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Full Length Research Paper

Effect of nano silver and silver nitrate on seed yield of borage

Seif Sahandi M[1], Sorooshzadeh A.[1]*, H. RezazadehS[2] and Naghdibadi H. A.[2]

[1]Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.
[2]Institute of Medicinal Plants, ACECR, Tehran, Iran.

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Borage plant (*Borago officinalis* L.) is an herbaceous annual plant. It was used as a medicinal plant since ancient times. Seed oil of this plant has been used to treat many skin disorders. It is the richest plant source of gamma-linoleic acid (GLA). However, seed production of borage is limited by flower and seed abscission. Ethylene is responsible for plant organs abscission which may be inhibited by silver ion (Ag+). Therefore, this experiment was designed to study the effect of nano silver and silver nitrate on abscission and yield of seed in borage. The study was carried out in a randomized block design with three replications. Four levels of either silver nitrate (0, 100, 200 and 300 ppm) or nano silver (0, 20, 40, and 60 ppm) were sprayed on borage plant at seed growth stage. The results showed that there was no significant difference between 100 ppm of silver nitrate and 60 ppm concentration of nano silver on the shoot silver concentration. However, increasing the concentration of silver nitrate from 100 ppm to 300 ppm caused a decrease in seed yield. On contrast, a raise in the concentration of nano silver from 20 ppm to 60 ppm has led to an improvement in the seed yield. Additionally, the lowest amount of seed yield was found with control plants. Finally, with increasing level of silver nitrate, the polyphenols compounds content were raised, but the enhancing level of nano silver resulting in the reduction of these components. In conclusion, nano silver can be used instead of other compounds of silver.

Key words: Borage, nano-silver, silver nitrate.

INTRODUCTION

Borage (*Borago officinalis* L.) is an herbaceous annual plant from Boraginaceae family which is used in traditional medicines. It is used to treat many disorders such as swelling of limbs, cough and other problems of breathing. Borage seed contain high amount of oil and crude proteins. Seed of borage is the richest plant source of gamalinoleic acid (GLA) (constituted 20 to 30% of seed oil). It is reported that seed production of borage is limits by flower and seed abscission. The average yield of the seed is estimated approximately 400 kg per hectare but about 320 kg of it is abscised therefore 80 kg of seeds is to be harvested (Naghdi badi et al., 2008).

*Corresponding author. E-mail: soroosh@modares.ac.ir. Tel: +98-21-44196522. Fax: +98-21-44196524.

Phytohormones and environmental stresses are the effective factors in controlling abscission process (Taylor and Whitelaw, 2001). It is demonstrated that the ethylene has an important role in initiation the abscission layer in different plants (Macnish et al., 2004). Ethylene activates the biosynthesis genes of hydrolytic enzymes e.g. cellulose and polygalacturonaze, which induces separation of plant organs from the main plant (Mishra et al., 2008; Wu et al., 2008).

In addition, the abscission process could be regulated by the other phytohormones such as auxin (IAA) and abscisic acid (ABA). The later induces the abscission process through stimulation of the ethylene biosynthesis while auxin is effective in delaying the abscission by reducing the sensitivity of cell to ethylene, (Taylor and Whitelaw, 2001; Mishra et al., 2008; Kushad and Poovaiah, 1984).

Table 1. Analysis of variance mean square testing traits

S.O.V	D.f.	LN	PH	DWP	DWI	SPAD	LL	LW	SY	WS	PP	TN	SC
R	2	66.89^{ns}	35.01^{ns}	5446.60**	1401.66**	7.61^{ns}	1.17^{ns}	1.87^{ns}	9.67**	0.061**	0.68*	0.15^{ns}	282.65*
T	6	316.87**	31.43*	3776.01**	796.02**	5.49^{ns}	0.46^{ns}	0.26^{ns}	30.41**	0.045**	0.36*	0.28**	5888.48**
E	12	31.39	10.45	779.46	117.32	2.65	0.99	0.58	0.94	0.003	0.104	0.045	65.018
CV		10.23	8.74	9.42	5.71	5.31	10.17	14.28	4.67	3.41	11.79	11.9	9.08

* and ** respectively, significant levels of 5 and 1 %. LN = Leaf number, PH = Plant height, DWP = Dry weight of plant, DWI = Dry weight of inflorescence, LL = Leaf length, LW = Leaf width, SY = Seed yield, WS = Weight of 100 seeds PP = Poly phenol, TN =Tannin, SC=Silver concentration.

Abscission may be delay by using some chemical components such as such as α-amino isobutyric acid and cobalt ions, (aminooxy) acetic acid, silver thiosulfate (STS) and sliver nitrate ($AgNO_3$) (Kushad and Poovaiah, 1984; Moore, 2006). Several studies demonstrated that spraying of silver ions decrease the flowers and flower buds abscission in orchid plant (Uthaichay et al., 2007). Additionally, Wagstaff et al. (2005) reported that silver ions decreased 100% flower abscission of Alstroemeria plant as compared to untreated flower within two first days. Moreover, Chang and Chen (2001) showed that ethylene is involved on senescence of flowers in Bougainvillea plant while this process may be postponed by spraying silver thiosulfat.

Nano silver solution consisting silver ions in the size range of 10 to 100 nm and it has more stability in comparison to other solutions. Nano silver particles, also, have more surface area in contact to outer space due to their small size. Thus, the amount of adhesion to the cell surface is increasing which lead to their higher efficacy (Shah and Belozerova, 2008). Additionally, nano-Silver may affect the metabolism, respiration and reproduction of microorganism (Lok et al., 2007). For example, the effect of nano-Silver on extend maintenance period of leaves (from 2 to 21 days) in asparagus plant is reported. Also, during this period the amount of ascorbat, chlorophyll and fiber were more in treated leaves (An et al., 2008).

The effect of silver nitrate in delaying the abscission has been studied (Labraba and Araus, 1991), however, the influence of nano-silver on seed abscission has not been reported. Therefore, this study was carried out to assess the possibility of using nano-silver and silver nitrate in delaying the time of seed abscission in borage plant.

MATERIALS AND METHODS

This study was carried out in a completely randomized block design with 3 replicates in the field research of medicinal plants institute. The data of this study was analyzed by SAS software and the comparison was done according to LSD test method. Four levels of either silver nitrate (0, 100, 200, and 300 ppm) or nano silver (0, 20, 40, and 60 ppm) were sprayed with 0.1% Tween 20 on borage plant at seed growth stage (that is 125 days after cultivation) and were repeated after 2 weeks. The nano silver solution with average particle diameter of 25 nm was obtained from Pars Nano Nasb Company.

Different parameters (that is, leave number, stem height, dry weight of plant, length and width of leaf, dry weight of inflorescence, seed yield, and weight of 100 seeds) were determined; greenness of leaves was measured by chlorophyll meter. The biochemical properties included polyphenol and tannin was determined according to Makkar (2000). Concentration of silver in the plant shoot was measured by “inductively-coupled plasma” according to the method described by Williams et al. (2005). The principal operating parameters of the instrument were as follows: argon gas flow: auxiliary, 1 L/min, nebulizer (crossflow), 0.8 L/min, sample uptake: 60 s. Measurements were carried out in the axial mode at 328.068nm.

RESULTS

Using silver (either as nano silver or silver nitrate) had a significant effect on silver concentration in the plant shoot, the number of leaves, the height of the plant, plant dry weight, inflorescence dry weight, seed yield, weight of one hundred seeds, polyphenol and tannin content in shoot (Table 1). In contrast, the length, width and greenness of leaves have not affected by silver treatment (Table 1). Silver concentration in shoot was increased by all treatments (either nano silver or silver nitrate) however, the higher concentration of silver in the shoot was obtained by silver nitrate treatment in comparison to others (Figure 1). There was no significant difference between 100 ppm of silver nitrate and 60 ppm concentration of nano silver on the shoot silver concentration (Figure 1).

The highest number of leaves was obtained by spraying 100 ppm of silver nitrate, while the lowest was seen in control plants (Table 2). The number of leaves and plant Height were improved by increasing the concentration of nano silver while they were reduced by rising in silver nitrate concentration. The longest height of the plant was obtained with the treatment of nano silver 60 ppm, while the lowest amount of this feature was seen with control (Table 2).

However, by going up the concentration of silver nitrate from 100 to 300 ppm caused a decrease in the dry weight of plant and dry weight of inflorescence. On contrast, a raise in the concentration of nano silver from 20 to 60 ppm has led to an improvement in those parameters

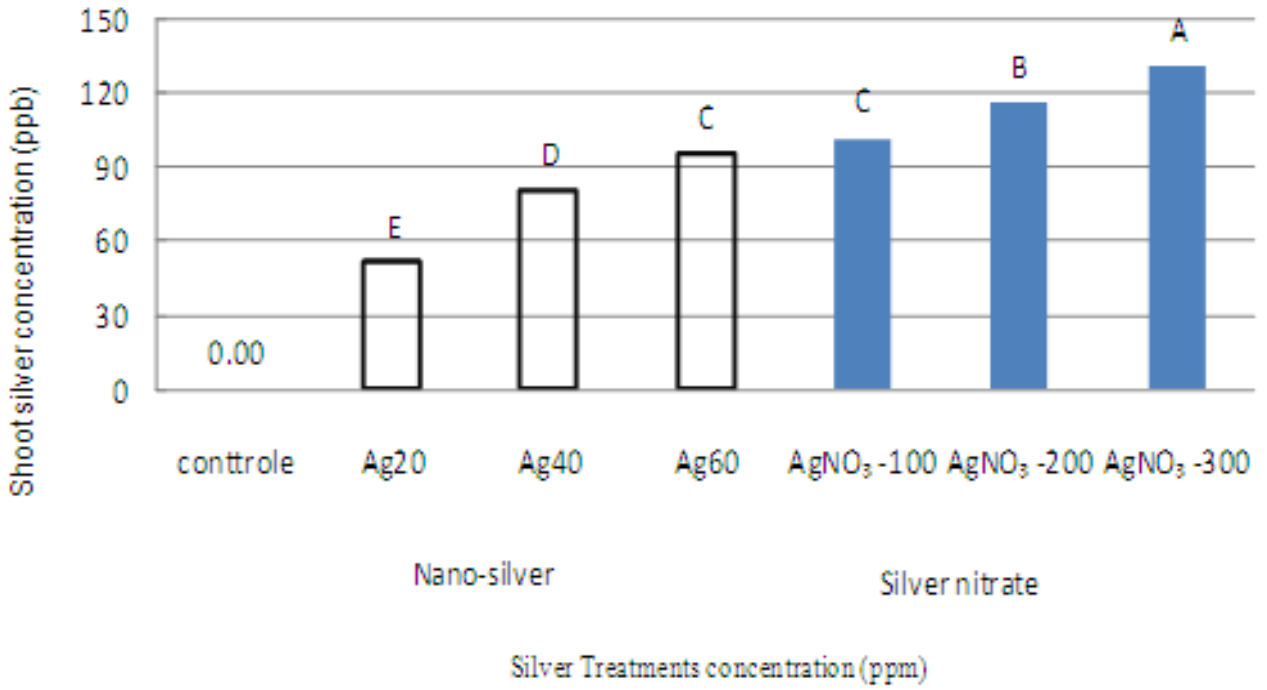

Figure 1. Effect of nano-silver and silver nitrate treatments on concentration of silver in plant tissue (µg/g dry tissue of plant).

Table 2. Comparison mean effect of different concentration of nano silver and silver nitrate on test traits

Treatments	LN	PH (cm)	DWP (g/m^2)	DWI (g/m^2)	SPAD	LL (cm)	LW (cm)	SY(g/m^2)	WS (g)	PP	TN
Control	41.533^{e}	31.977^{c}	247.98^{d}	162.91^{d}	29.857^{b}	9.303^{a}	5.043^{a}	15.64^{e}	2.00^{e}	3.163^{a}	2.223^{a}
NS 20 ppm	46.27^{de}	35.68^{abc}	269.39^{dc}	175.69^{cd}	30.813^{ab}	9.326^{a}	5.370^{a}	18.13^{d}	1.863^{b}	2.943^{ab}	1.986^{ab}
NS 40 ppm	50.067^{cde}	38.393^{ab}	301.36^{abc}	188.13^{bc}	30.837^{ab}	9.503^{a}	5.356^{a}	20.47^{c}	1.760^{bc}	2.783^{abc}	1.780^{bc}
NS 60 ppm	53.367^{cd}	41.447^{a}	348.06^{a}	202.4^{ab}	31.173^{ab}	9.913^{a}	5.703^{a}	22.63^{b}	1.71^{cd}	2.423^{ab}	1.606^{dc}
SN 100 ppm	68.133^{a}	39.813^{ab}	326.80^{ab}	210.47^{a}	33.120^{a}	10.330^{a}	5.730^{a}	24.92^{a}	1.616^{d}	2.210^{c}	1.267^{d}
SN 200 ppm	67.433^{ab}	7.027^{abc}	304.73^{abc}	198.8^{ab}	29.983^{b}	10.096^{a}	5.113^{a}	23.19^{b}	1.716^{cd}	2.596^{abc}	1.673$_{bc}$
SN 300 ppm	57.467^{bc}	34.500^{bc}	279.63^{bcd}	189.20^{bc}	28.783^{b}	9.910^{a}	5.030^{a}	19.76^{cd}	1.786^{bc}	3.050^{a}	1.963^{abc}
LSD	9.966	5.751	49.667	19.269	2.896	1.767	1.355	1.728	0.108	0.574	0.378

Mean values, followed by the same letters in each column are not significantly different (Duncan multiple range test at 5%). NS = Nano Silver, SN = Silver Nitrate, LN = Leaf number, LN = leaf number, PH = Plant height, DWP = Dry weight of plant, DWI = Dry weight of inflorescence, LL = Leaf length, LW = Leaf width, SY = Seed yield, WS = Weight of 100 seeds, PP = Poly phenol, TN = Tannin.

(Table 2). Raising the concentration of silver nitrate from 100 to 300 ppm caused a decline in the seed yield, but an increase in concentration on nano silver from 20 to 60 ppm, lead to arise in the seed yield. The lowest amount of seed yield was found with control samples and the highest was obtained by silver nitrate 100 ppm treatment. However, seed yield was found to be more than the control sample with different concentration of nano silver and silver nitrate (Table 2). The highest weight of 100 seeds was observed in the control sample, while this weight was decreased when either nano silver or silver nitrate was applied (Table 2).

The highest content of polyphenol and tannin was observed in control, the lowest content was observed with silver nitrate 100 ppm (Table 2). The polyphenol and tannin content was raised with the increasing level of silver nitrate, but was decreased with the increase in nano silver. The greenness, length and width of the leaves were not affected by nano silver and silver nitrate treatments (Table 1).

DISCUSSION

Application of silver ions can displace copper ions from the receptor proteins. Consequently, block ethylene perception, since copper ions have a critical role in ethylene binding upon receptors (Khan, 2006; Means et al., 2005; Hedden and Thomas, 2006). This effect of silver ion on ethylene was reported by several researchers (Chamani et al., 2005; Gad and Atta-Aly, 2006; Eo and Lee, 2009; An et al., 2008; Wagstaff et al., 2005; Strader et al., 2009; Reggiani., 2006). Therefore, some results in this study can be referred to the effect of silver in preventing the ethylene action.

Decrease of plant's height due to ethylene is proven. For example, mutants of tobacco and arabidopsis, which synthesize a high concentration of ethylene, have lesser height as compare to their wild species (Pessarakli, 2001). Furthermore, Nichols and Kofranek (1982) reported that silver ion cause increase in stem height of tulips and rose plants. Ouma et al. (2004) reported that under the *in-vitro* conditions, silver nitrate inhibited biosynthesis of ethylene, and caused regeneration of multiple shoots from hypocotyl sections of cotton. Another researcher reported that silver nitrate is effective on increasing wet weight of tobacco (Tso et al., 1973). Purvis (1980) reported that ethylene caused an increase in enzyme activity of chlorophyllase and destruction of internal membrane of chloroplast, while that 100 ppm of silver nitrate caused decreasing the production of ethylene and destruction of chlorophyll in calamondin fruit.

Labraba and Araus (1991) reported that spraying silver nitrate cause improvement of seed yield in wheat. In the present experiment, foliar application of either nano silver or silver nitrate cause increase in seed yield as compare to control (Table 2). In borage plant, rise in seed yield can be due to increase in the number of inflorescence in unit area, number of seed in the inflorescence, weight of seeds and decrease in seed abscission. Considering that the seed yield has a significant positive correlation with dry weight of inflorescence ($r = 0.808$, $p < 0.0001$), but it has a significant negative correlation with weight of 100 seeds ($r = -0.835$, $p < 0.0001$). Consequently, it can be concluded that the increase of seed yield in this study due to increase of the inflorescence number in unit area and decrease in seed abscission.

Seed abscission is one of the main factors in reducing seed yield in borage plant. It is proven that one of the reasons for plant organ abscission is imbalance between phytohormones. Ethylene is playing an important role in this process. Furthermore, it is proven that silver ions, inhibit the ethylene action by preventing its connection to its receptors in plant cells (Mishra et al., 2008; Kushad and Poovaiah, 1984; Taylor and Whitelaw, 2001). Thus the increase in the seed yield was a result of reducing the seed abscission due to the inhibitory effect of silver on ethylene action. These results are confirmed by results obtained from other studies (Eo and Lee, 2009; Wagstaff et al., 2005; Uthaichay et al., 2007; Labraba and Araus, 1991). There are many reports about the use of silver nitrate in decreasing the abscission but this study is the first research which is about nano silver effect on decreasing the abscission of reproductive organs of plants.

Increasing silver concentration in aerial organs of sprayed plants with nano silver, caused decreasing polyphenol and tannin content. While there was an increased in polyphenol and tannin content by spraying silver nitrate due to rising the silver concentration in aerial organs of plant. There are many reports available about ethylene effect on increasing the phenol content (Cajuste, and Lafuente, 2007; Heredia and Cisneros-Zevallos, 2009; Park, 2008; Lavid et al., 2001). On the other hand, high concentration of heavy metals (that is silver) in plant tissue causes polymerization of phenol by peroxidase enzyme which chelate the heavy metals (Elzaawely et al. 2007; Backor, 2009; Heredia and Cisneros-Zevallos, 2008; Madhavarao et al., 2006; Shi et al., 2006). Therefore, by increasing the level of silver nitrate more than 100 ppm, phenol and tannin content enhance due to toxicity effect of silver on plant cells.

There was no significant difference between 100 ppm of silver nitrate and 60 ppm concentration of nano silver on the shoot silver concentration. Therefore, permeability of nano-silver is far greater than silver nitrate. The reason of this matter is the small size of nano particle, which causes more adhesion of nano particles to plant tissues. By considering the lesser use of silver in nano silver, this treatment can be used instead of other combinations of silver. However, the nano silver effect compared with other silver combinations on reducing the ethylene effect needs more researches.

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