Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 5, p. 144-153, 2015
http://www.innspub.net

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RESEARCH PAPER

OPEN ACCESS

Effect of fresh pollen pellets and pollen balls coated with and without beeswax on the life history parameter of bumblebee (*Bombus terrestris)*

Muhammad Imran[1*], Munir Ahmad[1], Muhammad Naeem[1], Shafqat Saeed[2], Muhammad Farooq Nasir[1], Asif Ahmad[3], Muhammad Nasir[1] and Umer Ayyaz Aslam Sheikh[1]

[1]*Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Murree Road, Rawalpindi, Pakistan*

[2]*Department of Entomology, Faculty of Plant Protection, University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan*

[3]*Department of Food Technology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Murree Road, Rawalpindi, Pakistan*

Article published on May 18, 2015

Key words: *Bombus terrestris*, Pollen ball, Beeswax coated, Colony development.

Abstract

This investigation deals with the possible effect of fresh pollen pellets and pollen balls coated and without coated with beeswax on the life history parameter of bumblebee and their consumption rate. At the colony initiation stage it found that mother queen start egg laying earlier (6.1±0.82 days) at pollen patties without coated with beeswax as compared to at coated with beeswax and fresh pollen pellet. Similarly emergence of the first worker from the first batch of the colony also early (26.3±0.83 days) at pollen patties without coated of beeswax. Other two stages of the colony i.e. at colony foundation stage and the colony maturation stage it found that the pollen patties with one coat of beeswax had best for bumblebee rearing. According to the consumption rate of pollen during the 24hr of observation it found that at the colony initiation stage, colony foundation stage and colony maturation stage large amount of pollen consumption was found in fresh pollen pellets because to attack of wax moth on fresh pollen was also high. The best consumption rate was found at pollen patties with one coat of beeswax. So it found that at colony initiation stage pollen patties without beeswax coat was best and at colony foundation stage and colony maturation stage pollen patties with one coat of beeswax was best.

***Corresponding Author:** Muhammad Imran ✉ imranmalikvirgo@gmail.com

Introduction

Bees are in the right place to the large and very successful insects order Hymenoptera, also diverse type of insects like sawflies, wasps and ants belonging this order. There are about 25000 species of bees identified that belong to 4000 genera. All bees are phytophagous, feeding mainly on pollen and nectar during their lives obtain form different plants (Michener, 1974). Pollination is the transfer of pollen grain from stigma (male part of plant) of one plant to style (female part of plant) of another plant and mostly by insects and wind (Stern, 1994). Farmers had been using honey bees in glasshouses or greenhouses but it decreased due to lower availability of food source for the development of honey bee colonies (Williams *et al.*, 1991). Bumblebees are consider the most important pollinator and very effective from all other pollination due to their morphological character (Umer *et al.*, 2014; Imran *et al.*, 2015). These rank among the most copious and conspicuous of flower visitors in alpine, temperate and arctic environments, providing the vital ecosystem services of pollination in both natural and managed cropping systems (Corbet *et al.*, 1991). Highly commercial crops (cruciferous family crops) like tomatoes, peppers, strawberry, cucumber, brinjal, peaches and apple grown commercially in greenhouse, tunnels and field and they need buzz pollinators unlike honeybees (Goulson, 2003).

From majority of the bees species bumblebees are in large size and most of the body is covered with dense hair called fur. Due to this size and body hair, bumblebees are competent pollinators and also have ability to survive in cool condition (Heinrich, 1993). So it is not astonishing that these bees are largely restricted to alpine, temperate and arctic zones and they are found in all North America, Europe and Asia. In the Himalayas range bumblebee are found at altitudes above about 1000 m from the sea level (Williams, 1994). They are much extra effectual than honeybees credit to their longer tongue and their faster foraging speed (Chittka *et al.*, 2004). In addition, they are able to forage under uneven environment such as stormy weather and low temperatures. Among the 250 species of bumblebees one of the most important species is the *bombus terrestris* found very commonly in West-Palaearctic region, distributed in Mediterranean (Rasmont *et al.*, 2008).

Special environmental conditions are required for their laboratory rearing as they generally have one generation per year and spend harsh winters in diapause under natural environments. In spring, the queens emerge after diapause break and starts for colony initiation. Nest seeking queens are most frequently found along forest boundaries and field boundaries while least in forest and clearings (Sevensson *et al.*, 2000). Laboratory rearing of bumblebees have long history, Sladen, e.g. worked on rearing of bumblebees in 1912 and published his worked in Chapter VII of his famous book *The Humble-bee*. During laboratory rearing he noted some problems, like hibernation and mating. Many researchers worked on domestication of bumblebee under laboratory conditions, calculating each step of the bumblebee's life cycle, but it was not until the 1970s that domestication became a fact (Röseler, 1977). When bumblebees were domesticated, consideration on the potential for commercial rearing began (Röseler, 1979; Plowright and Laverty, 1987).

Previous researcher use pollen and honey solution obtain from honeybee hive for the artificial mass rearing of bumblebees (Griffin *et al.*, 1991; Tasei and Aupinel, 1994; Ono *et al.*, 1994; Hannan *et al.*, 1998), but the supplying method was different. Yoon *et al.*, 2005b investigate that 40% sugar solution was more effective than 50% for the laboratory rearing and if antiseptic was added in 40% sugar solution than it was most effective for the development of bumblebee's colonies. So 0.3% sorbic acid with 40% sugar solution and fresh pollen were used as food of *bombus ignites* (Yoon *et al.*, 2005a)

Along with sugar solution pollens is also important source of food but pollens are not energetic resource

that only provides primarily proteins for the ovary development of queens that help for the eggs production (Plowright and Pendrel, 1977). In the worker of European bumblebee *bombus terrestris* proteins are required for oogenesis and also have influence on the size of adult workers (Duchâteau and Velthuis, 1989; Sutcliffe and Plowright, 1988).

Body size of bees is strongly associated to the dietary conditions under which individuals are reared. The immature that are feed with non-nutritional diets or less protein and carbohydrate content of pollen can affect the resulting adult's size (Roulston and Cane, 2000; 2002). In the case of social bees, the colony's dietary conditions under which males are reared may also be significant for successful growth, given that spermatogenesis occurs during the pupal stages and is usually completed at and after the emergence of the imago (Pereira-Lima *et al*., 2006).

Dietetic necessities of proteins, vitamins, lipids and carbohydrates for bees have been reviewed by Haydak (1970). Proteins play very important role in the development of sexual organs and hypopharyngial glands of adults and larvae nutrition. The main source of protein for the bumblebee development is the pollen collected from different flowers (Groot, 1953). Nutritive value of pollen varies from plant to plant and which is within the range of 2.5-61% (Roulston *et al*., 2000). Quantity, quality and composition of pollens used in the mass rearing of *B. terrestris* in controlled environment have affected the colony development (Roseler, 1985; Ribeiro *et al*., 1996).

The present study was performed to examine that how to increase shelf life of pollen in the colony and minimize the attack of insects pest on the pollen as well as on the colony by coating pollen balls with beeswax.

Materials and methods

The present worked was conducted to study the effect of fresh pollen pellets and pollen balls coated and without coated with beeswax on the development of bumblebee colonies under controlled laboratory conditions in Non-Apis Bee Laboratory, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi during 2013-2014. Experimental daughter queens and males of *Bombus terrestris* (Hymenoptera: Apidae) were obtained from bumblebee hives imported from Koppert Biological Systems, Netherlands and Biobest, Belgium and rear on fresh and frozen honeybee collected pollens and commercial grade sucrose sugar as desired concentration solution. These bee hives were assigned hive numbers for identification and collection of sexual for further multiplication. Daughter queens were mated with the males of same species belonging to different bumblebee hives to avoid inbreeding problems. After mating in special desire mating cages, daughter queens were feed for about a week period and kept for diapause in refrigerator at 4 degree centigrade for 2.5 months period after CO_2 exposure (Tasei and Aupinel, 2008). Experiment was performed after completion of diapause to improve colony development through different pollen diet optimization.

Laboratory rearing condition

To minimize the risk of fungus inside, rearing boxes were checked and cleaned every day with a piece of cotton soaked with 75% ethyl alcohol. Glass plates were uses as lids and covered with black sheet. The climatic conditions were kept at $25\pm2^{o}C$ and 65% relative humidity (Duchateau and Velthuis, 1988).

Feeding

In laboratory mass raring of *bombus terrestris* all previous researcher have used sugar and honey obtain form honeybee hive as a nectar and pollen (Hannan *et al*., 1998) although the supplying method varied. I have studied the effect of fresh pollen pellets and pollen balls coated and without coated with beeswax on oviposition and colony development.

This experiment was performed by making pollen patties with 40% sugar solution and then coat with beeswax. To improve the maximum utility and benefit

of pollens and reduced disturbance to the colony, we compare fresh pollen pellets and pollen balls with one coat and two coat of beeswax and without coated to increase the life of pollens and also decrease the attack of wax moth on pollen after selection of best pollen patti and nectar concentration. The impact of coated Patti was observed on all three stages i.e. at colony initiation, colony developmental stages and colony maturation stage

Consumption of pollen balls coated and without coated with beeswax

Fresh pollen pellets and Pollen balls coated and without coated with beeswax were weighted and kept in rearing boxes at three stages and these balls were changed every 24 hr and weight again remaining balls to calculate the total consumption during after 24 hr.

Data analysis

Means of different life history parameters were subjected to statistical methods using means ± SD and compared with ANOVA at 5% probability for comparison of percentage values. Chi-squares Analysis was performed using SPSS 16.0 software (NorusICE SPSS Inc. 2007).

Results and discussion

This experiment was design by coating pollen balls with one and two coat of beeswax and compare with without coated and fresh pollen pellets to investigate which one is best. Basically the purpose of this experiment was to increase the shelf life of pollen in the colony and minimize the attack of insects pest on the pollen as well as on the colony due to pollen pest and also very important purpose was to decrease the economic cost for commercial rearing because the pollen cast is very high and by this study to investigate how to increase the shelf life of pollen. Another factor that we observed was the disturbance of colony by change diet on daily basis so to minimize the disturbance this study was plan.

Effect of fresh pollen pellets and pollen balls with one, two coat of beeswax and without coat of beeswax on the life history parameters of bumblebee (*Bombus terrestris)* was observed. The observed life history parameter was pre-oviposition period, first worker emergence day and colony foundation days. Consumption of fresh pollen pellets and pollen balls coated with bees wax was also observed at before emergence of first worker, after emergence of first worker and pollen patties consumption at colony foundation stages.

Pre-oviposition period

Non-significant difference exists among the fresh pollen pellets and pollen ball coated and without coated with beeswax on the per-oviposition period of *B. terrestris*. Minimum pre-oviposition period (6.1±0.86 days) was observed at pollen ball without coat and maximum per-oviposition period (9.1±1.64 days) was observed at pollen balls with two coat of bees wax (Table 1: Fig. 1). Overall result reveled that pollen balls without coat of beeswax was most effective ball for mother queen to lay eggs to decrease the oviposition period of foundation queen than other pollen balls coated with wax and fresh pollen pellets (Table 1. Fig. 1).

Table 1. Effect of pollen balls coated with beeswax and fresh pollen pellets on the life history parameter of bumblebee (*bombus terrestris)*.

Pollen balls coated with wax	Pre-oviposition period	First worker emergence days	Colony foundation days
One coat	6.7±1.12	26.5±1.83	51.4±1.71
Two coat	9.1±1.64	30.3±1.97	72.2±2.22
Without coat	6.1±0.82	26.3±0.83	55.0±1.36
Fresh pollen	6.3±0.86	27.1±1.31	53.5±1.61

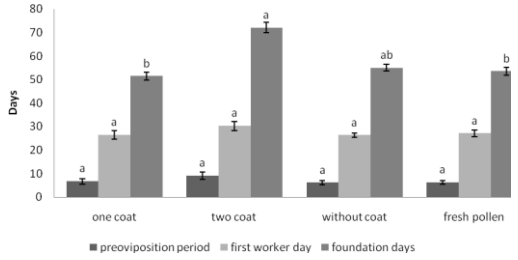

Fig. 1. Effect of pollen balls coated with wax and fresh pollen pellets on the life history parameter of bumblebee (*bombus terrestris)*.

First worker emergence day

Statistical analysis showed that non-significant difference exists among the fresh pollen pellets and pollen ball coated with bees wax on the emergence of first worker at colony initiation stage (Fig. 1). Minimum days (26.3±0.83 & 26.5±1.83 days) for the emergence of first worker at colony initiation stage were observed at pollen balls with one coat of beeswax and without coated. And maximum days for the emergence of first worker were observed form those colonies which were reared on pollen balls with two coat of beeswax (Table 1; Fig. 1). Overall comparison revealed that pollen ball coated with one coat of beeswax and without coat were most effective to increase the shelf life of pollen and also effective on earlier emergence of first workers in the colony as compare to other pollen patties and fresh pollen (Table 1; Fig. 1).

Colony foundation days

Significant difference exists among the fresh pollen pellets and pollen balls coated with beeswax on the period at which colony reached at foundation stage (workers strength reached at 50). Minimum colony foundation days (51.4±1.71 days) were observed from those colonies which were fed on pollen balls with one coat of beeswax and maximum colony foundation days was observed from those colonies reared at pollen balls with two coats (Table 1). Overall comparison showed that a pollen balls with one coat of beeswax was most effective to decrease the colony foundation days as compare to fresh pollen and other pollen balls with two coats and without coats separately (Table 1; Fig. 1).

Pollens consumption before and after emergence of first worker

Statistical analysis showed that significant difference exists among the fresh pollen pellets and pollen balls coated with beeswax on the consumption rate of pollen before the emergence of first worker. Results showed that maximum pollen consumption (1.67±.15 gm) was observed at fresh pollen pellets and minimum pollen consumption was observed at pollen balls with two coats of beeswax (Table 2; Fig. 2). Overall comparison reveled that at the pre-oviposition stage pollen balls without beeswax coat is most effective for the foundation queen as compare to other pollen balls coated with one and two beeswax (Table 2; Fig. 2). Significant difference also exists among different pollen balls coated with beeswax and fresh pollen on the pollen consumption in the colony after the emergence of first worker. Results showed that maximum pollen consumption was observed in fresh pollen pellets (2.42±0.12gm) and minimum pollen consumption (1.09±0.5gm) was observed on pollen balls with two coat of beeswax after the first emergence of worker in the colony (Table 2; Fig. 2). Overall result reveled that at this stage pollen balls with one coat of beeswax is most effective that queen and first worker consumed as compare to other pollen balls with two coated (Table 2; Fig. 2).

Table 2. Consumption Rate of fresh pollen pellets and pollen balls coated with and without beeswax during the colony initiation stage and colony foundation stage of bumblebee.

Pollen balls coated with wax	PCBFW	PCAFW	PCAFS
One coat	0.86±0.09	1.65±0.12	7.0±0.65
Two coat	0.44±0.062	1.09±0.5	2.7±0.37
Without coat	1.08±0.08	1.96±0.18	5.4±0.51
Fresh pollen	1.67±.15	2.42±0.12	8.8±0.5

Pollens consumption at colony foundation stage

According the statistical result data showed that significant difference exists among the fresh pollen pellets and pollens balls coated with beeswax and without beeswax on their consumption at colony foundation stage. Maximum pollen consumption (8.8±0.5gm & 7.0±0.65gm) was observed in fresh pollen pellets and pollen balls with one coat of beeswax and minimum consumption (2.7±0.37gm) was observed on pollen balls with two coats beeswax (Table 2; Fig. 2). Overall result reveled that at colony foundation stage pollen balls with one coat of beeswax was most effective because the shelf life of pollen was also higher than other fresh pollen pellets

and balls and consumption rate was also higher than other balls with two coats and with outs coat of beeswax (Table 2; Fig. 2).

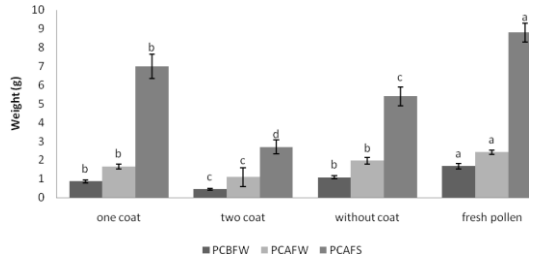

Fig. 2. Effect of pollen balls coated with wax and fresh pollen pellets on the life history parameter of bumblebee (*bombus terrestris)*.

PCBFW: Patties consumption before first worker emergence.

PCAFW: Patties consumption after first worker emergence.

PCAFS: Patties consumption at colony foundation stage.

According to above mention results of this study showed that at colony initiation stage of bumblebee rearing the most effective was pollen balls without beeswax. As it discussed earlier that this stage is very important stage in the bumblebee rearing process and this is the first step which lead to success. This step showed the sign of queen fertility and at this stage our study showed that the foundation queen or mother queen prefer pollen ball without coat of beeswax to make first eggs cup than other pollen balls with coat of beeswax and fresh pollen pellet. There are many possible reason exist that softness stuff of pollen balls as compare to other because at colony initiation stage only queen struggle no one is there at this stage to help her to build up colony so she need soft pollen to build up eggs cup. No related research to pollen balls coated with beeswax is available to compare result but there are many possible reason the main possible reason if we compare with fresh pollen pellets than there was the attack of pest on fresh pollen pellets the main pest which attack the colony on all of its stage was Indian meal moth which feed on pollen and reproduce on the pollen (Kwon *et al.*, 2003). Another possible reason was the foundation base for eggs cup i.e. pollen patties provide foundation base for the mother queen to make first egg cup so that queen like pollen patties as compare to fresh pollen pellets. By comparing with other balls with beeswax coated the main reason was the hard stuff because when pollen ball was coated with beeswax it become hard as compare to simple pollen ball so that reason may also exist that queen not prefer the coated ball for egg laying.

We observed that after the selection of best pollen balls by mother queen for eggs laying than queen prefer both balls i.e. with one coat of beeswax and without coating of beeswax at this stage. As our study showed that best result was found on these both balls one coated and without coated. If we discuss our result on the base of attack of disease on the pollen as discussed earlier which is main factor which affect on the growth of colony than we found that many researcher worked on the pollen pest, one of the main disease is the attack of fungus on the pollen. If storage and collection is not appropriate fungi might develop on the pollen pellet (Magan and Lacey, 1988; Lacey and Magan, 1991). Fungi grow on the surface of pollen effectively and use solid substrates by growing over their surfaces and then penetrating into their matrices. Many fungi species which are toxic and cause acute and chronic affect on all living organism (Marasas and Nelson, 1987; Moss, 1996). So that this possible reason may also exist which effect on the late and earlier emergence of first worker in bumblebee colony because in fresh pollen pellet fungus growth chance is high as compare to make pollen ball and attack of fungus decrease by coating with beeswax one time. And by coating pollen ball with more than one coat it make it more hard which as observed earlier that colony not prefer hard stuff of pollen.

Colony foundation stage is consider judgment stage on that stage colonies was separated by observing either it is used for pollination purpose or for collecting sexual for next rearing by observing the trend of colony either colony showed worker trend or

showing the trend toward switch point. In the sight of above mention result we found the pollen patties with one coat of beeswax showed the best result at colony foundation stage and also pollen ball without coat of beeswax also show good result. There are many possible reason for this result in the sight of scientist is the attack of wax moth and there are two main wax moth that feed on pollen one is lesser grain moth which attack mostly in honey bee hive to feed on pollen and also damage the hive and another important is Indian meal moth (*Plodia interpunctella*) (Ahmed, 1981; Caron, 1992).

Several measure are used to control this pest in the colony either by storing the fresh collected pollen in low temperature to kill the eggs or applying the chemical to control but chemical also have negative effect on the bumblebee colony (Mailer, 1994; Arthur and Johnson, 1995). But during our study we observed that the best technique to control the attack of pest on the colony and increase the growth of colony by providing the pollen ball with one coat of beeswax instead of using fresh pollen pellet. So in our study this possible reason may exist which make pollen ball with one coat of beeswax most effective and at that stage colony reached at foundation stage earlier as compare to other colonies which were fed with fresh pollen pellet and also this possible reason may exist that pollen ball with two coat of beeswax was not effective due to its hard stuff which already discuss that queen dislike.

According to above study result showed that pollen ball without beeswax coat is suitable before emergence of first worker in the colony and after the emergence of first worker pollen ball with one coat of beeswax is suitable. If we compare these result with above parameter result we found that these pollen ball with coat and without coat showed best result at Pre-oviposition stage and at emergence of first worker. Our next important purpose was of this experiment was to decrease the cast of pollen and as well as decrease the attack of pest. So from possible technique for this was to coat the pollen ball with beeswax to increase the shelf life of pollen and protect from degradation and also protect from the attack of Indian meal moth. If we compare our result with (Gradish *et al.*, 2012) they also used the pollen ball coat with beeswax to maintain their integrity and stimulate oviposition.

To increase the growth of colony and also decrease the cost of pollen for mass rearing process pollen ball with one coat of beeswax was most effective as compare to fresh pollen pellets and pollen ball with two coated. As discussed earlier and also observed from above study that by coating pollen ball with one time beeswax their shelf life increase and one things is also very important for mass rearing is that this stage is very susceptible to pest attack because the population density is high and also at developing stage supply of pollen increased which lead more chance of attack of pest on the colony. Some research article also showed that during the mass rearing of bumblebee the moisture level in the rearing room is 65%RH and at this moisture level chance of fungal attack on the pollen which lead the colony toward dead point earlier (Magan *et al.*, 2002, 2003). So possible reason may also exist and also proved from our previous study that by coating the pollen balls with beeswax the moisture level of pollen remain constant so we can decrease the chance of attack of fungi on pollen.

Overall purpose for planning this whole experiment was to protect the colony from attack of insect pest of pollen and bumblebee colony and also decrease the pollen cost by increasing the shelf life of pollen by coating with beeswax. So we found that at Preoviposition stage pollen ball without beeswax coat was the best and after the emergence of first worker in the colony than pollen ball with one coat of beeswax was most effective also effective at colony foundation stage. We also conclude from this study that to decrease the cost of rearing colony of bumblebee pollen ball with one coat of beeswax was most effective to increase the shelf life of pollen and also decrease the colony disturbance by change the

diet on daily bases in the colony which increase the colony growth.

Acknowledgement

The present studies were kindly financed by the Higher Education Commission of Pakistan under Bumblebee laboratory rearing project and indigenous fellowship for the first author of his PhD studies. We are also thankful for PMAS-Arid Agriculture University Rawalpindi to provide proper laboratory conditions for experimentation. We are also thankful to our anonymous reviewers for their kind inputs and improvement suggestions for this manuscript.

References

Ahmed R. 1981. A guide to honey bee management in Pakistan. Pakistan Agricultural Research Council Miscellaneous Publication. Islamabad: Pakistan Agricultural Research Council.

Arthur F, Johnson HL. 1995. Development of aeration plans based on weather data: a model for management of corn stored in Georgia. American Entomologist **41**, 241–6.

Carbet SA, Williams IH, Osborne JL. 1991. Bees and the pollination of crops and wild flowers in the European Community. Bee World **72,** 47-59.

Caron DM. 1992. Wax moth. American Bee Journal **132**, 647–9.

Chittka L, Ings T, Raine NE. 2004. Chance and adaptation in the evolution of island bumblebee behavior. Population Ecology **46,** 243-251.

Duchateau MJ, Velthuis HHW. 1988. Development and reproductive strategies in *Bombus terrestris* colonies. Behaviour **107,** 186-207.

Duchâteau MJ, Velthuis HHW. 1989. Ovarian development and egg laying in workers of *Bombus terrestris*. Entomologia Experimentalis et Applicata **51**, 199-213.

Goulson D. 2003. Effect of introduced bees on native ecosystems. Annual Review of Ecology, Evolution, and Systematic **34,** 1-26.

Gradish AE, Scott-Dupree CD, Frewin AJ, Cutler GC. 2012. Lethal and sublethal effects of some insecticides recommended for wild blueberry on the pollinator *Bombus impatiens*. The Canadian Entomologist **144,** 478-486.

Griffin RP, Macfarlane RP, van den Ende HJ. 1991. Rearing and domestication of long tongued bumblebee in New Zealand. ActaHortic **288,** 149-153.

Groot AP. 1953. Protein and amino acid requirements of the honeybee (*Apis mellifera* L) Laboratory of Comparative Physiology Utrecht, 197–285 pp.

Hannan MA, Maeta Y, Hoshikawa K. 1998. Feeding behavior and food consumption in *Bombus ignitus* under artificial condition (Hymenoptera: Apidae). Entomological Science **1,** 27-32.

Haydak MH. 1970. Honey bee (Hymenoptera: Apidae) nutrition, The Annual Review of Entomology **15,** 143–156.

Heinrich B. 1993. The Hot-Blooded Insects: Strategies and Mechanisms of Thermoregulation. Berlin: Springer-Verlag.

Imran M, Munir A, Farooq MN, Shafqat S. 2015. Effect of Different Nest Box Materials on the Mating of European Bumblebee, *Bombus terrestris* (Hymenoptera: Apidae) under Controlled Environmental Conditions. Pakistan Journal of Zoology **47,** 241-247.

Kwon YJ, Saeed S, Duchateau MJ. 2003. Control of *Plodia interpunctella* (Lepidoptera: Pyralidae), a pest in *Bombus terrestris* (Hymenoptera :Apidae) colonies. The Canadian Entomologist **135,** 893-902.

Lacey J, Magan N. 1991. Fungi in cereal grains: their occurrence and water and temperature relations J Chelkowski (Ed.), Cereal grain-mycotoxins, fungi and quality in drying and storage, Elsevier Science B.V, Amsterdam 77-118pp.

Magan N, Hope R, Cairns V, Aldred D. 2003. Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. European Journal of Plant Pathology **109,** 723– 730.

Magan N, Hope R, Colleate A, Baxter ES. 2002. Relationship between growth and mycotoxin production by Fusarium species, biocides and environment. European Journal of Plant Pathology **108,** 685– 690.

Magan N, Lacey J. 1988. Ecological determinants of mouldvgrowth in stored grain. International Journal of Food Microbiology **7,** 245– 256.

Mailer DE. 1994. Chilled aeration and storage of US crops, a review. In: E Highly EJ, Wright HJ, Banks BR. Champ (Eds), Proceedings of the 6th International Working Conference on Stored Product, 300–311pp.

Marasas WFO, Nelson PE. 1987. Mycotoxicology, Pennsylvania State University, University Park, PA.

Michener CD. 1974. The Social Behavior of the Bees: A Comparative Study. Second Edition. Harvard University Press (Belknap Press), Cambridge, 404 pp.

Moss MO. 1996. Mycotoxins. Centenary review. Mycological Research **100,** 513– 523.

NorusICH MJ. 2007. SPSS 15.0 guide to data analysis Prentice Hall SPSS Inc.

Ono M, Suzuki M, Sasaki M, Matsuka M. 1994b. Food processing strategy of bumblebees (Hymenoptera: Apidae); in Les InsectesSoxiaux. Lenoir A, Amold G, Lepage M. (eds), Publ. Univ. Paris Nord, 487pp.

Pereira-Lima A, Lino-Neto J, Oliveira CL. 2006. Sexual maturation in Meliponamondury males (Apidae: Meliponini). Journal of Braz. Morphological Science **23,** 369-375.

Plowright RC, Laverty TM. 1987. Bumble bees and crop pollination in Ontario, Proceedings of the Entomological Society of Ontario **118,** 155–160.

Plowright RC, Pendrel RA. 1977. Larval growth in bumblebees (Hemenoptera: Apidae). Canadian Entomologist **109,** 967-973.

Rasmont P, Coppee A, Michez D, De-Meulemeester T. 2008. An overview of the *Bombus terrestris* (L.1758) subspecies (Hymenoptera: Apidae). Annales de la Societe Entomologique de France **44,** 243-250.

Ribeiro MF, Duchateau MJ, Velthuis HHW. 1996. Comparison of the effects of two kinds of commercially pollen on colony development and queen production in the bumble bee *Bombus terrestris* L (Hymenoptera, Apidae). Apidologie **27,** 133-144.

Röseler PF. 1977. Rearing bumblebee colonies, Proc. 8th Int. Congr. IUSSI, Wageningen 312p.

Röseler PF. 1979. Hummelzucht für den Erwerbsobstbau? Erwerbsobstbau **21,** 177-178.

Röseler PF. 1985. A technique for year-round rearing of *Bombus terrestris* (Apidae, Bombini) colonies in captivity. Apidologie **16,** 165-170.

Roulston TH, Cane JH, Buchmann SL. 2000. What governs protein content of pollen: pollinator Major and minor floral choices of bumble bees 105 preferences, pollen-pistil interactions, or phylogeny? Ecological Monographs **70,** 617–643.

Roulston TH, Cane JH. 2000. The effect of diet breadth and nesting ecology on body size variation in bees (Apiformes). Journal of the Kansas Entomological Society **73,** 129-142.

Roulston TH, Cane JH. 2002. The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). Environmental Ecology **16,** 619-622.

Sevensson B, Lagerlof J, Sevensson BG. 2000. Habitat preference of nest seeking bumblebees (Hymenoptera: Apidae) in an agricultural landscape. Agriculture, Ecosystems and Environment **77,** 247-255.

Stern RK. 1994. Introductory Plant Biology. Wm. C. Brown Publisher, New York, USA. 537 pp.

Sutcliffe GH, Plowright RC. 1988. The effects of food supply on adult size in the bumblebee *Bombus terricola* Kirby (Hymenoptera: Apidae). Canadian Entomologist **120,** 1051-1058.

Tasei JN, Aupinel P. 1994 Effect of photoperiodic regimes on the oviposition of artificially over wintered *Bombus terrestris* L. queens and the production of sexuals. Journal of Apiculture Research **33,** 27-33.

Tasei JN, Aupinel P. 2008. Nutritive values of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers. Apidologie **39,** 397-409.

Umer AAS, Munir A, Imran M, Nasir M, Shafqat S, Imran B. 2014. Distribution of bumblebee, *Bombus haemorrhoidalis* Smith and its association with flora in lower northern Pakistan. Pakistan Journal of Zoology **46,** 1045-1051.

Williams IH, Cobert SA, Osborne JL. 1991. Bee keeping, wild bees and pollination in European community. Bee World **72,** 170-180.

Williams PH. 1994. Phylogenetic relationships among bumble bees (*Bombus* Latr.): a reappraisal of morphological evidence. Systematic Entomology **19,** 327–344.

Yoon HJ, Kim SE, Lee KY, Lee SB, Park IG. 2005a. Oviposition and colony development of the bumblebee, *Bombus ignites* and *B. terrestris* depending on different pollen. International Journal of Industrial Entomology **11,** 99-105.

Yoon HJ, Kim SE, Lee SB, Seol KL. 2005b. The effect of antiseptic and sugar solution on colony development of the bumblebee, *Bombus ignitus* and *B. terrestris*. International Journal of Industrial Entomology **11,** 43-48.