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Use of chemical and hormonal agents for changing sex expression of cucumber for breeding programs

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Abstract. This study was conducted to investigate the effects of chemical and hormonal applications, leaf stage, and number of sprays on the sex expression flowers for breeding programs in gynoecious plants such as cucumber that need to have both male and female flowers. Different concentrations of chemicals and hormones including Gibberllic acid (GA_3) (1000 and 1500 ppm), silver thiosulphate ($Ag\ (s_2o_3)_2^{-3}$) (200, 300, and 500 ppm), and silver nitrate ($AgNO_3$) (200 and 300 ppm) were applied at 5, 10, and 15-leaf stages with single and double sprays. The experiment was conducted as a factorial (three factors) based on a Completely Randomized Block Design with three replications. Analysis of variance showed that different chemical and hormone applications (GA_3, $AgNO_3$, and $Ag\ (S_2O_3)_2^{-3}$) had significant effects on the sex expression of gynoecious cucumbers. All the substances used were found to have significant effects on male flowering period, male flower formation, and number of male nodes. Increasing doses of $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ were observed to lead to significant increases in the number of male flowers and male nodes. Comparison of the chemicals applied showed no significant differences among them with respect to female flower formation. Finally, double sprays of the highest doses of the chemicals applied at the 5-leaf stage were identified as the best treatments for male flower induction.

Key words*:* flowering, hormone, sexuality, spraying, vegetable.

Introduction

Cucumber (*Cucumis sativus* L.), the fourth most cultivated vegetable around the world (Plader et al. 2007, Innark et al. 2013), is one of the most economically important cucurbit vegetable plants (Robinson & Walters 1997). The genus cucumis includes more than 30 species including cucumber which is the one with a high demand for its good quality and high yield varieties. Its high demand has also made it an important crop to be widely grown in glasshouses or plastic houses (Sarkar & Sirohi 2011). The vegetable is mainly used in salads or as pickling, but young and ripe fruits are also used as cooked vegetables (Robinson & Walters 1997). Cucumber has a diverse array of unisexual or bisexual flowering sex phenotypes (Nam et al. 2005). However, the majority of greenhouse cucumber hybrids are gynoecious (Wang et al. 2011). On the other hand, sex expression is an important factor that has a positive effect on yield and that constitutes a major component of cucumber improvement programs (Serquan et al. 1997). In gynoecious cucumbers, male flower induction is necessary for production of F_1 hybrid seeds (Wang et al. 2011). The sex appearance of cucumber is closely connected with its genetics as well as its chemical and environmental conditions (Apan1974, Karakaya & Padem 2011). Sex type in cucumber is under the genetic control of three major genes (M, A, and F) (Trebitsch et al. 1997, Wang et al. 2007). For F_1 hybrid seed production, it is essential to engender male and female flowers in parental genotypes. Moreover, the need for male flowers as a pollen source requires the application of different chemicals to induce male flowers (Karakaya & Padem 2011). Gynoecious sex expression has been responsible for phenomenal development and quicker exploitation of hybrid vigor in cucumber which has attained a high degree of perfection (More & Munger 1986). Some researchers have reported the effects of plant growth regulators on the modification of sex expression in cucumber flowers (Vadigeri et al. 2001, Rafeekher et al. 2002, Bano & Khokhar 2009). Among the plant growth hormones, Gibberllic acid and ethylene have had the greatest effects on sex expression in cucumber (Perl-Treves 1999). Gibberllic acid, as an inhibitor of ethylene production, is reportedly capable of increasing the number of male flowers (Jutamanee et al. 1994). The major site of bioactive GA is the stamen that influences male flower production (Gupta & Chakrabarty 2013). It is well established that exogenous gibberellin increases maleness in cucumbers or delays female flower formation (Peterson & Andher 1960). Aisha & Chaudhry (2006) found that application of 400 ppm GA_3 led not only to precocious flowering, but also to increased number of pistillate and staminate flowers in *Cucumis sativus* L. and *Momordica charantia* L. Self-pollination female cucumber lines has been found to respond to repeated GA_3 treatment (as an inhibitor of ethylene synthesis) to such an extent that the continuous female phase could be prevented (Gupta & Chakrabarty 2013). In addition to plant growth hormones, chemicals such as silver nitrate and silver thiosulphate appear to be powerful chemical inducers of male flowering in gynoecious cucumbers (Karakaya & Padem 2011). Maintenance of the gynoecious lines has been possible through the exogenous application of GA_3 (Peterson & Andher 1960), silver nitrate (Beyer 1976), and silver thiosulphate ($Ag(S_2O_3)_2^{-3}$) (Den Nijs & Visser 1980). Kallo & Franken (1978) reported that $AgNO_3$ yielded more male flowers than GA_3 on two gynoecious and two predominantly female determinate breeding lines. Karakaya & Padem (2011) reported the positive effects of $AgNO_3$ on male flower production in cucumber. Little effort seems to have been directed toward the study of the simultaneous effects of hormones and chemicals on the morphologic traits and the number of male flowers at different growth stages with different numbers of spray applications. Even more so is the case with practical application of research findings in this area. The present study aims to evaluate the effects of different kinds of chemical compounds [$AgNO_3$ and $Ag(S_2O_3)_2^{-3}$] and hormones at different leaf growth stages with different numbers of spay events to identify the best treatment for male expression in cucumber. The best selection could be important with respect to longer male flowering period, high numbers of male flowering, cost-effectiveness, and organic agriculture. This is because some agents are more expensive than others while still others are aversive to organic agriculture.

Materials and methods

Plant material and planting procedure

The seeds of "Adrian" cultivar as a gynoecious genotype were sown in the spring of 2013 at the Research Greenhouse of the Department of Agriculture, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran (51º36´ longitude and 32º63 latitude). The soil used was loam with a pH of 7.7. The spaces between and within the rows were 90 (cm) and 50 (cm), respectively, and 180 (cm) was left between every couple of rows. Different fertilizers including potassium nitrate, ammonium nitrate, magnesium nitrate, iron, and such other mineral fertilizers as sulphate dissolved in water were used based on soil analysis. Standard production practices for cucumber fields were used throughout the growing season. Different doses of GA_3 (1000 and 1500 ppm), $AgNO_3$ (100, 200 and 300 ppm), and Ag $(S_2O_3)_2^{-3}$) (200 and 500 ppm) were applied. Spraying was accomplished at 5, 10, and 15-leaf growth stages. Chemical and hormonal treatments were employed in single and double sprays at intervals of one week. $AgNO_3$ application was conducted early in the morning (before sunrise) to avoid plant sunburn. Each treatment was applied with adequate amounts of the solutions so that all the leaves were wetted in each spray event.

Measured traits

The traits of interest were measured on three plants per replication and included DMF: days to male flowering; NNMF: number of nodes of the first male flower; NMF: number of male flowers; MFD: male flower diameter; NNF: number of normal fruits; NAF: number of abnormal fruits; IL: mean value of internode length; FL: fruit length; NFN: number of female nodes (nodes with only female flowers); NMN: number of male nodes (nodes with only male flowers); and NMFN: number of male and female nodes (nodes with both male and female flowers). X_9, X_{10}, and X_{11} were measured in the 20 nodes between nodes number 10 and 30. MFP represented male flowering period; FW, fruit weight in every picking; and FNP, number of fruits in each picking.

Statistical analysis

The experiment was conducted as a factorial (three factors: hormonal and chemical treatments, leaf stage, and number of spray events) based on a Completely Randomized Block Design with three replications. The data collected were subjected to analysis of variance (ANOVA) using the general linear model (GLM) of Statistical Analysis System Program (SAS Ver. 9). 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.

Results

Analysis of variance

The results of analysis of variance are presented in Table 1. Clearly, chemical and hormone applications (A) had significant effects on all the traits studied, except for the number of abnormal fruits, fruit length, fruit weight per picking, and fruit number per picking. Number of sprays (single or double) also had significant effects on the studied traits, except for number of male nodes, male flower diameter, and number of normal fruits (Table 1). The leaf stage of spraying showed significant differences on days to male flowering, node number of the first male flower, male flower diameter, male flowering period, fruit weight in each picking, and fruit number per picking (Table 1).

The interaction effect of chemical and hormonal application × number of sprays had a significant effect on the number of male flowers (Table 1). Interaction of chemical and hormonal application × leaf stage of spraying was significant for the node number of the first male flower, number of female nodes, number of male nodes, number of male and female nodes, and male flowering period. Interaction of number of sprays × leaf growth stage had a significant effect on the number of normal fruits (Table 1). The interaction effect of chemical and hormonal application × leaf stage × number of sprays (triple interactions) was not significant for any of the traits studied (Table 1).

Effects of chemical and hormonal applications on the traits studied

In this study, eight different doses of the hormone (GA_3) and the chemicals [$AgNO_3$ and $Ag(S_2O_3)_2^{-3}$)] were used. The mean comparisons showed that chemical and hormonal sprays increased the mean values of all the studied traits compared to the control treatment, except for the number of female nodes (Table 2). The highest doses of $AgNO_3$(300 ppm) and $Ag(S_2O_3)_2^{-3}$ (500ppm) increased the number of male flowers, the number of male nodes, male flowering period, and number of male and female nodes (Table 2). The mean values for the studied traits under the application of silver ions were significantly higher than those for GA_3 application (Table 2). The chemical and hormonal applications had similar effects on increasing internode length, which was greater than that of the control treatment (Table 2). The highest mean for days to male flowering was obtained at 200 ppm of $Ag(S_2O_3)_2^{-3}$ (Table 2). Generally speaking, application of $Ag(S_2O_3)_2^{-3}$ and $AgNO_3$ led to higher mean values than GA_3 did for all the evaluated traits, except for days to male flowering and node number of the first male flower (Table 2). Fruit weight showed no significant differences under different treatments (Table 2). Fruit number was similar in all the treatments, except for 1500 (ppm) of GA_3 (Table 2). Finally, the chemical and hormonal treatments showed no significant effects on female flower production (Table 2). This result confirms the inducing effects of the chemical compounds and hormones used on male flower production.

Effects of number of sprays on the traits studied

Number of sprays (single or double) showed significant effects on days to male flowering, number of male flowers, number of female nodes, number of male nodes, number of male and female nodes, and male flowering period (Table 3). The single spray led to higher mean values of days to male flowering and number of female nodes, which is logical because the plant has had more time for sex expression. In contrast, the double spray treatment gave rise to higher mean values of number of male flowers, number of male nodes, and male flowering period.

Table 1. Analysis of variance for different studied traits in greenhouse cucumber.

		Mean Squares of studied traits													
S.O.V	df	DMF	NNMF	NMF	MFD	NNF	NAF	IL	FL	NFN	NMN	MFN	MFP	FW	FNP
Rep.	2	35.18**	0.86^{ns}	15.82^{ns}	4.11**	2.32*	1.49**	16.43**	7.77^{ns}	0.13^{ns}	1.18*	0.49^{ns}	0.39^{ns}	302.96*	0.69**
(A)	7	178.6**	22.74**	349.4**	14.11**	2.21**	0.26^{ns}	7.61**	2.95^{ns}	16.53**	1.64**	17.67**	24.55**	57.6^{ns}	0.13^{ns}
(B)	1	27.63 *	0.05^{ns}	142.5**	0.49^{ns}	0.02^{ns}	0.004^{ns}	0.46^{ns}	7.53^{ns}	6.87**	3.91**	1.81*	3.86**	148.78^{ns}	0.03^{ns}
(C)	2	26.92*	33.08**	11.7^{ns}	8.24**	0.39^{ns}	0.05^{ns}	3.11^{ns}	8.2^{ns}	0.04^{ns}	6.16**	1.95**	1.72**	46.91^{ns}	0.12^{ns}
(AB)	7	10.06^{ns}	0.59^{ns}	17.42*	0.66^{ns}	0.47^{ns}	0.22^{ns}	1.09^{ns}	2.2^{ns}	0.53^{ns}	0.21^{ns}	0.24^{ns}	0.35^{ns}	33.01^{ns}	0.09^{ns}
(AC)	14	10.81^{ns}	1.13**	10.21^{ns}	0.37^{ns}	0.39^{ns}	0.19^{ns}	2.63^{ns}	2.09^{ns}	1.77*	1.05**	0.82*	0.68*	109.47^{ns}	0.03^{ns}
(BC)	2	1.69^{ns}	0.35^{ns}	3.37^{ns}	0.16^{ns}	2.87*	0.07^{ns}	3.31^{ns}	0.65^{ns}	0.03^{ns}	0.29^{ns}	0.11^{ns}	0.01^{ns}	183.01^{ns}	0.004^{ns}
(ABC)	14	4.09^{ns}	.026^{ns}	3.82^{ns}	0.29^{ns}	0.68^{ns}	0.23^{ns}	1.38^{ns}	2.68^{ns}	0.31^{ns}	0.24^{ns}	0.39^{ns}	0.27^{ns}	88.24^{ns}	0.06^{ns}
Error	94	7.25	0.34	7.2	0.55	0.69	0.29	2.27	2.93	0.49	0.29	0.38	0.28	104.88	0.11

*., ** and ns significant at $P < 0.05$, $P < 0.01$ and not significant, respectively.

A: Chemical and hormonal application [Gibberllic acid(GA_3), Silver nitrate ($AgNO_3$) and Silver thiosulphate $Ag(s_2o_3)_2^{-3}$); B: number of spraying (Single and Double); C: Leaf growth stage (5, 10 and 15)

¥: Abbreviations: DMF: days to male flowering; NNMF The node number of male flower; NMF:The number of male flower; MFD: male flower diameter, NNF: The number of normal fruit; NAF: The number of abnormal fruit; IL: The mean of internode length; FL: Fruit length NFN: The number of female node NMN: The number of male node, NMFN: The number of male and female node; MFP: male flowering period; FW: Fruit length in every picking node FNP: Fruit number per picking.

Table 2. The mean comparison of different chemical and hormonal application on studied traits in cucumber.

Traits	Control	G_1	G_2	S_1	S_2	N_1	N_2	N_3
DMF	0^{c}	24.05^{ab}	24.33^{a}	24.83^{a}	23.96^{ab}	23.61^{ab}	24.11^{ab}	22.42^{b}
NNMF	0^{c}	16.83^{a}	16.69^{a}	13.28^{b}	12.64^{b}	17.08^{a}	16.67^{a}	11.85^{b}
NMF	0^{e}	23.36^{d}	34.39^{d}	69.08^{c}	203.36^{a}	33.78^{d}	110.14^{b}	181.97^{a}
MFD	2.77^{d}	4.55^{c}	4.72^{c}	5.23^{ab}	5.48^{a}	4.91^{bc}	4.85^{bc}	5.61^{a}
NNF	23.53^{c}	25.69^{bcd}	22.78^{c}	30.71^{ab}	33.57^{a}	29.61^{ab}	28.17^{abc}	31.47^{abc}
IL	6.22^{b}	7.4^{a}	7.45^{a}	7.58^{a}	8.17^{a}	7.58^{a}	8.29^{a}	8.04^{a}
NFN	20^{a}	13.47^{b}	12.81^{b}	8.03^{c}	3.21^{d}	11^{b}	7.46^{c}	3.36^{d}
NMN	0^{d}	0.44^{cd}	0.66^{bcd}	1.33^{bc}	3.39^{a}	1.11^{bc}	1.61^{bc}	2^{ab}
NMFN	0^{d}	6.5^{c}	6.53^{c}	10.75^{b}	14.33^{a}	7.89^{c}	11.06^{b}	14.25^{a}
MFP	^{e} 0	11.86^{d}	12.58^{cd}	14.99^{bc}	18.32^{a}	11.83^{d}	16.44^{ab}	18.39^{a}

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

N1, N2 and N3 are 100, 200 and 300(ppm) of AgNo3, respectively.

S1 and S2 are 200 and 500 (ppm) of $Ag(s_2o_3)_2^{-3}$;respectively.

G_1 and G_2 are 1000 and 1500 (ppm) of Gibberllic acid (GA), respectively.

¥: Abbreviations: DMF: days to male flowering; NNMF: The node number of male flower; NMF: The number of male flower; MFD: male flower diameter, NNF: The number of normal fruit; NAF: The number abnormal fruit; IL: The mean of internode length; FL: Fruit length; NFN: The number of female node; NMN: The number of male node, NMFN: The number of male and female node; MFP: male flowering period.

Table 3. The mean comparison of single and double spraying for some studied traits in cucumber.

	Traits					
Number of Spraying	DMF	NMF	NFN	NMN	NMFN	MFP
Single spraying (SP)	20.49^{a}	59.99^{b}	11.01^{a}	0.85^{b}	8.32^{b}	11.84^{b}
Double spraying (DP)	21.34^{b}	104.03^{a}	8.87^{b}	1.78^{a}	9.51^{a}	14.26^{a}

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

DMF: days to male flowering; NMF: The number of male flower; NFN: The number of female node; NMN: The number of male node; NMFN: The number of male and female node; MFP: male flowering period.

Table 4. The mean comparison for some of the studied traits under different leaf growth stages in cucumber.

	Traits					
Developmental stages	DMF	NNMF	MFD	NMN	NMFN	MFP
Five leaf stage(LS_1)	20.14^{b}	6.78^{c}	5.22^{a}	0.63^{b}	9.7^{a}	14.7^{a}
Ten leaf stage(LS_2)	20.97^{ab}	13.67^{b}	4.68^{b}	0.61^{b}	9.61^{a}	12.19^{b}
Fifteen leaf stage(LS_3)	21.93^{a}	18.95^{a}	4.41^{b}	2.72^{a}	7.42^{b}	12.27^{b}

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

DMF: days to male flowering; NNMF: The node number of male flower; MFD: male flower diameter, NMN: The number of male node; NMFN: The number of male and female node; MFP: male flowering period.

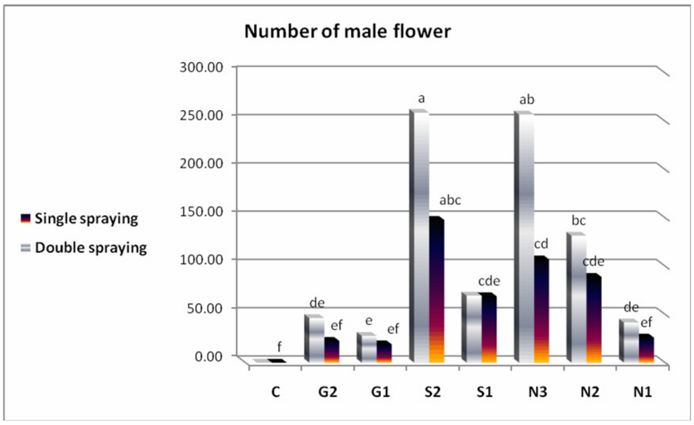

Figure 1. The mean comparison for interaction effects of chemical and hormonal application and number of spraying for number of male flower.

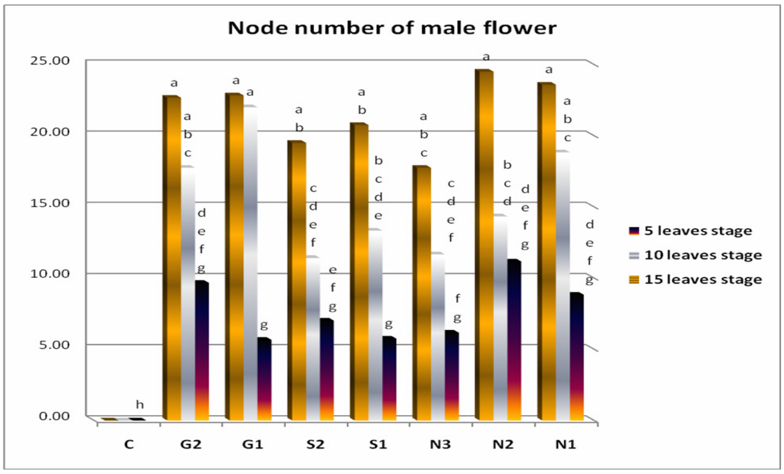

Figure 2. The mean comparison of node number of male flower for interaction effect of chemical and hormonal application× leaf growth stage.

<u>Effects of leaf stage on the traits studied:</u>

The mean values obtained for the studied traits at different stages of leaf growth [5: LS_1, 10: LS_2, and 15: LS_3] are presented in Table 4. Mean comparison showed that the highest mean values for days to male flowering, node number of the first male flower, and number of male nodes were obtained at the LS_3 stage (Table 4). Also, the highest values for male flower diameter, number of male and female nodes, and male flowering period were obtained at the LS_1 stage.

<u>Interaction effects of chemical and hormonal applications × number of sprays on studied traits:</u>

The interaction effect of chemical and hormonal applications × number of spray events showed that the double spray treatment increased male flower production in all the treatments compared to the single spray treatment (Fig. 1). More specifically, the highest doses of $AgNO_3$ (300 ppm) and $Ag(S_2O_3)_2^{-3}$ (500 ppm) led to a significant increase in the number of male flowers (Fig. 1).

<u>Interaction effect of chemical and hormonal applications × leaf stage of spraying:</u>

The interaction effects of chemical and hormonal applications × leaf stage showed that the highest doses of $AgNO_3$ (300 ppm) and $Ag(S_2O_3)_2^{-3}$ (500 ppm) increased the node number of the first male flower, the node number of male and female flowers, and the period of male flowering (data not shown). Interaction of chemical and hormonal applications × leaf growth stage led to the highest node number of the first male flower at LS_3 with 200 ppm of $AgNO_3$, followed by LS_3 with 100 ppm of $AgNO_3$, and with 1000 and 1500 ppm of GA_3 (Fig. 2). A dose of 500 ppm of $Ag(S_2O_3)_2^{-3}$ resulted in the highest number of male nodes and male flowers at LS_3 (data not shown). The longest period of male flowering was observed with 500 ppm of $Ag(S_2O_3)_2^{-3}$ at LS_1 (data not shown). Also, application of 200 and 300 ppm of $AgNO_3$ at LS_2 and LS_3 stages led to longer periods of male flowering.

<u>Interaction effect of leaf stage × number of sprays on the studied traits:</u>

Interaction of leaf stage × number of sprays revealed that the single spray treatment at the 5-leaf stage had the highest effect on the number of normal fruits. Moreover, no significant differences were observed between this treatment and the 15-leaf one (Fig. 3). This result confirms the non-synergic effect of chemicals on the formation of female flowers. In the present experiment, the 5-leaf stage and the single spray led to the lowest number of abnormal fruits.

Discussion

Identification of the best treatment for the number of spraying events, leaf growth stage, and chemical dosage (GA_3, $AgNO_3$, and $Ag(S_2O_3)_2^{-3}$) on sex expression and some horticultural traits in cucumber is essential for formulating

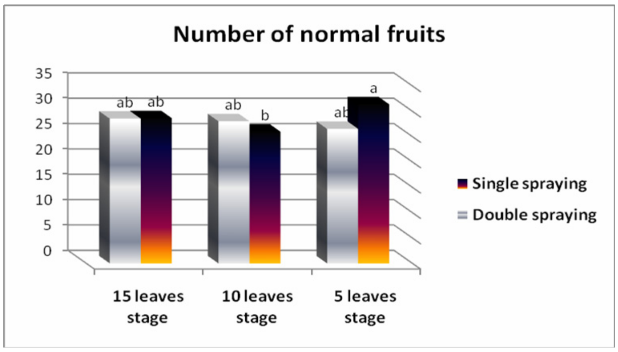

Figure 3. The mean comparison for number of spraying × leaf growth stage interaction for number of normal fruits.

new practical recommendations for cucumber breeding programs. Overall, despite the availability of reports published independently on the individual effects of each of the different chemical and hormonal agents, the different stages of growth period, or the number of spraying events, no study has been reported on the cumulative and simultaneous effects of all these factors and the assessment of their interactions for selecting the proper combination of all the factors for different purposes. The present study is an ensemble attempt to achieve these objectives. The highest number of male nodes and number of normal fruits were achieved with 500 ppm of $Ag(S_2O_3)_2^{-3}$ at the 15-leaf stage. Another finding of the study was that the number of male flowers also increased with increasing doses of AgNO3, $Ag(S_2O_3)_2^{-3}$ and GA_3 applied on the 'Adrian' variety in the spring season. A new aspect of this finding is that, similar to $AgNO_3$, application of $Ag(S_2O_3)_2^{-3}$ had better induction effects than did GA_3. On the other hand, while it is more expensive to use GA_3 is than Ag ions, especially in hybrid seed production progarns, the fruits produced in this treatment are safer for human consumption than the silver ion treatments on grounds of organic agriculture. $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ were found to have similar and positive effects on the male expression of cucumber. Ethylen, as one of the growth regulators in plants, has been found to induce feminization (Abeles et al. 1992, Gupta and Chakrabarty 2013). Most of the effects of ethylene can be antagonized by specific ethylene inhibitors. Silver ions (Ag^+) restrain the physiological effects of ethylene by blocking the ethylene receptors (Hirayama & Alonso, 2000). Silver ions (Ag^+) applied as silver nitrate ($AgNO_3$) or as silver thiosulfate ($(Ag(S_2O_3)_2\,^{3-})$) replace copper ions (Cu^+) which are part of the ethylene receptor preventing the receptor from responding to ethylene (Abeles et al. 1992). This finding is in agreement with that of Law et al. (2002) who reported an increasing effect of silver nitrate on male flower production in cucumber. Den Nijs & Visser (1980) and Yongan et al. (2002) proposed that $AgNO_3$ produced more male flowers than GA_3 treatment in cucumber and summer squash, respectively, a finding that is similar to our results. Similar to these findings, Kalo & Franklen (1978) reported that different doses of $AgNO_3$ (50, 200, and 500 mgl^{-1}) led to greater effects on male flower production than those of different doses (100, 500, and 1500 mgl^{-1}) of GA_3. Hallidri (2004) suggested that 400 and 500 ppm of $AgNO_3$ produced the highest number of male flowers in cucumber. Stankovic & Prodanovic (2002) reported that increasing concentrations of $AgNO_3$ from 0.01% to 0.04% enhanced the number of male flowers in gynoecious lines of cucumber, which is in agreement 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 Abscisic Acid). Rafeekher et al. (2002) reported that GA_3 and Naphthalene Acetic Acid (NAA) increased internode length. Meanwhile, Jutamanee et al. (1994) reported that the effects of different doses of GA_3 and $AgNO_3$ on flower sex expression depended on both the genotype and the photoperiod.

In our study, however, spraying was performed at different growth stages (5, 10, and 15-leaf stages), among which the 5-leaf stage produced the highest number of male flowers although the capacity for male flower production were also credible in the other leaf-stage treatments. Male flower diameter was greater with the 5-leaf stage, which might lead to higher pollen production and increased capacity for crossing in breeding programs. On the other hand, all the treatments at LS_1 led to earlier production of male flowers on the lower nodes, while in some instances of cucumber breeding programs, the breeder needs to select gynoecious plants before using chemical or hormonal agents for selfing and line production. Therefore, chemical treatment in the 15-leaf stage would be suitable. 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. 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. Den Nijs & Visser (1980) reported that application of chemicals at the 15-leaf stage reduces the male flowering period, a finding that is similar to our results. Selection of the growth stage for the spraying event depends on the type and concentration of the chemical used. As female flowers continue to appear in gynoecious cucumber, long periods of male flowering production could be useful for seed production.

Double spray treatment was found to affect only sex expression while it had no effect on the traits related to morphology and fruit yield. This can be beneficial for breeding programs that need high numbers of male flowers and long flowering periods, especially for parents that have different periods of growth. In contrast, the number of nodes with female flowers was found to be higher under the single spray treatment; this can be expected while it also shows that the low number of flowers changes their sex expression. Therefore, the double spray treatment is proposed for male flower production in greenhouse situations. Hallidri (2004) reported that the increased number of spray events had significant effects on male flower production, and that the greatest number of staminate nodes was produced on plants sprayed two or three times of $AgNO_3$ at concentrations of 400 to 500 ppm. Also, Nagar et al. (2014) used 200 and 400 (ppm) of $AgNO_3$ and 2 and 4(mM) of silver thiosulphate in one and two applications and showed that high and low concentrations of $AgNO_3$ and silver thiosulphate, respectively were better in the double treatment. It may, therefore, be claimed that high doses of the chemical agents can be used with the double spray treatment if only the male parents are used for male flower production in breeding programs, and further if their female flowers are not needed for reciprocal crosses or fruit yield.

Finally, the two chemical compounds containing silver ions ($AgNO_3$ and $Ag(S_2O_3)_2^{-3}$) were found to be superior to GA_3 with respect to male flower production; hence, they could be recommended for enhancing male flower production in cucumber. The findings showed that the highest number of male flowers was induced by applying $Ag(S_2O_3)_2^{-3}$ (500 ppm) and $AgNO_3$ (300 ppm). Based on the results obtained for the different chemical treatments, the double spray is recommended to start at the 5- and 15-leaf stages.

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