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CHALKBROOD TOLERANCE IN THE EGYPTIAN BEES, *Apis mellifera lamarckii* AND THE CARNIOLAN BEES, *A. m. carnica* IN EGYPT

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Abstract: The purpose of the present study is to compare the chalkbrood tolerance of two stocks of honeybee commonly used in Egyptian beekeeping, Egyptian bees, *Apis mellifera lamarckii* Cockerell and Carniolan bees, *A. m. carnica* Pollmann. Chalkbrood infection percentages were measured in both races as indicator to chalkbrood tolerance. Mean baseline chalkbrood infection percentage was determined for each stock, followed by three chalkbrood inoculations, each one week apart. Chalkbrood mummies were counted one week after each inoculation and removed. Results exhibit highly significant differences in chalkbrood tolerance between the two stocks. Egyptian race was the highest tolerant one with an average infection percentage of 0.229% after the three inoculations. Oppositely, Carniolan race was the lowest tolerant with an average infection percentage of 0.853%. Generally, tolerance to chalkbrood does occur in some honeybee stocks can be selectively bred.

Key words: honeybee, *Apis mellifera lamarckii*, *A. m. carnica*, chalkbrood, *Ascosphaera apis*, tolerance

Introduction

Most of the important economic features in beekeeping are the result of the behaviour of the whole colony. Fungi are common saprophytes of bees and combs. Chalkbrood, a highly infectious disease that affects honeybee brood, is caused by ingestion of the heterothallic fungus *Ascosphaera apis* (Maassen ex Claussen) Olive and Spiltoir. The disease is characterized by the presence of mummified larvae (Mehr *et al.*, 1976 and Gilliam and Vandenberg, 1990). Losses to honey production resulting of chalkbrood infection have proved significant and may be high as 10-15% (Kleinschmidt, 1996).

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Frequent food and water-sharing among nestmates contributes in distrbutinn the natural infection of the disease. However, chalkbrood is also stress related and certain predisposing physiological and environmental conditions are required for the development of infection (Health, 1982). Further, it is clear that some stocks are far less affected by *A. apis* than others (Gilliam, 1986). Chalkbrood cannot be effectively treated by the chemotherapeutic or comb sterilize-ation techniques often employed to treated other brood diseases and even if these methods were developed, problems with residues in honey might preclude the use of chemical treatments in the commercial sector (Gilliam, 1990).

Over the past two decades, reports of chalkbrood resistant bee strains have been made. Most of these reports have focused on hygienic behaviour as the cause of resistance (Milne, 1982; Gilliam *et al.*, 1983 and Spivak and Reuter, 1998).

Gilliam *et al.*, (1983) mentioned that, the hygienic behaviour is highest importance in the dynamic of population of the Carniolan bees because it can avoid or hinder the development of brood disease, being considered the primary defense of honeybee against AFB, EFB, chalkbrood and varroa. Honeybee hygienic behaviour is considered as mechanism of tolerance for many diseases. It includes the uncapping of the cells and the removal of dead or damaged brood from the colonies (Palacio *et al.*, 2001).

The purpose of the present study is to compare the tolerance of two stocks of *Apis mellifera* commonly used in Egyptian beekeeping, to the infestation with chalkbrood. First stock was *Apis mellifera lamarckii* Cockerell, is the endomic bee of Egypt and is well adapted to the local conditions and pests of the region. Second stock was a large population of honeybees, *A. m. carnica* Pollmann and is maintained commercially in Egypt.

Materials and Methods

The present work was carried out in the apiary yard at Mousha location, Assiut Governorate, Upper Egypt, throughout March and April, 2008, whereas, most chalkbrood existing during this period in Assiut region, , containing two stocks. First was ten colonies of Egyptian bees, *Apis mellifera lamarckii* Cockerell and second was ten colonies Carniolan bees, *A. m. carnica* Pollmann.

Egyptian bee colonies were collected from mud tube hives at March, 2007, then transferred into modified wooden moveable frame hives. Carniolan bee queens were produced from Al-Dakhla, New Valley at May 2007, then introduced into honeybee colonies.

The tested colonies were inoculated with a chalkbrood inoculation mixture prepared according to Koeing *et al.*, (1987).

Approximately 5 sporulating black mummies were pulverized, suspended in 75 ml. of a 50% sucrose solution, and poured into a 100 ml. handle sprayer. The inoculation mixture was sprayed directly onto the combs. Tested colonies were re-inoculated at one week intervals. In addition to counting of the mummies in the brood cells, the hive debris was collected and the fallen mummies were counted on the bottom board by placing a strong white paper on the hive floor. A wooden and wired (3 mm mesh) frame on the top of the paper prevented bees for coming in contact with debris. Three inoculations were performed, and a final mummy count was performed one week after each inoculation. During the final mummy count, the total capped workers brood area was determined using a standard frame divided into square inches. Brood area was then converted to total numbers of a capped workers brood cells. It was (brood area in sq. inch. × 33) in case of Egyptian bees while, concerning Carniolan bees the total numbers of capped workers brood cells was (brood area in sq. inch. × 26) (Abdel-Rahman, 2004). The infection percentage of chalkbrood was determined according to Fassbinder-Orth and Rinderer (2005).

% chalkbrood infection = the number of mummies ÷ capped brood cells

Means and standard deviation are given. The infection percentages of the two stocks were tested for differences using T-Test ($p < 0.01$).

Results and Discussion

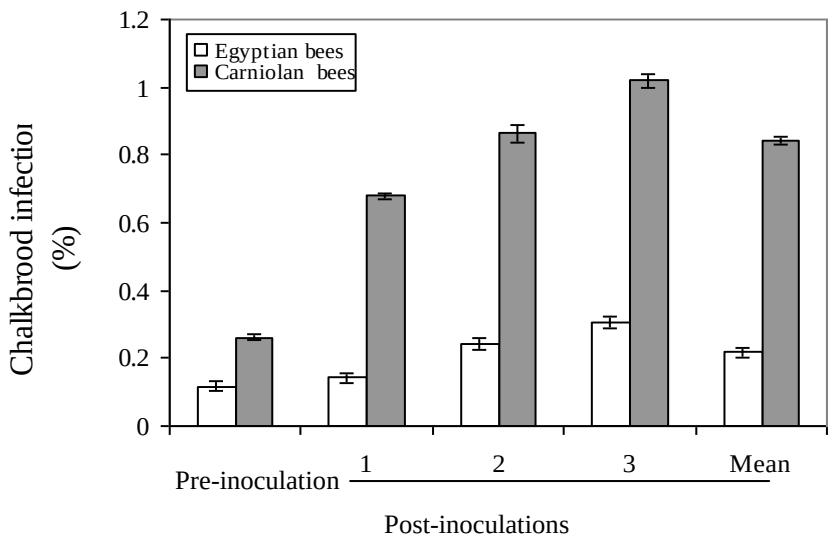
Data illustrated in Table 1 and Figure 1 indicated that, there were highly significant differences between the mean percentages of chalkbrood infection pre-inoculation ($p<0.01$). Mean percentages of chalkbrood infection pre-inoculation 0.118% ± 0.014 and 0.263% ± 0.008 were found in Egyptian bees and Carniolan bees, respectively.

Table(1): Mean percentages of chalkbrood infection pre-and post inoculation.

Infection \ Strains		Egyptian bees±SD	Carniolan bees±SD	T. Value	
				Tab.	Cal.
Pre-infection		0.118%±0.014	0.263%±0.008	2.878	8.4382
Post-Inoculation	First Inoculation	0.142%±0.015	0.678%±0.011	2.878	13.0312
	Second inoculation	0.24% ±0.017	0.863%±0.028	2.878	10.8493
	Third inoculation	0.305%±0.017	1.019%±0.019	2.878	16.5245
	Total	0.687%	2.560%		
	Mean	0.229%	0.853%	2.878	18.5547

All inoculations have the same trend of highly significance of mean percentages of chalk-brood infection of post-inoculation ($p<0.01$).

After the first inoculation, the mean percentages of infection in Egyptian and Carniolan bees were 0.142% ± 0.015 and 0.678% ± 0.011, respectively. The mean percentages of chalkbrood infection after the second inoculation were 0.240% ± 0.017 and 0.863% ± 0.028 in Egyptian and Carniolan bees, respectively. After the third inoculation, the mean percentages of infection in Egyptian and Carniolan bees were 0.305% ± 0.017 and 1.019% ± 0.019, respectively.



Fig(1): Mean percentages of chalkbrood infection pre- and post-inoculations vertical bars denote SD for bee strains.

The mean percentages of chalkbrood infection after the three inoculations 0.229% ± 0.014 and 0.853% ± 0.011 were recorded in Egyptian bees and Carniolan bees, respectively.

Obtained results can be explained as hygienic behaviour in Egyptian bee colonies is higher than those in Carniolan colonies. Kamel *et al.*,(2003) and Abdel-Rahman (2004) found that the Egyptian colonies have a higher significant level of hygienic behaviour than the Carniolan colonies. The complex of behaviours that result in hygienic bees has been implicat-

ed in resistance to various bee diseases including American foulbrood, chalkbrood and the parasitic mite, *Varroa destructor* (Spivak &Gilliam, 1998a,b and Boecking & Spivak, 1999).

Good hygienic behaviour inhibits the survival of the fungus *A. apis* (Gilliam *et al.* 1983) and is correlated with resistance to chalkbrood (Palacio *et al*., 2000). Rothenbuhler, (1964) mentioned that the resistant colony removed the dead brood completely, while the susceptible colony allowed some damaged brood to remain in the cells.

This discussion is in consistent with those of Schmid-Hempel (1998) and Glinski & Buczek (2003) who stated that the protection of the bee colony to fungi was realized by hygienic behavior.

Obtained results also can be explained as, Egyptian bees renew combs continually and secrete wax more than those in Carniolan bees. Abdel-Rahman, (2004) recorded that Egyptian race was more active in wax secretion than the Carniolan race. This explanation agrees with Nelson & Gochnauer (1982) and Koeing *et al.*, (1986) who found that chalkbrood infestations were several times greater in hives with old comb than those with new comb. They presumed that this was due to old comb serving as a reservoir for the disease organism. Bailey and Ball (1991) mentioned that old combs have increased disease problems due to accumulations of microorganisms, such as fungi, bacteria, protozoa and viruses.

The honeybee immune system depends on two main categories of defense reactions: the cell-mediated responses such as phagocytosis and encapsulation of foreign objects and cell-free defense mechanisms represented by the antimicrobial immune proteins. Phagocytosis and encapsulation are the most common mechanisms in bees against entomopathogenic fungi (Glinski & Buczek, 2003).

Thus, it may be that some characteristics that have a strong influence on conferring tolerance towards the fungus in some bee races may not have a high influence in others.

It is critical to determine which and what extent different characteristics and mechanisms confer tolerance to honeybee colonies toward *Ascosphaera apis* to facilitate the development of successful breeding programs for fungus tolerant honeybees. However, to accomplish this goal, studies are needed to develop direct and reliable techniques for measuring presumed tolerance characteristics. Therefore, additional studies are necessary to confirm the actual contribution and importance of different bee characteristics in

different environments with different races of honeybees.

Finally, it can be concluded that significant differences in chalkbrood tolerance were found between Egyptian and Carniolan stocks. Tolerance to chalkbrood that occurs in some stocks can be selectively bred.

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تحمل الإصابة بمرض الحضنة الطباشيرية في النحل المصري والنحل الكرنيولي في مصر

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الهدف من هذه الدراسة هو المقارنة بين تحمل الإصابة بالحضنة الطباشيرية في سلالتين من نحل العسل يستخدمان بصفة شائعة في تربية النحل في مصر وهما النحل المصري والنحل الكرنيولي. تم قياس نسبة الإصابة بالحضنة الطباشيرية في كلا السلالتين كمدلول لتحمل الإصابة. وتم تقدير متوسط نسب الإصابة في بداية التجربة في كل سلالة وعقب ذلك تم إحداث عدوى صناعية بالحضنة الطباشيرية ثلاثة مرات وذلك مرة كل أسبوع. وتم عد مومياوات الحضنة الطباشيرية بعد أسبوع من كل عدوى ثم إزالتها. ولقد أظهرت النتائج أن هناك اختلافا معنويا جدا بين السلالتين في تحملهما للإصابة بالحضنة الطباشيرية. حيث كانت السلالة المصرية هي الأعلى تحملا وكان متوسط نسبة الإصابة بعد الثلاثة عدوات 0.229% بينما وجد النقيض في السلالة الكرنيولي التي كانت الأقل تحملا حيث كان متوسط الإصابة 0.853%. وعموما يمكننا القول بأن ظهور صفة التحمل للإصابة بالحضنة الطباشيرية في بعض سلالات نحل العسل يمكن الإستفادة منه في الانتخاب وبرامج التربية.