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Effect of different physio-chemical factors on sex expression and fruit yield in greenhouse cucumber

M. Golabadi [1], **P. Golkar** [2 (*)], **A.-R. Eghtedari** [3]

[1] *Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, 81595158 Isfahan, Iran.*

[2] *Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, 8415683111 Isfahan, Iran.*

[3] *Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, 81595158 Isfahan, Iran.*

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(*) Corresponding author:
golkar@cc.iut.ac.ir

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

Abstract: Male flower expression is considered an important aim in greenhouse cucumber breeding for creating paternal lines as a base for hybrid progeny. The study was carried out to evaluate the effects of different treatments on sex expression and fruit yield of cucumber in two different season (autumn-winter and spring-summer), in particular this research focuses on the influence of 1) usage of two chemical agents: silver thiosulphate [$Ag(S_2O_3)_2^{-3}$] and silver nitrate ($AgNO_3$) at different concentration, respectively 200 and 500 ppm and 100, 200 and 300 ppm 2) plant development stages at the moment of the treatment (5, 10, and 15-leaf growth stages) and 3) application of single or double sprayings. Analysis of variance showed that season, chemical applications and number of spaying had significant effect on the induction of a higher number of male flowers. A positive significant effect of season suggested that longer days and higher temperature promote the formation of male flowers in cucumber. This study showed that male flower production was induced by all concentrations of silver ions, especially high concentrations. Important traits related to change of sex expression such as the number of days to male flowering and the number of male flowers are more affected by different interactions of studied factors in contrast to vegetative and yield related traits. Also, the quadruple interaction effects indicated that silver ion could change sex expression at higher temperatures and longer days (second season in summer) with high concentration when applied in 15 leaf stage for $AgNO_3$ and 5 and 15 leaf growth stage for $Ag(S_2O_3)_2^{-3}$ with double spraying. Consequently, female lines can be induced to male flowering with silver ions, thus increasing the feasibility of large scale seed production of gynoecious × gynoecious cucumber hybrid.

1. Introduction

Commercial cucumber (*Cucumis sativus* L., 2n= 2x= 14) is a member of cucurbitaceae that is indigenous to India (Renner *et al.*, 2007). It is one of

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the most economically important cucurbit vegetable plants (Tatlioglu, 1993, Robinson and Decker-Walters, 1997) and among the most widely-grown vegetable crop in the world, after tomato, onion and cabbage (Plader *et al.*, 2007). Cucumber is used in different types (Harvesting, slicing and fresh eating) that are used as fresh or processed vegetable (Shetty and Wehner, 2002). The cucumber is a thermophilic and frost- susceptible crop, usually cultivated in fields during the spring-summer period. Its high demand has also made it an important crop to be widely grown in glasshouses or plastic houses (Sarkar and Sirohi, 2011). Sex expression is an important factor that has a positive effect on yield and that constitutes a major component of cucumber improvement programs (Yamasaki *et al.*, 2003). In gynoecious cultivars, all flowers are female, so, male flowers are required for new line production via female flowers crossing. The sex expression of *Cucumis sativus* L. is determined by genetics as well as environment (e.g. photoperiod, temperature) (Yamasaki *et al.*, 2003; Bano and Khokhar, 2009). Change from vegetative growth to reproductive stages is a complex process regulated by many factors, and could be influenced by the application of plant growth regulators (Ainsworth, 1999; Sure *et al.*, 2013). Growth regulators have tremendous effects on sex modification and flowering in cucurbits that leading to either suppression of male flowers or an increase in the number of female flowers (Al-Masoum and Al-Masri, 1999). Some researchers have already reported the effects of plant growth regulators on modification of sex expression in cucumber flowers (Vadigeri *et al.*, 2001; Rafee kher *et al*., 2002; Bano and Khokhar, 2009).

Line production is important in breeding programs, because many cultivars in cucumber are hybrids (Golabadi *et al.*, 2015). In gynoecious cucumbers, male flower induction is necessary for production of F_1 hybrid seeds (Yamasaki *et al.*, 2003; Wang *et al.*, 2011). Therefore, change of sex expression in gynoecious cucumber is necessary.

In general, auxin, ethylene and cytokinins promote female sex expression in various monoecious and dioecious systems (Mohan Ram and Sett, 1982). Exogenous application of plant growth regulators could alter the sex ratio if applied at the two- or four-leaf stage, which is the critical stage at which the suppression or proportion of either sex is possible (Hossain *et al.*, 2006). The effect of ethylene can be antagonized by specific ethylene inhibitors (Hirayama and Alonso, 2000). Silver ions (Ag^+) restrain the physiological effect of the ethylene by blocking the ethylene receptors (Hirayama and Alonso, 2000). It is known that silver ions (Ag^+) applied as silver nitrate ($AgNO_3$) or as silver thiosulfate [$Ag(S_2O_3)_2^{-3}$] replace copper ions (Cu^+) which is a part of the ethylene receptor preventing the receptor from responding to ethylene (Abeles *et al.*, 1992). Another inhibitor of the ethylene action, $AgNO_3$, suppresses the development of female flowers and induces the male ones (Kumar *et al.*, 2009; Stankovic and Prodanovic, 2002). Male flower formation increased proportionally with the concentration of $AgNO_3$ applied (Stankovic and Prodanovic, 2002).

According to previous reports, $AgNO_3$ induced more male flowers than GA_3 on gynoecious breeding lines of cucumber (Kalloo and Franken, 1978) and summer squash (Yongan *et al.*, 2002). The positive effects of $AgNO_3$ on male flower production in cucumber have reported by Karakaya and Padem (2011). Thappa *et al*. (2011) used different plant growth regulators (ethephon, naphthalene acetic acid and maleic hydrazide) at the two, four and six-leaf and full-bloom stages in cucumber to induce male flowers. Law *et al*. (2002) reported that silver thiosulfate, an ethylene inhibitor, enhanced stamen development in female white campion (*Silene latifolia*). However, little effort seems to have been directed toward the study of the effects of silver ions on male flower production sprayed at different growth stages and if the numbers of spray applications could improve the effectiveness of the induction. On the other hand, it seems that there are few studies that correlate the effect of environmental conditions, especially season, to the number of spraying and leaf-stage growth on male flower induction of plant treated with silver ions.

Although silver is a toxicity ion, however the amount of its toxicity depends on species, silver concentration, times of application and plant growth stage spraying and exposure media. For exmple Fuente *et al*. (2014) applied different concentrations of $AgNO_3$ (0, 30, 60, 90, 200 mg l^{-1}) at intervals of 8 days throughout the crop cycle (90 days) in watermelon. Their results showed that silver accumulate in root more than other parts of plants and antioxidant in fruits increased in plants exposed to 30 mg l^{-1} $AgNO_3$, but lycopene content decreased.

Therefore the main objectives of this study was to investigate the effects of chemical application [$AgNO_3$ and $Ag(S_2O_3)_2^{-3}$], different leaf growth stages and number of spraying on flower and fruit characteristics of greenhouse cucumber in two different seasons to identify the best condition and the optimal level of chemical application to boost the formation of male flowers. Our findings will help to develop practical recommendations on these issues for improve male flowers production in cucumber breeding programs. Furthermore, this work aimed to evaluate the stability of sex expression of a gynoecious cultivar Adrian in different seasons. The present investigation was done as a first step to a hybridization program of cucumber.

2. Materials and Methods

Planting material and field evaluation

The seeds of cv. Adrian as a gynoecious genotype were sown at the mid autumn of 2014 and mid spring of 2015 at Research Greenhouse of Agriculture Department at Islamic Azad University Isfahan Branch, Isfahan, Iran (51° 36´ longitude and 32°63 latitude). The soil used was loam with pH 7.7 and Electrical conductivity (EC) of 4.1 (ds/m). Seeds were planted to soil directly and covered with peat moss and coco peat. Plants were disposed in two rows, where the spaces between cucumber seedling was 50 cm and within the rows were 90 cm respectively, and 180 cm was left between every couple rows. The greenhouse air temperature at the growing period was maximum 29°C/18°C (day/night) in first season (autumn-winter) and 34°C/20°C (day/night) in second season (spring-summer) with a relative humidity of about 55% and 60%, respectively. Nutrient levels in the irrigation solution water were N:216 (mg l^{-1}), P:58 (mg l^{-1}), K:286 (mg l^{-1}), Ca:185 (mg l^{-1}), Mg:185 (mg l^{-1}), S:43 (mg l^{-1}), Fe:5.59 (mg l^{-1}), Mn:1.97 (mg l^{-1}), B:0.7 (mg l^{-1}), Zn:0.2 (mg l^{-1}), Cu:0.07 (mg l^{-1}) and Mo:0.05 (mg l^{-1}). Different fertilizers were used based on soil analysis that included: potassium nitrate, ammonium nitrate, magnesium nitrate, iron and other mineral elements such as sulphate dissolved in the irrigation water. Dichlorvos, Trigard, Abamectin and organic neem oil were applied for insect control. The same fertilizers and pest management were used for all plants in the same time. Drip irrigation was applied when needed. The source of water was urban water with electrical conductivity (EC) of 0.4 (ds/m). The pH of the irrigation water was adjusted to 6.5 by adding nitric acid.

Silver application

Different chemical treatments including $AgNO_3$ [h_1=100 ppm (0.59 mM), h_2=200 ppm (1.18 mM) and h_3=300 ppm (1.77 mM)], and $Ag(S_2O_3)_2^{-3}$ [h_4=200 ppm (0.6 mM) and h_5=500 ppm (1.5 mM)] and control (h_6) were applied to induce changes in sex expression. $AgNO_3$ was purchased from Merck Chemical Company and $Ag(S_2O_3)_2^{-3}$ was synthesized according to Law *et al.* (2002). The solutions were applied as a spray on the whole plant. Water was sprayed on the control plants. Spraying was accomplished at 5, 10, and 15-leaf growth stages (five leaves stage: LS_1, ten leaves stage: LS_2, and fifteen leaves stage: LS_3). Chemical treatments were employed in single or double spraying at one week interval. $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ applications were conducted early in the morning (before sunrise) to avoid plant sunburn. Each treatment was applied with adequate amounts of the solutions to assure that all the leaves were completely wetted in each spray event. The total time of experiment in every season was about 4 months after sowing and measurement of traits were done in this period.

Studied traits

The traits of interest were measured on eight plants per replication and included: days to male flower expression (days to first male flower appearance after sowing) (DMF); the node number that the first male flower was appear (NNMF); the mean of internode length (the mean of five internode from node number 15 to 20) (IL); the number of male flower (total male flower that opened in whole plant) (NMF), male flowering period (days from observation the first male flowering until the last male flowering observation (MFP); male flower diameter (was recorded at the widest points) (MFD); single fruit weight (from dividing total fruit weight to total fruit number in every harvesting) (SFH); fruit number per harvesting (FNH); fruit weight per harvesting (FWH) (the numbers and the weights of all the fruits harvested in each plot in each harvesting).

Statistical analysis

The experiment was conducted as a combined analysis of variance with three different factors (chemical treatments, leaf growth stage, and number of spraying) under two different seasons. The data collected were subjected to analysis of variance (ANOVA) based on a completely randomized block design with three replications using the general linear model (GLM). Statistical analysis system program (SAS Ver. 9) was used for data analysis. The differences between applications were decided on the basis of the Least Significant Difference (LSD) test

(P<0.05) according to their importance at the 0.05 confidence level.

3. Results and Discussion

The result of analysis of variance based on a factorial experiment is presented in Table 1. According to Table 1, the season (S) had significant effect on all of the studied traits, except for single fruit weight. This result is logical, since phenotypic expression of sex is strongly modified by environmental and hormonal factors (Mohan Ram and Sett, 1982). Long days, high temperature, and silver ion promote formation of male flowers, whereas short days and low temperature promote the formation of female flowers (Perl-Treves, 1999).

Clearly, chemical treatments (CH) had significant effects on all the traits studied, except for fruit number per harvesting (Table 1). Number of spraying (single or double) also had significant effects on the days to male flowering, the node number of first male flower, the mean of internode length, the number of male flower and male flowering period (Table 1). Hallidri (2004) reported that increased number of spraying events had significant effects on male flower production. He suggested that doses of 400 and 500 ppm of $AgNO_3$ produced the highest number of male flowers. The leaf growth stage (LGS) of spraying showed significant differences on days to male flowering, the node number of first male flower, male flowering period, male flower diameter and fruit weight per harvesting (Table 1).

The interaction effect of environment × silver application had a significant effect on all traits with the exception of male flower period and fruit number per harvesting (Table 1). Interaction of season × leaf stage was significant for days to male flowering, the node number of first male flower, the number of male flower and male flower diameter. Interaction of number of spray events × chemical application had a significant effect on days to male flowering, the node number of first male flower, the number of male flower and single fruit weight (Table 1). Interaction of chemical application × leaf stage of spraying showed significant differences on days to male flowering and the node number of first male flower (Table 1). The interaction of environment × chemical application × leaf stage of spraying showed significant difference on days to male flowering, the node number of first male flower, the number of male flower and period of male flowering. Study of these interactions

Table 1 - Analysis of variance for single and combined effects of different studied traits in greenhouse cucumber

Source of variation	Mean squares of studied traits									
	DF	DMF	NNMF	IL	NMF	MFP	MFD	SFW	FNP	FNH
Season (S)	1	**	**	**	*	**	**	NS	**	**
Replication (season)	4	NS	NS	**	NS	NS	NS	**	NS	NS
Chemical treatments (CH)	5	**	**	**	**	**	**	**	NS	**
Number of spraying (S0)	1	**	*	**	**	**		NS	NS	NS
Leaf growth stage (LGS)	2	**	9**	NS	NS	**	**	NS	NS	NS**
SP × CH	5	**	**	*	**	NS	**	**	NS	NS
S×SP	1	NS	NS	NS	*	NS	*	NS	NS	**
S×LGS	2	**	**	NS	*	NS	**	NS	NS	NS
CH×SP	5	*	*	NS	**	NS	NS	*	NS	NS
CH ×LGS	10	*	**	NS	NS	NS	NS	NS	NS	NS
SP×LGS	2	NS	NS	NS	NS	*	NS	NS	NS	NS
S×CH×SP	5	NS	NS	NS	NS	NS	NS	NS	NS	NS
S×CH×LGS	10	**	**	NS	**	*	NS	NS	NS	NS
CH×SP×LGS	10	NS	NS	NS	NS	NS	NS	*	NS	NS
S×SP×LGS	2	NS	NS	NS	NS	*	NS	NS	NS	NS
S×CHSP×LGS	10	NS	NS	NS	NS	NS	NS	NS	NS	NS
Residual	140	NS	NS	NS	NS	NS	NS	NS	NS	NS

DF= Degree of freedom; S= season; CH= Chemical treatments [Silver nitrate ($AgNO_3$) and Silver thiosulphate $Ag(S_2O_3)_2^{-3}$); SP= number of spraying (single or double); LGS= Leaf growth stage (5, 10 and 15).
DMF= days to male flowering; NNMF= the node number of first male flower; IL= the mean of internode length (cm); NMF= the number of male flower; MFP= male flowering period; MFD= male flower diameter (cm); SFW= single fruit weight (g); FNH= fruit number per harvesting; FWH= fruit weight per harvesting (g).
NS= non significant. *, ** significant at P<0.05 and P<0.01, respectively.

showed that important traits related to change of sex expression such as days to male flowering, node number of first male flower, and number of male flower more affected by different interactions in contrast to vegetative and yield related traits. Therefore selection of best treatments should be done based on other treatments. For example numbers of male flower are affected by silver application, leaf stage, number of spraying and all their interactions. There was no significant difference for other triple interactions and environment × chemical application × leaf stage of spraying × number of spray events interaction in all of the studied traits.

Effects of season on the studied traits

The comparison between two seasons showed that the first season had superior mean on days to male flowering, the node number of first male flower, male flower diameter and single fruit weight. It could be concluded that in the first season male flowers expressed later than second season and therefore DMF and NNMF were increased (Table 2). On the other hand, in second season the number of male flower was increased (from 36 to 100 male flowers) and also the time of male flowering was earlier that is related to high temperature and long days. This result showed that environmental conditions, especially low temperature, could retard male flower formation and confirmed also that cucumber is a thermophilic plant. Accordingly, Stankovic and Prodanovic (2002) reported that sowing season affected the number of male flowers. The interaction effect of season × chemical application × leaf stage was significant for number of male flower, days to male flowering and period of male flowering that all of them are related to sex expression, even though this interaction was not significant for vegetative related traits (Table 2).

Effects of silver application on the studied traits

In this study, different doses of chemical treatments [$AgNO_3$ and $Ag(S_2O_3)_2^{-3}$] were used. The mean comparisons showed that chemical application increased the mean values of days to male flowering (Table 3). There was a narrow variation in days to male flowering between different chemical treatments. The least day to male flowering was at a high concentrations of $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ (Table 3). This result showed that high concentrations of different chemicals agent caused earlier male flowering.

Table 2 - The mean comparison of different studied traits in cucumber in different seasons

Season	Traits								
	DMF	NNMF	IL	NMF	MFP	MFD	SFW	FNH	FWH
S1	23.08 a	20.17 a	7.13 b	36.24 b	12.20 b	5.46 a	73.17 a	1.93 b	138.80 b
S2	19.89 b	11.96 b	7.63 a	99.78 a	13.35 a	4	74.95 a	3.60 a	272.96 a

DMF= days to male flowering; NNMF= the node number of first male flower; IL= the mean of internode length (cm); NMF= the number of male flower; MFP= male flowering period; MFD= male flower diameter (cm); SFW= single fruit weight (g); FNH= fruit number per harvesting; FWH= Fruit weight per harvesting (g).
Means followed by the same letter in each column were not significantly different at 0.05 level using LSD test.

Table 3 - The effects of various chemical treatments application on studied traits in cucumber

Traits	Control	Chemical treatments				
		$AgNO_3$			$Ag(S_2O_3)_2^{-3}$	
		100 ppm	200 ppm	300 ppm	200 ppm	500 ppm
Days to male flowering	0.23 c	26.41 a	26.08 a	25.04 b	26.02 a	25.17 b
Node number of male flower	0.13 b	20.18 a	19.11 a	19.23 a	19.08 a	18.68 a
The mean of internode length	6.25 b	7.38 a	7.8 a	7.64 a	7.28 a	7.65 a
The number of male flower	0.18 d	20.89 cd	68.07 b	129.76 a	44.79 bc	144.4 a
Male flowering period	0.074 e	10.16 d	16.13 bc	17.366 ab	14.71 c	18.21 a
Male flower diameter	3.29 d	5.36 c	5.45 bc	5.65 ab	5.36 c	5.71 a
Single fruit weight	84.7 a	73.9 b	70.95 b	72.15 b	70.68 b	71.89 b
Fruit number per harvesting	2.69 a	2.71 a	2.65 a	2.88 a	2.84 a	2.84 a
Fruit weight per harvesting	219.74 a	205.88 a	190.87 a	212.9 a	199.72 a	206.22 a

Means followed by the same letter in each row were not significantly different at 0.05 level using LSD test.

The highest value for male flower diameter observed at 500 (ppm) of $Ag(S_2O_3)_2^{-3}$ and 300 (ppm) of $AgNO_3$ (Table 3). Therefore these concentrations of $Ag(S_2O_3)_2^{-3}$ and $AgNO_3$ could affect both vegetative and reproductive growth of flower. However there was no significant difference between all treatments for internode length. Hallidri (2004) reported that the greatest number of staminate nodes was produced on plants sprayed two or three times with 400 to 500 (ppm) of $AgNO_3$. Rafee Kher *et al*. (2002) reported that 200 (ppm) of GA increased the length of internode in cucumber. According our results the highest value for the number of male flower was denoted at 300 (ppm) of $AgNO_3$ and 500 (ppm) of $Ag(S_2O_3)_2^{-3}$ about 130 and 144 male flower, respectively. Law *et al.* (2002) reported that application of $Ag_2S_2O_3$ produced stamens in female flowers of white campion (*Silene Latifolia)* with longer filaments and larger anther locules. Yongan *et al*. (2002) reported that $AgNO_3$ had more significant effects than GA_3 on male flower production in summer squash. Stankovic and Prodanovic (2002) reported that increasing concentrations of $AgNO_3$ from 0.01% to 0.04% enhanced the number of male flowers in gynoecious lines, which is similar with the results obtained in the present study. Jadav *et al.* (2010) reported that ethrel (200 ppm) had the greatest effect on male flower production among the hormones they investigated (GA_3, ethrel, naphthalene acetic acid, and absisic acid). Jutamanee *et al.* (1994) reported that the effects of different doses of GA_3 and $AgNO_3$ depended on the genotype and photoperiod. Similar to their results, $AgNO_3$ in the present study induced the formation of male flowers in all concentration. Hallidri (2004) reported the concentration of 100 (ppm) of $AgNO_3$ was ineffective on male flower induction. Kalloo and Franklen (1978) reported that different doses of $AgNO_3$ (50, 200, and 500 mg l^{-1}) led to non-significant effects on male flower production, fruit weight and fruit number. They showed similar trends in all the treatments. However in this study all concentrations of $AgNO_3$ (100, 200, 500 ppm) produced male flower with significant differences. One explanation which might account for disparities observed between experiments was the difference in average quanta of solar radiation received by the plants during two different environmental conditions in two seasons. This result confirms the inducing effects of different chemical compounds used on male flower production. The highest value for single fruit weight was observed at control. Mean comparison showed that chemical application has reduced the mean of single fruit weight in comparison to control, because production of male flower in every treated plant will reduce female flower number in contrast to control. There were no significant differences between all treatment for fruit number per Harvesting and fruit weight per harvesting (Table 3). So, this result could demonstrated that chemical application has only effects on flower number and its sex expression.

Effects of number of sprays on the traits studied

Number of sprays (single or double) showed significant effects on days to male flowering, the node number of first male flower, the number of male flower and period of male flowering (Table 4). According to Table 4, double spraying treatment gave rise to higher mean values for days to male flowering, the node number of first male flower, the number of male flower and period of male flowering. On the other hand, double spray treatment was found to affect only sex expression while it had no effect on the traits related to morphology and fruit yield.

Effects of leaf stage on the studied traits

The comparison of the mean values for the studied traits at different stages of leaf growth [Five leaf

Table 4 - The effects of single and double spraying on studied traits in cucumber

Number of Spraying	Traits								
	DMF	NNMF	IL	NMF	MFP	MFD	SFW	FNH	FWH
Single spraying (SP)	20.85 b	15.49 b	7.31 a	49.15 b	11.61 b	5.11 a	74.04 a	2.76 a	205.96 a
Double spraying (DP)	22.13 a	16.64 a	7.44 a	86.86 a	13.94 a	5.16 a	74.08 a	2.77 a	205.82 a

DMF= days to male flowering; NNMF= the node number of first male flower; IL= the mean of internode length (cm); NMF= the number of male flower; MFP= male flowering period; MFD= male flower diameter (cm); SFW= single fruit weight (g); FNH= fruit number per harvesting; FWH= fruit weight per harvesting (g).
Means followed by the same letter in each column were not significantly different at 0.05 level using LSD test.

stage: LS_1, Ten leaf stage: LS_2, and Fifteen leaf stage: LS_3] is presented at Table 5. The highest mean for DMF and NNMF were observed at LS_3 stage (Table 5).This logical result is resulted from the most distance (days) between planting time to fifteen leaf stage. On the other hand, the highest DMF at 15 leaf growth stage belonged to 200 ppm $Ag(S_2O_3)_2^{-3}$ treatment (Fig. 1). El-Ghamriny *et al.* (1988) confirmed that sex differentiation in cucumber takes place at the 2-true leaf stage and that this was the best time for studying the effects of growth regulators on sex expression. There was no-significant difference between three leaf growth stages for number of male flower (Table 5). Therefore use of silver ions at different three growth stages had similar effects on modification of flower sex type in cucumber. Therefore, if it is necessary to separate monoecious or androecious from gynodioecious plant types in breeding programs (for example in line production), breeder could apply these chemical agents at 15 leaf stage, when sex form of plant has been expressed. Raymond (2004) proposed that 1000 ppm of GA_3 at the 2-leaf stage had the best effect on male flower production with 3 hormone applications at 2-week intervals in cucumber. Also, the highest values for period of male flowering and male flower diameter were obtained at the five leaf stage; that is logical, since the period between male flower observation and end of male flowering in LS_1 was higher than LS_2 and LS_3. As already mentioned, male flower diameter was greater at the 5-leaf stage, which might be caused by the higher production of pollen and the increased capacity for crossing in the breeding programs.

Effect of different factor interactions on number of male flower

As the number of male flower trait is the most important trait related to male flower induction in cucumber, the different significant interaction effects

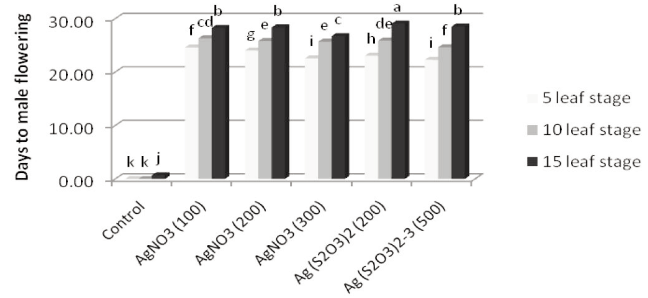

Fig. 1 - The interaction effect of chemical treatment × leaf growth stage on the days to male flowering in cucumber

in this trait was assayed (Table 2). The highest number of male flower was recorded at double spraying in 500 ppm of AgS_2O_3 and double spraying at 300 ppm of $AgNO_3$, respectively of 187.36 and 179 flowers (Fig. 2 a). In the two chemical treatments at higher concentration, double spraying caused increasing of male flower number, although differences between one and two spraying in low concentrations of chemical agents was not significant. The highest (119.2) and the least (24) number of male flower was observed at second season and the first season at fifteen leaf stage, respectively (Fig. 2 b). In all stages, high number of male flower was observed in second season and pointed out significant differences with first season. Also, the most number of male flowers was obtained in high concentration of chemical treatments at the second season, however in all chemical treatments the number of male flowers was more than at second season (Fig. 2 c). Again, the second season showed the highest number of male flower with double spraying in contrast to first season, although double spraying in two seasons was more than single spraying (Fig. 2 d). The triple interaction effects of season, leaf growth stage and chemical treatment on male flower number showed that male flower number in all three leaf growth stages and all chemical treatments under second season were more than first season, specially high concentration

Table 5 - The mean comparison for some of the studied traits in different leaf growth stages in cucumber

Developmental stages	Traits								
	DMF	NNMF	IL	NMF	MFP	MFD	SFW	FNH	FWH
Five leaf stage (LS1)	19.44 c	8.73 c	7.55 a	68.94 a	14.21 a	5.40 a	74.63 a	2.88 a	216.06 a
Ten leaf stage (LS2)	21.40 b	17.10 b	7.40 a	63.47 a	12.40 b	5.05 b	73.00 a	2.69 a	195.85 a
Fifteen leaf stage (LS3)	23.62 a	22.38 a	7.18 a	71.60 a	11.71 b	4.95 b	74.55 a	2.73 a	205.76 a

DMF= days to male flowering; NNMF= the node number of first male flower; IL= the mean of internode length (cm); NMF= the number of male flower; MFP= male flowering period; MFD= male flower diameter (cm); SFW= single fruit weight (g); FNH= fruit number per harvesting; FWH= fruit weight per harvesting (g).
Means followed by the same letter in each column were not significantly different at 0.05 level using LSD test.

of chemical treatments in fifteen leaf growth stage under second season (Table 6). These results demonstrated that the second season had progressive effect on induction and development of male flower and environmental condition could be having a significant effect on this phenomenon. As mentioned before, in second season the temperature of greenhouse was higher than first season and also days were longer. These two reasons changed sex expression in second season more than first season.

4. Conclusions

Phenotypic expression of sex determining loci in cucumber is strongly modified by environmental, chemical and hormonal factors. The results of the experiment revealed that $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ have similar and positive effects on the male expression of cucumber. This study showed that male flower induction was induced by all concentrations of silver ions. These two chemical agents could induce a high number of male flowers in 300 and 500 ppm concentration [$AgNO_3$ and $Ag(S_2O_3)_2^{-3}$], respectively. Chemical treatments with silver ions as $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ application can, therefore, be recommended for enhancing male flower production in cucumber, if further study will confirm the accumulations of silver in the fruit is negligible for the human health. As the time of $AgNO_3$ spraying in this experiment was only three times, so the toxicity of this ion was low in a short time of period. On the other hand, as this chemical agent only was applied in experimental greenhouse, therefore the fruits of treated

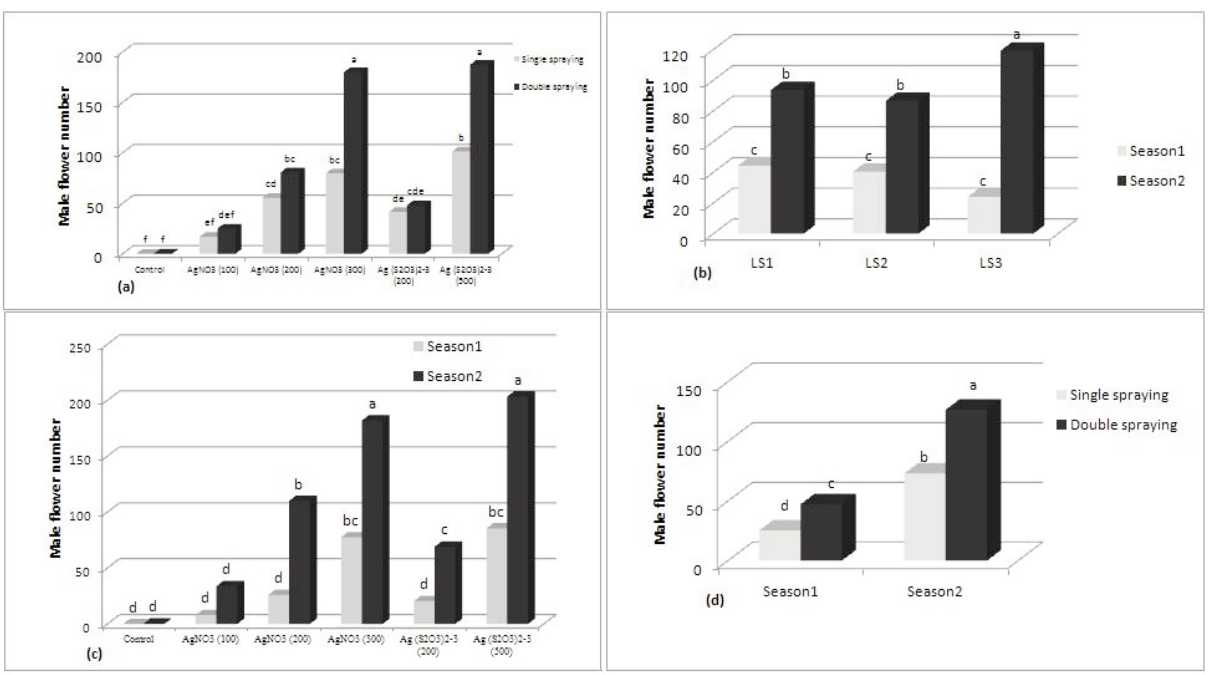

Fig. 2 - The effect of different interactions (a, b, c and d) on the number of male flower in cucumber.

Table 6 - The effects of interaction between chemical treatment, seasons and leaf growth stage on male flower number in cucumber

Chemical treatment	Seasons					
	Season 1			Season 2		
	LS1	LS2	LS3	LS1	LS2	LS3
Control	0.00 n	0.00 n	0.00 n	0.00 n	0.00 n	0.00 n
$AgNO_3$ (100 ppm)	6.83 mn	8.00 mn	9.17 mn	42.42 i-n	31.75 j-n	27.17 k-n
$AgNO_3$ (200 ppm)	43.00 i-n	20.33 lmn	14.67 mn	64.33 h-m	123.33 d-h	142.75 d-g
$AgNO_3$ (300 ppm)	122.33 d-h	91.00 e-j	19.33 lmn	145.33 def	147.42 cde	253.17 a
$Ag(S_2O_3)_2^{-3}$ (200 ppm)	16.331 mn	26.00 k-n	19.00 lmn	84.58 f-k	38.00 i-n	84.67 f-k
$Ag(S_2O_3)_2^{-3}$ (500 ppm)	77.33 h-l	97.17 e-i	81.83 g-k	224.83 ab	178.75 bcd	206.5 abc

LS1= Five leaf growth stage; LS2= Ten leaf growth stage; LS3= Fifteen leaf growth stage.
Means followed by the same letter were not significantly different at 0.05 level using LSD test.

plants were disposed from toxic effects of Ag ion. Also, the morphologic appearance of the cucumbers showed no abnormality after ion application. Overall, this result indicates that silver ion could change sex expression in higher temperatures and longer days with high concentration and when applied in 15 leaf stage for $AgNO_3$ and in 5 and 15 leaf stage for $Ag(S_2O_3)_2^{-3}$, although this ion is able to modify sex form in lower concentration and earlier growth stage in cucumber. Although double spraying led to late male flowering, however this treatment could increase number of male flower significantly. Therefore, the double spray treatment is proposed for male flower production in greenhouse conditions. This can be beneficial for breeding programs that need high numbers of male flowers and flowering periods, especially for parents that have different periods of growth stage.

The interaction effect showed that the highest number of male flowers was induced by applying $Ag(S_2O_3)_2^{-3}$ (500 ppm) in 5 and 15 leaf stage in high temperature about maximum 35°C at day and minimum 20˚C at night. Another finding of this study was that the number of male flowers showed significant increase with increasing doses of $AgNO_3$ from 100 ppm to 300 ppm and $Ag(S_2O_3)_2^{-3}$ from 200 ppm to 500 ppm. Therefore, high dosages of $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ should be selected for inducing male flowers in cucumber. The highest dosage of chemical agents led to decrease in days to male flowering and the mean of internode length that is related to the effect of silver ion on sex form earlier than low dosage of this ion. Leaf stage of the spray event was found to have a toxic effect on fruit. Over all, this result indicates that high concentration of silver ion could change sex expression at higher temperatures and longer days in 15 leaf stage for $AgNO_3$ and in 5 and 15 leaf stage for $Ag(S_2O_3)_2^{-3}$. Because the highest level of number of male flower and male flower period and the lowest level of days to male flowering were obtained in these treatments. Finally, male flowering started about 3 weeks after treatment and lasted for a period of up to 3 weeks thereafter. Plants treated with silver ions did not elongate more than normal plants and grew normally; effective concentrations of $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ did not proved phytotoxic effects in growing conditions.

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