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Egg laying in workers of *Bombus terrestris* L. (Hymenoptera, Apidae) out of colony in laboratory conditions

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A b s t r a c t : Workers of *Bombus terrestris* in different number and age taken out of their mother colony put in separated boxes and examined for their potential of egg laying. Workers fed by sugar solution (1:1) and pollen. Workers made eggcups prior to egg laying. Results shown that each worker when separated from mother colony can lay eggs with a delay of 7 to 20 days depend on age of worker. Analysis of variance for delay in first eggcup making and laying egg of workers based on single and multiple factorial experiment shown that just age of workers is the most important factor which affect on making egg cups and egg laying. Statistical analyses have been shown that number of workers is not a significant factor. Percent of egg laying in workers with one day, one week and two weeks ages, were correspondingly, 46.66 %, 40 %, 33.33 %. Mean comparison of different treatments and levels for delay in first egg cup making of workers using LSD test ($P< 0.05$) shown interactions between age and number of workers. Despite what known in honeybees which workers that are isolated from their colonies will die after short periods of time, this research documented that in bumblebees it is different: workers can continue to live if they be away from their colonies and can lay eggs.

K e y w o r d s : *Bombus terrestris* L., Worker, Egg laying, Making Egg cup.

Introduction

Workers in social bees are very important which function to colony survival. Although there are documents shown that workers in bumblebees reproduce males from unfertilized eggs but there were no qualification test on male egg laying potential in controlled conditions. True bumblebees are among social bees with queen, workers and males. Queen has the main role in the colony (SLADEN 1912, ALFORD 1975). Young mated queen survive during winter and after overwintering starts colony individually, makes honey pots, pollen pots and egg cups, lay eggs, incubate broods, do foraging and feed first broods by the first batch of workers appeared. After that, queen continues to lay eggs but workers forage and help in nest works. The most important role for males is reproduction and by now there is not many publications indicates that males do any works in the colony although some believe that males sometimes incubate broods (CAMERON 1985). Workers do different functions in the colony; foraging and prepare pollen and nectar for broods, feeding them, defense of colony and making honey pots, pollen pots and egg cups are the main works of them in social life (SLADEN 1912,

ALFORD 1975). Like honeybees, workers of bumblebees can reproduce males from unfertilized eggs but there is a clear difference that workers of bumblebees can do this in queen right as well as queen less colony while workers in honeybees reproduce males only when there is not queen in the colony (CNAANI et al. 2002, GOULD & GOULD, 2005). Through eusocial Hymenoptera, queens and workers differ morphologically. Such differences extended to the reproduction system: workers are morphologically incapable of mating. If such workers retain ovaries they can still reproduce, however, because in all Hymenoptera males arise parthenogenetically from unfertilized eggs (BOURKE 1988). A review of former researchers carried out by BOURKE (1988) shown that worker reproduction known as a part of eusocial evolution chain and is prior to queen control behavior. Worker reproduction is widespread in natural colonies of bumblebees in the nature, but the proportion of males produce is greatly different. Sometimes male produce in bumblebee colonies have been reported up to 98 % (KERR 1969), apparently in presence of queen. In fact, similar to some other eusocial Hymenoptera, especially honey bees, workers reproduction in queen less colonies known as a common phenomenon. In honeybees, workers that are isolated from their colonies will die after short periods of time, but some observations indicated that in bumblebees it is different: workers can continue to live if they be away from their colonies. They can even start a "new life" in a different colony, where they will sometimes lay unfertilized eggs. In the wild (in late summer), it has been seen worker spending the night in flowers, and these bees did not appear to be ill. In some cases, workers can even start their own colonies. At least this happened in "artificial" conditions, like flight arenas that used for sensory ecology experiments, where workers will sometimes start building honey pots and lay eggs (lab observations). Some late returning workers would find that their nest had disappeared, and would then start building a new nest in whatever cavity they could find nearby (field observations). This suggests that, if a natural nest is destroyed by natural enemies, workers can still build their own nests and thus redeem some of the colony's fitness. These observations indicated that workers in bumblebees potentially have reproduction in solitary life as well as in queenless and queen right colonies. No pheromone which control of queen on worker's egg-laying known in bumblebees (DUCHATEAU & VELTHUIS 1988, RÖSELER & RÖSELER 1974, RÖSELER 1985, PAXTON et al. 2001 and MÜLLER et al. 1992). But in which conditions workers of *B. terrestris* lay eggs individually and which internal or external factors are affected on this behavior is not studied by now. The problem was that there were no controlled data on this - we didn't know how frequently this happens and other questions like these: Do all workers have this potential or only worker of a certain age, size, etc. Does it depend on how old the colony of origin is? We didn't know whether workers are more successful if there is more than one worker? What the actual fitness of workers is? Also, we encounter with this question? 1. Workers in which age can start to reproduction? 2. Is there any inter or intra colonial competition between workers to egg-laying which selected from different colonies or the same colony? 3. How many egg, eggcup, honey pot, pollen pot and male can a worker produce individually? 4. Is there any different in shape of colony in this kind of life? 5. How is brood care in this kind of life?

Material and Methods

Workers

Coloney of *B. terrestris* for this experiment provided from representative of Koppert Co. in Turkey. Workers at emergence tagged by labels and the date of their appearance in colonies recorded.

Nest boxes and food

Nest boxes from plastic sized 10*12*8 cm, bottom covered by cardboards. Chamber usually contains an Eppendorf tubes cut from bottom put up down and filled with bee feeding solution. This was sugar solution but since this was rapidly used to dry up, hence the special commercial solution was used. In addition, the bees were given by a pollen ball, which was actually a mixture of ground up pollen and sucrose solution and honey (or sugar solution) roughly of the consistency of paste (like toothpaste). Since this material also tended to dry, the remained (not eaten) was replaced frequently every day.

Setting up the experiments

The temperature of the bee room was 25 ± 2 ºC, Humidity kept fixed 70 % ± 10 by using the air humidifiers. Colonies put in dark room, just a red light used in feeding and data writing times. We collected callow workers (0–12 h after emergence from pupa cells and before the appearance of yellow stripes on the thorax and abdomen) and marked each bee with an individual color tag. Labels on all the boxes showed date of their set up, from which colonies the workers picked up, mother colonies, date of entrance of colony in the lab. Then the boxes checked daily, and monitored to know how many workers start building honey pots, how many laid eggs, from each colony. Workers in all experiments selected from one which had arrived in lab on the same day and so these workers were the same.

Treatments

The main question in this experiment was; "is there any chance for workers picked from their mother colonies to egg laying when confined in boxes?" and also if yes, which factors can affect egg laying of workers out of colony. Its believed that size of workers cannot be important in this regards and have no effects on egg laying, so we examined two other factors of Age and number of workers on their laying egg. Two factors were raised for study. 1) Age of workers with four levels of 1, 7 and 14 days old; the main question was "Do age of workers influence on egg-laying or not?" and 2) Number of workers with three levels including 1, 2 and 3 individuals; with another question "Do number of workers make competition or stimuli for laying egg or not?" Experiment started by taking 115 workers out of their main colony and confined in boxes (table 1). We also keep monitoring of behaviors of workers and notice to survival of workers out of colonies and other behaviors of them like making honey pots, egg cups, egg eating and other competitions between them. A completely randomized design with 5 replications was used and the data were analyzed based on both factorial and non-factorial methods. The factors and their different derived treatments used in this experiment have shown in table 1.

Results

Statistical Analysis

Analysis of variance for delay in first 'Egg Cup Making' and 'Laying Eggs' by workers based on 'Multiple Factorial Experiment' shown significant differences ($p<0.01$) for factor of 'Age' while 'Number' and interactions between 'Age cross Number' were not significant (Table 2 and 3). When just treatments considered in analysis with 'Single Factoriol Experiment' for both first 'Egg Cup Making' and 'Laying Eggs' were significant (Table 4 and 5). in a complicated comparing based on 'Mean Comparison' of different treatments for first 'Egg Cup Making' and 'Laying Eggs' by workers however, shown significant differences ($p<0.05$) between various composition of treatments e.g. 'Age' and 'Number' (Table 6). Moreover, the most important factor 'Age', using LSD test shown the same result not only for delay in first 'Egg Cup Making' but also for 'Laying Eggs'(Table 7 and 9). in Table 8 again various compositions of comparing sub factors of treatments whit significant differences shown. All statistical analysis indicated that the main factor is the 'Age'. It's not strange because 'Egg Laying' is a character related to internal conditions of workers and would naturally affected by growth of bees. Just the age of workers was the factor could affect egg laying and in spite of our expectation; the number factor (which indirectly represented coloney size) had no effect on egg laying.

Results shown that more than 70 percent of workers can survive when separated from their mother colony, and even they can lay eggs individually and reproduce unfertilized eggs (Fig.1). Making honey pots is one of the duties of workers in colony. We expected when workers confined in boxes, starting to make honey pots means they continue life in colony. Observations shown that most workers (about 65 %) did not make any honey pot. The other workers made honey pots in boxes with 1 to 5 weeks delay. One reason we guess, is the ready dishes full of sugar were available for them all the time. In all experiments workers improved entrance of feeding tube as places for saving sugar other than honey pots they make by secreting wax (fig. 4.).

About 30-100 % of workers in various treatments could make first egg cups and lay eggs; this shown in Fig.1. From all survived workers; Percent of egg laying in workers which remove from their mother colony shown in Fig. 2. which the highest rate in known age group was for workers which removed at appearance day. The maximum number of eggs in the first eggcups was 14. We considered onset or delay time of egg laying after separation from mother colonies as an index of reproduction potential in workers. The highest delay which shown number of days lasting to making egg cup and egg laying in lab was about 18-20 days (Fig. 1).

Behaviors of workers out of colony

- Priority in making honey pots or egg cups: Workers individually showed behavior when confined in boxes. At first, they start to feeding from sugar and secreting some wax on ceiling of boxes irregularly everywhere like a surface of wax layer then they select a position for making egg cups and honey pots in box. Priority of making honey pots or egg cups depended on being available sugar in the box. Most of them

firstly, started to make egg cups then developed around the entrance of feeding tube by secreting wax to change them to honey pots to save more sugar (Fig. 2). Egg cups made by workers were the same as made by queens in the colony but in smaller size. They made a cylinder of wax height 4-5 mm then put eggs in them and closed their caps by secreting wax. In some cases we observed a worker opened egg cup by chewing the cap and laid one egg more then closed it again.

- Competition by Egg eating: like in natural colony, workers showed aggressive behavior and sometimes more aggressive than in natural colonies. After some days when a worker could not lay egg it killed by others especially in groups of 3 workers. Workers ate eggs of each other in boxes. Usually workers who could not lay egg ate the others eggs. In one case when a worker put an egg mistakenly an egg out of egg-cups this egg was eaten by another worker in the box. In order to avoiding eat by other workers one worker used to close the egg cup immediately by secreting wax. Sometimes workers opened others egg cups and ate the eggs.
- Feeding and incubating broods; the most important reason of development of Lego's holes to save sugar is for feeding broods, like in the colony workers continued to feeding broods and incubated the broods most of the time by putting their abdomen on them.
- Reaction of workers to opened egg cups; Workers leaved egg cups which opened by others. In one case we opened an egg cup to counting the number of eggs in it, it had 4 eggs, and then we closed the cap and left it in box but worker did not accept it again and did not feed or incubate this broods.

Discussion

Reproduction system functions in social bees including laying eggs, determination of sex ratio, queen and worker reproduction conflict, morphological and physiological differentiation of the castes, especially in honeybees and bumblebees have been studied by many researchers (RÖSELER & RÖSELER 1974, OWEN & PLOWRIGHT 1982, BOURKE 1988, DUCHATEAU & VELTHUIS 1988, BEEKMAN & VAN STRATUM 1998, PAXTON et al. 2001, GOULD & GOULD, 2005). Worker reproduction is known widespread in the social insects (BOURKE 1988). While in honey bees egg laying is the main function of the queen and in presence of the queen, workers cannot lay eggs in contemporary, bumblebee workers are not sterile and as HONK et al. (1981) stated that workers produced up to 82 % of adult males in their laboratory populations. In this experiment we tested ability of workers to laying eggs especially in solitary life. OWEN & PLOWRIGHT (1982) found that 20 % of males produced in queen right colonies were worker-produced. Although workers' reproduction in bumblebees in queen right and queenless colonies suggested by some researches (DUCHATEAU & VELTHUIS 1988, RÖSELER & RÖSELER (1974), ALAUX et al. 2004) but in this experiments we studied solitary life of workers out of coloney individually which not tested by none of researchers previously. As we observed in the field, sometimes while we extracted a coloney of bumblebees from ground, workers which came back to coloney's place, start repairing nest again. As we observed in our experiments, workers of bumblebees, like a solitary bee, starting to secrete wax, making honey pots and eggcups, laying eggs and naturally they could feed male broods. Results shown that coloney size has no effect on egg lying, making egg cup in workers and we

believe that egg laying is an innate characters of workers which depend on internal conditions and physiology of workers. There are many controversial points in workers' egg laying in *B. terrestris* while they are social and there are various stages and phases including switch phases and competition phase. Certainly, in coloney of bumblebees as an organization, reproduction of workers need to be part of social life and would be under control, although mechanisms influence might b e not known as well. Using microsatellites, PAXTON et al. 2001, found no evidence for worker reproduction in free-flying (but captive) colonies of *B. hypnorum* in Scandinavia. In general, theory predicts successful worker reproduction in bumble bees (RATNIEKS 1988). In BROWN et al. 2003 colonies, they observed no queen-worker aggression, as was also found in a previous behavioral study (AYASSE et al. 1995). Worker reproduction may depend upon variation in resource availability, colony demography and phonology (BOURKE & RATNIEKS 2001). They suggested that further studies of both laboratory and field colonies under various resource regimes would be clearly needed to determine accurately when, how and why worker reproduction occurs in queen-right bumblebee colonies. BROWN et al. 2003 suggested that sexual productivity increased with colony size, and this relationship persisted for both males and new queens when considered independently. In our experiment, queenless workers, coloney size no has effect on reproduction rate of workers. May we should consider more number of workers in future in our experiments. Our results have shown workers can dependently make egg cups and laying eggs even when removed at appearance day in their mother colony. About physiological characters of workers in egg laying, GEVAA et al. 2005 suggested that the social environment strongly influenced both behavior and physiology of workers, including ecdysteroid levels in the ovaries and hemolymph. However, in evolution of socialism in insect, bumblebees could be a good case study. We suggest more biological and physiological tests on bumblebees workers reproduction, especially in a simulation system very close to nature conditions.

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References

ALAUX C., JAISSON P. & A. HEFETZ (2004): Queen influence on worker reproduction in bumblebees (*Bombus terrestris*) colonies. — Insectes Sociaux **51**: 287-293.

ALFORD D.V. (1975): Bumblebees, Davis-Poynter, London. xii+352 pp.

AYASSE M., MARLOVITS T., TENGO J., TAGHIZADEH T. & W. FRANCKE (1995): Are there pheromonal dominance signals in the bumblebee *Bombus hypnorum* L (Hymenoptera, Apidae)? — Apidologie **26**: 163-180.

BEEKMAN M. & P. VAN STRATUM (1998): Bumblebee sex ratios: Why do bumblebees produce so many males? — Proceedings of the Royal Society of London Series **B 265**: 1535-1543.

BOURKE A.F.G. (1988): Worker reproduction in the higher eusocial hymenoptera. — Quarterly Review of Biology **63**: 291-311.

BOURKE A.F.G. & F.L.W. RATNIEKS (2001): Kin-selected conflict in the bumble bee *Bombus terrestris* (Hymenoptera: Apidae). — Proceedings of the Royal Society of London B **268**: 347-355.

BROWN M.J.F., BAER B., SCHMID-HEMPEL R. & P. SCHMID-HEMPEL (2003): Queen-controlled sex ratios and worker-reproduction in the bumble bee *Bombus hypnorum*, as revealed by microsatellites. — Molecular Ecology **12**: 1599-1605.

CAMERON S.A. (1985): Brood care by male bumble bees. — Proc. Natl Acad. Sci. USA **82**: 6371-6373.

CNAANI J., SCHMID-HEMPEL R. & J.O. SCHMIDT (2002): Colony development, larval development and worker reproduction in *Bombus impatiens* CRESSON. — Insectes Soc. **49**: 164-170.

DUCHATEAU M.J. & H.H.W. VELTHUIS (1988): Development and reproductive strategies in *Bombus terrestris* colonies. — Behaviour **107**: 186-207.

GEVAA S., HARTFELDERB K. & G. BLOCH (2005): Reproductive division of labor, dominance, and ecdysteroid levels in hemolymph and ovary of the bumble bee *Bombus terrestris*. — Journal of Insect Physiology **51**: 811-823.

GOULD J.L. & C.G. GOULD (2005): The honey bee. — New York: Scientific American Library. 460 pp.

HONK VAN C.G.J., VELTHUIS H.H.W, ROSELER P.F. & M.E. MALOTAUX (1981): The mandibular glands of *Bombus terrestris* queens as a source of queen pheromone. — Entomol. Exp. Appl. **28**: 191-198.

KERR W.E. (1969): Some aspects of the evolution of social bees (Apidae). — Evol. Biol. **3**: 119-175.

MÜLLER C.B., SHYKOFF J.A. & G.H. SUTCLIFFE (1992): Life history patterns and opportunities for queen-worker conflict in bumblebees (Hymenoptera: Apidae). — Oikos **65**: 242-248.

OWEN R.E. & R.C. PLOWRIGHT (1982): Worker-queen conflict and male parentage in bumble bees. — Behavioral Ecology and Sociobiology **11**: 91-99.

PAXTON R.J., THORÉN P.A., ESTOUP A. & J. TENGÖ (2001): Queen-worker conflict over male production and sex ratio in a facultatively polyandrous bumble bee, *Bombus hypnorum*: the consequences of nest usurpation. — Molecular Ecology **10**: 2489-2498.

RATNIEKS F.L.W. (1988): Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. — Am. Nat. **132**: 217-236.

RÖSELER P.F. (1985): "A technique for year-roundrearing of *Bombus terrestris* (Apidae, Bombini) colonies in captivity." — Apidologie **16**: 165-170.

RÖSELER P.-F. & I. RÖSELER (1974): Morphological and physiological differentiation of the castes in the bumblebee species *Bombus hypnorum* (L.) and *Bombus terrestris* (L.). — Zoologisches Jahrbuch. Allgemeine Zoologische, Physiologie Tiere: Physiologie **78**: 175-198.

SLADEN F.W.L. (1912): The humblebee, its life history and how to domesticate it. — Logaston Press, 273pp.

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Table 1: Treatments legends; Age and number of workers of *B. terrestris* and total of individual used in experiment.

Treatments	**Age (day)**	**Group size**	**Sum** (Workers)
D_1N_{1*}	1	1	5
D_1N_2	1	2	10
D_1N_3	1	3	15
D_2N_1	7	1	5
D_2N_2	7	2	10
D_2N_3	7	3	15
D_3N_1	14	1	5
D_3N_2	14	2	10
D_3N_3	14	3	15
Total			**90**

* D = Age (including, D_1: one day, D_2: one week and D_3: two weeks old), N = Number of worker(s) in treatments, (N1: one worker, N2: two workers, N3: three workers).

Table 2: Analysis of variance of delay in first eggcup making of workers *B. terrestris* based on multiple factorial experiments.

Sources of variation	df	MS
Age	2	269.13**
Number	1	2.27
Age × Number	2	1.52
Error	12	5.70

Table 3: Analysis of variance of delay in laying egg of workers *B. terrestris* based on multiple factorial experiment

Sources of variation	df	MS
Age	2	222.96**
Number	1	1.41
Age × Number	2	2.15
Error	7	1.86

Table 4: Analysis of variance of delay in first eggcup making of workers *B. terrestris* based on single factorial experiment

Sources of variation	**df**	**MS**
Treatment	7	117.04**
Error	16	12.73

Table 5: Analysis of variance of delay in laying egg of workers *B. terrestris* based on single factorial experiment

Sources of variation	df	MS
Treatment	7	68.78**
Error	9	2.83

Table 6: Mean comparison of different treatments for delay in first egg cup making of workers *B. terrestris* using LSD test

treatment	mean	D_1N_1 17.4	D_1N_2 16.00	D_1N_3 18.33	D_2N_1 6.50	D_2N_2 7.00	D_2N_3 6.67	D_3N_1 5.00
D_1N_2	16.00	1.4†						
D_1N_3	18.33	-0.933	-2.33					
D_2N_1	6.50	10.90**	9.50**	11.83**				
D_2N_2	7.00	10.40**	9.00**	11.33**	-0.50			
D_2N_3	6.67	10.73**	9.33**	11.67**	-1.67	0.33		
D_3N_1	5.00	12.40**	11.00**	13.33**	1.50	2.00	1.67	
D_3N_2	3.67	13.73**	12.33**	14.67**	2.83	3.33	3.00	1.33

† The numbers in the table are the difference of means of treatments in rows and columns of corresponding number.

Table 7: Mean comparison of different levels of age (D) factor for delay in first egg making of workers *B. terrestris* using LSD test

		D_1	D_2
	mean	**16.70**	**6.75**
D_2	**6.75**	9.95**†	
D_3	**4.33**	12.37**	2.42

† The numbers in the table are the difference of means of treatments in rows and columns of corresponding number

Table 8: Mean comparison of different treatments for Delay in lying egg of workers *B. terrestris* using LSD test

		D_1N_1	D_1N_2	D_1N_3	D_2N_1	D_2N_2	D_2N_3	D_3N_1
	Mean	21.00	22.50	18.00	13.50	15.00	12.50	8.00
D_1N_2	22.50	-1.50†						
D_1N_3	18.00	3.00	4.50*					
D_2N_1	13.50	7.50**	9.00**	4.50*				
D_2N_2	15.00	6.00**	7.50**	3.00*	-1.50			
D_2N_3	12.50	8.50**	10.00**	5.50**	1.00	2.50		
D_3N_1	8.00	13.00**	14.5**	10.00**	5.50**	7.00**	4.50*	
D_3N_2	7.00	14.00**	15.50**	11.00**	6.5**	8.00**	5.50**	1.00

† The numbers in the table are the difference of means of treatments in rows and columns of corresponding number

Table 9: D level (Delay in laying egg) of workers *B. terrestris*

		D_1	D_2
	Mean	21.75	14.25
D_2	14.25	7.50**†	
D_3	7.50	14.25**	6.75**

† The numbers in the table are the difference of means of treatments in rows and columns of corresponding number

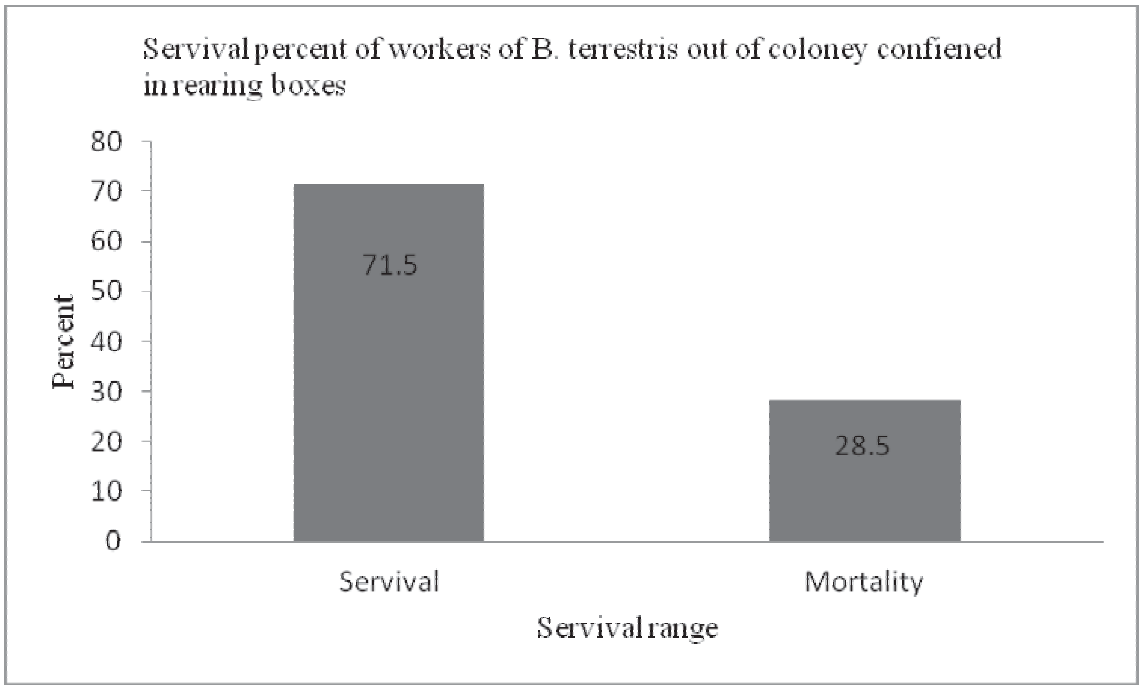

Fig. 1: Survival range in workers of *Bombus terrestris* out of mother coloney.

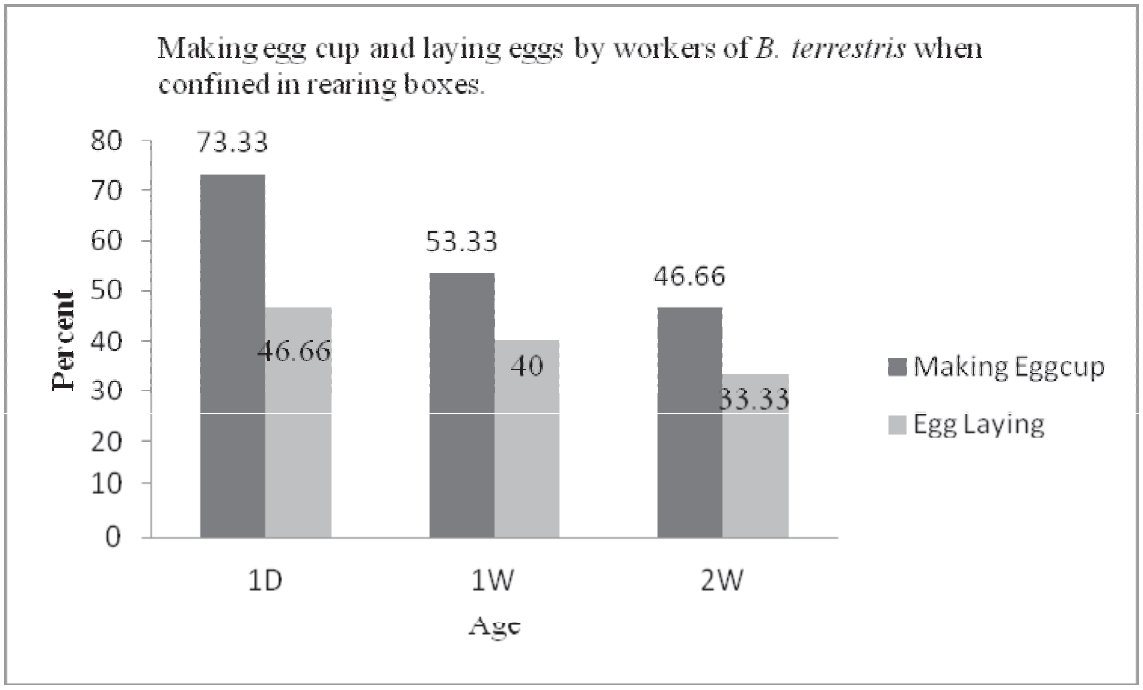

Fig. 2: Making eggcups and laying eggs percents in workers of *Bombus terrestris* out of mother coloney with different ages.

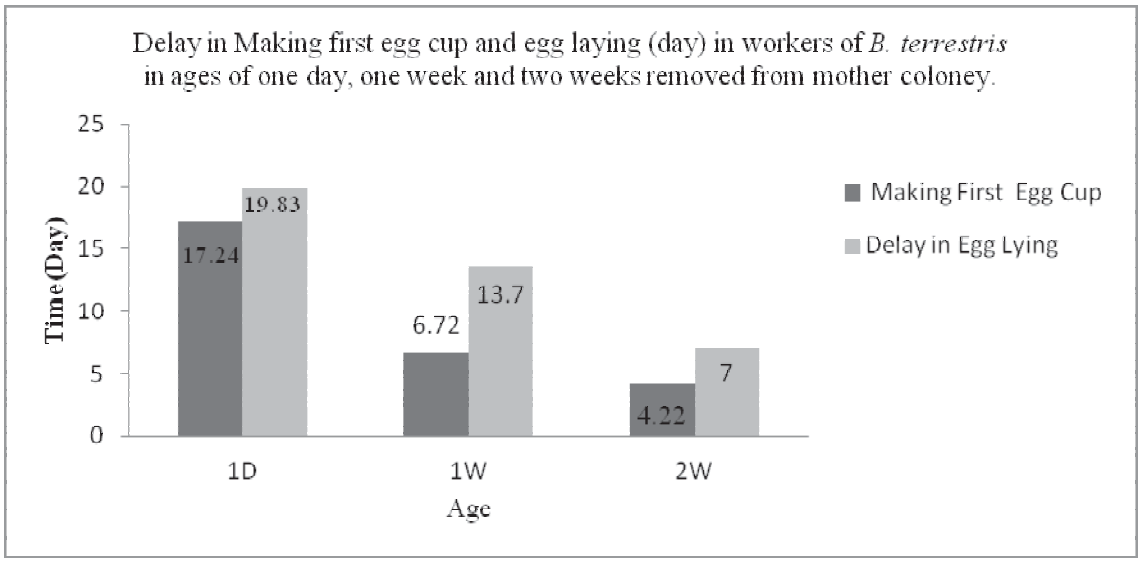

Fig. 3: Delay in making eggcups and laying eggs in workers of *Bombus terrestris* out of mother coloney with different ages.

Fig. 4: Workers of *B. terrestris* out of their mother colony confined in rearing boxes. Broods and honey pots made by workers. Honey pots were made by workers even when there were feeding dish were full of honey.