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Are colony developmental stages of bumblebee, *Bombus terrestris* (hymenoptera: apidae) affected by different concentrations of sugar and honey solutions?

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Abstract

Bumblebees, more efficient than honeybees, provide important services for pollination especially in tomato, pepper, cucumber, strawberries and other crops grown under tunnel farming or glasshouse conditions to yield maximization. These bees require pollen and nectar to meet their dietary needs and maintain their colony structure, development and reproduction. Keeping in view their economic importance, the effect of five concentrations of sugar and honey solutions (1:1, 1:1.5, 1:2, 2:1,1.5:1) each as alternative to nectar were used to observe their effect on life history parameters of *Bombus terrestris*. The 1:1 ratio of sugar solution was found most effective followed by 1.5:1, 1:1.5, 1:2 and 2:1 and also more effective of all five concentrations of honey solutions on all three stages of colony development i.e., at colony initiation, colony development and colony maturation stages. At colony initiation stage, early pre-oviposition period (6.40± 0.97 days), early emergence of first worker in the first batch (25.40±1.21 days) and maximum numbers of workers (6.20±0.24) emergence in the first batch were observed at 1:1 ratio of sugar solution. Colonies reared on 1:1 ratio of sugar solution reached earlier (52.13±1.28 days) at colony foundation stage with minimum mortality (3.27±0.54 workers). At colony maturation stage, maximum numbers of workers, sexual (males, queens) and maximum mother queen longevity was observed at the same 1:1 ratio of sugar solution. It can be suggested from present study that sugar solution as alternative of nectar at 1:1 ratio was better than other sugar concentration levels and also from those of honey solution.

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Introduction

Pollination is the movement of pollens from the male part of the flower to female part usually by arthropods and air (Stern, 1994). Farmers are using honeybees for years but their use has decreased due to lower availability of food sources (Williams *et al.,* 1991). Bumblebees have been observed to be the most efficient pollinators from all others, used to pollinate various crops and fruits (Pinchinat *et al.,* 1979). These rank among the most abundant flower visitors in alpine, temperate and arctic

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regions providing pollination services (Corbet *et al.,* 1991; Sheikh *el al*., 2014). Greenhouse crops, mostly of the cruciferous family need buzz pollination to shake their pollen and pollinate the flower which is usually done by bumblebee (Goulson, 2003). Bees, the most successful Hymenopterans insect are phytophagous, mainly feeding on pollen and nectar of flowers (Michener, 1974).

To meet the need of greenhouse and glasshouse tomato growers European countries started rearing bumblebees commercially at mass scale since 1987. Different techniques were used to establish their year-round mass rearing (Alford, 1975; Pouvreau, 1976). Since 1996, many laboratories in China, Korea, Israel and Mexico have been rearing bumblebee for the researcher to study the behavior, pollinating capability and biology with remarkable progress (An *et al.,* 2001; Peng *et al.,* 2003; Zhan-bao *et al.,* 2003). In nature, these bees live in small colony of 200-300 individuals (Dramstad and Fry, 1995). Most of these species are social and their colonies consist of almost always single mated queen and usually a few hundred workers (Richards, 1973).

During the mass rearing of bumblebees on commercial scale, colonies are fed with sugar syrups obtained from different plant sources such as *Zea mays*, *Saccharum* spp. and *Beta vulgaris* because of their low costs and liquid structure. It is estimated that about one million kilogram of sucrose syrup is used by bumblebees rearing industry and another two million kilogram of sucrose solution to feed bumblebees in glasshouses (Velthuis and Doorn, 2006). Pollen, an important source of food which not only supply proteins for the development of queen ovary ((Plowright and Pendrel, 1977) but also help to increase the adult workers body size (Sutcliffe and Plowright, 1988; Duchâteau and Velthuis, 1989).

Previous researchers used different concentrations of honey solution and pollen for bumblebee rearing under laboratory condition, obtained from honeybee (Hannan *et al.,* 1998; Griffin *et al.,* 1991; Ono *et al.,* 1994; Tasei and Aupinel, 1994) with different methods of their supply to bees. Caste specific distinction in feeding frequencies is result, but not the reason, of differences in development (Pereboom *et al.,* 2003). Because influx of pollen and nectar affect the reproduction, development and survival of bumblebee (Spaethe and Weidenmüller, 2002). Short term food shortfall affects colony growth as they may boost the susceptibility of colonies to parasites and predators (Cartar, 1991). In the earlier work conducted by Yoon *et al.* (2005), different concentration of sucrose solution as nectar with addition of antiseptic to this solution, were compared. They found that different type of sugar solutions and antiseptic concentrations showed different effects on oviposition and development of *Bombus terrestris* colony.

Social insects have complex life like division of labour which greatly influences their successful developmental and reproductive processes (Wilson, 1985). This not only include the stereotyped behavioural development process rather has plasticity for short and long term needs of colony survival for environmental cues previously observed in bumblebees and honeybees as well (Camazine 1993). However, status of colony nectar resources can only be achieved by interaction in foraging and nest food storing bees. Considering the importance of carbohydrates as important food source, for early development of workers, drones and queen larvae, glucose was the main sugar component while fructose was major constituent in older larval stage determining in caste determination (Brouwers, 1984). Kaftanoglu *et al*. (2011) highlighted sugar contents importance for metamorphose and pupation stages in laboratory development of *Apis mellifera*. These sugar contents not only affected the normal larval development but also increased the overall numbers with high queen and intercastes numbers. Like biological characters behavioural development does get affected by colony nutritional needs as important social interaction like early foragers development in starved colonies, such social polytheism looks more temporal (Schulz *et al*., 1998).

Power of insect flight muscles is proportional to muscle temperature up to certain limits (Coelho, 1991; Esch, 1976). This regulation of temperature plays important role in those insects that forage for carbohydrates and proteins (Heinrich, 1993). Bumblebees required 30^{0}C (Heinrich, 1979) while honeybees 38^{0}C for flight (Woods *et al.,* 2005). However, bumblebee foragers showed increase in their flight muscles temperature with increased concentration of sugar solution. Carbohydrate foraging provided only part of colony needs but for the normal development of the colony, protein foraging as for pollen is essential (Sagili and Pankiw, 2007; (Wolf *et al.,* 1989)). Bees body size strongly affected by dietetic conditions under which they were reared. Less carbohydrate and protein content of pollen also affect the immature resulting adult size (Roulston and Cane, 2000). The objective of this study was to investigate the effect of different concentrations of sugar and honey solution on colony growth of European bumblebee *bombus terrestris*

Materials and Methods

Research was conducted in Non-Apis Bee Laboratory, Department of Entomology, PMAS-Arid Agriculture University Rawalpindi during 2013-2014. Colonies of bumblebees (*Bombus terrestris* L.) were imported from Netherland, Koppert Biological Systems. Colonies were reared on company supplied nectar and pollens. Specific identification numbers were assigned to all imported hives for collection of new daughter queens and males separately. Newly emerged males and queens were separated from hives and placed separately in different cages. Same age queens were placed in separate box in rearing room illuminated with red bulb and males were placed in different chambers illuminated with UV light (Kwon *et al.,* 2003). Single time mating was done with the same species of bumblebee but from different hives to prevent inbreeding problem. Mating was carried out in a special polycarbonate cage to get maximum copulation (Imran *et al.,* 2015). Mated daughter queens were fed one week on fresh pollen and 50% sugar solution with distilled water and then placed for hibernation at 0-4^{0}C for 2.5 months (Tasei and Aupinel, 2008). After the completion of hibernation period, experiments were performed through food to improve colony development.

Artificial rearing of the bumblebees

European bumblebee, *Bombus terrestris* L. which is known as large bumblebee has been artificially reared in many countries of the world (Yoon *et al.,* 1999, 2002, 2004). The selection of this specie for artificially rearing under laboratory conditions was due to its best results both in pollination ability and also on artificial multiplication under laboratory (Velthuis and van Doorn, 2006).

After the completion of hibernation, queens were introduced into flight cage (0.5×0.5×1.2 m^{3}) for one week before each was placed into rearing boxes (starter box). After one week, each queen was separately placed in starter boxes with two small *B. terrestris* L. workers as helpers and randomly divided into five groups which were replaced after one week with new ones. Hard cardboard was used to place in the box with fixed artificial bumblebee pupa made of styrofoam coated with beeswax and provided with artificial beeswax honey cup. Small plastic petri dishes (35 mm × 10 mm) with absorbent wicks were placed in each starter box to fed queens on sugar and honey solution. Solution was prepared with distilled water, and pollens were also supplied in similar plastic petri dishes with diet changed after every 24 hour.

Conditions in rearing room

To reduce the chance of fungus attack inside, boxes were monitor and cleaned with a piece of cotton soaked with 75% ethyl alcohol every day. Laboratory conditions were set at 25±2^{o}C and 65% RH and covered with dark black sheet. (Duchateau and Velthuis, 1988). Holmes Warm Mist Humidifier HM5082 was used to maintain relative humidity and Dual System Mode Cooling and heating unit was used to maintain the temperature. Thermo-hygrometer (CH-AZHT02/CLOCK bench mount BIG digit indicator for RH/T with clock and MIN/MAX) was used to measure temperature and relative humidity. Red light was used in the rearing room because bees are red color blind and it is easy to work under red light, caused minimal disturbance and reduced attempts to fly.

Effect of different concentrations of sugar and honey solutions on life history parameters of bumblebees

Basic rearing technique of bumble bee, *B. terrestris* was followed as described by Yoon *et al.* (2002). Three different types of boxes were used according to their three different development stages of colony initiation, foundation and colony maturation stages (Imran *et al.,* 2017). Five concentrations levels i.e., 1:1, 1:1.5, 1:2, 2:1 and 1.5:1 of sugar and honey with distilled water were used separately on three colony developmental stages. Pre-oviposition period (time taken by queen to laid first egg), first worker emergence time and total workers emerged at this stage were observed at colony initiation stage. At colony foundation stage, observed parameters were: time taken by the colony to reach at 50 individual and mortality at this stage. Total emerged males, daughter queens, workers and mother queen longevity (mother queen longevity was calculated from introduction of queen in starter box to its death) were observed at colony maturation stage.

Data analysis and statistics

Statistical analysis was done by using SPSS software (Norus, SPSS Inc. 2006). Different bumblebee colony growth parameters were compared with t-test and DMRT at 5% probability for comparison of percentage values.

Results and Discussion

Colony initiation stage

Significant difference existed among different concentrations of sugar and honey solution on per-oviposition period ($F_{(4, 74)}$ = 45.3, P< 0.001; $F_{(4, 74)}$ = 3.40, P= 0.0134) (Table 1). Early egg laying period (6.40± 0.97 days) was observed at 1:1 (W: S) ratio of sugar solution and late (20.13±0.80 days) at 2:1 ratio of sugar solution. Similarly at honey solution, minimum oviposition period (13.80±1.23 days) was observed at ratio of 1:2 and maximum (20.70±2.23 days) at ratio of 1.5:1 (Table 1).

Effect of different tested nectar concentration on first worker emergence in the first batch of colony showed early emergence of worker at 1:1 ratio of sugar solution (25.40±1.21 days) and late emergence (38.93±2.71 days) at 2:1. For honey concentrations, minimum time period (32.53±1.22 days) for emergence of first worker was observed at ratio of 1:2 and maximum (42.33±1.82 days) at 1.5:1 (Table 1). Overall statistical analysis showed that significant was observed on first worker emergence during the first batch ($F_{(4, 74)}$ = 37.2, P<0.001; $F_{(4, 74)}$ = 8.57, P= 0.0134) (Table 1).

Similarly maximum workers (6.20±0.24) emerged from colonies feed at 1:1 ratio of sugar solution and minimum (5.0±0.4) at 2:1. At honey solutions, maximum number of workers (5.46±0.27) emerged at ratio of 1:1 and minimum (3.80±0.42) at 2:1 (Table 1). Non-significant differences were observed among different concentrations of sugar ($F_{(4, 74)}$ = 2.37, P= 0.060) while these were significant between honey solutions ($F_{(4, 74)}$ = 2.94, P= 0.0262)(Table 1).

Colony foundation stage

Colony foundation stage was considered when the workers strength in the colony reached at 50 individuals. Significant difference existed among five concentrations of sugar and honey solutions ($F_{(4, 74)}$ = 6.94, P= 0.0001; $F_{(4, 74)}$ = 5.49, P= 0.007) (Table 2). Colonies feed at 1:1 ratio of sugar solution reached early at foundation stage (52.13±1.28 days) and late (63.27±2.33 days) at 2:1 ratio of sugar solution. Similarly, minimum time period (62.4±2.03 days) of colony foundation on honey solution was observed at ratio of 1:2 and maximum (73.06±2.54 days) at 1.5:1 (Table 2).

Table 1. Effect of different concentrations (Mean± SE) of sugar and honey solutions at colony initiation stage under controlled laboratory conditions

		----------Sugar/Honey Solution Concentrations----------						
	Observed Parameters	1:1	1:1.5	1:2	2:1	1.5:1	F	P
Sugar Solution	Pre-oviposition period	6.40± 0.97	9.53±0.75	12.26±0.79	20.13±0.80	8.73±0.56	45.30	0.001
	1st workers emergence day	25.40±1.21	27.60±0.75	29.46±0.86	38.93±0.70	27.86±0.69	37.20	0.001
	Total workers emerged in 1st batch (n)	6.20±0.24	5.40±0.25	5.20±0.29	5.0±0.40	5.80±0.34	2.37	0.060
Honey solution	Pre-oviposition period (days)	17.13±1.33	14.60±1.6	13.80±1.23	19.93±1.79	20.70±2.23	3.40	0.0134
	1st workers emergence day	36.20±1.34	33.86±1.60	32.53±1.22	41.26±1.39	42.33±1.82	8.57	0.001
	Total workers emerged in 1st batch (n)	5.46±0.27	4.73±0.40	4.93±0.38	3.80±0.42	4.06±0.44	2.94	0.0262

Table 2. Effect of different concentrations (Mean± SE) of sugar and honey solutions at colony foundation stage under controlled laboratory conditions

		----------Sugar/Honey Solution Concentrations----------						
	Observed Parameters	1:1	1:1.5	1:2	2:1	1.5:1	F	P
Sugar Solution	Colony foundation stage (days)	52.13±1.28	55.33±1.19	60.26±2.37	63.26±2.33	53.26±1.44	6.94	0.001
	Mortality at foundation stage	3.26±0.54	6.13±1.05	7.13±0.93	8.66±0.92	5.46±0.76	5.47	0.007
Honey solution	Colony foundation stage (days)	64.06±3.56	65.80±1.62	62.40±2.03	71.86±1.94	73.06±2.54	5.49	0.007
	Mortality at foundation stage	6.60±1.01	4.93±0.64	4.13±0.73	9.60±1.36	8.47±1.07	5.34	0.0008

*Mortality in numbers

Mortality at foundation stage showed minimum (3.26±0.54) at 1:1 ratio of sugar solution and maximum (8.66±0.92) at 2:1. Effect of five concentrations of honey solution showed minimum mortality (4.13±0.73) at ratio of 1:2 and maximum (9.60±1.36) at 2:1, ($F_{(4, 74)} = 5.47$, P= 0.0007; $F_{(4, 74)} = 5.34$, P= 0.0008) (Table 2).

Colony Maturation Stage

Maximum males emerged (156.60±12.8) from colonies reared at 1:1 ratio and minimum (99.07±8.18) at 2:1 of sugar solutions. Colonies reared on honey solutions showed maximum males (97.13±4.96) emergence at 1:1.5 and minimum (56.46±3.64) at 1.5:1 ratio of honey solutions. Significant differences existed in term of males emergence in the colony ($F_{(4, 74)} = 4.88$, P= 0.0016; $F_{(4, 74)} = 16.6$, P<0.001) (Table 3).

Five concentrations of sugar and honey had significant effects on the emergence of total daughter queens. Maximum numbers of daughter queens (70.20±10.4) were observed at 1:1 while minimum (22.26±3.68) at 2:1 ratio of sugar solutions. Similarly results of different concentration of honey solution showed that maximum number of queens (16.30±3.87) emerged at ratio of 1:2 and minimum (0.60±0.41) at ratio of 1.5:1 (Table 3).

Significant differences were observed among different concentrations of sugar and honey solutions on total number of worker emergence during the whole life cycle of the colonies ($F_{(4, 74)} = 20.4$, P<0.001; $F_{(4, 74)} = 3.85$, P= 0.007) (Table 3). Maximum numbers of workers emerged (158±7.86) at ratio of 1:1 and minimum (83.33±4.44) at 2:1 of sugar solution. Similarly, at honey solutions, maximum numbers of workers emerged (80.40±4.18 and 80.33±6.03) at ratio of 1:1.5 and 1:2 while minimum (63.03±2.47) at 1.5:1 (Table 3).

Mother Queen (foundation queen) life was calculated from the introduction of queen in to the starter box till its death. Highly significant differences were observed between five concentrations of sugar and honey on foundation queen life ($F_{(4, 74)} = 12.1$, P<0.001; $F_{(4, 74)} = 9.46$, P<0.001) (Table 3). Maximum queen life duration (107.27±4.0 and 106±4.42 days) was observed at ratios of 1:1 and 1.5:1 while minimum (76.4±3.89 days) at 2:1 of sugar solution respectively. For honey solutions, maximum queen life duration was observed (86.13±2.22 days) at 1:2 and minimum (63.20±2.05 days) at 1.5:1 ratio (Table 3).

Overall results of five concentrations of sugar and honey solution showed that 1:1 ratio of sugar was the most effective concentration level with best performance on all observed parameters at colony initiation, colony foundation and colony maturation stages.

Most important character at colony initiation stage of *B. terrestris* L. is the laying of first egg batch showing the reproductive success of the foundation queen (Yoon *et al.,* 2004). This provides the basis for successful rearing under controlled conditions (Yoon *et al.,* 2004). Queen that starts early egg-laying produced stronger colonies in development which is necessary to meet the need of higher number of workers at colony development stages. Variation in nectar concentrations may vary with different *Bombus* species as 1:1.5 ratio of sugar solution as nectar was found best for early oviposition in *B. ignitus* (Park *et al.,* 2004). Different physical characters like viscosity of nectar and sugar concentration levels influenced the performance of workers

Table 3. Effect of different concentrations (Mean± SE) of sugar and honey solution at colony maturation stage under controlled laboratory conditions

		----------Sugar/Honey Solution Concentrations----------						
	Observed Parameters	1:1	1:1.5	1:2	2:1	1.5:1	F	P
Sugar Solution	Total emerged males	156.60±12.8	143.60±11.14	125.0±7.04	99.07±8.18	144.10±10.43	4.88	0.0016
	Total emerged queens	70.20±10.4	55.73±8.82	36.33±6.15	22.26±3.68	68.30±3.49	8.64	0.001
	Total emerged workers	158.0±7.86	122.60±7.37	115.86±3.9	83.33±4.44	138.60±6.22	20.40	0.001
	Mother queen longevity	107.27±4.0	91.73±2.72	89.46±3.07	76.40±3.89	106.0±4.42	12.10	0.001
Honey solution	Total emerged males	83.8.0±4.18	97.13±4.96	90.13±4.85	63.86±3.49	56.46±3.64	16.60	0.001
	Total emerged queens	12.06±3.20	14.2.0±3.33	16.33±3.87	1.330±0.64	0.6±0.41	7.42	0.001
	Total emerged workers	75.53±5.25	80.40±4.18	80.33±6.03	64.66±2.08	63.03±2.47	3.85	0.0070
	Mother queen longevity	77.40±3.49	79.86±2.08	86.13±2.22	76.86±3.39	63.20±2.05	9.46	0.001

bees which might vary for their morphological characters of shape and size of the tongue (Borrell, 2004, 2007, Kim *et al.,* 2011). Plowright and Pendrel (1977) found increased sugar concentrations affected the larval growth of bumblebees which sometimes lead to their earlier mortality because when sugar concentration increased viscosity of solution also increased and difficult for larval stage to digest highly viscous solution.

Viscosity of solution and imbibition ratio due to increased sugar concentrations led to death occurrence at larval stage. This ultimately decreased the number of workers emergence (Nardone *et al.,* 2013). Lower concentration and content of nectar in the flowers is a huge draw of most pollinators and showed pollinators' visitation preference due to easy suction (Kim *et al.,* 2011: Kaur *et al.,* 2013), also bees spend more time on those flowers which have higher concentration of nectar (Wolf *et al.,* 1989).

Strength of workers in the colony reached fifty earlier at 1:1 ratio of sugar solution which coincide with previous work of Park *et al.* (2004) who found 40-50% sugar solution to be the best in rearing of *B. terrestris* and *B. ignitus* at colony foundation stage. Most physiological functions like thermoregulation help maintain the strength and size of bumblebee workers which is under influence of nectar concentrations (Bishop and Armbruster, 1999; Heinrich and Heinrich, 1983). Optimum viscosity of sugar solution depends on feeding style, with higher for viscous dippers than suction feeders. However, increased concentrations of sugar decreased the imbibition rate and subsequently led to death of workers (Tezze and Farina, 1999).

Sexual emergence (males and daughter queens) is important in the colony for continuous commercial rearing. Gurel *et al.* (2012) used three type of syrup i.e., sucrose syrup, industrial bee feeding syrup and high fructose corn syrup (Fructose 42-45%, Glucose 50-54%). They found the highest colony production rate observed at high fructose corn syrup. Low pH in HFCS resists solution for bacterial contamination and fermentation (Ruiz-Matute *et al.,* 2010). Result of (Velthuis and Doorn, 2006; Gurel and Gosterit, 2008a) showed that foundation queen observed environmental conditions including the food quantity and quality and then switched to produce sexual. When the food quality is good according to her requirement, more number of sexual are produced. Similarly, Gurel and Gosterit, (2008b) who found that maximum number of males are produced at ratio of 50% sugar solution (W: S) with normal pollen cake.

Yoon *et al.* (2012) used different types of sugars i.e., white, brown and dark brown. They found the white sugar showed with the best results of all others types regarding the emergence of progeny. Colonies of *B. ignitus* reared on 50% sugar solution produced more numbers of progeny (new daughter queen) as compared to reared at 40% sugar solution and 40% sugar solution+ antiseptic. Their work on *B. terrestris* showed that maximum numbers of progeny queens are produced at 40% sugar solution + antiseptic as against those colonies fed with Beehappy® and 40% sugar solution (Yoon *et al.,* 2005).

Changing of diet and environmental conditions of an organism leads to stress with adverse effect on further survival and reproductive success (Oster and Wilson, 1978). Longevity of bumblebee queen and workers, also effect by the pollen and nectar, was observed (Smeets and Duchateau, 2003) that nutrimental deficiency led to a strong decline in the survival. Park *et al*. (2004) showed that maximum mother queen longevity at 50% sugar solution than at 40% and 60%. They also found that at mixed honey, the queen lived maximum days but preferred sugar solution. For queen life as compare to sugar solution it was observed by Yoon *et al.* (2005) that longevity of foundation queen was more from those colonies reared at 40% sugar solution for *B. ignitus* and *B. terrestris*. Later Yoon *et al.* (2012) found that queen's longevity period was maximum of all those colonies reared at dark brown sugar. Carbohydrates always influence on larval provisions, their function and their caste specific development (Pendrel and Plowright, 1981). As the important of carbohydrate fructose was major constituent for bees larvae at older stage for caste determination and glucose was considered the main sugar component (Kaftanoglu *et al*., 2011)

Conclusion

In conclusion, colony growth of bumblebees in relation to different concentrations of sugar and honey solution helped for commercial breeding systems of these bees. Sugar solution with 50% concentration proved the most suitable for bumblebee in enclosed laboratory rearing programs. We believe such studies will be helpful regarding the colony development to get maximum numbers of new progeny for efficient rearing programs and enhancing their role in crop pollination of enclosed farming systems with good economic return.

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Authors' Contributions

M. I. Reared bees and conducted experiments, M.A. and M.N. designed and supervised the research, K.M. reviewed the article, and M.N and U.A.A.S. help in bees rearing.

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