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Comparison of Hygienic Behavior of Exotic Honey Bee *Apis mellifera L.* and Indigenous Honey Bee *Apis cerana* of Pakistan

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Abstract

Indigenous and exotic honey bee species were evaluated for their hygienic behavior in the climatic condition of Peshawar Khyber Pakhtunkhwa, Pakistan. Colonies of equal strength from indigenous (*Apis cerana*) and exotic (*Apis mellifera*) species were selected for the study. The same colonies were tested in two seasons. Sealed brood were killed with different methods i.e pin killed and freeze killed. The uncapping of cells and brood removal was recorded at different intervals. Significant differences were recorded between hygienic behavior of both species of honey bees. *Apis cerana* showed significantly superior hygienic behavior than *Apis mellifera* in both seasons. At different intervals in both species significant differences were recorded. A significant difference was recorded after 12 and 24 hours between the species in both seasons. No significant differences were recorded after 48 hours in both species. From the study it is concluded that indigenous honey bee species has superior hygienic behavior than exotic species.

Introduction

Honey bees have an instinct behavior of removing infested, diseased or dead broods, clean their cells and take these diseased or dead broods out of their hives, which is called hygienic behavior of honey bees (Rothenbuhler, 1964 a,b). Hygienic behavior in the honey bee species *A. mellifera* was noticed and reported in earlier years (Park, 1936). Honey bees with a higher hygienic behavior level in their colonies are more resistant towards two brood diseases i-e American foul brood (Spivak, 1996; Spivak & Reuter, 2001) and Chalkbrood (Invernizzi et al., 2011). Selectively bred honey bees for hygienic behavior shows higher level of hygienic behavior while non selective bred honey bees show lower level of hygienic behavior (Masterman et al., 2000). Hygienic behavior is one of the most desirable traits for selective breeding of the honey bees by the breeders (Spivak, 1996).

Natural physical defense mechanism, i-e Grooming and Hygienic behaviors of *A. mellifera* and *A. cerana,* play a main role in their protection against brood diseases and *Varroa* mites (Boeking & Spivak, 1999).The extent of damage caused by *Varroa* to its original host *A. cerana* is less as compared to western honey bee *A. mellifera* colonies (Jong et al., 1982). The main reason for lower *Varroa* mite population in *A. cerana* colonies is their behavior of efficiently attacking and killing the introduced *Varroa* mite individuals in their colonies (Peng et al., 1987). In *A. cerana* colony, the *Varroa* mites prefer to infest drone cells while in *A. mellifera* colonies the *Varroa* mites have adapted to infest both drone and worker cells, and this adaptation to reproduce in worker cells in *A. mellifera* has led to greater damages due to disease transmission from *Varroa* that ultimately lead to lower bee population (Koeniger et al., 1981, 1983). Application of pesticides/Acaricides for controlling *Varroa* mites in the

honey bees hive cause contamination of honey with chemical residues and resistance to these pesticides by *Varroa* mites (Lodesani et al., 1992& 1995).

Selective breeding of honey bees for higher level of hygienic behavior by the breeders may act as an alternate to pesticide usage for *Varroa* mite infestation and other brood diseases in honey bee hives (Spivak, 1996). The comparison of African honey bee *A. mellifera scutellata* with the different hybrids of European honey bee *A. mellifera* showed that the first mentioned honey bees showed higher levels of hygienic behavior than the second one (Nganso et al., 2017). Studies showed that *A. cerana* showed higher level of hygienic behavior in removing artificially killed broods in a specific area of comb than *A. mellifera* in two seasons of southern part of China (Lin et al., 2016).

Our experiments in this research aim to compare the level of hygienic behavior of exotic honey bees *A. mellifera* and native honey bees *A. cerana* of Pakistan in different seasons, and provide a baseline for future related studies.

Materials and Methods

Selection of study site and determination of Hygienic behavior of *A. mellifera* and *A. cerana*: Experiments on Hygienic behavior of *A. mellifera* and *A. cerana* were conducted on apiaries located at medicinal plants garden of Khyber Pakhtunkhwa Forest Department. Strong honey bee hives consisted of langstroth design with 10 frames of combs were selected for conducting experiments in the spring season of March and summer season of June, 2018. All the colonies were having a fertile queen, workers, broods, honey and pollen. Honey bee colonies were healthy and strong. There was no sign of any disease in the colonies. Frames with broods covered area were selected. Experiments on hygienic behavior were conducted on two methods i-e Freeze killing of broods (Reuter & Spivak, 1998)) and Pin killing of brood assay (Newton and Ostasiewski, 1986).

Freeze killing assay

Broods of *A. mellifera* and *A. cerana* were killed by liquid nitrogen treatment to find out difference between their rates of dead brood removal in the spring season of March, 2018. 5 hives of honey bees of both species were selected. One frame having most of the sealed brood cells present was selected from each hive and was tagged. An area on each frame with most of the sealed brood cells was selected by pressing with a hardboard cylinder with a diameter of 6.5 cm and height of 8 cm. About 10 ml of liquid nitrogen was poured in to cylinder through volumetric flask for freeze killing the broods present in area covered by hardboard cylinder, containing a total of 150 cells. The cylinder was removed till the liquid nitrogen was fully evaporated. The frames were returned back to their respective hives and were kept at their original position. After 12 hours, 24 hours and 48 hours the data were recorded by counting the dead eradicated and remaining broods by honey bees. The parameter of number of dead broods removed during 24 hours was considered as hygienic behavior of both species of honey bees.

Pin killed brood assay

For determination of hygienic behavior of *A. mellifera* and *A. cerana* pin killed brood assay was conducted in summer season of June, 2018. A total of 5 hives were selected from each species of honey bees for conducting experiment. The frame having most of the sealed brood cells was selected from each hive. Area having the most of sealed brood cells was selected and was pressed through hardboard cylinder (covering a total of 150 cells). The brood present in the area covered by hardboard cylinder was killed through a sterile metal pin. The frames were then returned back to their original position at their respective hives. The number of dead broods removed by both species of honey bees in their hives during 12, 24 and 48 hours were recoded.

Statistical data analysis

The total number of cells that was covered under hardboard cylinder in both species was 150 capped brood cells. The data was recorded by comparing the freeze uncapped dead brood removed cells with capped dead brood cells in hives of both species. The percentage of uncapped dead broods removed in both species of honey bees after 12 hrs, 24 hrs, 48 hrs was calculated. The percentage of uncapped dead broods removed was compared with capped dead broods to evaluate hygienic behavior of *A. mellifera* and *A. cerana*. For comparison of means of both variables (capped and uncapped cells) student's t test was used for correlation and significance of means. All the statistical analyses were conducted through statistical package SPSS (version 20.0).

Results

Freeze Killed Brood Assays

The results in Fig 1 shows that there is significant difference ($P<0.05$) of hygienic behavior level between *A. mellifera* and *A. cerana* in the spring season. The trend of hygienic behavior of *A. cerana* was significantly ($P<0.05$) higher than *A. mellifera* and the number of uncapped cells and number of dead brood eradication during 12 hrs, 24 hrs was higher. No significant differences were recorded after 48 hrs in both exotic and indigenous species.

Similar kind of behavior was recorded in the summer season as well (Fig 2). *A. cerana* showed significantly ($P<0.05$) higher hygienic behavior than *A. mellifera* and the number of uncapped cells and number of dead brood removal during 12 hrs, 24 hrs was higher. Both species showed similar hygienic behavior and no significant differences were recoded after 48 hrs.

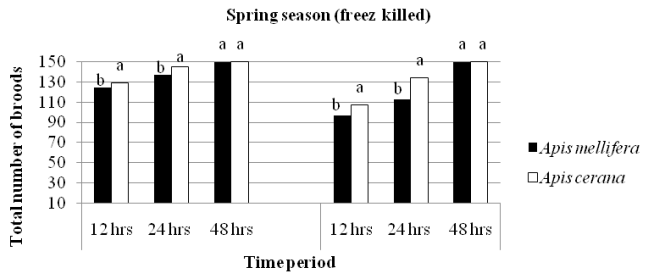

Fig 1. Comparison of hygienic behavior of *A. mellifera* and *A. cerana* in freeze killed brood assay during spring season.
Means followed by same letter are non-significant at 5% significant level.

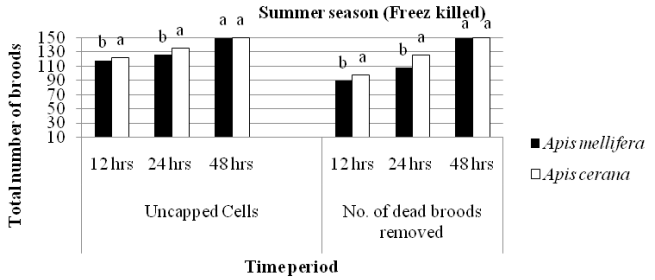

Fig 2. Comparison of hygienic behavior of *A. mellifera* and *A. cerana* in freeze killed brood assay during Summer season.
Means followed by same letter are non-significant at 5% significant level.

Pin Killed Brood Assays

The results in Fig 3 shows the comparison of hygienic behavior level between *A. mellifera* and *A. cerana* in spring season by pin kill brood assay method. A significantly ($P<0.05$) lower number of uncapped cells and dead brood removal was recoded in *A. mellifera* than *A. cerana* after 12 and 24 hours. However, no differences were recorded after 48 hours.

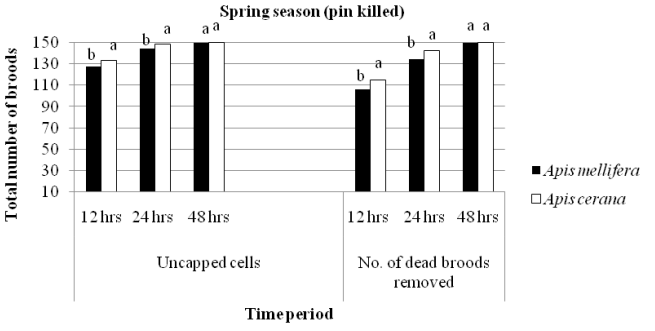

Fig 3. Comparison of hygienic behavior of *A. mellifera* and *A. cerana* in pin killed brood assay during spring season.
Means followed by same letter are non-significant at 5% significant level.

During the summer seasons the assays were repeated and similar results like springs was recorded Fig 4. Both species showed no significant differences after 48 hours in their hygienic behavior but they showed significant ($P<0.05$) difference after 24 and 12 hours. *A. cerana* hygienic behavior was significantly higher after 12 and 24 hours in removal of dead brood and uncapped cells.

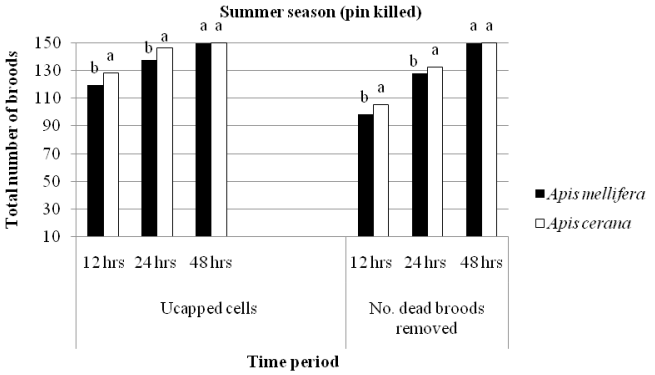

Fig 4. Comparison of hygienic behavior of *A. mellifera* and *A. cerana* in pin killed brood assay during Summer season.
Means followed by same letter are non-significant at 5% significant level.

Discussion

Results of freeze killed brood assay show that overall hygienic behavior of *A. mellifera* was slightly lower than *A. cerana* in both spring and summer seasons, however *A. cerana* showed slightly higher hygienic behavior than *A. mellifera* in spring than summer season. *A. cerana* expresses higher level of hygienic behavior than *A. mellifera* in autumn season than spring season in uncapping and removal of dead broods during freeze killed brood assay (Lin et al., 2016).

Hygienic behavior is expressed variably among different species, subspecies and races of honey bees irrespective of different seasons and environmental conditions (Liliaet al., 2016; Spivak & Galliam, 1998 a, b; Spivak & Reuter, 1998; Rasolofoarivao et al, 2015; Athreya & Reddy; 2013). Our results show that *A. cerana* hygienic behavior is higher in spring than summer season. This difference between both species may be attributed to the higher floral resources and lower temperature in spring as compared to summer season. Our results are in conformity with (Lin et al., 2016) in which *A. cerana* showed higher level of hygienic behavior compared to *A. mellifera*. Bee population in hive didn't have effect on their hygienic behavior which shows that higher efficiency of hygienic behavior is instinct to *A. cerana* in comparison to *A. mellifera* (Lin et al., 2016).

There are genetic reasons for phenotypic expression of the hygienic behavior (Moritz, 1988). The instinct nature of higher level of hygienic behavior in *A. cerana* is the presence of seven genetic loci controlling hygienic behavior (Lapidge et al., 2002). All the colonies selected for assessing hygienic behavior of *A. cerana* and *A. mellifera* were naturally bred and the queen naturally mated in our experiment. The results suggested that slight difference of bee population in colony did not have significant effect on their hygienic behavior in both species of honey bees. Since *A. cerana* is more efficient at removing the dead broods from their cells, the prevalence of deadly viruses like deformed wing virus and sac brood virus carried by *Varroa destructor* is lower in *A. cerana* colonies in

comparison to *A. mellifera* (Highfield et al., 2009; Mondet et al., 2016). Boecking and Drescher, (1992, 1999) demonstrated that Africanized bees were more efficient in hygienic behavior as compared to western honey bees and removed broods faster in plastic combs than wax combs.

Our results are in conformity with (Palacio et al., 1996, 2000; Spivak & Downey, 1998) research demonstrating that honey bees were faster at removing pin killed broods as compared to freeze killed broods. The reason for this behavior may be due to easy detection of pin killed broods because of the hole made in the wax seal of the cell having brood by honey bees through olfaction of pheromones released by broods (Palacio et al., 1996). The indigenous honey bee species of Pakistan, *A. cerana* showed significant higher hygienic behavior than the exotic species untill 24 hours. This could also be the reason explaining less pests and diseases attack in *A. cerana* than in *A. mellifera*. These are confirmed by some earlier studies of (Lint et al., 2016).

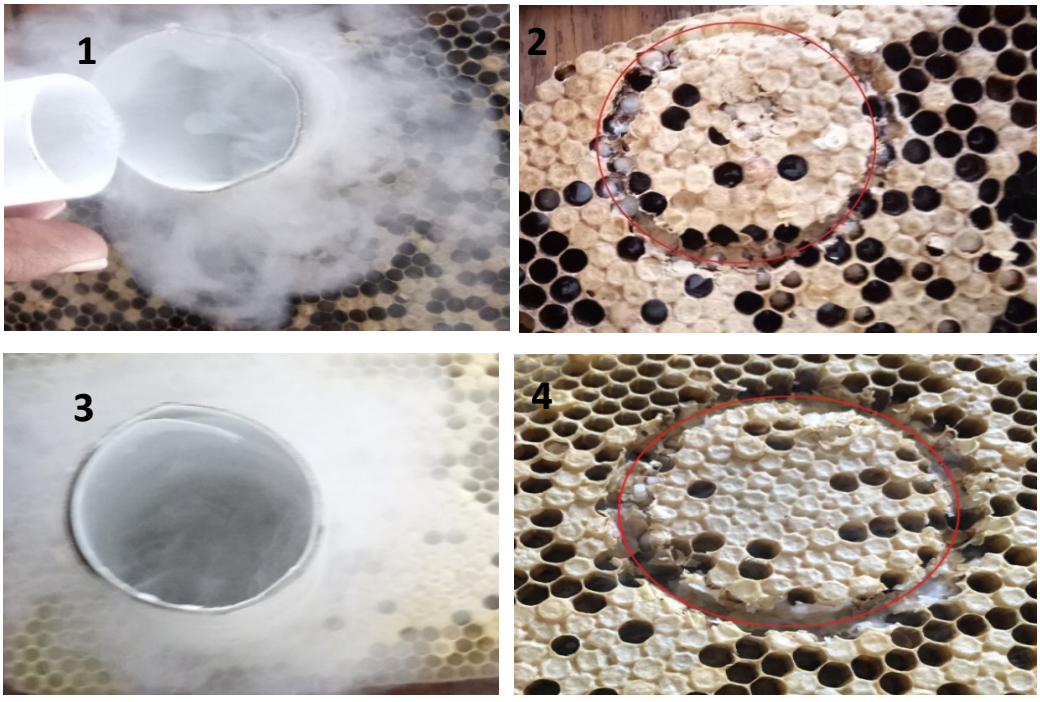

Fig A. Freeze killing of broods with liquid nitrogen 1 and 2 *Apis mellifera*, 3 and 4 *Apis cerana*.

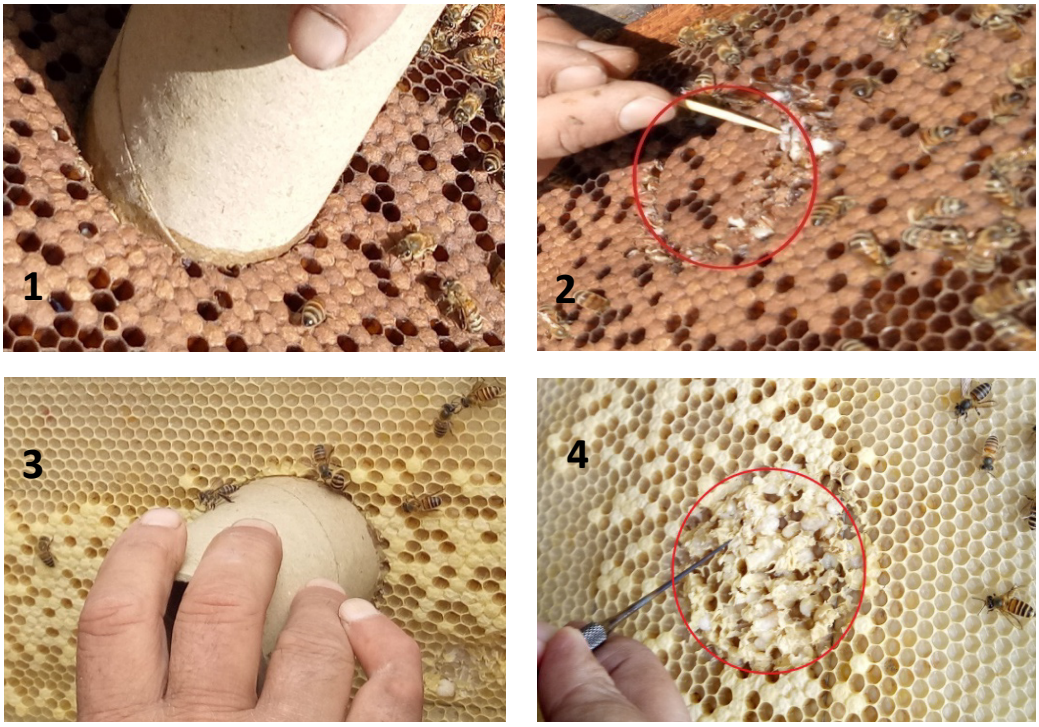

Fig B. Pin killing of broods in the selected area 1 and 2 *Apis mellifera*, 3 and 4 *Apis cerana*.

Acknowledgments

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Authors' contributions

Muhammad Shakeel, Hussain Ali and Sajjad Ahamd Planned the experiments, MS and HA conducted the experiments, SA and HA analyzed the data, MS and HS wrote the text. SA Checked the version and corrected the text. HA submitted the paper.

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