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Original article

Foraging behavior and visit optimization of bumblebees for the pollination of greenhouse tomatoes

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ABSTRACT

The tomatoes grown under greenhouse conditions require supplemental bee pollination for the better fruit set. The present study was conducted to evaluate the optimized role of bumblebees (*Bombus terrestris* L.) for tomato pollination under greenhouse conditions. The impact of increasing number of floral visits (i.e. 1 to 5) on physical and biochemical properties of tomato was studied on tomato variety 'Grande' grown on an area of 500 m². The self-pollination and hand vibration treatments were maintained for the comparison. The foraging behavior in terms of colony traffic, stay time and visitation rate was also studied. The maximum average outgoing bumblebees (7.38 individuals) were recorded at 10:00 while the maximum average incoming (6.75) were recorded at 2:00 pm. The three visits of bumblebees on a single flower resulted in the maximum improvement in physical (higher fruit length, fruit weight, fruit weight, number of seeds per fruit, weight of 100 seeds) and biochemical properties (vitamin C, shelf life) as compared to hand vibration and self-pollination treatments. There was no improvement in physical and biochemical properties in fourth or fifth visit. Bumble bee pollinated fruits had low TSS, pH and postharvest weight loss than that of self-pollinated and hand vibrated treatments. Therefore, three visits of bumblebees per flower are enough to get the optimum production of tomato under greenhouse conditions.

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1. Introduction

Tomato *Lycopersicon esculentum* Mill (Solanaceae) is the most grown vegetable in the world with annual production of 177 million tons. Tomato is self-fertile however owing to the position of style, the structure of anther cone and the mode of dehiscence,

it needs some vibration to ensure adequate pollination (Raymond, 1985; Rylski et al., 1994). The tomatoes which are grown in greenhouse therefore, require supplemental pollination for better fruit setting (McGregor, 1976). For this purpose, growth regulators, plant or truss vibration, bumblebees and honeybees are frequently used (Pak & Kim, 1999).

Honeybees, however, are also considered as nectar robbers of tomatoes as they cannot buzz pollinate which bumblebees can (Winston, 2001; Depra et al., 2014). Using vibrators and growth regulators on the other hand, are labor intensive whilst later also gives low quality fluffy fruits (Ravestijn & Sande, 1991). Bumblebees are important pollinators of greenhouse grown crops, because they have the ability to forage in low light intensity and low temperature. As compared to honey bees, they are robust, larger and furrier (Winston, 2001). Using their long tongue, they obtain pollen

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from tomato flowers by grabbing and vibrating them. The sonication of bumblebees allows for shedding of pollen grains through apical pores in tomatoes (Buchmann, 1983). About 95% of commercially-reared colonies are being used in production of greenhouse tomatoes and sweet peppers across the world (Ercan & Onus 2003; Velthuis & van Doorn, 2006).

Since the foraging behavior of a pollinator can influence its pollination efficiency; understanding such behavior can help in effective pollination management. The important foraging behavioral parameters of bumblebee include its colony traffic, stay time on a flower, visitation rate and abundance. Ahmad et al., (2015) noticed higher floral visitation of bumblebees on tomato crop in the morning hours. Yankit et al., (2018) recorded greater stay time of bumblebees on flowers in the morning hours. Similarly, Kwon and saeed, (2003) observed decreased colony traffic in afternoon due to increase in temperature. Zaitoun et al., (2006) reported higher foraging rate of bumblebees on strawberry flowers than that of honeybees.

It is well described that tomatoes, pollinated by bumblebees, exhibit higher yield, better weight, fruit firmness, higher number of seeds and higher specific gravity as compared to vibration and plant growth bio-regulators (Nazer et al., 2003). However, no effectors have been made in knowing the optimized number of bumblebee visits for better fruit set in tomatoes. Number of honeybee visits for normal development of watermelon and white clover, for example, has shown to have positive relationship with the yield parameters (Adlerz, 1966; Goodwin et al., 2011). Only one previous study (i.e. Morandin et al., 2001) has shown a positive relationship between intensity of bumblebee pollination and bruising level on tomato flowers under greenhouse conditions. However, the effect of number of bumblebee visits on tomato physical, biochemical properties and shelf life is poorly known.

The aim of the study was to know the optimum number of bumblebee visits for the optimum production of tomatoes. Therefore, current study was undertaken to evaluate the effect increasing number of bumblebee visits on tomato fruiting in terms of their physical and biochemical properties under hydroponic conditions. Some other foraging behavioral parameters of bumblebees were also studied.

2. Materials and methods

2.1. Study Site:

The current study was conducted in a greenhouse hydroponic unit (1000 m²) of MNS-University of Agriculture Multan, Pakistan. The daily relative humidity and temperature were maintained “between” 60–80% and 18–35 °C, respectively with cooling pad wall and exhaust fans. The tomato variety ‘Grande’ was sown. One bumblebee colony was placed in the area of 500 m² of hydroponic unit while in another 500 m² plot separated by plastic sheet, vibration and self-pollination methods were applied.

2.2. Bumblebee foraging behavior:

The colony traffic (outgoing and incoming individuals during 15 min at the entrance of hive), stay time (length of time spent by a bee on a single flower) and visitation frequency (number of individuals visiting a flower during one minute) was observed at 8 am, 10 am, 12 pm, 2 pm and 4 pm. The data was recorded twice a week during the whole study period (February-April 2018).

2.3. Pollination experiments:

Three different methods of pollination were evaluated i.e., hand vibration (two and four minutes), increasing number of bumblebee

visits (one to five visits on a single flower) and self-pollination (caged pollination). For hand vibration, forty near to open flower buds were covered with nylon mesh bag in the evening and then uncaged on the very next day. Twenty flowers were vibrated with hand for 2 min and other 20 for 4 min and then re-caged. The bags were permanently removed upon the confirmation of fruit set.

For the evaluation of increasing number of bumblebee visits (i.e. 1 to 5 visits) on tomato pollination, 25 floral buds (each on a different plant) were caged with nylon mesh bags one day before opening and then uncaged on very next day to receive one to five bumblebee visits (5 flowers per treatment) in five replications. The flowers were re-caged and tagged. Besides this, 20 flowers (each on a different plant) were also caged with nylon mesh bag for entire flower life to exclude from insect visits and ensure self-pollination.

2.4. Physical and biochemical fruit traits:

Upon ripening the fruits were harvested from each treatment. The physical properties recorded were fruit dimension, fruit weight, number of seeds per fruit, seed weight per 100 seeds and shelf life. The dimensions (length and width) of freshly harvested fruits were measured with the help of a digital Vernier caliper. Fruits were weighed with the help of an electronic balance. The number of seeds per fruit was counted manually. The weight of 100 dried seeds was recorded with the help of an electronic balance.

The biochemical properties noted were Titratable Acidity (TA), Total Soluble Salts (TSS), Vitamin C, and pH. For this purpose, only those pollination treatments were selected which exhibited the best physical properties i.e. three visits, two minutes hand vibration and self-pollination. The TA was determined according to the method proposed by Horwitz (1960). Similarly, TSS was measured according to the method of AOAC (1990). A method described by Ruck (1961) was used to determine vitamin C contents of the juice. A pH meter was used to measure the pH of the juice.

2.5. Shelf life

The shelf life (in terms of daily weight loss) was determined by keeping the fruits of each pollination treatment at room temperature. The percentage of daily weight loss was calculated by following formula:

$$\% \text{ daily weight loss} = \frac{(\text{weight on first day}) - (\text{weight on next day})}{(\text{Weight of first day})} \times 100$$

2.6. Statistical analysis

The data for bumblebee colony traffic, abundance, stay time and visitation rate was expressed graphically. Moreover, data regarding physical (Fruit length, width, weight, number of seeds/fruit, weight of 100 seeds), bio-chemical parameters (T.A, TSS, Vitamin C and pH) and shelf life (shelf life, daily weight loss percentage) was analyzed by using analysis of variance (ANOVA). All the statistical analysis was performed by using the software XLSTAT (2019).

3. Results

3.1. Bumblebee foraging behavior

The incoming and outgoing of bees at the entrance of hive is presented in Fig. 1. The maximum outgoing was recorded at

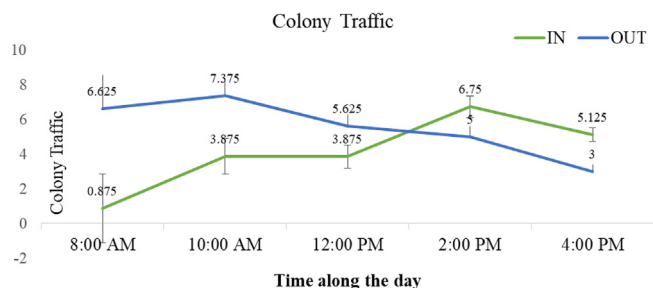


Fig. 1. Colony traffic of Bumblebee on Grande tomato flowers.

10:00 am (i.e. 7.38 individuals per 15 min) which gradually decreases until 4:00 pm (i.e. 3). On the other hand, the maximum incoming was recorded at 2:00 pm (i.e. 6.75) and the minimum at 08:00 am (i.e. 0.87). The mean stay time of bumblebees on tomato flowers was maximum (7.25 s per flower) at 02:00 pm and it was minimum (5.95) at 04:00 pm (Fig. 3). While the mean visitation rate of bumblebees was maximum (5.01 flowers 60 s) at 02:00 pm and it was minimum (4.25) at 04:00 pm (Fig. 2).

3.2. Physical parameters

The maximum fruit length, fruit width, fruit weight, number of seeds per fruit and weight of 100 seeds was obtained from three bumblebee visits i.e. 40.54 mm, 54.26 mm, 107.77 g, 59 seeds and 0.50 g, respectively. Three visits of bumblebees resulted in best physical fruit properties. Two minutes of hand vibration gave better results than one minute of vibration in terms of fruit width, number of seeds per fruit and weight of 100 seeds. Therefore, biochemical analysis was only performed for the fruits obtained from three visits of bumblebees and two minutes of hand vibration. The self-pollination produced only a few seeds per fruit (Table 1).

3.3. Biochemical properties

The average TA value was similar in fruits obtained as a result of three visits of bumblebees (i.e. 0.61%) and two minutes of hand vibration (i.e. 0.60%). TSS was greater in two minutes of hand vibration (6.10) than three visits of bumblebees (4.40). On the other hand, vitamin C was higher in three visits of bumblebees (425.40 mg/100 ml) than two minutes of hand vibration (401.59 mg/100 ml). In case of pH, self-selected fruits showed to have more pH (6.22) than two minutes of hand vibration (6.15) and three visits of bumblebees (4.16) (Table 2).

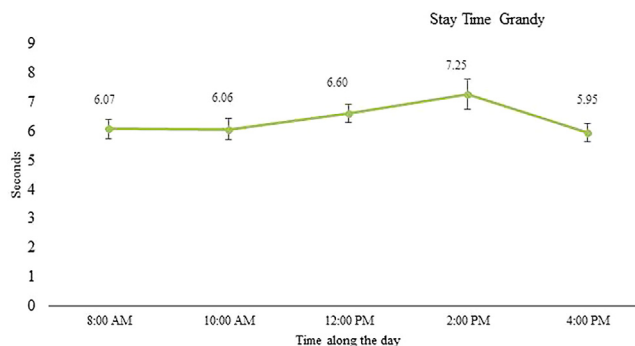


Fig. 3. Stay time of Bumblebee on Grande tomato flowers.

3.4. Shelf life

The average shelf life of the tomatoes was the greatest for the fruits resulted from three visits of bumblebees (14.20 days) followed by two visits of bumblebees (10.66 days) and two minutes of hand vibration (10.33 days). Similarly, daily weight loss was highest for the fruits resulted from self-pollination (9.71%) and lowest for three visits of bumblebees (0.98%) (Table 3).

4. Discussion

In this study, the colony traffic and foraging activity varied along the days. The maximum outgoing was recorded at 10:00 am (i.e. 7.38 individuals per 15 min) and the minimum at 4:00 pm (i.e. 3). The maximum foraging activity was recorded at 2:00 pm and the minimum at 4:00 pm. The colony traffic (exiting and entering) and foraging activity decrease significantly with the increase in temperature under greenhouse conditions (Kwon, & Saeed (2003). Bumblebee activity increases from 5 to 250C mainly due to decreased thermoregulation costs (Heinrich 1979). Chen and Hsieh (1996) found that external temperature also affects bumblebee activity as it significantly reduced in the noon of summer as a result of extreme temperatures (up to 40 °C) but, in our case, the temperature of greenhouse did not exceed 35 °C. Yankit et al. (2020) observed decreased foraging activity at noon because of high temperature as compared to evening hours.

Our results showed that there was no increase in fruit weight, seed weight and number of seeds after three visits of bumblebees. With the increase in total frequency of visits the proportion of damaged pistils is increased and this trend is more obvious for *B. terrestris* than *A. mellifera* (Sáez et al. 2014). Another possible reason is that during first three visits bumblebees remove significantly larger amount of pollen grains than the subsequent visits.

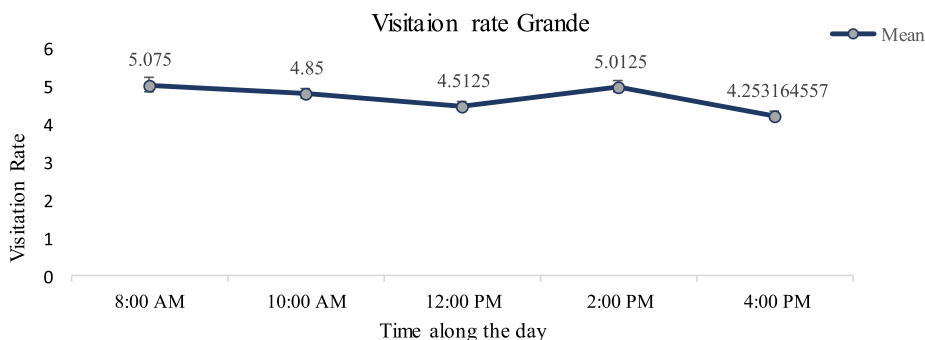


Fig. 2. Visitation rate of Bumblebee on Grande tomato flower.

Table 1
Comparison of physical parameters for different treatments of Grande tomato.

	Length (mm)	Width (mm)	Weight (mm)	No. of seeds per fruit	100 seed weight (g)
1 min. of HV	34.42 ± 1.4b	42.6 ± 1.86 bc	63.51 ± 4.00c	78.50 ± 5.7c	0.31 ± 0.0c
2 min. of HV	35.47 ± 1.0b	45.80 ± 1.52b	65.68 ± 5.00c	121.60 ± 4.3b	0.42 ± 0.0 ab
1 visit of BB	34.42 ± 1.9b	47.07 ± 4.15 ab	74.00 ± 4.97 bc	143 ± 6.00 ab	0.35 ± 0.2 bc
2 visits of BBs	36.56 ± 4.3 ab	50.03 ± 4.31 ab	96.40 ± 4.01 ab	145.33 ± 4.3 ab	0.36 ± 0.0 bc
3 visits of BBs	40.54 ± 1.7 a	54.26 ± 5.89 a	107.77 ± 3.9 a	159 ± 5.16 a	0.50 ± 0.1 a
4 visits of BBs	37.00 ± 1.3 ab	48.33 ± 4.37 ab	63.00 ± 3.85c	116 ± 3.51b	0.39 ± 0.1 abc
5 visits of BBs	35.03 ± 1.7b	43.26 ± 2.35 bc	71.86 ± 2.57 bc	110.67 ± 6.0b	0.41 ± 0.1 abc
Self-pollination	30.20 ± 0.3c	39.02 ± 0.79c	60.59 ± 2.59c	45.90 ± 3.70 d	0.30 ± 0.1c
ANOVA results	P = 0.0000 F = 11.6 DF = 7	P = 0.0000 F = 5.91 DF = 7	P = 0.0000 F = 5.38 DF = 7	P = 0.0000 F = 54.1 DF = 7	P = 0.0000 F = 11 DF = 7

Table 2
Comparison of biochemical properties of different treatments of Grande tomato.

	Titratable acidity (%)	Total soluble solid	Vitamin C(mg/100 ml)	pH
3 visits of BBs	0.61 ± 0.30 a	4.40 ± 0.15b	425.40 ± 4.19 a	4.16 ± 0.01c
2 min. of HV	0.62 ± 0.23 a	6.10 ± 0.05 a	401.59 ± 3.17b	6.15 ± 1.85b
Self-pollination	0.13 ± 0.12b	6.20 ± 0.05 a	114.29 ± 5.50c	6.22 ± 0.01 a
ANOVA results	P = 0.0000 F = 146 DF = 2	P = 0.0000 F = 102 DF = 2	P = 0.0000 F = 1552 DF = 2	P = 0.0000 F = 9207 DF = 2

Table 3
Comparison of Shelf life of Grande tomato in different treatments.

	Shelf life (days)	Daily weight loss (%)
1 min. of HV	8.33 ± 0.88 cd	8.89 ± 3.05 ab
2 min. of HV	10.33 ± 1.20 bc	7.30 ± 2.48 abc
1 visit of BB	9.75 ± 0.25 bcd	1.119 ± 0.11 bc
2 visits of BBs	10.66 ± 0.88b	1.11 ± 0.94 bc
3 visits of BBs	14.20 ± 0.37 a	0.98 ± 0.61c
4 visits of BBs	10.00 ± 0.00 bcd	1.24 ± 0.18 ab
5 visits of BBs	9.00 ± 0.57 bcd	1.71 ± 0.19 abc
Self-pollination	8.00 ± 0.57 d	9.71 ± 2.93 a
ANOVA results	P = 0.0000 F = 10.5 DF = 7	P = 0.0002 F4.32 DF = 7

This elevated pollen removal may leads to high pollen deposition during the first three visits (Zhang et al. 2019). Nunes-Silva et al., (2013) therefore recommend that higher intensity of bumblebees should be avoided because it damages the reproductive part of tomato flower. Morandin et al., (2001) observed that more than two times bruising had no impact on diameter, weight and number of seeds in tomato fruit. Keeping this finding in view they suggested 2,000 bumblebee trips per hectare per day as adequate to achieve the best pollination of tomatoes under greenhouse conditions and to ensure these trips, at least seven average sized colonies –60 individuals in each- are required in one hectare. Managing the frequency of bumblebee visits is important for tomato production as more visits can damage reproductive organs of flowers and cause their abortion (Morandin et al., 2001; Morse 2009).

In this study, three visits of bumblebee pollination increased the number and weight of seeds significantly as compared to hand vibration and self-pollination. These findings are in line with those of Yankit et al., (2018) and Ahmad et al., (2015) who reported greater number of seeds per fruit and seed weight per fruit in bumblebee pollinated plants as compared to caged pollination. Insufficient pollination due to absence of wind in greenhouse is an important challenge for tomato pollination (Free, 1970; Banda and Paxton, 1991). The results of this study showed that fruit size was the maximum in bumblebee pollinated fruits than hand

vibrated and self-pollinated fruits. High speed of Bumblebee foraging, its buzzing behavior and efficiency at low sunlight and temperature make them reliable pollinators of greenhouse crops (Paydas et al., 2000; Winston, 2001). The fruit size of tomato is the function of number of pollen grains transferred from anther to stigma of flower (Morandin et al., 2001). Bumblebee pollination exhibit affirmative results on yield parameters including fruit weight and fruit shape (Hatami et al., 2013; Ahmad et al., 2015; Yankit et al., 2018).

Our results suggest that bumblebee visited fruits showed higher vitamin C and acidity, while total soluble solid and pH of fruit juice was higher in self-selected fruits. The results were in agreement with the results of Bashir et al., (2017) who found positive impact of bee pollination on physicochemical properties of tomatoes in terms of TA, TSS and pH of tomato juice. The results of this study are also in line with the study of Ikada & Tadaichi (1995). They also reported the positive effect of bumblebee pollination in terms of vitamin C.

In conclusion, three visits of bumblebees on a single flower are enough to get the optimum production of tomatoes under greenhouse conditions. Future studies should optimize the number of visits of bumblebees and other wild pollinators in terms of physical and biochemical properties of tomatoes under open field conditions.

5. Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Syed Usama Zameer, Mudassar Ali, Asif Sajjad and Amar Matloob. The first draft of the manuscript was written by Syed Usama Zameer and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CRedit authorship contribution statement

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The first draft of the manuscript was written by Syed Usama Zameer and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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