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Original Research Article

Effect of queen age on hygienic and grooming behavior of *Apis mellifera Ligustica* against *Varroa destructor* (Anderson and Trueman)

Saboor Ahmad, Muhammad Asif Aziz, Munir Ahmad, Imran Bodlah
Department of Entomology, PMAS Arid Agriculture University, Rawalpindi, Pakistan

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**Corresponding author email:*
asifaziz@uaar.edu.pk

Abstract

The studies were conducted to compare the hygienic and grooming behavior of honey bees (*Apis mellifera Ligustica*) headed by new (0-year) and old queens (1-year) against *Varroa* mites at Apiculture Research Farm, PMAS-Arid Agriculture University Rawalpindi during 2013-14. Worker bees removed 91.56, 95.11 and 98.52 % dead brood in colonies headed by new queens, while 75.22, 82.78 and 88.78%, in colonies headed by old queens; after 24, 48 and 72 h, respectively. Similarly, significant differences were observed regarding the removal of artificially introduced *Varroa* mites between both types of colonies; bees in colonies headed by new queens removed 74.67 and 84.67%, while the bees headed by old queens removed 52.67 and 66.67% artificially introduced *Varroa* mites from brood cells after 5 and 7 days, respectively. The mean percentage of leg deformed *Varroa* mites due to grooming behavior of adult bees in colonies with new and old queens was 67.45 and 57.83%, and mites with deformed dorsal shield was 27.03% and 20.71%, respectively. Colonies with new queens exhibited better hygienic and grooming behavior against the *Varroa* mite as compared to those having old queens. Replacing old queens with new queens every year is suggested for better management of *Varroa* mite in Pothwar region of Punjab.

Keywords: Hygienic behavior, *Apis mellifera Ligustica*, *Varroa* mite, Pothwar, Punjab

Introduction

Varroa mites (Anderson and Trueman, 2000) are basically ectoparasites of the Asian honey bee, *Apis cerana*. These mites were transferred to *Apis mellifera* from the intercontinental trade of bees, and now a day have become severe threat to bees all over the world. They can cause colony collapse within two to three years (Morse and Nowogrodzki, 1999) and large numbers of *A. mellifera* colonies have been destroyed in last century in the both Europe and North America (Oldroyed, 2007; Van Engelsdorp et al., 2007) due to infestation of these mites and their related viruses. *Varroa destructor* is a carrying agent of 18 different viruses in honey bee colonies (Chen and Siede, 2007). The most important of these viruses are; Acute bee paralysis virus, Deformed wing virus, Kashmir bee virus, Israeli acute paralysis virus, Sac brood virus, Chronic bee paralysis virus and Black queen cell virus (Boecking and Genersch, 2008; Mohammadreza G et al., 2017). Different acaricides have been used to control *Varroa* mites but their trouble is increased even more due to the development of acaricide resistance against these chemicals (Thompson et al., 2002).

However, honeybees have naturally evolved some special features (hygienic and grooming behavior) to defend themselves against these dreaded ectoparasitic

mites. Some worker bees in the colony detect the diseased or infested pupae, uncap the cells and remove the effected brood from the hive (Peng et al., 1987; Boecking and Spivak, 1999). Due to which the numbers of fertile and immature mites are reducing in honey bee colony. This is called hygienic behaviour. Although it is basically considered as a character of resistant against American foulbrood and chalkbrood diseases (Cremer et al., 2007), however hygienic colonies are also reported to have less mite population as compared to others (Boecking and Ritter, 1993). Therefore this behavior is considered important to develop social resistance among *A. mellifera* colonies (Wilson-Rich et al., 2009).

Another defensive mechanism is grooming behavior; in which the adult bees remove feral mites stuck on their bodies (auto-grooming) or attract other workers by doing special movements to remove them (all-grooming). During this action, mites are injured from legs (amputation) or idiosoma due to bites of worker bees (Vaziritabar et al, 2016) and fall down on bottom board. This behviour can also be considered as possible limiting factor to mite population. Expression of behavioral defense varies depending on bee species, location, weather conditions, colony strength, inbreeding of queens and even in the colonies kept in same apiary headed by queens of different ages (Akyol et al., 2007).

The present study was planned with the hypothesis that hygienic and grooming behavior *Apis mellifera* colonies against *V. destructor* mite is effected with the age of queen in the colony and attempt was made to explore the difference in workers' performance in colonies headed by new and old queens. So that the beekeepers may use the findings of these investigations in maintaining queens stocks of required age to combat *Varroa* problem in their apiaries.

Materials and Methods

The experiments were conducted at Apiculture Research Farm, Koont, PMAS-Arid Agriculture University Rawalpindi, Pakistan from September, 2013 to February, 2014. Six bee colonies of equal bee strength were selected and separated into two groups on the basis of their queens i.e., one group contained the old queens (of previous year) of Italian bees and second with new ones (introduced in spring season). The tested bee colonies were infected by nature with *Varroa* mites and no chemical treatment were used to control *Varroa* mite. Sealed brood area was selected from the middle of the frame and killed with the fine wooden pin and placed back in their respective locations. The percentage of brood, removed from the frames was recorded after 24h, 48h and 72h. The experiment was repeated three times. Six colonies of honey bees were selected (three colonies of new Italian queen race and three colonies of old queen) to compare the hygienic behavior of two queen races against artificial infested brood cell with *Varroa* mites. For this purpose we selected 25 worker sealed brood cells from middle frames in each colony and inserted one female *Varroa* mite with the help of fine camel hair brush in each cell. After inserting the mite, the cells were again capped with the help of wax. In case of control treatment, the worker cells were perforated with help of small needle pin and again closed without inserting the *Varroa* mites. The number of brood cells emptied by bees in treated and control treatment were counted after 5 and 7 days interval during winter season.

Grooming behavior was compared between two honey bee races colonies by conducting the experiments for six times after one week interval. For this purpose a plastic sheet coated with vaseline was inserted in each bottom board of each hive. After one week dropped mature female mites were separated depending on color, counted and examined for any deformity using a microscopic magnification at Biosystematics Lab. Department of Entomology, PMAS-Arid Agriculture University Rawalpindi. Grooming behavior was expressed as percentage mean of deformed *Varroa* female mites in each colony. The mean percentage of legs and dorsal shield deformed female *Varroa* mites were counted in each colony.

Statistical analysis

Data were analyzed through analysis of variance and means were compared by the Student-Newman-Keuls Test at 0.05 by using SPSS software (SPSS, 2001).

Results and Discussion

Removal of artificially killed brood cells

The comparison of hygienic behavior between colonies headed by new and old queens after 24, 48 and 72h is illustrated in (Fig 1). Colonies headed by new queens removed 91.56% artificially killed brood as compared to 75.55% in those headed by old queens after 24 h. After 48h, worker bees in colonies headed

by new queens removed 95.11% dead brood as compared to 82.78% by those headed by old queens.

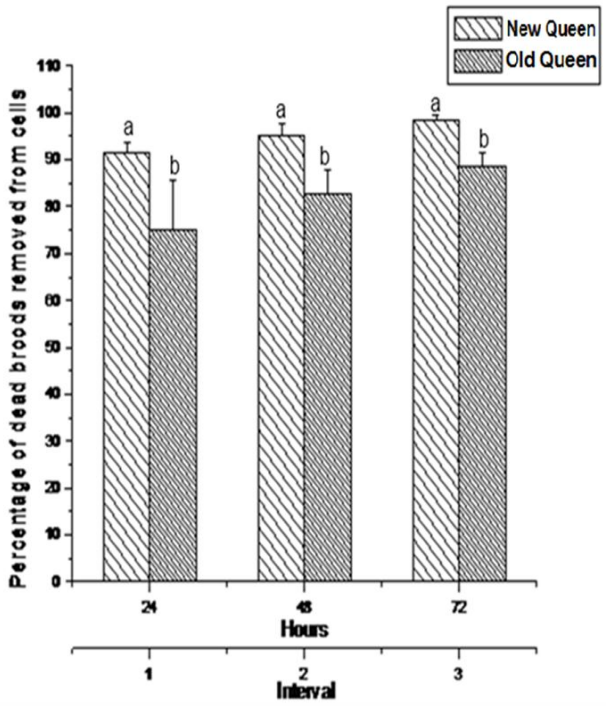

Figure – 1: Comparison of Hygienic behavior (expressed as % of mean removal of killed brood) of *Apis mellifera* colonies with new queen verses old queen

Similarly after 72h, significant differences were found between both type of colonies with 98.52 and 88.78% dead brood removal, in colonies headed by new and old queens, respectively. According to Tanja G et al. (2016) improved hygienic behaviour colony working was mainly accomplished by a great number of honey bees retaining in the hygienic responsibilities and molecular manners in the brain of honey bee may disturb quantitative changes of hygienic behavior. Rosenkranz et al. (2010) demonstrated that several resistance traits have been recognized in the honey bees. Removal of artificially killed brood cells is considered an important indicator of hygienic behaviour (Balhareth et al., 2012). Alejandra CS et al. (2016) observed hygienic behavior of honey bees was asymmetrical regarding the age-distribution and larger numbers of honey bees performing hygienic activities early in their life. Removal of dead brood is performed by nurse bees. Arathi et al. (2000) reported that middle aged bees (nurse bees) perform the hygienic activities in the colony before becoming foragers and about 18% of bee population is engaged in this task at any given time. High rate of dead brood removal in colonies headed by new queens may be due to strong population of nurse bees (Thompson, 1964). Akyol et al. (2007) also reported low level of *Varroa* infestation in colonies headed by new queens as compared to old ones, and also recorded about 40% more population of adult bees in colonies with new queens. In our studies, colonies headed by new queen had also more population of bees as compared to those headed by old ones. Which may be regarded as main factor affecting the performance of both type of colonies.

Removal of artificially infested brood cells with *Varroa* mites

Comparison of *A. m. Ligustica* colonies headed by new and old queens regarding removal of artificially infested brood cells with *Varroa* mites is presented in Fig 2.

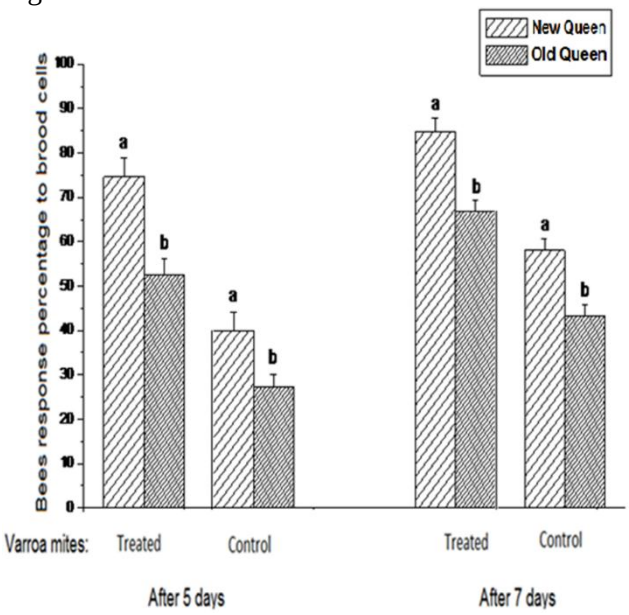

Figure – 2: Hygienic behavior of new and old Italian honey bee with respect to cleaning of worker brood cells containing the *Varroa* mite

Significant differences were found regarding removal of artificially introduced *Varroa* mites i.e., 74.67 and 84.67 % in colonies headed by new and 52.67 and 66.67 % in those headed by old queens after 5 and 7 days of artificial infestation, respectively. In control colonies the removal percentage of infested pupae in colonies headed by new and old queens was 40.00, 58.00 and 27.33, 43.33 %, respectively after 5 and 7 day of artificial infestation. In addition, we observed that colonies headed by new queens had more pollen storage. According to Janmaat and Winston (2000),

workers in colonies with suboptimal conditions become less efficient in performing colony tasks as compared to those colonies having strong worker population. Cakmak et al. (2009) investigated that severely infested worker bees with *Varroa* mite were introduced into remark hives earlier and during winter has also revealed compact their lifecycle. Otten and Fuchs (1990) reported that the bees having high pollen storage had more efficiency to detect and remove *Varroa* mites from infested cells as compared to those having less pollen storage; probably due to the fact that the main focus of bees remains pollen collection cues as colony task cues. In current studies, it was observed that the removal percentage of mites in the control sealed cells was low in both type of colonies, this phenomenon may be explained on the basis of findings of Rosenkranz et al. (1993), who reported that bees are more efficient to detect and remove of the mites artificially introduced from any foreign source as compared to those living in the same colony, on the base of their different odour.

Grooming behavior of *Apis mellifera linguistica* colonies headed by new and old queens

Means comparison of data regarding population of mites with deformed legs collected at weekly interval showed significant differences between both type of colonies (($F_{1,35}$=26.88, P=0.0001) (Fig 3).

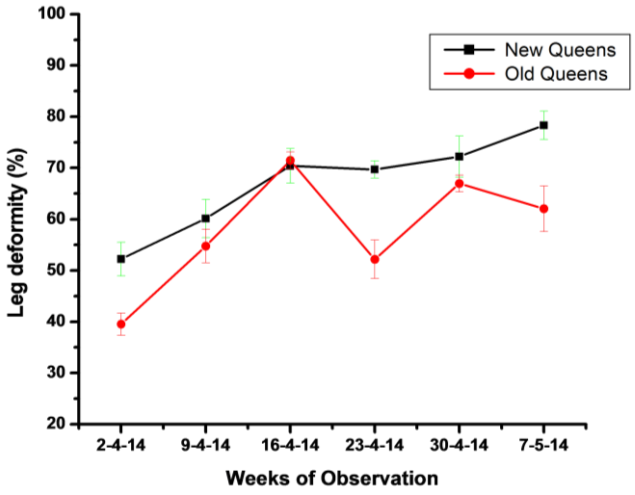

Figure – 3: The percentage of leg deformed *Varroa* mites collected in the debris of colonies containing new and old honey bee queens

Overall, mean percentage of fallen leg deformed mites fallen was 67.45 and 57.83% in colonies headed by new and old queens, respectively. The deformed mites fallen at bottom board of colony may be regarded as indicator of grooming behviour of bees (Balhareth et al, 2012); which has important role in reduction of *Varroa* mites population within the hive (Mondragon et al., 2005). Variable percentage of leg deformity has been reported in literature in different sub species of *A. mellifera*. Omran (2004) found 67.2 to 77.2% leg deformity of *Varroa* mites in two consecutive years in *A. m. lamarkii*. Zaitoun and Al-Ghazawi (2009) reported 19.75, 11.50 and 10.75% leg deformity in *A. m. syriaca*, *A. m. carnica* and *A. m. lingutica*, respectively. Balhareth et al. (2012) observed 62.94 and 56.29%, leg deformity in *A. m. jemenitica* and *A. m. carnica*, respectively. Correa-Marques et al. (2002) found 47.4 and 46.00 % leg deformity in Italian and Carniolan bees, respectively. Al-Medani (2004) observed the percentage of leg-deformed was 62.5% in debris of honey bee colony. According to Rosenkranz et al. (1997) amputation of leg or legs is the main indicator of successful grooming, in our studies the leg deformity in colonies headed by new queens varied in different weeks of observation, but remained statistically similar with those headed by old queens. However both types of colonies showed better grooming performance as compared to those reported by Correa-Marques et al. (2002).

The comparison of data regarding percentage of mites with deformed dorsal shields collected from colonies headed by new and old queens is shown in (Fig 4).

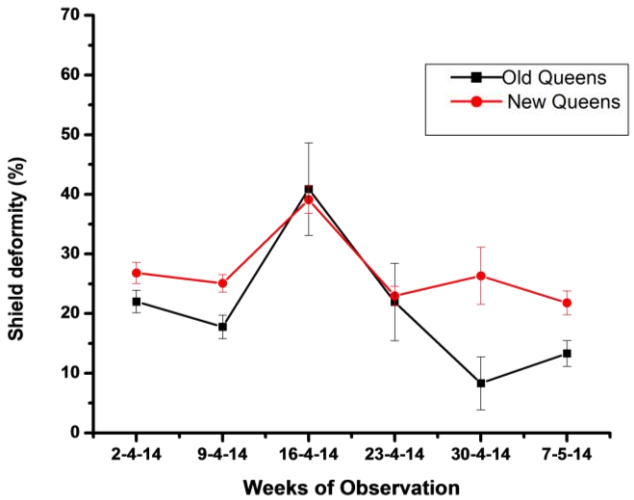

Figure – 4: The percentage of dorsal shield deformed *Varroa* mites was collected in the debris of colonies containing new and old Italian honey bee queens

The figure depicted significant differences for both type of colonies ($F_{1,35}$=21.73, P=0.0001). The overall mean dorsal shield deformity was found 27.03 and 20.71% in colonies headed by new and old queens respectively. Balhareth et al. (2012) reported 37.18 and 36.32 % dorsal shield deformity in *A .m. jemenitica* and *A. m. carnica*, respectively. Correa-Marquez et al. (2002) found 40.0 and 36.7%, dorsal shield deformity in Carniolan and Italian bees, respectively. In current studies, over all means of dorsal shield deformity was less as compared to those reported in previous studies. Moreover, significant differences in leg and shield deformity of fallen phoretic mites shows that grooming behaviour of experimental colonies also altered with the queen age heading the colony.

Conclusion

Present studies revealed that colonies headed by new queens performed comparatively better as compared to those headed by old queens particularly with respect to hygienic behavior and grooming behavior. These findings reveal better performance of bees headed by new queens and advocate the need to replace the queens every year keeping in view the particular environmental conditions of Pothwar region to get stronger population of worker bees and better colony performance against *Varroa* mites.

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