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Effect of Honey Bee Hybrid, Season and Position of Collecting Plate inside the Hive on the Quantity of Harvested Dry Venom in Egypt

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ABSTRACT

This research was conducted to evaluate the biological effect (bees hybrids), environmental (collection season) and technical method (location of collecting plate within the hive) on the amounts of harvested dry venom using electrical chock method in Giza, Egypt. Ninety-six honeybee colonies in homogenous strength were selected from Carniolan and Italian hybrids (48 colonies each). Each hybrid group was equally divided into six subgroups, which were distributed from March to August. The venom was collected only once from each subgroup. The results showed that the upper frame position of collecting plate was the best with significant amounts of venom (29.4 mg/col.), higher than putting the plate beneath the frames (14.6 mg/col.). The colonies of Carniolan hybrid produced significant quantity of dry venom (26.5 mg /col.) compared with Italian hybrids (17.6 mg /col.). Also, the mean amount of harvested venom during spring season was significantly higher (23.1 mg/col.) than that produced during summer season (20.8 mg/col.). A pronounced quantity of dry venom could be monthly produce throughout the period from March to August, ranging from 21.4 to 34.1 mg/col., with the upper frames collecting plate.

Key words: *Apis mellifera*; hybrid; harvested; venom; Carniolan bees, Italian bees.

1. INTRODUCTION

Benton *et al.* (1963) made a device that provides an electric shock which makes it possible to collect pure venom from several thousands honey bees (*Apis mellifera*). The collection apparatus fits underneath the brood chamber of a colony of bees and could be moved between hives. Each colony is "milked" for 5 minutes. An average of 20 hives must be "milked" to obtain 1 gram of venom. This quantity of venom is produced by 10,000 worker bees, under optimum conditions. Maschwitz (1964) stated that races of honeybee differ in their temper defense behavior and the type of

stimulant, whereby once the sting is deposited, alarm pheromones are suddenly liberated from glands associated with their sting apparatus and their mandibles. Eslam (2020) reported that the defensive response depended on the colony strength and time of collection during the season. When the number of stings per colony was determined, the stings number was related to the number of frames covered with bees. Bee venom amount extracted weakly significantly differed during the summer months (July and August). The variability of extracted venom amounts, indicated that the main peak of production was recorded during July. Negative correlation

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coefficients were recorded between defensive response and collected bee venom from three strength levels from honey bee colonies. Benton (1965) described a device for chocking bees electrically at a standard hive, making it possible to collect venom from several thousand honeybees. The device consists of wooden frame with copper or steel wires stretched across it at 3.18 mm. intervals. Alternate wires carry an electric charge. The circuit is closed when a bee comes into contact with two adjacent wires. The bees sting through a piece of nylon sheet which is stretched over a glass plate and is fitted under the wires. The collecting apparatus is placed underneath the brood chamber of the bee colony and may move from hive to hive. Each colony is "milked" for 5 min. using a 12V wet cell battery in conjunction with a converter (12V dc to 115V ac). An electric timer is used to break the circuit for 4 sec. at 3 sec. intervals. An average of 20 bee hives must be milked to obtain 1 gm. venom. Benton and Morse (1966) reported that venom from other *Apis* species is similar, but the venoms from various races within each species were slightly different. The toxicity of *Apis cerana* venom was reported to be twice as high as that of *A. mellifera*. Miao (1983) reported a new apparatus that passes alternating impulses along an electric wire placed at the entrance of the hive so that the workers touch it when entering or leaving thus, the electric shock (20-40 volts) causes a bee to eject venom. There were differences in the presence of 5-hydroxytryptamine in honeybee venom collected by electrical milking from bee workers, which was determined by liquid chromatography with electrochemical detection. In the venom gland and reservoir of newly emerged bees, no 5-hydroxytryptamine was present, but at 5, 10, 20 and 40 days old, the content of venom system was 2.4, 8.7, 22.1 and 37.2 mg, respectively. The content of 5-hydroxytryptamine was much higher in 10-day-old workers (173-176 mg).

2. MATERIALS AND METHODS

To study the effect of electric collecting plate position inside the colony on the quantity of dried venom (in mg) gathered from Carniolan and Italian hybrid bees during spring and summer of 2023. Ninety-six honey bee colonies at same strength were used in this experiment and two hybrid bees, Carniolan and Italian bees, were divided into two groups:

1. Forty-eight colonies for Carniolan bees

2. Forty-eight colonies of Italian bees, each was divided into six groups over six months of spring and summer. Each group of them contained eight colonies, which was divided to two groups:

1. Four colonies used to collect venom from upper of the frames

2. Four colonies used to collect venom from beneath of the frames.

3. RESULTS AND DISCUSSION

3.1. Determining the best position inside the colony to collect venom, and the best hybrid bee for venom production.

Data in Table (1) show that the best position of plate to collect venom from Carniolan and Italian hybrid bee's colony was the upper frame position, which was higher than that from under frame position with significant differences, where 29.4 and 14.6 mg /colony for the two positions, respectively. Regarding Carniolan hybrid, in successive spring and summer seasons, there was a small non-significant difference in collected venom amount (27.0 ± 2.7 and 25.9 ± 4.9 mg /colony, respectively), where the general mean was 26.5 ± 2.5 mg /colony. On the contrary, in the same hybrid and same seasons, upper frames position had a significant difference from beneath frame position where it was 36.4 and 16.4 mg /colony, respectively. While, for the Italian hybrid in successive spring and summer seasons, there was a significant difference in collected venom amount (19.4 ± 1.1 and 15.8 ± 2.0 mg /colony, respectively). The general mean was 17.6 ± 1.3 mg /colony. In addition, in same Italian hybrid and same seasons, upper frames position had a significant difference from beneath frame position; it was 22.2 and 12.7 mg /colony respectively as shown in Table (10 and Fig. (1).

Moreover, in Italian hybrid there was a significant difference between the collected venom amounts in the same successive seasons, being 19.4 ± 1.1 and 15.8 ± 2.0 mg/colony, respectively. In general, there was a clear significant difference in collected venom amounts, with mean \pm SE of 23.1 ± 2.1 and 20.8 ± 5.2 mg /colony in successive season's spring and summer, respectively.

At any case, when removing the outer cover of honeybee colony, the workers move to the upper side to defend the colony,

Table (1): Effect of the position of electric collecting plate inside the colony on the quantity of dried venom (mg) gathered from Carniolan and Italian hybrid bees during spring and summer of 2023.

Race Month and Season	Carniolan bees			Italian bees			Upper frames	Beneath frames	Mean ±SE /Month
	Upper frames	Beneath frames	Mean ±SE	Upper frames	Beneath frames	Mean ±SE			
March	30.1	13.3	21.7	21.3	11.3	17.3	25.7	12.3	19.0
April	41.4	19.8	30.6	26.7	15.3	21.0	34.1	17.6	25.8
May	39.3	18.2	28.8	25.8	14.1	20.0	32.6	16.2	24.4
Mean/spring	36.9	17.1	27.0±2.7a	24.6	13.6	19.4±1.1b	30.8	15.4	23.1±2.1a
June	52.7	18.5	35.6	25.1	14.5	19.8	38.9	16.5	27.7
July	29.5	15.6	22.6	17.3	11.4	14.4	23.4	13.5	18.5
August	25.5	13.2	19.4	17.2	9.3	13.3	21.4	11.3	16.3
Mean/summer	35.9	15.8	25.9±4.9a	19.9	11.7	15.8±2.0c	27.9	13.8	20.8±5.2b
Mean/race	36.4 A	16.4 B	26.5±2.5A	22.2 A*	12.7 B*	17.6±1.3 B	29.4A	14.6B	21.9±1.883

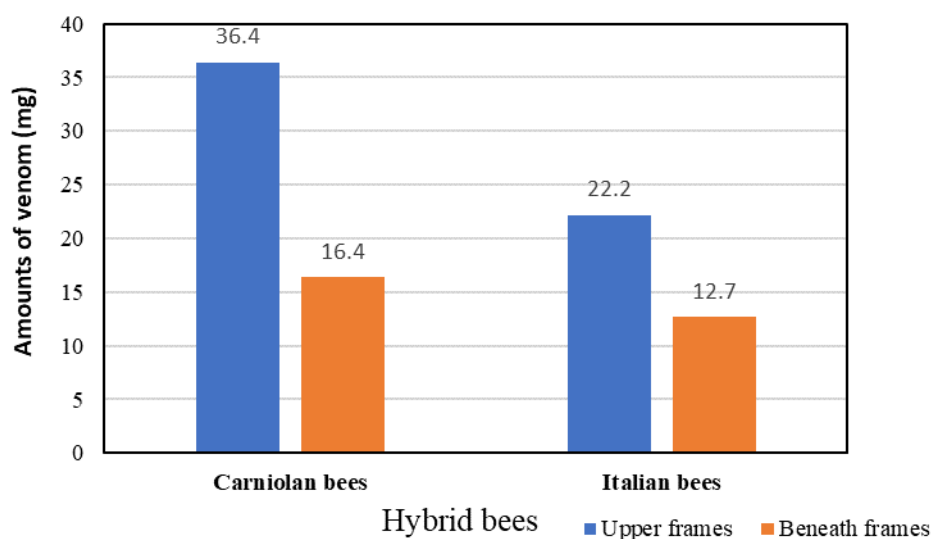


Fig. (1): Effect of electric collecting plate position inside the colony on the quantity of dried venom (mg) collected from Carniolan and Italian bees during spring and summer of 2023.

which allows a large number of workers to collect venom. This is in contrast to the case when we collect venom from the bottom side. Moreover, comparing the two hybrids under investigation, the Italian hybrid produces less amount of venom because of its higher aggressiveness than Carniolan. Finally, there was a rapid increase in colony activities in the spring season, reflected in honeybee colony young workers population and glands growth especially venom glands. Thus, in spring season we can collect more amount of venom. These findings agreed with those reported by Benton *et al.* (1963) who made a device that provides an electric shock which makes it possible to collect pure venom from several thousand honey bees (*Apis mellifera*).

The collection apparatus fits underneath the brood chamber of a colony of bees and may be moved from hive to hive. Each colony is "milked" for 5 minutes. An average of 20 hives must be "milked" to obtain (1gram) of venom. Under optimum conditions this quantity of venom is produced by 10,000 bee workers. This group also reported that they had to dry the nylon sheet for a few minutes before inserting it into the next hive. If this was not done, the bees would become extremely irritable and the operator might suffer several stings. In addition, Badawy *et al.* (2022) found that the Italian

hybrid showed significant sugar feed intake after bee venom collection than the Carniolan hybrid during winter season, followed by the autumn while it showed the lowest intake rate. The high hording behavior rate in winter may be due to the severe weakness of the colonies and their severe need for the sugary food in such period due to the lack of flowering crops at this time of the year. Furthermore, Benton (1965) described a device for chocking bees electrically at a standard hive, making it possible to collect venom from several thousand honeybees. The device consists of wooden frame with copper or steel wires stretched across it at 3.18 mm. intervals. Alternate wires carry an electric charge. The circuit is loosed when a bee comes into contact with two adjacent wires. The bees sting through a piece of nylon sheet, which is stretched over a glass plate and is fitted under the wires. The collecting apparatus is placed underneath the brood chamber of the bee colony and may move from hive to hive. Each colony is "milked" for 5 min. using a 12V wet cell battery in conjunction with a converter (12V dc to 115V ac). An electrical timer is used to break the circuit for 4 sec. at 3 sec. intervals.

An average of 20 bee hives must be milked in to obtain 1 gm. of venom. Furthermore, Omar and Shorate (1991) stated that the longest sting acid glands were recorded in the Carniolan

workers and the shorter in the Egyptian ones, in each hive there was a low percentage of bees whose acid glands do not split. The percentage of unsplit acid glands were significantly higher in the Egyptian workers than in the Carniolan. No correlation was found between acid glands length and the size of venom sac in both split and unsplit. In addition, Skubida *et al.* (1995) compared the amount of venom collected when venom-collecting frames were inserted (1) in the lower hive body, or (2) in the upper hive body, or (3) in an empty body placed between the upper and lower bodies. A fourth technique involved a super with a fixed set of 6 venom collecting frames (incorporating removable glass plates for scraping off the venom). Venom collection had no adverse effects on colony strength, brood rearing and productivity of honey, pollen and beeswax. However, it affected winter performance, with colonies in group (3) most affected, and those in group (1) least affected. Overall, colonies in group (2) gave the best results for total colony productivity. Also, Simics (1995) described a modern frame, which was placed on top of the frames in a hive. When collectors were put in each hive, 20 - 40 hives are connected together and the electric impulses are passed through for 30 min. The venom, scraped from this device in dried form, and uncontaminated. He added that the colonies were relatively unaffected by the procedure. Furthermore, Omar (2011) used three positions for venom collection boards attachment to honey bee hives (at hive entrance, beside the hive frames, over frames top), the venom extraction achieved the highest amount (93.22 mg /colony) when the venom collection board was attached over the colony frames. The venom quantity increased significantly with 37.41% when compared by the position of boards at the hive entrance, Also Hussein (2013) stated that three positions were applied for venom collection board attachment to honey bee hives (Under frames down, over frames and Hive entrance) during two seasons 2011-2012. The venom extraction achieved the highest amount (77.26 mg dry venom) during 2012 when the venom collection board was attached over the colony's frames. The venom quantity increased significantly with (+50.19%) when compared by the position of boards at the hive entrance. But, the Less productive of bee venom was (38.33 mg dry venom) during 2011 when the venom collection board was near of hive entrance. In this manner Kolekole *et al.* (2021) stated that

statistically significant differences in the amounts of analyzed components were not dependent on harvesting time, collection site on the beehives or season. On the other hand, region samples significantly differed in the amounts of all three components, ranging from 1.28% to 3.81% for apamin, 19.51-64.03% for melittin and 7.22%-28.18% for PLA2. However, beekeepers' improper practices during harvesting and storing might be the most critical parameters that determine the quality of HBV.

Authors' contributions

All authors contributed in conceptualization, methodology, software, validation, formal analysis investigation, resources, data curtain, writing the original draft preparation, writing, review, editing, supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Competing interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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تأثير هجين نحل العسل والموسم ومكان وضع لوح جمع السم داخل الطائفة علي كمية السم الجاف المجموع في مصر

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ملخص

تم إجراء هذه التجربة لتقييم التأثير البيولوجي (نوع هجين النحل) ، والتأثير البيئي (موسم الجمع) والتأثير التقني (مكان وضع لوح الجمع داخل الطائفة) على كميات السم الجاف التي يتم جمعها باستخدام طريقة الصدمة الكهربائية وذلك في منطقة الجيزة، مصر. تم استخدام عدد 96 طائفة نحل عسل متساوية القوة من هجين النحل الكرنولي والإيطالي (عدد 48 طائفة لكل هجين)، كل مجموعة من نفس الهجين تم تقسيمها بالتساوي إلى عدد 6 تحت مجموعة على مدار شهر مارس وحتى أغسطس. أوضحت النتائج أن وضع ألواح جمع السم فوق إطارات الطائفة كانت الأفضل وبفارق معنوي في كمية السم المجموع حيث أنتجت 29.4 ملجم/للطائفة عن وضعها تحت الإطارات حيث كانت الكمية المنتجة 14.6 ملجم/للطائفة. سجلت طوائف الهجين الكرنولي إنتاج أعلى وبفارق معنوي عن الهجين الإيطالي حيث كانت وزن الكمية المجموعه 26.5 و 17.6 ملجم /للطائفة على التوالي. أيضا متوسط كميات السم المجموعه خلال موسم الربيع كانت أعلى وبفارق معنوي عن الكميات التي تم جمعها في موسم الصيف حيث كانت 23.1 و 20.8 ملجم/للطائفة على التوالي. يمكن انتاج كميات واعدة من السم الجاف شهريا خلال الفترة من مارس وحتى أغسطس وذلك بمتوسط من 21.4 إلى 34.1 ملجم/للطائفة باستخدام ألواح الجمع الفوقية.

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