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Host Preference Evaluation of *Varroa destructor* on *Apis mellifera* by Air-Flow Olfactometer

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ABSTRACT

Four arm air-flow Olfactometer was used to study the behavior and host preference of *Varroa destructor* on different stages of *Apis mellifera* L. Four arm Olfactometer studies revealed that among adult castes, *V. destructor* exhibited strong orientation towards drone and recorded maximum mite population and percentage preference followed by nurse bees with no significant difference (p= 0.158). However, varroa mites were less attracted to foragers accounting mite population of 10.5 with percentage preference of 21%. Further, it was determined that the among all the larval stages, the odor produced by drone larvae elicited strong orientation behavior by recording a mite population of 18.5 mites with percentage preference of 37% followed by 18-20 hr old larvae with 12.25 mite population and were significantly different (p=0.015). Our results showed that the newly-emerged 2-3 hr old worker larvae were less attractive to mites. Based on the present studies it was evaluated that drone brood was preferred by Varroa mites among all the hosts. Drone brood trap method can be used for the efficient management of varroa in an integrated approach for the sustainable development and wellbeing of honeybees by reducing the application of chemical methods and resistance development in this mite.

Keywords *Apis mellifera*, *Varroa destructor*, Host preference, Olfactometer, Different stages.

INTRODUCTION

Honeybees are important pollinating agents and about 80% is contributed solely by *Apis mellifera* (Smart *et al.* 2018) but are facing huge colony losses due to abiotic and biotic factors including pesticides, pathogens, climate change and parasites (Goulson *et al.* 2015, Steinhauer *et al.* 2018) thereby threatens the production of many bee-pollinated crops. The parasitic mite, *Varroa destructor* has most economic importance in beekeeping (Goulson *et al.* 2015, Liu *et al.* 2016). The Varroa mite feeds on bee haemolymph and reproduces in brood cells, thereby spends its whole life on hosts without any free living stage (Nazzi and Le Conte 2016). Over the years, a variety of acaricides have been developed and applied to control varroa (Pinnelli *et al.* 2016, Ziegelmann *et*

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al. 2018). The injudicious use of acaricides for controlling this pest has accelerated the development of resistance to virtually all synthetic miticides (Thompson *et al.* 2002, Goodwin *et al*. 2005). The acaricides also deleteriously affects the market for bee products by contaminating honey and wax. The limitations of chemical methods lead to the development and application of physical and biological methods for the control of varroa (Guichard *et al.* 2020). Due to high host specificity varroa mites prefer hosts at a particular stage. The high degree of host specificity is exhibited by the presence of kairomones that are used by the mite to recognize and parasitize hosts of larva and adult. Due to different host odors, Varroa is able to distinguish between different honeybee stages (Donze *et al.* 1998). To test the orientation behavior of *V. destructor* to semiochemicals several bioassays have been used either by using simple petri dish or glass plate assays (Aumeier *et al.* 2002, Calderone *et al.* 2002). The orientation in mites is governed by stage-specific odor differences of live hosts. Host finding behavior of Varroa mites is also influenced by chemical components of honey bee pheromones. Semiochemicals is thought to be one of methods for controlling varroa mites either by disrupting their host locating behavior or by attracting and capturing a portion of the population within a hive. Keeping in view the disastrous effects of Varroa mites in colonies of *A. mellifera*, the investigations were carried out to study the host finding behavior and host preference of *V. destructor* on different stages of *A. mellifera*.

MATERIALS AND METHODS

Host preference of *V. destructor* on adult stages of *A. mellifera*

To study the host preference of *V. destructor* on adult stages of *A. mellifera*, four- armed Olfactometer (Fig. 1) was used, previously described by Vet *et al.* (1983) and Kaiser *et al.* (1989) with certain modifications. The Olfactometer was placed inside the laboratory with dimensions of exposure chamber 56 mm by 2 mm and the arms having diameter of 2 mm. In the star-shaped exposure chamber (area for introduction of mites), air was sucked out through the hole in center to create four odor fields. The device consisted of four arms and three 50 ml glass vials were connected to each arm. The vial nearest to the exposure chamber acted as a trap to capture the varroa. The odor source was furnished by the second vial and the outer vial was provided with distilled water to create uniform humidity in device by the passage of incoming air flow. Freshly killed host bees were weighed and equal weight (g) of each host was placed in respective glass vials as odor source. An air-flow of 0.7 ± 0.1 liters per hour was regulated in each of the fields of Olfactometer by flow meter and was passed gently from the corners towards the center. The first arm of the Olfactometer produced odor of nurse bees, second and the third arm produced odors of drone and forager bees, respectively and the fourth arm was kept blank (control). The whole experiment was replicated four times.

Host preference of *V. destructor* on larval stages of *A. mellifera*

Another experiment was carried out in which three different larval stages viz, drone larvae, newly emerged 2-3 hr and 18-20 hr old larvae were collected by opening the sides of brood cells and were removed and placed in plastic vials with the help of forceps for carrying them up to the laboratory. Freshly killed hosts were weighed and equal weight (g) of each host was placed in three different vials (second) of each arm of Olfactometer as odor source and the fourth arm was kept blank (control) and was replicated four times. The further procedure was followed same as in case of adult stages.

Release of mites in an Olfactometer

Fifty mites of Varroa females were taken and released in the center of an exposure chamber. In the four odour fields mites moved freely and their behavior was recorded for 20 minutes. Prior to enter into odorized and the control arms, the mites explored the central zone (exposure chamber) for quite some period of time. Some individuals remained within a particular arm during the whole experiment others explored the four arms. The number of mites in each field was evaluated by counting the number of mites after 20 minutes. The whole experiment was replicated four times following the same procedure. After every 20 minutes, the exposure chamber was cleaned with

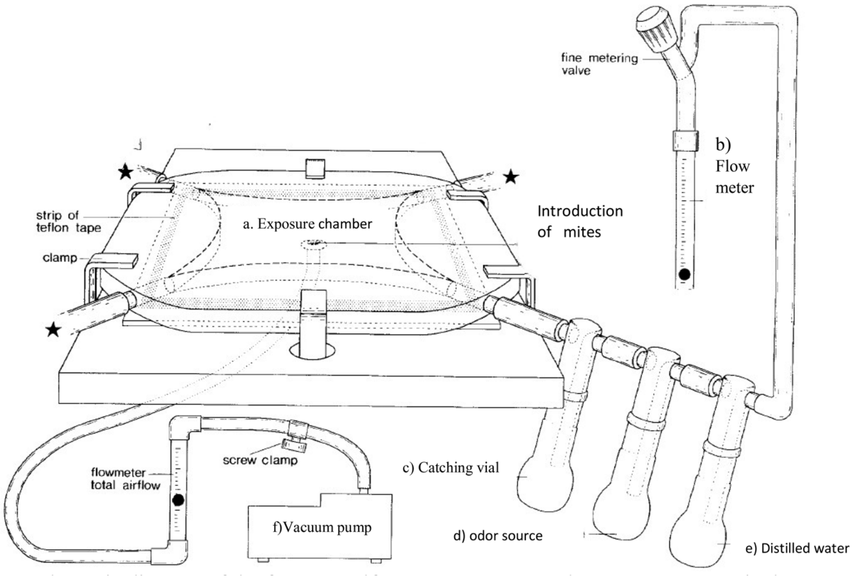

Fig. 1. A schematic diagram of the four-arm olfactometer set up used to assess Varroa mite host preference a) Area for introduction or release of varroa mites, b) Uniform airflow was maintained at 0.7 ± 0.1 liters per hour, c) Vial for catching varroa mites, d) Vial containing different stages of *A. mellifera* as hosts to produce odour, e) Maintained uniform humidity in device, f) Air was drawn through the four glass arms towards the center for attracting mites, g) Stars represent the rest of three arms.

75% ethanol to eliminate any possible bias. All the experiments were run at 30° ± 1°C and at a relative humidity of 75 ± 5%.

Statistical analysis

All the data presented is mean of four replications. Data was subjected to analysis of variance and Tukey's Test at p≤ 0.05 by SPSS Software. Preference percentage of mites was calculated by number of mites in a particular field/total number of mites introduced in device ×100.

RESULTS AND DISCUSSION

Host finding behavior by *V. destructor* is clearly a complex process. Our results (Table 1) showed that all the treatments were significantly superior to control and significant difference existed between them. *Varroa* mites were strongly oriented towards the odor produced by adult drones as maximum population of varroa mite was observed in arm containing drones (16.0) followed by nurse bees (14.5) with preference percentage of 32 and 29, respectively . However, these two treatments showed no significant difference (p= 0.158) with each other and were significantly superior over rest of the treatments. Minimum population of mites was recorded in arm containing foragers and there was significant difference between the drone and forager bees (p= 0.002). Moreover, the mean number of 4.5 and 4.5 mites remained in control and center (exposure chamber), respectively and were not attracted to any of the odors placed in the Olfactom-

Table 1. Host preference of *V. destructor* on adult stages of *A. mellifera*. Data is mean of four replications ± SD, different letters show significant difference between the treatments at p<0.05, Control= Blank arm of olfactometer, Center= Area of introduction of mites in device.

Treatment (odor source)	Mean number of mites in each field	Preference percentage (%)
Nurse bees	14.5^{c} ±2.38	29
Drone	16.0^{c} ±1.15	32
Foraging bees	10.5^{b} ±2.08	21
Control	4.5^{a} ±1.29	9
Center	4.5^{a} ±0.57	9
CD (p< 0.05)	0.37	

Table 2. Host preference of *V. destructor* on larval stages of *A. mellifera*. Data is mean of four replications ± SD, different letters show significant difference between the treatments at p<0.05; Control= Blank arm of olfactometer, Center= Area of introduction of mites in device.

Treatment (odor source)	Mean number of mites in each field	Preference percentage (%)
Drone larvae	$18.5^c \pm 2.643$	37
Newly emerged larvae 2-3 h old	$10.75^b \pm 2.21$	21.5
Newly emerged larvae 18-20 h old	$12.25^b \pm 1.70$	24.5
Control	$3.75^a \pm 1.70$	7.5
Center	$4.75^a \pm 2.21$	9.5
CD (p<0.05)	0.54	

eter and showed no significant difference (p=1.00).

Investigation carried out for evaluation of mite preference towards larval stages of *A. mellifera* showed (Table 2) that Varroa mites were strongly attracted to drone larvae with mean population of 18.5 mites resulted in preference percentage of 37% and was significantly superior over rest of treatments followed by 18-20 hr old larvae accounting 12.25 mite population and were significantly different (p=0.015). The minimum mite population was observed in arm containing 2-3 hr old newly emerged larvae and showed significant difference from 18-20 hr old larvae (p= 0.002). However, there was no significant difference (p= 1.00) in mite population that was observed in control and center.

It is depicted from the results that Varroa mites showed strong orientation towards the odor produced by drone larvae followed by drone and nurse bees. The mite enters 40 and 20 hrs before capping in drone and brood cells, respectively for multiplication (Rosenkranz *et al.* 2010). Drone larvae are preferred over workers and drone brood is often 8-10 times more infested than worker brood (Al Toufailia *et al.* 2018). Drones have longer pupal developmental stage which increases the parasitization of mites on drone brood over the workers (Rosenkranz *et al.* 2010, Traynor *et al.* 2020). Drone bee brood is preferred 8-fold (Traynor *et al.* 2020) contrary to 1.6 times (Gunesdogdu *et al.* 2021). The infestation rate of mite varies with periods of year as higher preference for the drone bee brood was in May, June, and July (Gunesdogdu *et al.* 2021). Similarly, Al-Toufailia *et al.* (2018) identified that Varroa prefers drone bee brood 13 times more than the worker brood. Drone brood suffers higher Varroa parasitism than worker brood because of active choices by the mites based on brood or food odors or slower drone development and more nurse bee visits providing more opportunities to infest drone cells (Calderone and Kuenen 2003). Present findings conform to previous studies showing Varroa's ability to recognize and preferentially parasitize nurse bees over foragers (Kraus 1993). Varroa mite has more preference for nurse bees and very little for newly emerged bees and foragers (Xie *et al.* 2016). Nurse bees have substantially larger and more nutritionally dense fat body which Varroa mites prefer than other stages of the worker bee caste (Keller *et al*. 2005). The high degree of host specificity exhibited by Varroa suggests that kairomones are used by mites to locate and parasitize larval and adult hosts (Pernal *et al.* 2005).

CONCLUSION

Our results showed that Varroa mites prefer drone brood compared to other stages of *A. mellifera*. For mass trapping of varroa population, extra drone brood combs can be utilized in colonies and removal of these combs before the emergence of drones would prove an effective way for controlling varroa mite as it has been observed that it reproduces mostly in drone cells. Though, there are many options available for managing varroa but to understand the application of best option is an important part of decision making. To evaluate the use of drone brood trap method in an integrated approach for efficient management of honeybee colonies and for reduction of insecticide resistance in pests is the need of hour.

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