^{-1} ; Indole-3-butyric-acid (IBA) ($C_{12}H_{13}NO_2$) MW=203.24 g mol^{-1}; Zeatin ($C_{10}H_{13}N_5O$) MW=219.2 g mol^{-1}; Picloram ($C_6H_3Cl_3N_2O_3$) MW=241.5 g mol^{-1}] were purchased from chemical componies. Used plant growth regulators were dissolved first in requisite solvents then after completed to 1 liter with distilled water. To each sub-parcel of treatments 1 liter solution and to each control parcel 1 liter distilled water (equal to 3.8 m^3 da^{-1}) was applied directly to the soil at once as soil drench method 45 days after corm planting when initially plant growth observed. At the application time the rooted plants consisted at least 2 leaves, 1 nodium and plant height was approximately 10 cm (Figure 1.b). The treated plots were regularly irrigated with tap water in the same amount weekly.

Arial part characteristics, except flower, as plant height, leaf number and active nodium were recorded every month and data were evaluated when reached the maximum values. Aboveground characterisitics as number, weight, diameter and yield of daughter corm (bigger than 10 mm in diameter) were recorded at normal corm lifting time when leaves completely turned yellow, at 1st week of May 2012 and 2013. Data were statistically analyzed for variance (ANOVA) and mean separation comparisons at $P \leq 0.05$ level performed by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

According to data, characters of aerial and aboveground part of *Crocus sativus* showed different responces to the treatments. Paclobutrazol concentrations had positive responce on daughter corm diameter but had negative effect on plant height. Additionally at concentration of 10 mg L^{-1} paclobutrazol caused an increase in daughter corm number and healthy daughter corm yield statistically than control and the other treatments (Table 1, 2; Figure 1.g). The results are supported by Devi et al. (2011). In their study 1.7 µM and 3.4 µM paclobutrazol gave higher saffron corm production respectively than the other treatments in *in vitro*. The mentioned study was the rare one about paclobutrazol effect on *Crocus sativus*. On the other hand there are some studies on the other cormous, tuberous and bulbous plants. Francescangeli (2009) found that at certain concentration of paclobutrazol had negative effect on bud appearance in *Iris x hollandica* Tub. by immersion methods. Steinitz et al. (1991) studied on *Gladiolus grandiflorus* x *Gladiolus trisitis* hybrid and it was emphasized that paclobutrazol responded limited leaf elongation and caused corm growth at 10 mg L^{-1} paclobutrazol by liquid-shake medium. Nagaraju et al. (2002) also found that effectiveness of 10 mg L^{-1} paclobutrazol and sugar in MS medium on formation of bigger corms in *in vitro* study in gladiolus. In the study on *Caladium bicolor* (Krug et al., 2007) it was found that paclobutrazol provided height control and shorter plants than untreated control by substrate drench method after initially plant development. The used method and result supported to the our experiment.

Indole-3-butyric acid (IBA), is one of the auxin type growth regulators and also showed effects in the saffron growth experiment. Although 1 mg L^{-1} IBA showed more plant height (Figure 1.e), active nodium number, daughter corm number and daughter corm yield than control, its effect was medium when compaered with the other used plant growth regulators (Table 1, 2). According to *in vitro* studies in saffron, when IBA used with 6-benzyladenine (BA), it was resulted that larger leaf lenght (Bhagyalakshmi, 1999). In another study, multiple shoot formation was high at secondary when 3 mg dm^{-3} IBA used with certain BA (Sharma et al., 2008). According to another study 1 mg L^{-1} IBA showed the best corm initiation and corm number than 2 mg L^{-1} IBA and control (Zeybek et al., 2012). Similarly in our study while 1 mg L^{-1} IBA gave the second best responce on corm yield, 3 mg L^{-1} IBA did not showed remarkable results. Application method as soil drench of our study was completely in differ from the most of saffron studies in which IBA was used.

Zeatin is a cytokinin and had remarkable effects on aerial part of saffron in the experiment. Especially 3 mg L^{-1} zeatin statistically effected nodium activation (Figure 1.d) than control and the other treatments (Table 1). Used zeatin concentrations gave lesser daughter corm weight and diameter than control and other used growth regulators when picloram treatments were ignored (Table 2). Plessner et al. (1990) found zeatin at 3 mg L^{-1} concentration effective on leaf number, leaf lenght and corm diameter in *in vitro* as well as other treatments.

Picloram has auxin-like properties (Colins et al., 1978) and mainly known with its herbicidal activities (WHO, 2009) especially on woody plants and broad-leaved weeds (IARC, 1991). At the same time picloram can be used in plant tissue culture tests for somatic embryogenesis in saffron (Devi et al., 2014) and in *Agapanthus praecox* ssp. *minimus* (Yaacob et al., 2012) for shoot proliferation in *Lilium michiganense* (Ault and Siqueira, 2008). Beside the monocots it also gave responce on somatic embryogenesis of *Theobroma cacao* L. (Zuyasna et al., 2012). According to our results used picloram doses showed the highest plant height, the lowest nodium activation and leaf number (Table 1). But according to personel observation plant aerial parts were not healthy looking (Figure 1.c). Growth of aboveground part of saffron also had the lowest values in number, weight, diameter and yield of daughter corm (Table 2). In addition daughter corms had dry-necrosis (Figure 1.f). It can be thought that this systemic chemicals strictly absorbed from soil and caused harmful effect at this concentrations under the used methods.

Table 1. Results of data analysis for effect of growth regulators on aerial part characters in saffron*

Plant Growth Regulators		Plant Height (cm)	Activated Nodium Number (number/corm)	Leaf number (leaves/corm)
Control		48.78 d	2.51 abcd	5.64 a
Paclobutrazol	10 mg L^{-1}	45.93 e	2.43 bcde	5.65 a
Paclobutrazol	20 mg L^{-1}	44.97 e	2.23 cde	5.32 a
IBA	1 mg L^{-1}	53.72 c	2.76 ab	5.62 a
IBA	3 mg L^{-1}	49.37 d	2.49 abcde	5.19 a
Zeatin	1 mg L^{-1}	50.08 d	2.56 abc	5.30 a
Zeatin	3 mg L^{-1}	49.68 d	2.81 a	5.62 a
Picloram	5 mg L^{-1}	57.37 b	2.15 e	4.64 ab
Picloram	10 mg L^{-1}	60.98 a	2.19 de	4.12 b
		LSD=2.134 Sx̃ : 0.7183	LSD=0.31116 Sx̃ : 0.1049	LSD=0.8907 Sx̃ : 0.2998

*Values followed by different letters differ from each other at $P \leq 0.05$ (Duncan's Multiple Range Test)

Table 2. Results of data analysis for effect of growth regulators on corm related characters in saffron*

Plant Growth Regulators		Daughter Corm Number (number/corm)	Daughter Corm Weight (gr/daughter corm)	Daugher Corm Diameter (mm/daughter corm)	Daughet Corm Yield (kg/da)
Control		1.53 bcd	2.46 a	15.26 ab	372 bc
Paclobutrazol	10 mg L^{-1}	1.79 a	2.60 a	15.57 a	469 a
Paclobutrazol	20 mg L^{-1}	1.11 bc	2.48 a	15.43 a	387 bc
IBA	1 mg L^{-1}	1.69 ab	2.47 a	14.82 abc	421 ab
IBA	3 mg L^{-1}	1.43 cd	2.40 ab	14.82 abc	342 cd
Zeatin	1 mg L^{-1}	1.35 d	2.25 bc	14.63 bcd	304 d
Zeatin	3 mg L^{-1}	1.71 ab	2.12 c	14.34 c	369 bc
Picloram	5 mg L^{-1}	0.71 e	1.35 d	13.37 d	96 e
Picloram	10 mg L^{-1}	0.43 f	1.26 d	11.94 e	55 e
		LSD=0.177 Sx̃ : 0.0595	LSD=0.1955 Sx̃ : 0.06580	LSD=0.7023 Sx̃ : 0.2364	LSD=48.21 Sx̃ : 16.23

* Values followed by different letters differ from each other at $P \leq 0.05$ (Duncan's Multiple Range Test)

Figure 1. Experimental steps; a) One of the experimental block of randomized design, b) Plant condition at application time of plant growth regulators, c) Effect of 5 mg L^{-1} picloram on saffron aerial part, d) Nodium activation at 3 mg L^{-1} zeatin, e) Plant height measurement at 1 mg L^{-1} IBA, f) Effect of 10 mg L^{-1} picloram on saffron underground (corm) part, g) Effect of 10 mg L^{-1} paclobutrazol on saffron underground (daugher corm) part

CONCLUSIONS

In brief the experiment can be concluded that the experiment is one of the rare studies in methodological terms that using directly in to the soil as soil drench method for some used the plant growth regulators. When all data taken into consideration; paclobutrazol has an effect on plant height control and maximum corm production at used concentration. IBA also have effect at certain concentration on corm yield. Zeatin can be used in nodium activation. Picloram was found harmful at the both of used concentrations on underground part. After the premise experiment, in future work, a series of concentration of the growth regulators in different growing media with different application methods should be studied without ignoring toxicity and translocation of the plant growth regulators in saffron especially in stigma that is mainly used part. In addition economic analysis should be worked for the used methods with the used plant growth regulators in saffron.

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