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Effects of Ethephon Application at Different Growth Stages on Male Flowers in Cantaloupe (*Cucumis melo* L.)

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Abstract: Field experiments were carried out to investigate the effect of Ethephon levels at different growth stages of plant on male flowers in cantaloupe (*Cucumis melo* L.) during 2013 and 2014 growing seasons. The tests were done as a split plot experiment in a randomized complete block design with three replications to randomize the different growth stages and Ethephon levels in the main and sub-plot, respectively. The experiments comprised of three growth stages (3 and 6-leaf stages and early reproductive growth) and four Ethephon levels (0, 100, 200 and 300 ppm of effective substance). Traits measured in the experiment were the appearance time of female flowers (ATFF), number of male flowers (NMF) and number of female flowers (NFF) recorded 7 and 14-days after spraying Ethephon and yield. Statistical results of study indicated that plant response to increasing levels of Ethephon was different among different growth stage. Moreover, increasing Ethephon level increased the appearance time of flowers (male and female). Also, the highest yield (25834 kg ha^{-1}) was obtained with consumption of 100 ppm Ethephon in trifoliate plants. A high level of Ethephon (200 ppm and more) at all growth stages, especially at the reproductive growth stage was associated with significant reductions of yield.

Key words: Cantaloupe · Female flower · Male flower · Ethephon · Growth stages · Yield

INTRODUCTION

Cantaloupe (*Cucumis melo* L.) is a valuable cash crop grown throughout the world, belonging to the cucurbitaceae family. In *C. sativus* and *C. melo* gynoecious plants produce more ethylene than their monoecious counterparts. In these species, floral primordia are initially bisexual with sex determination occurring by the selective developmental arrest of either the male stamen or female carpet organs, resulting in unisexual flowers [1].

It has been reported that andromonoecious plants (with male and hermaphrodite flowers) envolve low amounts of ethylene compared to monoecious plants (with male and female flowers) and hermaphrodite flowers [2]. Ethylene is a plant growth regulator known to alter sex expression in plants belonging to the cucurbitaceae family, increasing the number of pistillate flowers when applied to monoecios plants [3]. Ethylene is biologically active at very low concentrations measured in ppm and ppb ranges. Other molecules with specific configurations can mimic ethylene, but are less effective. For example, C_2H_4 analogs propylene (C_3H_6) and acetylene (C_2H_2) and requires 100- and 2700- fold, respectively, of C_2H_4 concentration to elicit the same effect [4]. Most plants synthesize small amounts of ethylene that appear to coordinate growth and development. Ethephon and similar C_2H_4 releasing chemicals, permit the commercial application of C_2H_4 in the field [5].

According to findings of the past three decades, researchers have emphasized that higher ethylene content is associated with female sex expression in plants. Exogenous application of plant growth regulators can alter a sex ratio and sequence if applied at a two or four-leaf stage, the critical stage at which either suppression or promotion of either sex is possible [6]. It has been demonstrated that Ethephon treatments with 100 ppm concentration caused a significantly earlier production of female flowers in cucumber compared with untreated plants [7]. Conversely, Ethephon at 300 ppm decreased

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the yield of mature fruit and caused stunted growth and a decrease in fresh and dry weights of treated plants. Similarly, in another study it was observed that increased applications of Ethephon increased the number of days to first flowering, 50% flowering, first fruit set and 50% fruit set. increasing the number of female flowers, therefore only one application of Ethephon, was adequate. It was found that one Ethephon application produced optimum percentage of culls and fruit quality rating, since two Ethephon applications resulted in a higher percentage of culls and lower rate fruit quality [8].

Therefore, the present study was planned to investigate the effect of Ethephon application at different stages of plant's development on male flowers in cantaloupe.

MATERIALS AND METHODS

Research Site: This study was carried out at the research site of Tehran Agricultural and Natural Resources Research and Education Center, Varamin, Iran on a clay loam soil for two succesive growing seasons (2013 and 2014). The research site is located at at latitude of 35° 19′ N and longitude of 51° 39′ E and is 1000 m above mean sea level, in arid climate (150 mm rainfall annually) in the center of Iran, where the summers are dry and hot while the winters are cool. The soil of the research site is classified as an Aridisol (fine, mixed, active, thermic, typic haplocambids). Details of soil physical and chemical characteristics are given in Table 1.

Weather Parameters: The mean monthly temperature of the research site during the years of study (2013 and 2014) is given in Fig. 1.

Soil Sampling and Analysis: To determine soil physical and chemical properties of the research site, a composite soil sample (from 12 points) was collected from 0-30 cm depth 30 days before planting during the study years. Soil samples were analysed in the laboratory for N, P, K, Fe, Zn, Cu, Mn, B, EC, pH, organic carbon (OC), particle size distribution and dry bulk density. Details of soil physical and chemical properties of the research site are given in Table 1.

Field Methods: A split plot experiment was laid out in a randomized complete block design (RCBD) with three replications to randomize the growth stages and Ethephon levels in the main and sub-plots, respectively. The experiment comprised of three growth stages, i.e. 3-leaf stage, 6-leaf stage and early reproductive growth stage (in the main plots) and four Ethephon levels, i.e. zero, 100, 200 and 300 ppm (in the sup plots). The treatments were carried out on the same plots in the 2013 and 2014 growing seasons. The size of each plot was 20.0 m long and 6.0 m wide. There were four furrows in each plot. The furrows had 20.0 m long, 75 cm wide and 50 cm depth. In both growing seasons, one of the most commercial varieties of cantaloupe cv. Semsoori was planted manually on one side of each furrow (totally four rows per plot) by keeping plant to plant distance 40 cm and row to row distance 150 cm on May 5th. Based on soil test results, recommended levels of N (200 kg ha^{-1}), P (150 kg ha^{-1}) and K (200 kg ha^{-1}) were used as urea, triple super phosphate (TSP) and sulfate of potassium (SOP), respectively. All P and K fertilizers and 20 percent of N fertilizer were added to the soil before planting. The rest of N fertilizer was added to the soil at the reproductive stage (after Ethephon application and fruit set). Irrigation was applied when the soil moisture in the crop root zone declined to 50-60% of the field capacity. Tensiometers were placed at depths of 15 cm and 30 cm for measuring soil water tension and determining irrigation time. Ethephon (2-chloroethyl phosphonic acid) was sprayed on seedlings at the 3-leaf stage, 6-leaf stage and early reproductive growth stage (tip over = five nodes). Foliar applications were carried out in the early mornings. Two sprays (intervals of five days), one each at the 3-leaf stage, 6-leaf stage and early reproductive growth stage (tip over) were applied using a hand-operated sprayer. Ethephon was applied at effective dose levels of zero, 100, 200 and 300 ppm.

Observation and Data Collection: Cantaloupes were harvested in the middle of July and standard procedures were adopted for recording the data on crop yield. Moreover, effects of Ethephon application on controlling the female and male flowers, gender and emergence of them were identified on plants nearly up to 15 days after flowering. Characteristics measured in the study were: appearance time of female flowers (ATFF), number of female flowers (NFF) and number of male flowers (NMF) 7 and 14-days after Ethephon spraying and yield.

Statistical Analysis: All collected data were subjected to the Analysis of Variance (ANOVA) following by Gomez and Gomez [9] using SAS statistical computer software. In addition, means of the different treatments were separated by Duncan's Multiple Range Test (DMRT) at P = 0.01.

Table 1: Soil physical and chemical characteristics of the experimental site

Soil characteristics	Values
Texture	Clay-loam
Sand (%)	24.0
Silt (%)	39.0
Clay (%)	37.0
EC (dS m^{-1})	1.70
pH	7.50
Organic carbon (%)	0.95
Available P (mg kg^{-1})	40.4
Available K (mg kg^{-1})	460
Available Fe (mg kg^{-1})	2.84
Available Mn (mg kg^{-1})	12.9
Available Zn (mg kg^{-1})	1.50
Available Cu (mg kg^{-1})	1.13

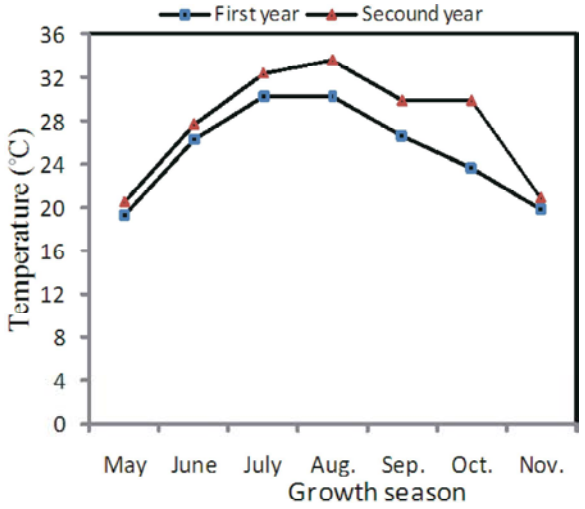

Fig. 1: The mean monthly temperature of the research site during the years of study (2013 and 2014)

RESULTS AND DISCUSSION

Year effect on all studied traits, except for the appearance time of female flowers (ATFF) was not significant (Table 2). This difference might be induced from the effect of temperature at the time of Ethephon application. In the second year, high temperature caused less absorption of Ethephon. In this case, the Ethephon volatilization of the leaf area was greater. Therefore, ATFF was less affected by Ethephon application (Fig. 1).

Effects of growth stages (GS) and Ethephon level (EL) were statically significant (P = 0.01) on all studied traits (Table 2). Foliar application of Ethephon affected number of male flowers (NMF) in the tip over stage significantly more than at the 3 and 6-leaf stages (Fig. 2). Processes for both application of Ethephon (7 or 14-days after spraying) were similar. Number of female flowers (NFF), 7 and 14-days after applications of Ethephon in the six-leaf stage and tip over stage, was significantly more than at the trifoliate stage (Fig. 2). Rudich *et al.* [10] indicated that ethylene application pre flowering may promote the production of pistillate flowers either directly or by reducing endogenous gibberellic acid and auxin levels, or by promoting the production of abscisic acid. Similar results with more emphasis on the shifts of ethylene and gibberellic acid have been reported in more recent research by Achard *et al.* [11].

Effect of Ethephon application on the number of female flowers (NFF) at the tip over stage was significantly higher than at the 3 and 6-leaf stages (Fig. 3). Ethephon application at the tip over stage, the female flowers formation compared to 3 and 6-leaf stages, were 15 and 10 days earlier, respectively. The number of days to anthesis of female flowers increased when Ethephon was applied at later stages of growth from the cotyledon to the third true leaf stage [5, 12].

The number of female flowers (NFF) in the control treatment (no application of Ethephon) was significantly more than those of 200 and 300 ppm of Ethephon applications, however had no significant difference with the 100 ppm Ethephon application although emergence (male and female) were delayed (Table 3). Inhibition of both cell division and cell elongation has been observed with the application of hormonal substances, resulting in production of shorter shoots and leaves in melon [13]. With consumption of 100 ppm Ethephon, cantaloupe yield was not significantly different than that of the control treatment, but this treatment produced 19 and 23 percent better performance, respectively than 200 and 300 ppm Ethephon applications (Table 3). Similarly, Shetty [14] found that Ethephon treatment significantly improved total yield and fruit quality traits evaluated in pickling cucumbers.

The interaction of plant growth stage (GS) and Ethephon level (EL) was statistically significant (P = 0.01) on numbers of male (NMF) and female flowers (NFF) (Table 2). The highest number of female flowers (NFF) was obtained at higher levels of Ethephon and in trifoliate plants (Fig. 4 A1, A2). With the increased age of six-leaf plants, the opposite trend was observed (Fig. 4 B1, B2). Fourteen days after foliar applications, at the six-leaf stage, with increasing levels of Ethephon the number of female flowers (NFF) was drastically reduced. In the tip over stage, increasing the number of flowers with increasing levels was significantly less than at the trifoliate stage and most in the six-leaf stage (Fig. 4 C1, C2). The interactions of growth stage and level of Ethephon on yield and the ratio of female to male flowers

Table 2: Combined analysis of variance (ANOVA) for flowering characteristics and total yield as affected by growth stage and Ethephon application

Source of variation	df	ATFF	NFF (7-day after spraying)	NFF(14-day after spraying)	NMF(7-day after spraying)	NMF (14-day after spraying)	Yield
Year	1	1369.38 **	28.12 ^{ns}	64.22 ^{ns}	231.12 ^{ns}	2005 ^{ns}	479^{ns}
Replication	4	5.72	439.91	1551.72	533.08	1168.5	71.3
Growth stages (GS)	2	1357.05 **	1635.29 **	19534.54 **	28097.04 **	15459.2**	1241**
GS × Year	2	6.72 ^{ns}	70.87 ^{ns}	200.34 ^{ns}	1012.87 ^{ns}	211.84 ^{ns}	301^{ns}
Ethephon level (EL)	3	879.81 *	731.53 **	8727.66 **	153955.3 **	284471.5**	156432**
EL × Year	3	4.16 ^{ns}	94.27 ^{ns}	57.00 ^{ns}	136.71 ^{ns}	937.14 ^{ns}	765.1^{ns}
GS × EL	6	237.20 **	862.53 **	5360.15 **	14704.33 **	4969.9 **	45674**
GS × EL × Year	6	5.16 ^{ns}	19.41 ^{ns}	267.01 ^{ns}	955.80 ^{ns}	297.94 ^{ns}	345^{ns}
Error	46	10.61	246.67	1623.88	367.94	385.1	875.5
CV%	---	8.6	15.06	23.08	22.19	19.25	24.2

* and ** = significant at 0.05 and 0.01 probability levels, respectively

ns = non-significant

ATFF = appearance time of female flowers

NFF = number of female flowers

NMF = number of male flowers

Table 3: Means comparison for yield and flowering characteristics as affected by Ethephon level using DMRT at 1% probability (average of two years)

Ethephon level (ppm)	ATFF (day)	NFF (7-day after spraying)	NFF (14-day after spraying)	NMF (7-day after spraying)	NMF (14-day after spraying)	Yield (kg ha^{-1})
0	29.44 c	116.1 a	203.4 a	224.1 a	304.8 a	25432 a
100	35.06 b	108.6 ab	180.1 ab	57.11 b	97.33 b	25834 a
200	42.17 a	96.22 b	159.2 b	34.06 c	46.33 c	21153 b
300	44.89 a	96.22 b	155.7 b	30.61 c	34.50 c	21005 b

Means in the same column with different letters differ significantly at 0.01 probability level according to DMRT

ATFF = appearance time of female flowers

NFF = number of female flowers

NMF = number of male flowers

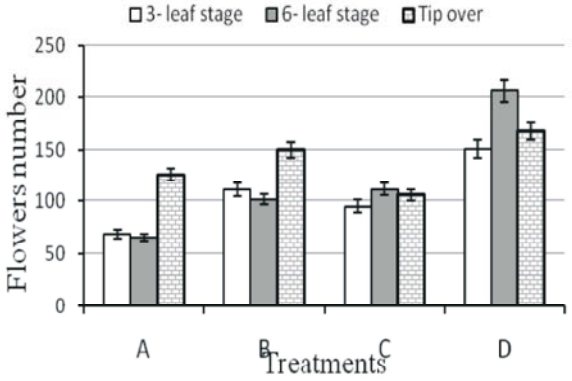

Fig. 2: Standard error of the means for number of male flowers 7 and 14-day after Ethephon spraying (A and B, respectively) and number of female flowers 7 and 14-day after Ethephon spraying (C and D, respectively)

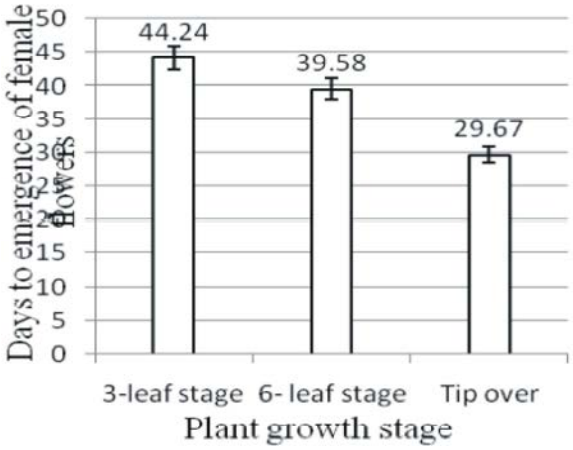

Fig. 3: Standard error of the means for effect of plant growth stage on days to emergence of female flowers (average of two years)

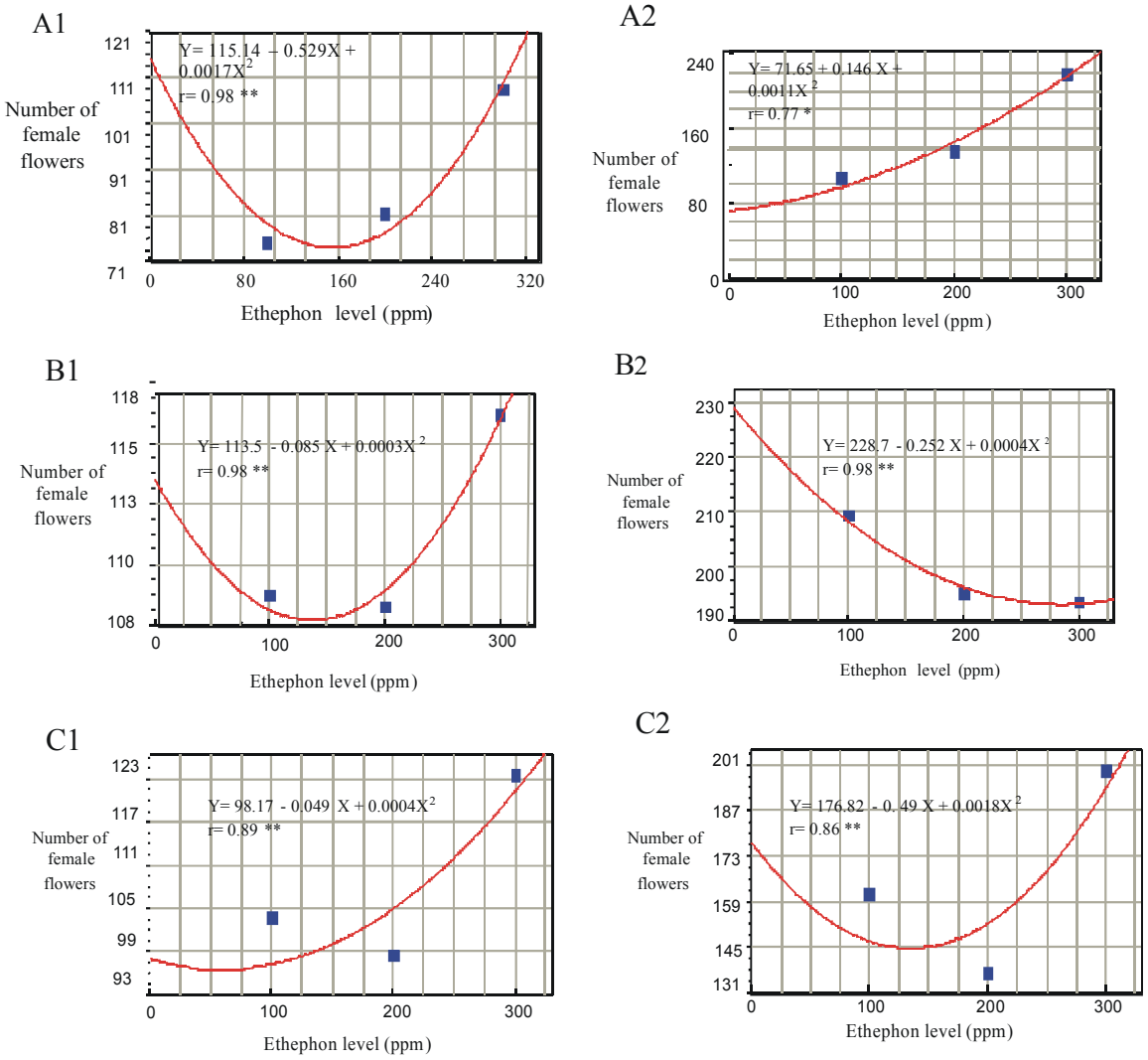

Fig. 4: Effect of growth stage and Ethephon application interactions on number of female flowers. A1 and A2 are three-leaf stage growths in 7 and 14-day after Ethephon application, respectively. B1 and B2 are six-leaf stage growths in 7 and 14-day after Ethephon application, respectively. C1 and C2 are tip over stage growths in 7 and 14-days after Ethephon application, respectively

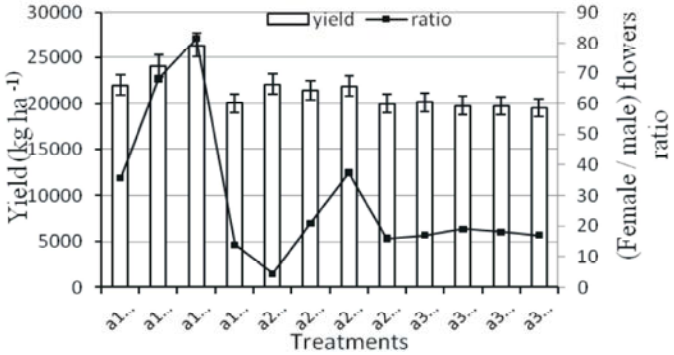

Fig. 5: Effect of growth stage and Ethephon interaction on yield and flowers ratio (female/male). a1, a2 and a3 are three-leaf, six-leaf and tip over stages, respectively. b1, b2, b3 and b4 are 0, 100, 200 and 300 ppm of Ethephon application (vertical bars are standard error of the mean)

are shown in the Fig. 4. In this study, the highest level of yield (25834 kg ha^{-1}) was obtained with consumption of 100 ppm Ethephon in trifoliate plants. A high level of Ethephon (200 ppm and more) at all growth stages, especially at the reproductive growth stage is associated with significant reductions of yield. Similar results have been reported with doses of 300 ppm of Ethephon by Radwan *et al.* [7].

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