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RESEARCH ARTICLE

Influence of natural pollen sources on the morphological parameters of honey bee, *Apis mellifera* L. sting apparatus

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Abstract

Natural pollen feeding induces a wide range of morphological and anatomical changes in honey bees. The worker honey bee is used to determine the effect of the natural feeding of two different types of pollen collected by the honey bee upon the development of the stinger, venom sac length, and bee venom production. This experiment was carried out in 2022 during the period from May to August in two private apiaries, where honey bee colonies are fed naturally on pollen collected from clover plants. In the present experiment, it was assessed how different plant impact the stinger and venom sac length. The results clearly showed significant differences in the stinger and venom sac parameters between the workers fed on clover plants and those fed on clover for the clover plant pollen. The present study showed that bees fed on clover pollen have longer stingers than that fed on corn pollen, also venom sac length (t=5.987; $p < 0.000$), and venom sac width (t=9.205; $p < 0.0001$) for worker bees fed on clover pollen compared to worker bees fed on corn plant pollen, the same for other parameters which showed significant differences in the Stylet length (t=7.216; $p < 0.0001$), and the lancets length (t=7.216; $p < 0.0001$), barbs lancet length (t=9.205; $p < 0.0001$. These results indicated that the development of the stinger, venom sac parameters, and quantity of collected bee venom is extremely sensitive to the type of nutrients.

Keywords: venom sac, stinger, honey bee, bee venom, apiary.

1. Introduction

European honey bee *Apis mellifera* (Hymenoptera: Apidae) is a social insect that lives in well-organized communities and is very important to a significant proportion of the world economy due to the key role they fulfill as pollinators in agriculture. Bees are economically beneficial insects, which have provided several products to humans, such as honey, beeswax, pollen, royal jelly, and propolis. They also pollinate a wide variety of crops. Although bees are extremely beneficial to crops and humans, they do present a danger due to their ability to inflict painful and toxic stings (de Graaf et al., 2021). The defining feature is that the ovipositor has been modified into a sting (Moreau, 2013). The stinger is a small, effective device that assists honey bees in two main physiological activities: defending against enemies and laying eggs for reproduction (Wu *et al.*, 2014). Both queens and workers have a barbed stinger that is torn, with the venom sac, from the end of their abdomen when they deploy the sting into a skinned victim. The sting of *Apis mellifera* consists of a motor apparatus (a pair of quadrate, oblong and triangular plates with two connecting rami) and a piercing instrument (a

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stylet and paired lancet), along with glands (acid and alkaline) associated at the sting base, and lubricating glands of the quadrate plate (Snodgrass, 1956).

The life, growth, and development of honey bees require specific nutritional needs for carbohydrates, proteins, vitamins, fats, and minerals available in natural foods. The development of colonies depends to a large extent on the quantities of nectar and pollen. Pollen represents the main source of protein and fatty acids necessary for the normal and healthy growth of bee bodies. The nutritional value of pollen from different plants varies greatly. Whereas Graham *et al.* (1992) stated that protein content is variable in collected pollen from different sources, ranging from 8 to 40%. The bee's body systems and activity are affected by the type of pollen that it feeds on and the appropriate amount for it. Moreover, pollen protein is critical for the development of honey bee muscles, glands, and other tissues (Dietz *et al.*, 1979). Also, Hosafy *et al.* (2021) reported that feeding honey bees a mixture of pollen diet and a broad bean pollen diet resulted in better results than others in the development of HPG and AG. Honeybees use their sting as a defense to inject approximately 0.1 mg of venom into the victim (Sanad & Mohanny, 2013).

As feeding supply can affect Acidic gland development (Nenchev, 2003), it can also affect stinger and venom sac parameter development and the production of bee venom quantity. The present work aimed to study the nutritional effects of two different natural pollen sources for honeybee (*Apis mellifera*) colonies on the stinger length parameter, venom sac width, and venom sac length.

2. Material and methods

2.1. Honey bee apiary

The present work was conducted in two private apiaries feeding on clover and clover plant located at kafr Elzayat and Qena, Egypt from May to August 2022.

2.2. Sample collection

Five colonies were randomly selected, where are 10 Individuals of *Apis mellifera* worker bees were collected from each hive (N=50). The sting apparatus was gently pulled out. Each sting apparatus was settled on a slide within a drop of physiological solution (sodium chloride 0.9%). The chitinous structures were carefully removed with a fine needle. The stinger and venom sac were separated with a blade. As well as, they were handled gently.

2.3. Measurement of venom sac volume (width and length)

The venom sacs of honeybee workers from the two apiaries were separated using a blade and placed into a droplet of physiological solution (sodium chloride 0.9%), and examined under Olympus szx streomicroscope. The development venom sac length and width were measured using a micrometer eyepiece (Fig. 1). The volume of the venom sac was evaluated by measuring the amount of the venom inside venom sac, The volume of the acid gland sac was calculated by using the following formula (Maurizio, 1954):

$$\text{Acinal surface area } \pi \frac{a * b}{2}$$

Where a = maximum length, b = maximum width, π = 3.14.

2.4. Measurement of stinger length

Stinger length measured in mm for each worker from the two apiaries under a stereomicroscope using a micrometer eyepiece as in Photo Stereoscope microscope provided with an ocular micrometer lens was used for this purpose as shown in Fig. (2).

2.5. Statistical analysis

Statistical analysis was performed with GraphPad Prism 9 software. In addition, residual analysis was conducted to ensure that the data conformed to parametric assumptions. Results are expressed as means ± standard deviation. The effect of different pollen sources on sting length venom sac (length and width), stylet length, lancets length, barbs lancet length, lancet tip length, and diameter lancet at last barb of was evaluated using t-test, the level of significance was determined at 5%.

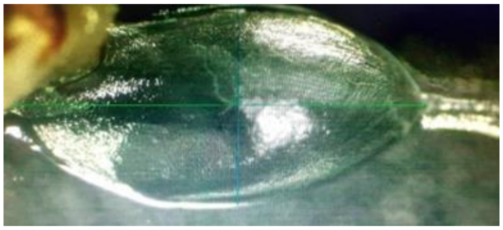


Figure 1. Venom sac of worker honey bee

Figure 2. Stereo microphotographs of a worker honey bee stinger

3. Results and discussion

The current study aimed to determine the effects of different pollen sources specifically, corn and clover plants on the growth and morphology of the stinger apparatus of the European honey bee *A. mellifera* workers, adult worker sting length, venom sac length and width, stylet length, lancets length, barbs lancet length, lancet tip length and diameter lancet at last barb were compared on two natural pollen corn and clover plants.

The development of stinger and venom sac parameters of worker bees fed on different natural pollen plants is shown in Table (1) illustrated with figs. 3and 4. The results clearly showed significant differences in the parameters of stinger and venom sac parameters between the workers in apiary no.1 fed on the clover plant and those fed on the corn Plant, apiary no.2.

Table 1. Effect of two different natural nutrients on stinger parameters of the worker honey bees.

Parameter (mm)	Apiary No.1	Apiary No.2	P. values
Stinger length	2.82 ± 0.16	2.34±0.17	$p < 0.0001$
Venom sac width	0.98± 0.06	0.72±0.07	$p < 0.0000$
Venom sac length	2.07± 0.17	1.06±0.18	$p < 0.0001$
Stylet length	2.50±0.12	2.0±0.18	$p < 0.0001$
Lancet length	1.5±.08	1.2±0.10	$p < 0.0001$
Barbs lancet length	0.67±0.098	0.48±0.08	$p < 0.0001$
Lancet tip length	0.07±0.02	0.04±0.009	$p < 0.0001$
Diameter Lancet at last barb	0.12±0.05	0.08±0.007	$p < 0.0006$)

The findings of this study shed light on the potential influence of pollen sources on the development and morphology of the stinger apparatus in honey bees.

3.1. Stinger length

Differences were found between the two apiaries table (1), in which worker bees fed on clover pollen (Apiary No.1) had Stinger with significant length (t=8.234; $p < 0.0001$) compared to those fed on corn plant pollen (apiary No.2) Fig.3.

The present study showed that bees fed on clover pollen have longer stingers than that fed on corn pollen. This finding suggests that the pollen source can influence the development and the morphology of the stinger length in honey bees. Feeding supply can affect Acidic gland development (Nenchev, 2003), consequently, it can also, affect stinger length development.

3.2. Venom sac length and width

There were statistically significant differences between the workers in terms of venom sac length (t=5.987; $p < 0.000$) and venom sac width (t=9.205; $p < 0.0001$) for worker bees fed on clover pollen Table 1 as compared to worker bees fed on corn plant pollen. worker bees fed on clover pollen (Apiary No.1), had the highest value in terms of venom sac length and venom sac width, respectively Fig. (4). This finding suggests that the pollen source can influence the size and development of the venom sac parameters in honey bees. Also, Hosafy et al., (2021) reported that feeding honey bees on a mixture of pollen diet and a broad bean pollen diet resulted in better results than others in the development of HPG and AG**.**

3.3. Stylet length

Results in Table 1 clearly showed significant differences in the Stylet length between the worker bees fed on clover pollen (Apiary No.1) and worker bees fed on corn plant pollen (apiary No.2), (t=7.216; $p < 0.0001$)) (Fig. 5). This finding suggests that the pollen source can influence the development and morphology of the stylet in honeybees.

The specific nutritional components present in clover pollen and corn plant pollen may contribute to the observed differences in style length. Previous research has shown that different pollen sources can affect the growth and development of anatomical structures in honey bees. For example, Roulston and Cane (2002) demonstrated that pollen's nutritional content can influence honey bee physiology and development.

3.4. Lancets length

Data in Table 1 clearly showed significant differences in the lancets length between the workers in apiary No.1 fed on the clover plant and those fed on the other Apiary No.2 Corn plant (t=7.216; $p < 0.0001$), As compared to apiary No. 2, Apiary No.1 workers had the longest lancets length (Fig. 6).

The data analysis from the study clearly demonstrated significant differences in the length of the lancets between worker honey bees from the apiary that were fed on clover plants compared to those fed on corn plants. Specifically, the workers fed on clover plants exhibited the longest lancet length compared to the workers fed on corn plants.

The lancets are crucial structures involved in the honey bee's stinger apparatus and play a vital role in honeybee defense mechanisms. The longer lancet length observed in the workers fed on clover plants suggests potential variations in stinging capabilities and defense mechanisms between the two groups. The lancet length may influence the penetration and effectiveness of the stinger during defensive actions.

3.5. Barbs lancet length

Results in Table 1 and illustrated in Fig.6, clearly showed significant differences in barbs lancet length, where the worker bees fed on clover plant apiary No.1 was 0.67 ± 0.098 mm, which was significantly longer than bees raised on corn (0.48 ± 0.08 mm) (t=9.205; $p < 0.0001$).

The results of the study clearly demonstrated significant differences in the length of barbs on the lancets between worker honey bees fed on clover plants and those raised on corn. Specifically, the worker bees fed on clover plants exhibited significantly longer barbs on the lancets compared to bees fed on corn.

While limited research has specifically examined the direct effects of pollen source on barb length in honey bees, studies on nutrition and honey bee anatomical structures provide relevant insights. Different pollen sources contain varying nutrient compositions that can influence honey bee physiology and development (Alaux *et al.*, 2010). These nutritional variations may contribute to the observed differences in barb length.

3.6. Lancet tip length

The results of the study indicate that there are statistically significant differences in lancet tip length between the apiaries under study, as the lancet tip length the apiary feeding on clover was longer than the lancet tip length in the bees feeding on corn (t=6.75; $p < 0.0001$), which is evident in Table 1. and Fig. (7).

The results of the study clearly demonstrated significant differences in lancet tip between worker honeybees fed on clover plants and those fed on corn . Specifically, the worker bees fed on clover plants exhibited significantly longer lancet tip compared to bees fed on corn . This finding suggests that the pollen source can influence the development and morphology of the lancet tip in honey bees.

3.7. Diameter Lancet at last barb

There were statistically significant differences between the workers in apiary No.1 fed on clover plant and those fed on Corn plant Apiary No.2 (t=3.753; $p < 0.0006$) Table1. As compared to apiary No.2, Apiary No. 1 workers had the highest value in diameter of lancet at the last barb Fig 7.

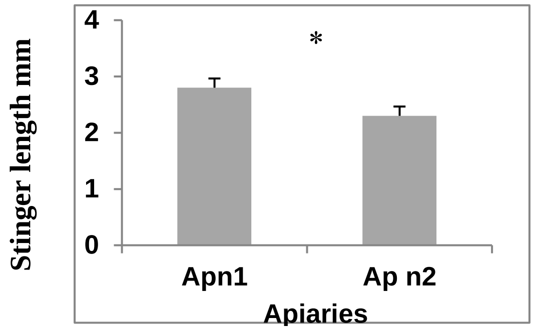
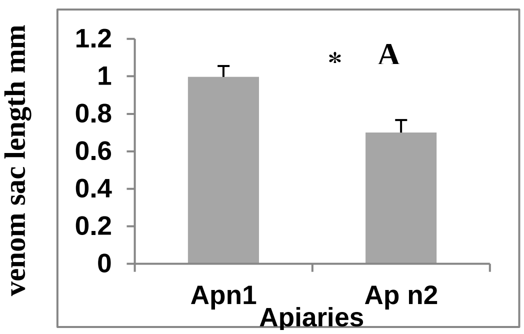


Figure 3. Effect of pollen source on stinger length



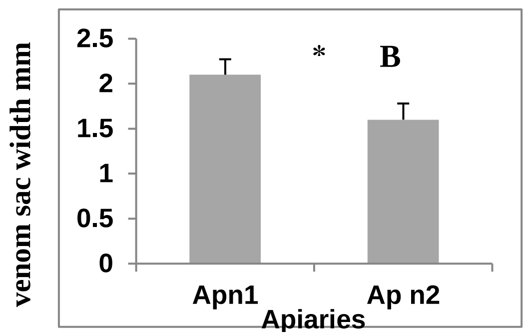


Figure 4. Effect of pollen source on venom sac parameters: A-venom sac length, B-venom sac width

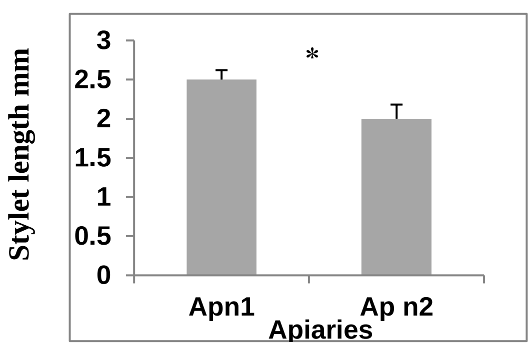


Figure 5. Effect of pollen source on stylet length

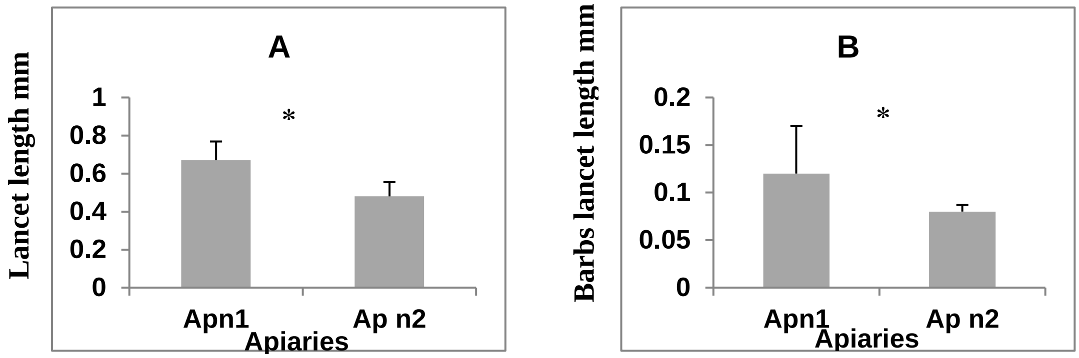


Figure 6. Effect of pollen source on lancet parameters: A-lancet length and B- Barbs lancet length.

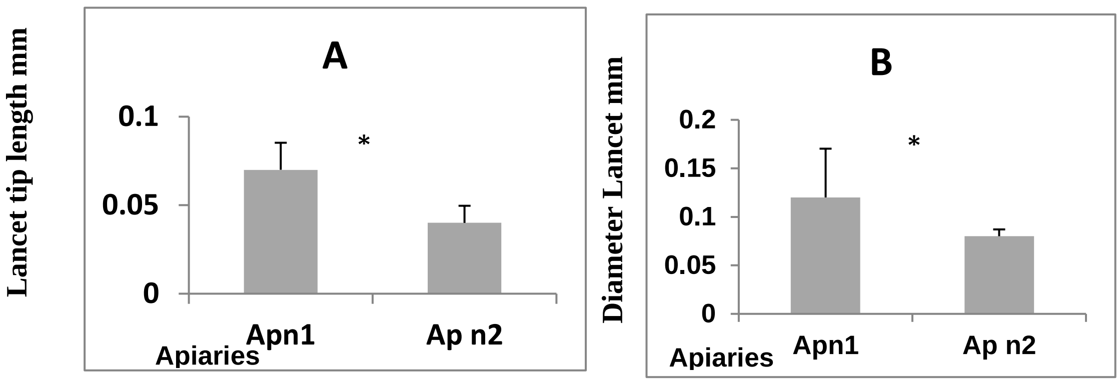


Figure 7. Effect of pollen source on: A- Lancet tip length and B- Diameter Lancet at last barb

Pollen sources gave significant differences in the development of stinger of workers. In this respect, results indicated that the pollen substitute gave significant increases in the parameters of honey bee worker's stinger parameters and venom sac parameters. This is consistent with many previous studies that explained the effect of protein feeding on the components of the venom and some characteristics of the stinger of honey bee workers. These results were confirmed by (Nowar, 2016) who concluded that feeding honey bee with pollen substitute increase all activities of honey bee colonies such as worker's sealed brood area and the produced venom amounts, venom gland, and venom sac.

4. Discussions

The protein content of pollen is important for certain aspects of bee physiology, such as development and survival (Bruna *et al.,* 2016; Katarína *et al.,* 2019; Makaylee *et al.,* 2021). Bees rely on pollen as their main source of protein, and the quality and digestibility of the pollen can impact their health and well-being (Aoi *et al.,* 2021).

Higher protein intake from pollen has been associated with increased bee survival and enhanced development of brood and population (Zheko, Radev, 2018). Additionally, the protein content of pollen can vary depending on the season, with spring pollen generally having higher protein content than summer and autumn pollen. These findings highlight the importance of protein nutrition from pollen for bee physiology and suggest that optimizing the protein content of pollen diets could contribute to the overall health and development of bee colonies. data obtained showed that the amount of total protein intake (16.10, 15.14, 14.74, 14.46, and 9.96 mg/bee/18 days) was determined in corn pollen, broad bean pollen, date palm pollen, pollen mix, and maize, respectively. Also, workers fed on mixed pollen diet had the best average acinal surface area of HPG followed by those fed on broad bean pollen and corn pollen with similar degrees then followed by those fed on date palm and maize, respectively. Whereas bees fed on sugar solution only (control) had the lowest acinal surface area of HPG compared with other pollen diets, with statistically significant differences (Eshbah *et al.,* 2021). also, Lan *et al.* (2021) Pollen sources have significant effects on the stinger apparatus in worker honey bees. Different pollen diets can influence the development of hypopharyngeal glands and ovaries, which are important for worker bee physiology.

Additionally, diets containing higher ratios of corn pollen, which has higher protein content, produced larger individuals with increased survival rates and faster development times in small carpenter bees (Sarah *et al.,* 2014). Pollen composition significantly impacts the development and body size of bees. T'ai *et al.* (2002) reported that Diets with higher ratios of clover pollen have been shown to produce larger individuals with increased survival rates and faster development times in small carpenter bees. In another study, offspring body size in sweat bees was found to increase with a shift to higher protein content in the pollen diet (Irina *et al.,* 2014). Additionally, the amount of pollen reserves in a colony has been found to have a major impact on male body size, sperm production, and speed of sexual maturity in stingless bees (F. *et al.,* 2012). These findings highlight the importance of pollen composition in determining the development and body size of bees.

5. Conclusion

In conclusion, the findings of this study provide evidence that feeding honey bees with a pollen substitute significantly influences the parameters of honey bee workers' venom stinger and venom sac. The morphological parameters of the sting apparatus, such as the length and width of the venom sac, were found to be significantly affected by the source of protein intake through honey bee feeding. Specifically, the consumption of clover pollen had a more pronounced effect on the morphological parameters of the sting apparatus compared to corn pollen. These results suggest the nutritional composition of pollen sources. Clover pollen, with its specific nutritional profile, appears to have a greater impact on the venom stinger parameters and venom sac parameters of honey bee workers. Understanding the relationship between pollen source, protein intake, and the morphological parameters of the sting apparatus is essential for gaining insights into honey bee biology and its defensive capabilities. This knowledge can have practical implications for beekeeping practices, such as optimizing pollen substitutes or manipulating the nutritional sources available to honey bees to enhance their overall health and productivity. Further research is warranted to explore the underlying mechanisms that mediate the influence of pollen sources on venom stinger and venom sac parameters. Additionally, investigating the functional implications of these morphological variations in terms of venom production, defensive capabilities, and overall colony health would provide a comprehensive understanding of the importance of pollen nutrition on honey bee physiology.

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Conflict of interest

The authors hereby declare that no competing and conflict of interests exist.

Author contributions

Conceptualization, K. S., M.Z., L.A., M.A., and O.S.;methodology, L.A., M.A., and O.S.; validation, M.Z., K.S., M.A., and O.S.; formal analysis, M.A., and O.S.; resources, L.A., M.Z., O.S.;M.A., and O.S.; data curation, L.A., M.Z., K.S., M.A., and O.S.; writing original draft preparation, all authors; writing review and editing, M.Z., K.S., M.A., and O.S.; visualization, *O.S.; supervision, M.Z., K.S., and O.S.; project administration, O.S.; funding acquisition, L.A.. All authors have read and agreed to the published version of the manuscript.*

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