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Effect of *Bombus terrestris* L. (Hymenoptera, Apidae) pollinating on flowering and fruiting trends of greenhouse tomato (*Lycopersicon esculentum*)

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A b s t r a c t : Efficiency of bumblebees in greenhouse tomato was compared to traditional pollination method by setting up two colonies of *Bombus terrestris* L. (Hymenoptera, Apidae) provided from Koppert company in a greenhouse with tomato planted in Gachsaran (Kohgiluyeh and Boyr-Ahmad Province, Iran) in four netted plots; each included 16 plants and some characteristics such as number of flowers from early flowering; flowers falling off rate, number of fruits, internal and external diameters of rape fruits, number of seeds or seed sets, in treatments of with and without bumblebees colony, trends of flowering and fruiting during sampling, were examined. Results based on measuring of mentioned characteristics, especially yield, in the two treatments of control and bumblebees pollinating showed highly significant ($p < 0.01$) differences. Tomatoes pollinated by bumblebees showed a 41.5 % increasing in yield (fruit weight), 15 % in external diameter and 30 % in seed production compare with controls. Using of Bumblebees, because of their high speed in pollinating, made flowers turn into fruits in a shorter time. Decreasing labor costs, time consuming and also high quantity of pollination by bees, by visiting all flowers, were advantages of this method and was economically reasonable.

K e y w o r d s : *Bombus terrestris* L., Greenhouse, pollination, Gachsaran, Iran.

Introduction

Tomatoes are commercially important crop in the world, and one of the most widely consumed vegetables. This product is available in a wide range of crops and greenhouse production in Iran. Recently greenhouse cultivation of this crop has been increased significantly in this country (ANONYMOUS 2012).

In Iran, Pollination of greenhouse tomatoes performs mostly by hand while workers attach plant stem around the string, so-called among greenhouse owners as in Persian "kham zadan" which means "bending the bush stem around the string". In developed countries, pollinating of this product done in greenhouse in 4 ways: hand pollination, electrical vibration, growth regulators, and use of bumblebees (DOORN 2006). Results are shown that bumblebees with hairy bodies, big size and long tongue done the pollinating work with more quality (PLOWRIGHT & LAVERTY 1987; VELTHUIS AND DOORN 2006). In farm, tomato pollination is made by insects, wind and shaking the bushes, but in greenhouse entrance of insect and wind is very limited and then, plants need other tools

for pollination. However, tomato flowers need pollination for fruit sets. Natural pollination alone is not sufficient to pollinate flowers and some of the flowers are imperfect or non-pollinated. So, the other activities can be used to release pollen (RAHEMI 2007). The results of the comparison between pollination by hand and bumblebees in a study in Canada (PLOWRIGHT & LAVERTY 1987) showed that these two methods have a different effect on the quantity and quality of tomatoes. This means that bumblebees have been more efficient as pollinators in commercial products. The quantities increase in the efficiency of tomato production means an increase in the yield as well as improving in the product quality standards including tomato pomace, marketability, ease of transport and increased soluble solid materials (MOUSAVIFAZL & MOHAMMADI 2005). Subsequent investigations proved that the bumblebees' pollination have the better quality than that done by hand, and the activity of bees generate more and higher quality product (BONDA & PAXTON 2008).

Today in the most countries, bumblebees are presented in the most greenhouse tomato. A suitable species of bee for using in greenhouses depends on the geographical location. There are 34 species of bumblebees in Iran, but they are generated just two kinds of them use in mass rearing (MONFARED et al. 2007). Researches on honey bees have suggested that these bees pollinate many various plants but they are less effective in greenhouse pollination, also, they are not suitable for the Solanaceae pollination especially in greenhouses because honey bees are much more aggressive and also having a longer flight radius than bumblebees, so they prefer flowers outdoors (NEISWEINDER 1956, CRIBB et al. 1991). Bumblebees are bigger and stronger than honey bees and also they have more dense hair superficially which help on transporting the pollen grains. Bumblebees can easily pollinate and fly in temperatures about 8-10 Celsius degrees. They grab and vibrate the pipe like tomato flowers and remove the pollens. Having long beak enables them to pollinate flowers with long corolla, easily (CERVANICA & BERGONIA 1991). High capacity of foraging in low light intensity and temperatures made bumblebees as important pollinators in the greenhouse. Although more than a century passes of discovery of the bumblebees biologyand their role in pollination of many plants (SLADEN 1912), Since the early 90_s these bees were developed in mass rearing and utilization for pollination of more than 30 greenhouse crop such as tomatoes, peppers and eggplants in America, and many other European countries including Netherland, France, Belgium and later in Asian countries such as south Korea, Japan, China and Turkey (RAVESTIJN 1990; SANDE 1990; KAFTANOUGHLU 1999; VELTHUIS & DOORN 2008). Bumblebees dangle from flowers by their jaws, stick to corolla and move their muscles to vibrate the flowers, this act is called vibrate pollination. Bumblebees pollinate flowers of the tomato and they make brown color spots on the flower. Many spots on the corolla of a flower determined visitation by bees and can be used as a criteria to control greenhouse needs to the required number of bumblebee colony. Symptoms of bee jaws on flowers very early remain on bloom and tomato blossoms as brown spots and the breeder confidents about visiting bees with flowers (CERVANICA & BERGONIA,1991). Bumblebees are more active in mornings and afternoons at temperatures between 10-30 $^{\circ}$C and have the best performance at temperatures between 5 and 25°C. Bumblebees able to pollinate crops such as peppers, cherry tomatoes, eggplants and blueberries (VELTHUIS & DOORN 2008). Hand pollination is costly and time consuming, yet maybe all flowers not pollinate perfectly in this method, while when we use bumblebees in greenhouse, these bees because of their need for pollen and nectar meet the flowers several times a

day which guaranty pollination perfectly. Although all stages of mass rearing of native species has been successfully carried out in Yasouj University (Unpublished) but by now bumblebees have not been used in Iran for commercial pollination purpose. The aim of this study was to investigate the feasibility of using bumblebees' colony in greenhouse tomatoes and study of bees' efficiency and impact of them on greenhouse tomato production.

Materials and Methods

Time and location of experiment

In the winter of 2011, this experiment was performed in one of the tomato planted greenhouse in industrial park of Chahar-Bishe in Gachsaran, Kohgiluyeh & Boyer-Ahmad Province, Iran. Cultivated area of greenhouse was 2000 m^2.

Culture in greenhouse

Soil used for the cultivation of plants was sandy-loam with completely rotten manure. For disinfectant, for every 500 square meters, 5 kg Granulated Radomyl with 1kg Benomyl was mixed and dispersed relatively uniform on surface of culture sites. Then land was tillage and made ready to planting operations. After 4-6 weeks sowing transplant tomatoes in disposable cups were planted and can be taken to the rows at 4-5 leaf stage. Transplants carefully sent out from plastic pots for avoiding to damage of root and root hairs and immediately were planted in two rows at depth of about 10cm. Schedule irrigation (amount and frequency of irrigation) was adjusted based on plants growth stage during experiment.

Selecting tomato plants and installation of nets

Experiment was carried out by selecting 4 plots in the greenhouse each one containing 16 tomato plant with the same terms and close to each other. Tomato cultivar called Falcato® which is a hybrid with early ripening process, bushes not dense with medium leaf size. Fruit clusters were the same, fruits were round and red with firm texture and easy to harvest. The tomato hybrid was resisted against diseases such as: Tomato Mosaic Virus, Fusarium, verticillium and Nematode, So, this hybrid has been taken as a witness in some experiments (HOBAN et al. 2008).

Before beginning of flowering stage, the bushes limited by nets (Fig. 1) so in a part of greenhouse, 4 nets with dimensions of 3×3×3 m was installed. Of these nets, two were placed with establishment of bee colony and 2 without bee colony. In each net, 16 bushes were considered for test plants. So a completely randomized design with 16 replications and 2 treatments was used in this experiment. In total, 64 plants were examined. For this purpose plants were labeled (Fig. 1B). Each net tagged with letters of A, B, C and D. Setting up the bee colony, counting the flowers and fruits were performed under the nets.

Fig. 1: Installation of nets (A) and bush label (B), a *Bombus terrestris* L. worker seen on flower.

Bumblebee Colonies

In early of flowering stage a bee colony provided from Koppert Company representing in Turkey placed in the middle of two of nets. After that the colonies were allowed to adjust with environment for two hours (Fig. 2). Then the bees were let to go out by opening the

cap slowly. After a few seconds bees got out of bee colony one by one to explore the place and flew under the net and sat on the flowers. Since tomato flowers do not produce nectar, we used a sugar solution with water (1:1) to compensate of shortage of power supply.

Fig. 2: Establishment of colonies in the middle of net (**A**), a box of *Bombus terrestris* colony bought from Koppert Co., representing in Turkey (**B**).

Notes and statistical analysis

There were brown spots on the flowers which showed the flowers get a sign up by bee jaws and they were pollinated by bumblebees. From these spots we find out the pollination of flowers. Parameters measured for comparing treatments were as follow: 1) counting the number of flowers on bushes on a daily basis. 2) Counting falling off of flowers on a daily basis, 3) Counting the number of formed fruits, 4) Measuring the inner and outer diameter of rape fruits, 5) Measuring the weight of rape fruits, 6) Measuring the number of seeds, 7) Comparison of yield between with and without bumblebees treatments, 8) Changes in the number of the flowers and fruits during sampling. In mid-June final counting was done about fruits in different treatments and were allowed all the fruits of treatments stay on the plant to the end of test and simultaneously picked up and number and weight of fruits were determined on a plant. Taking notes of the desired traits was done on a daily basis up to the end time of flowering and fruit maturation. Crop yields in all treatments were also measured by random sampling, and needed data derived for statistical analysis of plant. Data were recorded and classified. Statistical analysis of data was done by using MSTAT-C software. Comparison test was performed at the 5 % level with using Excel software.

Results

Falling rate of flowers

Mean squares derived from analysis of variance of data collected by the effect of treatments (pollinate and not pollinate by bumblebees) and the viability of the flowers on the plants calculated for 14 dates. Results indicated that Effect of treatment on 13,16,19,22 and 30 March was not significant while on the other dates showed in the table was significant ($p<0.01$) (Table 1). Falling rate of flower, in the control were less than treatments with bumblebees' colonies.

Table 1: Mean-squares of remaining flowers on bushes in different dates in greenhouse tomato with and without using of *Bombus terrestris* colony.

Average Number of Flower per plant (Mean squares)								
		March						
Sources of variation	df	3	13	16	19	22	30	
Treatment	1	0.020**	0.006	0.0001	0.001	0.012	0.001	
Error	30	0.002	0.002	0.002	0.003	0.005	0.012	
CV (%)		3.70	3.78	3.50	4.07	5.30	8.27	
Average Number of Flower per plant (Mean squares)								
		April						
Sources of variation	df	3	7	11	15	19	23	26	28

Treatment	1	0.054**	0.104**	0.128**	0.079**	0.125**	0.176**	0.119**	0.106**
Error	30	0.004	0.005	0.005	0.004	0.002	0.003	0.003	0.002
CV (%)		5.51	6.05	5.82	5.14	4.22	5.19	5.37	3.95

The results of the analysis of variance in number of flowers remaining on the plant showed that the use of bees and the lack of bees in the dates on the March, 3rd, and the dates of April had highly significant difference ($p<0.01$). The significance of the average number of flowers on March 3rd may refer to environmental condition, not to activity of bees, because in the other dates has not seen the same condition. The flowering process for two treatments in the experiments has been shown in Fig. 3.

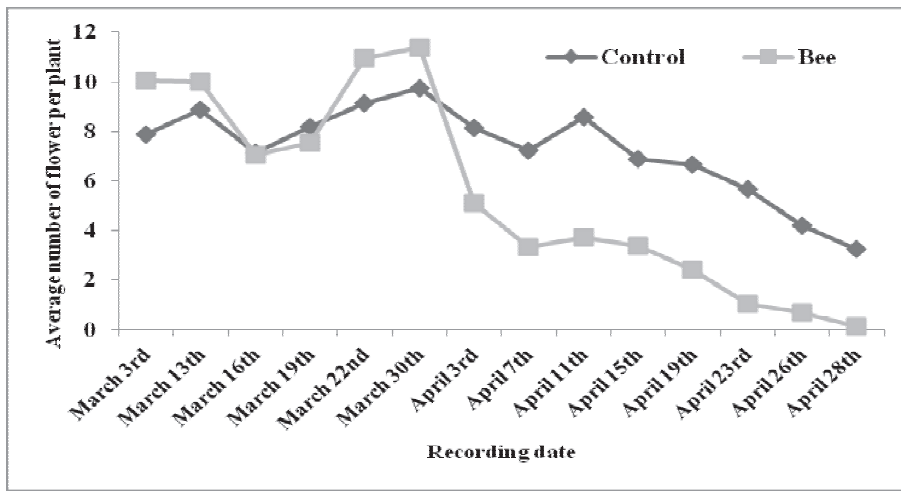

Fig. 3: The flowering process in greenhouse tomato bushes with and without *Bombus terrestris* colony pollination treatment

As seen in the fig. 3, the flowering process in control (not using bumblebees) and in the other treatment (using of bumblebees) have a trend of almost ascending by March 30th and then descending by April 28th. Descending trend observed for flowering means to increase the number of flowers turn to fruit in treatments with rather than treatment of control with no lack bee and not means the effect of treatment on number of flowering formed by plant. It can be said that, the treatment with bee, because of helping to pollination, flowers turn to fruits more quickly which show the decreasing process in flowers and not changing to fruits. The highest level of flowers on days 2 to 6 March (22 March to 26 March) have been seen in two treatments. It is perhaps due to the right temperature and the environment inside of greenhouse and increased plant growth and flowering.

Fruiting trend

Mean-square of variance analysis of the effect of treatments (pollinate and non-pollinate by bumblebees) on the fruiting, notes taken on 14 dates shown in fig. 2. Effect of treatment on the yield was significant except for March 19th, at the first trail.

Based on variance analysis of mean of fruits number per plants of each treatment pollinating by bumblebees shown significant differences and better quantity (table. 2.).

Changing in the average number of fruit in testing and differences in treatment of using and not using of bumblebees can be seen in figure 2. Ascending trends of fruits number for both treatments because of growth period of plants were expected. An important point to be note in table 2 and figure 2 simultaneously suggested that using of bumblebees for pollination in all dates cause that fruiting number significantly increased by plant. In other words, this result shows the effectiveness of bumblebees in increasing tomato yield.

Table 2: Mean square of **fruiting** in different dates in greenhouse tomato with and without using of *Bombus terrestris* colony.

Source of variation	df	March					
		3	13	16	19	22	30
Treatment	1	0.18**	0.33**	492.19**	561.12**	785.07**	1860.50*
Error	30	0.002	0.002	5.468	7.442	9.820	16.029
CV (%)		3.78	4.22	20.36	19.57	19.05	17.74

Source of variation	df	April							
		3	7	11	15	19	23	26	28
Treatment	1	1976.	2080.	2040.	2337.	2701.	2719.	2765.	2628.
Error	30	18.72	25.49	27.41	31.67	34.44	34.92	35.42	38.07
CV (%)		17.52	18.92	18.24	17.93	17.33	17.05	16.79	17.02

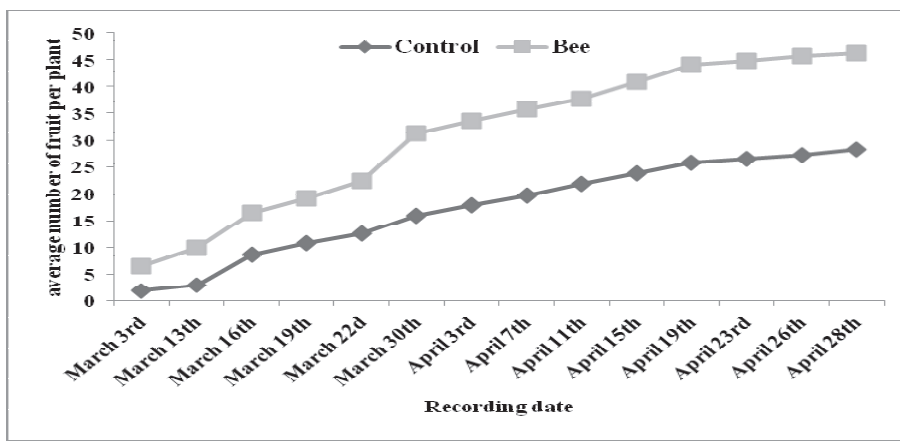

Fig. 4: The fruiting process in greenhouse tomato with and without bumblebees pollination treatments.

Quality traits

Statistics indicated that tomato fruit quality factors such as fresh weight, internal and external fruit diameters and average number of seeds per fruit with 99 % confidences

was affected by bumblebees pollination. So that, the bees make the 41/56 % increase in fresh weight, 15/79 % in diameter increases the external diameter, 24/14 % increase in internal diameter and 30 % increase in the average number of seeds for per fruit.

Mean squares of analysis of variance test were significant for all quality traits at the 1 % level. In other words, that can be said the presence of bumblebees and pollination by bees in visiting the flowers made different results than not using of the bee for fresh weight, inner and outer diameter and number of seeds. Based on results of test conducted in this study, the presence of bees significantly increased in outside diameter and produced a bigger fruit (table.3.)

Table 3: Analysis of variance of quality traits of greenhouse tomato with and without bumblebees pollinating.

Source of variation	df	Wet weight	External diameter	Internal diameter	Seed number per fruit
Treatment	1	9305.89**	572.911**	14.178**	4357.111**
Error	30	452.114	15.014	0.558	243.643
CV (%)		21.46	6.70	12.00	17.44

Also, results of the analysis of variance were shown a change in internal diameter and fruit characteristics of treatments with presence of bees like increase the inner diameter of fruit too.

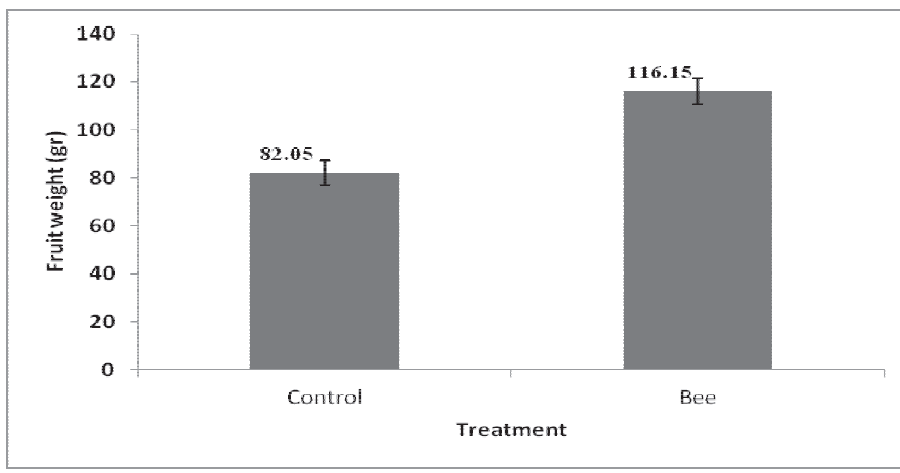

Fig. 5: Compare fruit weight in greenhouse tomato bushes with and without bumblebees pollination treatments.

Increase rate of yields in tomato pollinating by bumblebees was about %41/5 per fruit weight, see below equation:

$$Y_P = \frac{Y_2 - Y_1}{Y_1} \times 100$$

Y_P= yield production

Y_2= amount of yield in bumblebee pollination treatment

Y_1= amount of yield in treatment without bumblebee pollination.

With mentioned pattern in this equation the increasing in external and internal diameters can bee calculated and compared in treatments with or without pollination by bumblebees.

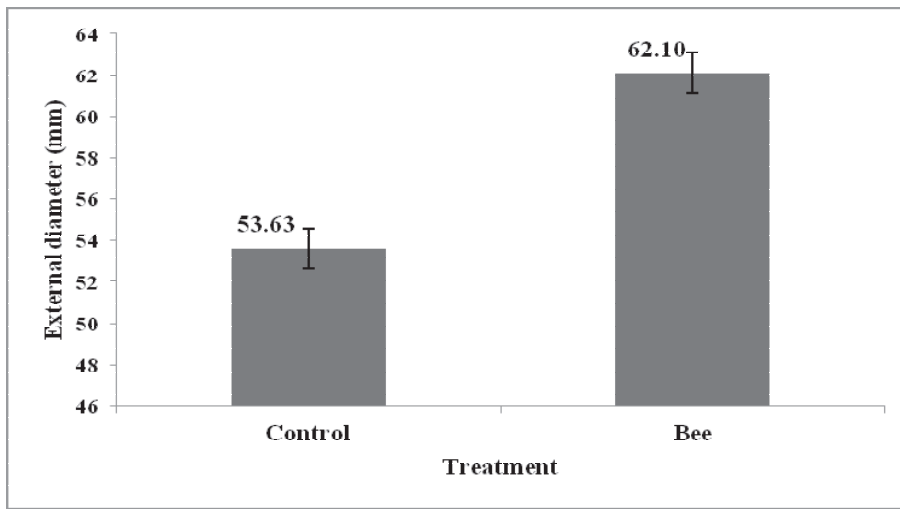

Fig. 6: Compare external diameter in greenhouse tomato bushes with and without bumblebees pollination treatments.

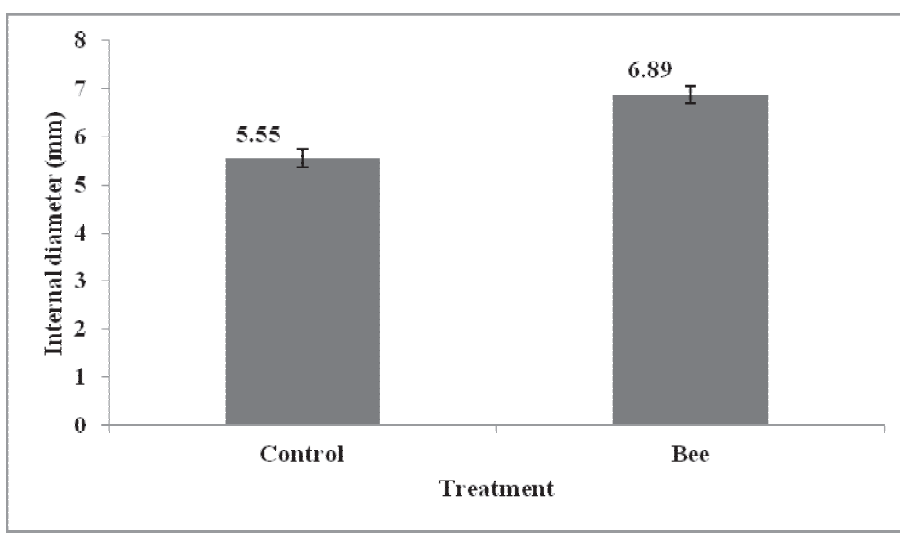

Fig. 7: Compare internal diameter in greenhouse tomato bushes with and without bumblebees pollination treatments.

Mean number of seeds per fruit has been shown the effect of presence of bee about 30 % increasing in the test.

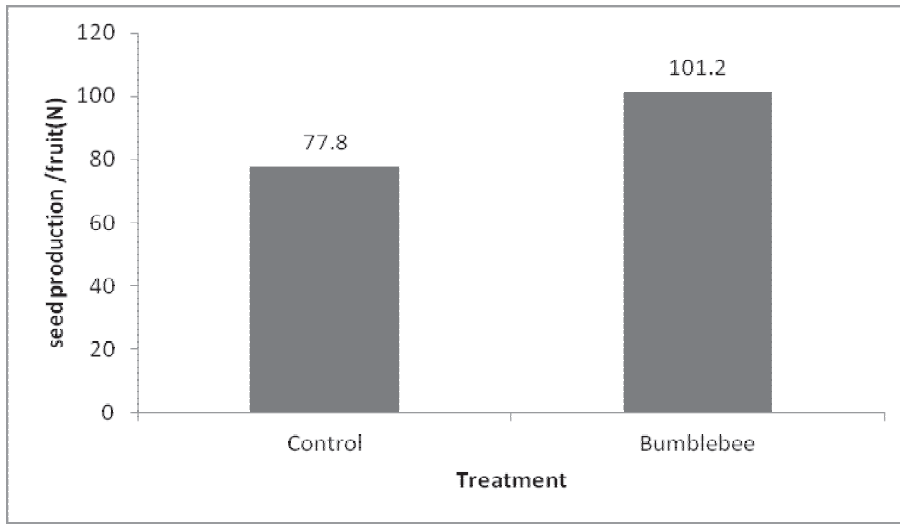

Fig. 8: Compare mean of seed production in greenhouse tomato bushes with and without bumblebees pollination treatments.

Discussion

Tomatoes pollinated by bumblebees in comparison to other methods such as handy works, growth regulators and manual vibration, showed a higher weight of fruit and product per bush in many researches (BANDA & PAXTON 1991; VELTHUIS & DOORN 2008, RAVESTIJIN & SANDE 1991). All found out that pollination by bumblebees with considering to tomato have the best mean pollination and more productivity than using of growth regulators and other methods. This increasing in yield by bumblebees is associated with a high number of seeds and better fruits quality too. AL-ATTAL et al. 2003 found out that temperature condition is effective on flowering in pollination and produce fruit. Since experiences on using bumblebees in tomato production in greenhouse have been successful in all over the world and we have native species of *Bombus terrestris* L. (MONFARED et al. 2007). It is possible we use this bees for enhance the crop productions too. In Iran, respect to lately increase of green house products especially of tomato this method been neglected seriously by farmers.

Tomato pollinated with bumblebees have more yield, high number seed set, solidarity weight and better size, relative higher density and firm fruit than other methods such as growth regulators and mechanical vibration and natural pollination. In a study by DEGTRUM et al. 1998 show that *B. vonesenskii* in Beefsteak tomato created more efficiency than manual pollination and non-pollination under greenhouse condition. In presence research which carried out for the first time in Iran, Weight, height, fruit diameter, seed number were more higher in pollinated flowers by bumblebees in comparison with pollinated flowers with non-bumblebees. IKEDA & TADAUCHI (1995)

found out tomato fruits by bumblebee pollination are flatter and full of vitamin C, acids and etc rather than the growth regulator method this was an interesting result too. According to results of variance analysis related to the mean number of fruits for per bush in our research, using of bumblebees were much more better than not using them and generated significant statistical difference. Although positive effect of using bumblebees has been proved in increasing yield of tomato in the world, also this project has been accepted in the greenhouse condition of Iran, but some points are important as followed:

1- Because these bees are commercial and used them in many productions, there is not sufficient data yet in Iran about applying them.

2- In our country bumblebees (commercial species) exist in many localities so the better way for increasing in yield and quality of crops productions especially in greenhouse could be encourage of rearing native one and introduction their advantages to farmers and producers.

It seems that increasing yield in different greenhouses has not the same productivity. So, it should be determined the effective factors on increasing yield simultaneously with using these bees in different weather conditions in Iran, type of greenhouse and way of using bee colony considered to the number of tomato and etc.

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Zusammenfassung

Vorliegende Arbeit behandelt den Vergleich von traditionell bzw. im Glashaus bestäubten Tomatenpflanzen (*Lycopersicon esculentum*) durch zwei Kolonien von *Bombus terrestris* L. (Hymenoptera, Apidae). Die Versuchsreihe fand in Gachsaran (Kohgiluyeh und Boyr-Ahmad Provinz) im Südwesten des Irans statt.

References

ANONYMUS (2012): Agriculture Statistic, second edition, Statistic office of Information of Ministry of Jahadkeshavarzi. — Available at; www.maj.ir. (Accessed, 14.V.2012).

BANDA H.J. & R.J. PAXTON (1991): Pollination of greenhouse tomatoes by bees. — Acta Horticulturae **288**: 194-198.

CRIBB D. (1990) Pollination of tomato crops by honeybees. — Bee Craft **72**: 228-231.

SANDE J. (1990): Bumblebees are good alternatives to truss vibration for beefsteak tomatoes. — Horticulture Abstract **60**: 506.

DOGTEROM M.H., MATTEONI J.A. & R.C. PLOWRIGHT (1998): Pollination of greenhouse tomatoes by the North American *Bombus vosnesenskii* (Hymenoptera: Apidae). — Journal of Economic Entomology **91**: 71-75.

GREENLEAF S.S. & C. KREMEN (2006): Wild bee species increase tomato production and respond differently to sorrnding land use in Northern California. — Biological Conservation **133**: 81-87.

HEINRICH B. (2004): Bumblebee economics. — Harvard University Press, Cambridge, Mass.

HOBAN A., LUCA E., SUSIU S, SALAGEAN T & N. POP (2008), the influence of the hybrid on the tomatoes production, cultivated in solarium irrigated. — Bulletin UASVM, Horticulture **65** (2): p. 648.

KAFTANOGLU O. & H. YENINAR (1996): Studies on the domestication of bumblebees (*Bombus terrestris*) and using them in green houses for the production of better quality vegetables. — Presented at the International Conference on Bee Products: Properties, Applications and Apitherapy, pp 26-30.

KEVAN P.G., STRAVER W.A. OFFER M. & T.M. LAVERTY (1991): Pollination of Greenhouse Tomatoes by Bumblebees in Ontario. — Proceedings of the Entomological Society of Ontario **122**: 15-19.

KEVAN P.G. (1999): Pollinators as bioindicators of the state of the environment: species, activity, & diversity. — Agriculture Ecosystems & Environment **74**: 373-393.

MICHENER C.D. (1962): An interesting method of pollen collecting by bees from flowers with tubular anthers. — Revista de Biologia Tropical **10**: 167-175.

MOUSAVI FAZL S.H. & A.R. MOHAMMADI (2005): Effect of water stress at different stages of growth on yield and fruit quality of two tomato varieties. — Journal of Agricultural Engineering Research **6** (22): 27-40.

MONFARED A., TALEBI A.A., TAHMASBI G., WILLIAMS P.H., EBRAHIMI E. & A. TAGHAVI (2007): A Survey of the Localities and Food Plants of the Bumblebees of Iran (Hymenoptera: Apidae: *Bombus*). — Entomologia Generalis **30** (4): 283-299.

PLOWRIGHT R.C. & T.M. LAVERTY (1987): Bumblebees & crop pollination in Ontario. — Proceedings of the Entomological Society of Ontario **118**: 155-160.

PTÁCEK V. (2001): Some biological aspects of Bumblebee (*Bombus*, Hymenoptera) management. — Acta Horticulturae **561**: 279-286.

PAXTON R.J. & H.J. BANDA (1991): Pollination of plastichouse tomatoes by bees. — Acta Horticulturae **288**: 194-198.

RAHEMI M. (2007): Pollination and Fruit Forming, (Translation from Englis), — 5th edition, Shiraz University publications. pp 120.

RAVESTIJIN W. & J. SANDE (1991): Use of bumblebees for the pollination of glasshouse tomatoes. — Acta Horticulturae **288**: 342-345.

VELTHUIS H.H.W. & VAN A. DOORN (2006): A century of advances in bumblebee domestication & the economic & environmental aspects of its commercialization for pollination. — Apidologie **37**: 421-451.

WINSTON M. (2001): Bees under glass. — Bee Culture **129**: 13-16.

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