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Impact of Bee Venom Collector Equipment Positions on The Productivity of Venom Extracted from Carniolan, Buckfast, and Italian Honeybee Hybrids in Upper Egypt

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ABSTRACT

The honeybee venom productivity was evaluated between three honeybee hybrids i.e. Carniolan (*Apis mellifera carnica*), Buckfast (*Apis mellifera buckfast*) and Italian (*Apis mellifera ligustica*) during 2022 summer season in Assiut area, Upper Egypt. Bee venom was extracted from colonies using bee venom collecting equipment with an electronic impulse generator for colony stimulation. The efficiency of three positions of venom collector trap namely, front drawer venom trap (FDVT), bottom brood nest venom trap (BBNVT) and above brood nest venom traps (ABNVT) were evaluated. The Carniolan bees showed a non-significant amount of produced venom compared with Buckfast. However, it showed a highly significant venom amount when compared with the Italian hybrid. The obtained data indicated that in the case of using ABNVT, a significantly high amount of bee venom was collected (2.30, 2.00, 1.78 g/colony/day) followed by FDVT with, (1.97, 1.88, 1.52 g/colony/day) while BBNVT, enables bees to collect the lowest amount of venom (1.03. 0.95, 0.64 g/colony) by Carniolan, Buckfast and Italian honeybee colonies, respectively. The highest amount of bee venom collected during summer season was recorded between 10-20 July and followed by 17-31 August. However, the lowest amount of bee venom collected by the traps was recorded during the first two weeks of September for all tested honeybee hybrids. In conclusion, reliance on Carniolan and Buckfast hybrid bees may meet the requirements of beekeepers to produce satisfied quantities of bee venom with a distinct preference for using pollen traps above the brood nest to enhance venom production.

INTRODUCTION

Due to its widespread medicinal applications and other commercial products, bee venom is one of the sources of economic gain for beekeepers (Kim, 2021; von Reumont *et al*., 2022 and Ullah *et al*., 2023). The venom glands of honeybees actively secrete a large amount of venom, which helps with colony defense and social immunity (Haggag *et al*., 2015; Omar, 2020 and Seyam *et al*., 2022). On the other side, bee venom causes painful toxic effects for humans and also has many beneficial biological ones e.g.: anti-inflammatory (Orsolic, 2012), anticoagulant (Zolfagharian *et al*., 2015), increase blood circulation (Abdela

& Jilo, 2016), lower cholesterol levels (Zahran *et al*., 2021), anti-rheumatic (Yousefpoor *et al*., 2022), anti-bacterial (Soltan-Alinejad *et al*., 2022) and immunosuppressive (Rosiek-Biegus *et al*., 2022).

Many factors affect the optimal quality and quantity of bee venoms such as the season of collection (Sanad & Mohanny, 2013), honeybee races (Haggag *et al*., 2015), purity and storage conditions (Danneels *et al*., 2015), feeding supply (Nowar 2016), method of collection (Omar, 2020) and age of bees (von Reumont *et al*., 2022). The effectiveness of producing bee venom as well as the quality of this production was studied in relation to the hybrids (Carniolian, Italian, Egyptian and Caucasian) that are most prevalent in Egypt (El-Shaarawy *et al.* 2007; Haggag *et al*., 2015; El-Bahnasy *et al*., 2017 and Zidan *et al*., 2018). Moreover, in Egypt, Khodairy and Omar (2003) studied the correlation between the characteristics of honeybee colonies and the bee venom produced by electrical impulses i.e. (bee population, brood, stored pollen, stored honey areas in addition to foraging activity). They measured the change in the amount of venom collected from colonies at distinct periods of the summer season and recorded significant variations in the quantity of venom collected throughout the active season. In the various geographic regions of Egypt, numerous researchers have documented that the highest venom production was recorded in the summer season (Sanad & Mohanny, 2013; Haggag *et al*., 2015 and Zidan *et al*., 2018).

Researchers have differing opinions about Italian and Carniolan honeybees in which hybrids should hold the top ranking in the production of bee venom (Haggag *et al*., 2015 and Hussein *et al*., 2019). Recently, attention was drawn to the Egyptian bee not only with respect to the productivity of the venom but also to its characteristics (Darwish *et al*. 2021). The Buckfast bee (*A. m. buckfast*) is considered one of the promising honeybee hybrids introduced to Egypt in the last decade, which known for being suited to cool, wet climates; overwintering well; good honey producers and have a low tendency to swarm (Olszewski *et al*., 2012; Ivancia *et al*., 2020 and Nimprom *et al*., 2022). However, few studies have been conducted on Buckfast bees locally in Egypt (Ghanim *et al*., 2021) and the ability of this hybrid to produce bee venom is still not well known.

Therefore, the objectives of this study are to evaluate the venom productivity of three honeybee hybrids (Carniolan, Buckfast, and Italian) in the summer season. Also, to assess the efficiency of three positions of bee venom collector traps on the yield quantity of bee venom in the Assiut region, Upper Egypt.

MATERIALS AND METHODS

Evaluating the Efficiency of Three Honeybee Hybrids on Venom Production:

The experiments were conducted in the summer season, from med June 2022 to med September 2022 in a private apiary at Bany-Ady, Assiut governorate, Assiut, Egypt (Elev. 250 ft., 82.20°N and 57.19°E). The experiment was carried out over 13 consecutive weeks. Three hybrids of Carniolan (*A. m. carnica*), Buckfast (*A. m. buckfast*) and Italian (*A. m. ligustica*) honeybees were used in the experiments.

Honeybee Colonies:

Forty-five colonies were used for this study, and fifteen colonies of each hybrid headed with sister queens were used in the study. The colonies were divided into 3 groups (5 colonies per group). Colonies were of similar strength at the start of the experiment. Each experimental colony had eight standard Langstroth frames.

Bee Venom Collection:

- Bee Venom Collector Device (Installation and specification):

Bee venom was extracted from colonies using bee venom-collecting equipment. Seven extractions were weekly applied for venom collection. An electronic impulses generator developed by Omar (1994) was used for colony stimulation (Electric shock device, VC-Starter kit) (Plate 1). Characters of the bee venom device are: - Input Voltage:13.5-16.5VDC - Frequency 50 HZ - Timer ON:0.5 - 2 sec.- Timer-OF:3 -5 sec. - Collector Frames: 40cm x50 cm - Operation Mode: semiautomatic - Temperature: -5 C˚ to 40 C˚ - Humidity (max): 95% at 40 C˚ - Max operating time: 8 hours. The venom collection boards were collected and the glass plate boards were held for 24 hours at room temperature for drying after the 30 minutes of venom extraction (Plate 2a). By scraping the glass plate's surface (Plate 2b), the total quantities of dry venom were preserved in Eppendorf tubes and calculated (Plate 2c). Each glass board's production of dry venom was individually weighed using an electrical balance.

Plate 1: Bee venom collecting equipment (electronic impulses generator).

Plate 2: a- venom collection boards, **b-** scraping the glass plate's surface and **c-** Preservation, storage and weighing of bee venom.

- Evaluating the Effectiveness of Three Venom Collector Positions on Venom Production:

The impact of the bee venom collector position on the quantities of produced venom (g./colony) for the three tested honeybee hybrids Carniolan, Buckfast and Italian bees was tested. Fourteen extractions (one extraction weekly) of venom were taken during the study period from mid-Jun till mid-September for each honeybee hybrid. Forty-five colonies were used for this study, fifteen colonies of each hybrid divided into 3 groups, five colonies for every group as follows:

- **Front Drawer Venom Traps (FDVT):** the venom collection boards located at the hive entrance (Plate 3a).
- **Bottom Brood Nest Venom Traps (BBNVT):** the venom collection board located at the lateral position and under the brood frames (Plate 3b).
- **Above Brood Nest Venom Traps (ABNVT):** the venom collection board located over the colony frames under the outer cover of the hive (Plate 3c).

Plate 3: Different bee venom collector positions: **a-** front drawer venom traps; **b-** bottom brood nest and **c-** above the brood nest.

Statistical Analysis:

All data were subjected to statistical analysis. The level of P < 0.05 was used as a criterion of statistical significance and subjected to the analysis of variance (ANOVA) at a 5% level of significance, under a complete randomized design. An unpaired T-test was used to compare the obtained results of each honeybee hybrid under different venom collection positions at a 5% level of probability (Student, 1908). Data were depicted as means ± standard deviation to show the data deviation. The statistical analysis was performed using SAS software (SAS Institute 2004).

RESULTS AND DISCUSSION

Evaluating the Efficiency of Three Honeybee Hybrids on Venom Production:

The fluctuation of bee venom production quantities (g./colony/day) in honeybee colonies of Carniolan, Buckfast and Italian hybrid in Assiut region during summer season of 2022 is presented in Figure 1. The Carniolan honeybee colonies showed superiority in venom production in comparison with Buckfast and Italian bees. The differences between the two hybrids, Carniolan and Buckfast considered statistically non-significant during the study period (P > 0.05). In contrast, the venom production from Italian honeybee colonies was significantly lower than the rest of the hybrids (P > 0.05). The three tested honeybee hybrids showed the same trend of bee venom production during the study periods (Fig. 1). Regardless of the tested honeybee hybrid, two peaks of bee venom production were observed throughout the entire study period. The first one was recorded on July 13, however, the second was recorded on August 31. It is important to note that the first peak revealed highly significant values than the last one (P > 0.05).

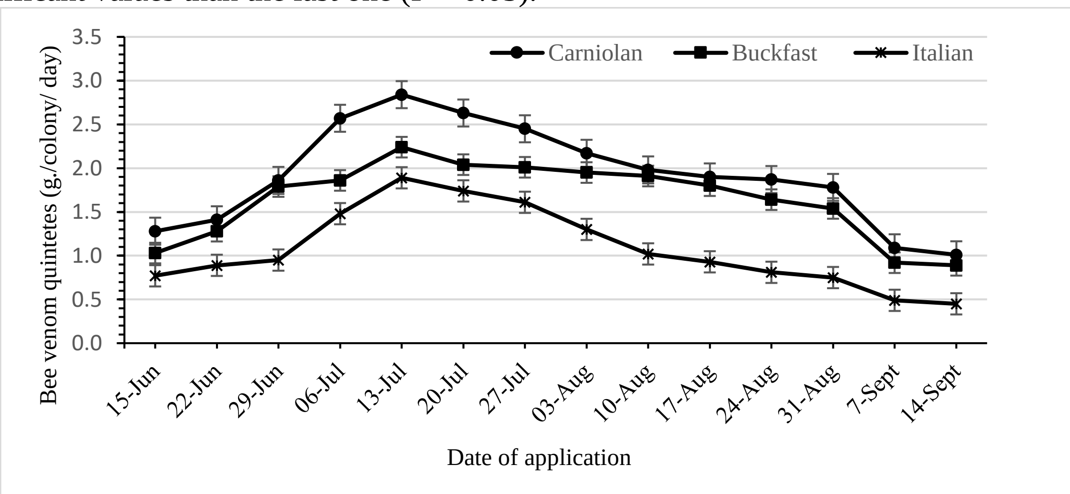

Fig. 1: Fluctuation of bee venom production quantities (g./colony/day) in honeybee colonies of Carniolan, Buckfast and Italian hybrid in Assiut region during summer season of 2022.

The majority of researchers emphasized the honeybee strain/hybrid, season, and collecting method as important variables impacting venom production both in quantity and quality. Our findings were in line with Omar (2017), who reported that both Carniolan and Italian hybrid honeybees produced the most bee venom in August, with 0.45 and 0.44 mg/colony, respectively in Benha governorate, Egypt. While the lowest monthly average of bee venom was recorded in February. Mohanny (2005) recorded the general mean of bee venom collected during the year by *A. m. caucasica* was 2.77 mg./colony, while *A. m. carnica* collected 2.86 mg./colony, and statistically no appreciable difference in the amounts of bee venom produced by the races. In addition, El-Shaarawy *et al*. (2007) recorded that the Egyptian race and Carniolan hybrid produced the highest venom quantities among other tested bee races during spring, summer and winter seasons by using an electrical shock device in El-Kalubia Governorate, Egypt.

Our statistical analysis (t-test) of the obtained data showed significant variations between produced venom quantities at different extraction times during the study period ($p > 0.05$). Like all living organisms, honeybees have distinct dietary needs. Natural resources like pollen and nectar provide the essential nutrients and vitamins that the body needs (Amro *et al*., 2020). However, Protein plays a major role in honeybee venom production which will be reduced when protein availability is inadequate (Nowar, 2016). Consequently, information on the dietary needs of honeybees and available pollen-forage plants in our research region must be readily accessible. Honeybees are the most efficient pollinator for enhancing most crop yields in Assiut region, Upper Egypt (Amro, 2021). The main pollen flow seasons in Assiut are citrus (*Citrus* Spp.) during March and April, Egyptian clover (*Trifolium alexnadrinum*) during April to June, and cotton (*Gossypium* Spp) during July and August (Hussein, 1982). In addition, two secondary pollen flow seasons: faba bean (*Vicia faba*) from January to March, and maize (*Zea mays*) during August and September (Hussein, 1983). Meanwhile, not all of these plant species are sufficiently high in pollen protein for honeybees (Hussein, 1982). This might be the underlying cause of the considerable variation in venom production between the extraction times.

Therefore, the results we have obtained can be interpreted in light of what has been observed by Omar (1989) in the Assiut area, Egypt. He indicated that the total protein content of bee bread collected from honeybee colonies during the active season ranged between 16.88 to 20.97%, and the water content varied from 18.50 to 22.46%. According to the protein fractions isolated from the bee bread, the bread collected in early summer was of better quality than the bread collected in late summer. The optimal total protein content and protein fractions were recorded during May-June, and they decreased significantly in August-September. Also, the results of Badawy *et al*. (2022) supported ours, they found Italian hybrid showed a significant sugar feed intake after bee venom collection than the Carniolan hybrid during the winter season followed by the autumn while summer showed the lowest intake rate.

Evaluation of the Effectiveness of Three Venom Collector Positions on Venom Production:

The impact of the tested bee venom collector position on the quantities of produced venom (g./colony/day) extracted by Carniolan, Buckfast and Italian hybrids in Assiut region during summer season of 2022 were depicted in Figure 2. It's worth mentioning that the vast majority of produced bee venom was extracted with ABNVT in all tested colonies regardless of the honeybee hybrid. Significantly, the ABNVT located above the brood nest was higher than FDVT and BBNVT as a general mean of venom production ($P > 0.05$) with, 6.03, 5.37, 2.62 g./colony/day, respectively all over the study period.

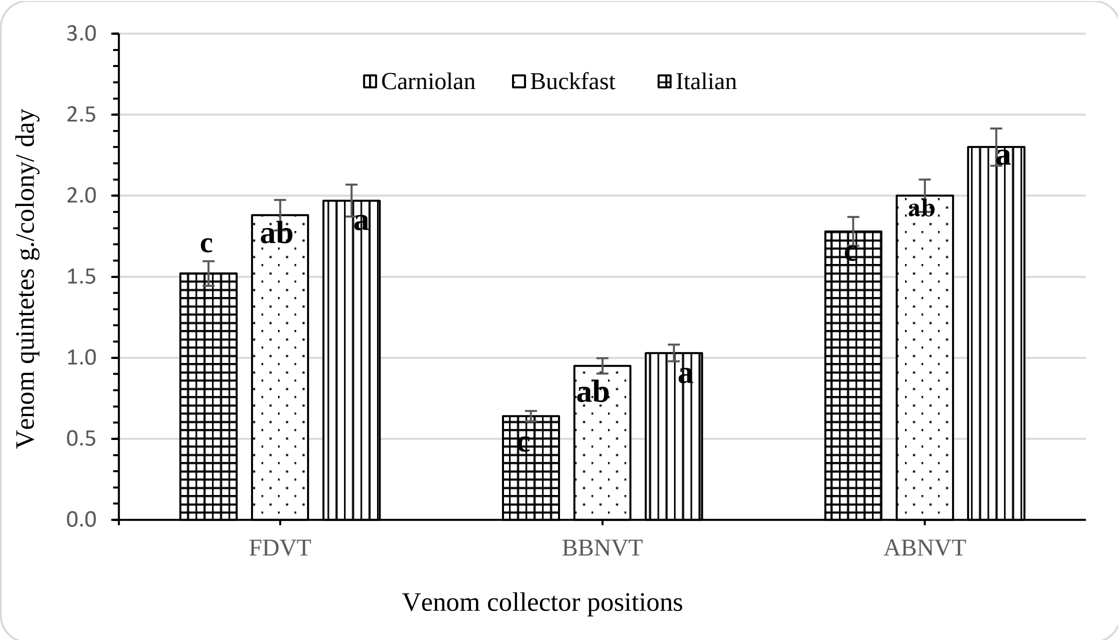

Fig. 2: Impact of the bee venom collector position on the quantities of produced venom (g./colony/day) for the three tested honeybee hybrids Carniolan, Buckfast and Italian in Assiut region during summer season of 2022. (FDVT= front drawer venom traps; BBNVT= bottom brood nest venom traps and ABNVT= above brood nest venom traps).

The early study by Benton *et al*. (1963) highlighted the role of venom collector positions on venom production and they found that using it underneath the brood chamber of honeybee colonies for 5 minutes was not satisfactory to obtain larger quantities of bee venom. Also, Omar *et al*. (1994), El-Saeady *et al*, (2016) and Omar (2020) used venom collectors at the hive entrance to avoid any harmful effect on honeybee activities inside the colony. Gholamian (2007) used two kinds of venom collector apparatuses one used outside and another inside the hive. The rate of venom production by the collector out of the hive was low and much trouble for the user during venom collection. However, the results of Mohanny (2005) indicated that the modified device of gathering bee venom from hives successfully gave adequate quantities of bee venom during the period of the experiment in Qenna – Egypt.

The obtained data revealed significant differences between Carniolan, Buckfast and Italian bees for the three venom trap positions according to the t-test (Figure 2). It can be noted that *A. m. carnica* ranked first in venom production for the ABNVT, FDVT and BBNVT recording 2.30, 1.97 and 1.03 g./colony/day, respectively, followed by *A. m. buckfast* (2.00, 1.88 and 0.95 g./colony/day) and the *A. m. ligustica* (1.78, 1.52 and 0.64 g./colony/day) which ranked third. The differences in venom production between *A. m. carnica* and *A. m. buckfast* are considered statistically non-significant in ABNVT, FDVT and BBNVT ($P > 0.05$). In contrast, the venom production from *A. m. ligustica* was significantly lower than the rest of the hybrids ($P > 0.05$) in all venom trap positions.

Several investigators highlighted the correlation between honeybee strain/hybrid and aggressive behavior which consider the key factor responsible for venom productivity e.g. (Roat *et al*., 2006; El-Bahnasy *et al*., 2017; Pucca *et al*., 2019 and Omar, 2020)**.** Our results were harmonious with Omar, (2011), who reported that venom production had a negative correlation with the colony's aggressive behavior because the honeybee workers' stingers were unstable on the collection boards over the colony frames, which produced 93.22% mg/colony and a significant increase in dry bee venom (37.31%) when compared to their position at the hive entrance. This might be the underlying cause of the considerable variation in venom production recorded with *A. m. buckfast* which are well-known as calm honeybees and stable in the hive (Olszewski *et al*., 2012). Various honeybee races have different temperaments, defense strategies, and stimulant preferences (Hussein *et al*., 2019). Also, the aggressiveness of defense behavior may be affected by weather, time of year, bee health and factors affecting foraging activity (Omar, 2020). In addition, the adult worker bee's venom productivity rises throughout the first two weeks of life and peaks when it begins to participate in hive defenses and foraging (Roat *et al*., 2006).

CONCLUSION:

Enhancing the efficiency of Carniolan, Buckfast and Italian honeybee hybrids to produce bee venom by comparing three positions of venom collector equipment was our goal. Carniolan honeybees showed superiority in venom production. Also, Buckfast is a promising hybrid gave reasonable quantities of bee venom. The venom collector position above the brood nest was the optimal position for producing satisfied quantities of bee venom in all tested hybrids and recommended methods for enhancing venom production. findings of the present study can be helpful to obtain higher bee venom production in Assiut region, Upper Egypt.

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