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Simple approaches for environmental and mechanical management of the Varroa mite, *Varroa destructor* Anderson and Trueman (Parasitiformes: Varroidae), on the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) in Egypt

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Abstract

Background: Varroa mite, *Varroa destructor* Anderson and Trueman (Parasitiformes: Varroidae), is an ectoparasitic mite of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), with a great economic importance. It is the major deadlock of apiculture development all over the world.

Results: This work aimed to assess the effect of bee house and dark bee house on numbers of Varroa mite on white card board sheets, worker broods, and alive bees during spring and autumn of 2018 and 2019. Two types of card board for sticking the fallen Varroa mite were evaluated through winter of 2019. Keeping honey bee hives in a dark room during March and September of 2018 and 2019 for a successive 3 days resulted in a great reduction in the number of Varroa inner bee hive, i.e., on the white card board sheets, area of broods, and alive honey bee. Highest number of fallen Varroa mite on the white card board sheets was obtained in the case of using the dark bee house during March and September in 2018 and 2019, followed by keeping in a normal bee house then those fallen in the case of the open apiary.

Conclusion: The dark bee house grooming behaviour increased through 3 days of dark. Environmental management of bee house and dark bee house can be promising in colony collapse disorder. Modified adhesive sheets were more efficient in this regard than the normal ones.

Keywords: *Apis mellifera*, *Varroa destructor*, Environmental management, Bee house, Grooming behaviour

Background

The ectoparasitic Varroa mite, *Varroa destructor* Anderson and Trueman (Acari: Varroidae), is a common devastating parasite for the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) causing serious losses to the beekeepers (Mabrouk et al., 2016). It feeds on the haemolymph of developing and

adult honeybees, where the infection results in carriage secondary diseases such as viral diseases (Yang and Cox-Foster, 2007 and Dainat et al., 2011). Continuous chemical management can lead to emergence of real estate resistance and contaminated products. The management of Varroa mite is mostly difficult as the plurality of mites live inside the closed brood for reproduction and are well protected from the different methods of management (Kirrane et al., 2018). Photoperiod and temperature strongly influence the

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rate of diapause development in animals (Danks, 2002). Honeybees have a great economic importance to agriculture not only for honey production, royal jelly, pollen, propolis, pharmaceutical purposes, food production, and other industrial products (Neumann and Carreck, 2010), but also for pollination of the plants.

In most cases, acaricides are effective in managing Varroa mite, but their application within the hives mostly contaminates bees' products. In addition, the Varroa mite can develop resistance to these chemicals (Ward et al., 2008). However, there is an urgent need, for alternative, sustainable forms for its management. *V. destructor* can reproduce on both male and female brood of *A. mellifera*, thus attaining a longer reproductive season and larger mite populations. Furthermore, without managing the infested colonies with Varroa mite, they may be die within 3 years (Fries et al., 2006; Allam and Zakaria, 2009; Rosenkranz et al., 2010 and Mondet et al., 2019).

Arechavaleta-Velasco et al. (2012) reported that several mechanisms may be involved in resistance of some honey bee lines to *V. destructor* including hygienic and grooming behaviour. Grooming behaviour is one of the most important mechanisms of honey bee defence against Varroa mites, and this affected with genetic characteristics of bee colonies as well as environmental conditions (Tahmasbi, 2009). In general, Noel et al. (2020) reported that due to a short history of coevolution, the host-parasite relationship between *A. mellifera* and *V. destructor* is unbalanced, with honey bees suffering infestation effects at the individual, colony, and population levels.

This study aimed to test a new simple approach of managing the Varroa mites by keeping honey bee hives (colonies) in a dark bee house and also assess the effect of this trial on the colony strength.

Main Text

Methods

Bee houses measurement

Normal room measured (3.5 × 5.5 × 3 m) was built from bricks of clay and covered with 3 layers: (1) a layer from jungle with clay mixed with wheat straw, (2) a plastic sheet, and (3) a layer from jungle with clay mixed with wheat straw, in an open field at Aga county, Dakahlia governorate, Egypt. The room was named normal bee house. It has a door (80 cm width × 1.9 cm height) and 2 opening windows, which roll polestar and plastic wire were used to close these opening, with pores permit the bees to go in. Inner this room, another room measured (1.8 × 1.8 × 2 m). A dark room was built by the same materials but without any opening, only has a door (80 cm width × 1.9 cm height) to close it.

Effect of keeping colonies of honey bees in the bee houses and open apiary on the management of Varroa mite

Honey colonies were divided into 3 groups, each contained of 6 hives. The first group was placed in the dark bee house, the second group was placed in the bee house, and the third group was placed in an open apiary.

Effect of keeping sites of honey beehives on the mortality of Varroa mite

The effect of keeping sites of honey bee colonies on the mortality of Varroa mites was assessed by adding a white card board sheet (coated with Vaseline), as well as counting the number of Varroa mite on random 20 broods and 100 alive bees in each hive, 3 days after placing the hives in the sites. This procedure was carried out during March (beginning of the active period of honey bees) and September (the time of high level of infection with Varroa mite) of 2018 and 2019. In addition, pure water was provided inner the dark and the normal bee house during the summer season.

Assessment of the efficiency of dark bee house levels

Infestation levels in all experimental colonies were recorded after each application. The following data were recorded:

- Number of dead mites fallen down on the white card board sheet (coated with Vaseline) located under the colony
- Number of mites in random sample of 100 alive bees
- Number of mites in 20 cells of workers

The efficiency of the treatment was calculated by using the following formula of (Allam et al., 2003 and Marinelli et al., 2004):

Rate of efficiency % = No. of dead mites*/Total No. of mites** × 100

where *dropped mites on a white card board sheet and **dropped mites + No. of mites on 100 alive bees + No. of mites in 20 brood cells.

Effect of using modified adhesive sheets on sticking Varroa mite

Normal adhesive sheets for mite traps (to increase the period of the validity of the used 45 sheets) or their modification by adding brood juice + royal jelly for sticking Varroa mite every 2 weeks (juice of five broods + 1 g royal jelly/colony) were used (to attract Varroa mite to the sheet). Due to the adhesive sheets for mite traps that have a high potential for sticking and makes bees adherent on these sheets, galvanized wire mesh was placed in each hive above the sheet to be a barrier for sticking bees on the sheet. Three hives were used for each kind

of adhesive sheets. The experiment was carried out during September 21 to December 21 of 2019 at Dakahlia governorate, Egypt, under bee house conditions. The number of the injured and fallen Varroa mite on both kinds of the adhesive sheets (in the bottom of the hive) was counted weekly, then removed from the sheets, in each inspection, using a forceps. The averages of injured and fallen Varroa mite of each inspection were recorded.

Statistical analysis

All the data were statistically processed by split design (Snedecor and Cochran, 1980) and the analysis of variance and by determining the significance threshold using Duncan's test (Duncan, 1955).

Results

Effect of keeping colonies of honey bees in the bee houses and open apiary on the management of Varroa mite

Tables 1 and 2 show that the population of the Varroa mite was lower during March of both 2018 and 2019 than the population of September of both years. Also, the number of the fallen Varroa mite on white card board sheets was significantly higher than those found in the case of the normal bee house and the open apiary. In addition, results revealed that there were higher numbers of Varroa mite fallen on the white card board sheets as well as found on the broods and alive bees during September of both 2018 and 2019 than those found in the case of March of both years.

Data presented in Table 1 and illustrated by Fig. 1 indicated that the highest number of the fallen Varroa mites on the white card board sheets was obtained in the case of the dark bee house during March of both 2018 and 2019, being 6.2 in both years, followed by keeping in normal bee house, being 4.23 and 4.5, respectively, then those fallen in the case of the open apiary, being 2.5 and 2.8, respectively. The same trend was found concerning the number of the Varroa mite on 20 broods and 100 alive bees.

Data shown in Table 2 and illustrated by Fig. 2 revealed that there were higher numbers of Varroa mites

fallen on the white card board sheets than those fallen in the case of the normal bee house and the open apiary during September of 2018, being 24.3, 12.2, and 11.8, and being 28.2, 13.5 and 12.2 during September 2019. On the other hand, the number of Varroa mites found on 20 broods and 100 alive bees was significantly lower in the case of the dark bee house than in the normal bee house and open apiary in descending order. In this respect, the numbers of Varroa mites on 20 broods and 100 alive bees in the case of the dark bee house were 10.3 and 9.2, 22.2, and 28.2 in normal bee house and 28.5 and 48.5 in open apiary, respectively.

The obtained data revealed that there were higher numbers of Varroa mites that fallen on the white card board sheets than those fallen in the case of the normal bee house and the open apiary during September of 2018 and 2019. On the other hand, the found number of Varroa mites on 20 broods and 100 alive bees was significantly lower in the case of the dark bee house than that found in the case of the normal bee house and open apiary in descending order.

Effect of using modified adhesive sheets on sticking Varroa mite

Results shown in Table 3 revealed that the modified mite trap was more efficient than normal mite trap for sticking Varroa mites. The number of the conjoined Varroa mites was gradually increased by prolonging the period of the experiment. In addition, the modified mite trap was more efficient than the normal mite trap in this regard. In this concern, the average number of the fallen Varroa mites at the beginning of the experiment (September, 21) was 13.3 and 15.7 in the case of the normal mite trap and the modified mite trap, respectively. These figures increased at the end of the experiment (December 21) to 17.3 and 19.7, respectively.

Discussion

Varroa mite, *V. destructor*, is the major causal of colony collapse in *A. mellifera* populations (Rosenkranz et al, 2010).

Table 1 Effect of keeping-bee hives in a dark bee house compared with keeping in bee house with windows and in open apiary on the average number of fallen Varroa mites on white card board sheets as well as on broods and alive bees, during March of 2018 and 2019 at Dakahlia governorate, Egypt

Site of keeping	March 2018			March 2019		
	Sheet	Brood ^a	Alive bee ^b	Sheet	Brood	Alive bee
Dark bee house	6.2a	1.2c	2.5c	6.2a	1.3c	2.3c
Bee house	4.3b	1.8b	5.7b	4.5b	2.2b	5.8b
Open apiary	2.5c	3.2a	7.3a	2.8c	3.7a	8.7a

^aNumber of Varroa mite on 20 broods

^bNumber of Varroa mite on 10 alive bees

Duncan multiple range was significant at 0.05. Means with the same letter are not significantly different. a, b, and c—values in the same column with different superscripts differed significantly

Table 2 Effect of keeping-bee hives in a dark bee house compared with keeping in a bee house with windows and in open air (beekeeping) on the average number of fallen Varroa mites on white card board sheets as well as on broods and alive bees, during September of 2018 and 2019 at Dakahlia governorate, Egypt

Site of keeping	September 2018			September 2019		
	Sheet	Brood ^a	Alive bee ^b	Sheet	Brood	Alive bee
Dark bee house	24.3a	10.8c	9.7c	28.2a	10.3c	9.2c
Bee house	12.2b	21.3b	27.5b	13.5b	22.2b	28.2b
Open apiary	11.8b	27.3a	40.8a	12.2c	28.5a	42.5a

^aNumber of Varroa mite on 20 broods

^bNumber of Varroa mite on 10 alive bees

Duncan multiple range was significant at 0.05. Means with the same letter are not significantly different. a, b, c—values in the same column with different superscripts differed significantly

The highest number of the fallen Varroa mites on the white card board sheets was obtained in the case of using of dark bee house during March of both 2018 and 2019, followed by keeping in normal bee house and then those fallen in the case of the open apiary. The same trend was found concerning the number of the Varroa mite on 20 broods and 100 alive bees. Obtained data are in agreement with those reported by Nürnberger et al. (2018) who concluded that timing of brood onset in late winter is mainly driven by temperature but modulated by photoperiod not effect on brood onset.

Rosenkranz et al. (2010) reported that the female of Varroa mites was more likely to lay eggs on drone brood than on worker brood (about 10–12 times). This may be due to drones being of a longer brood cycle. Therefore, this reason inspection of drone brood gives the best chance of detecting the infestation with Varroa mite. In addition, workers brood also reserved as an effective method of detection.

Allo- and auto-grooming contribute to Varroa resistance by both removing mites from adult bees and by physically damaging the mites, preventing them from seeking a new brood cell to infest (Pritchard, 2016).

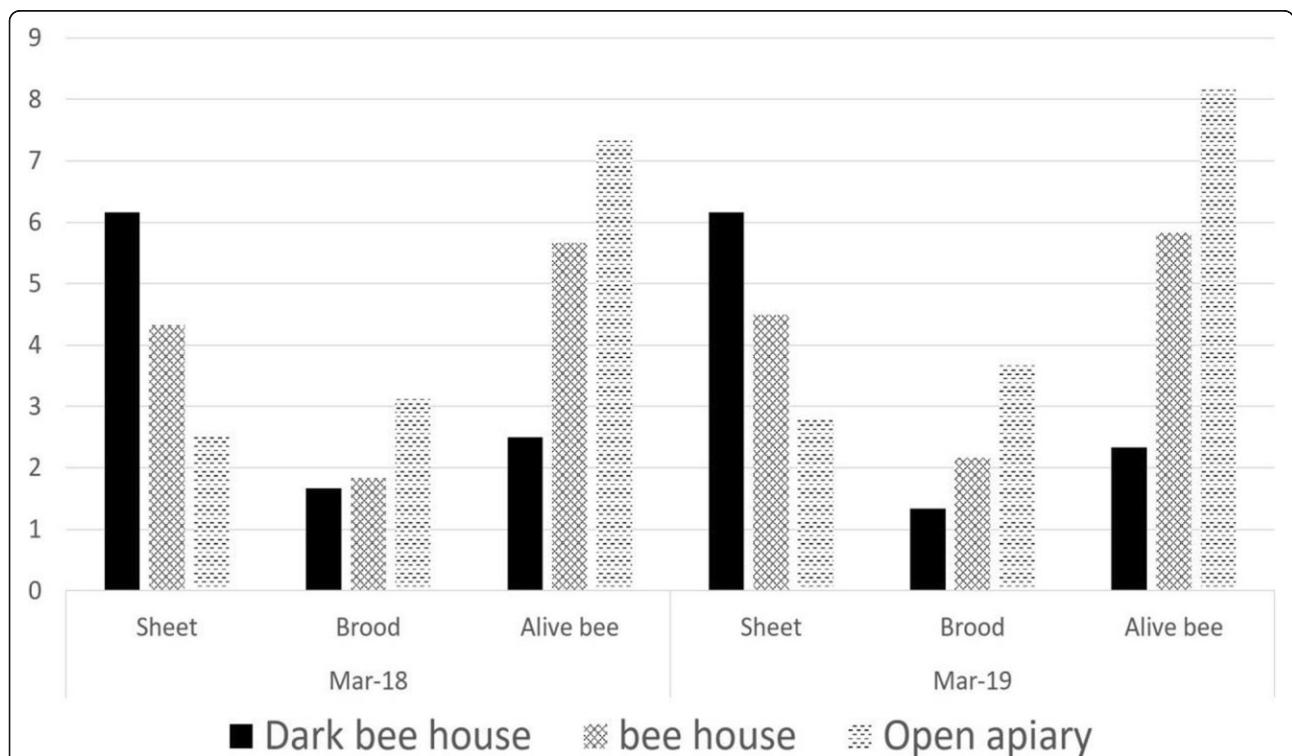


Fig. 1 Effect of keeping-bee hives in a dark bee house compared with keeping in normal bee house has windows and in open apiary on the average number of fallen Varroa mites on white card board sheets (coated with Vaseline) as well as found on broods and alive bees, during March of 2018 and 2019 at Dakahlia governorate

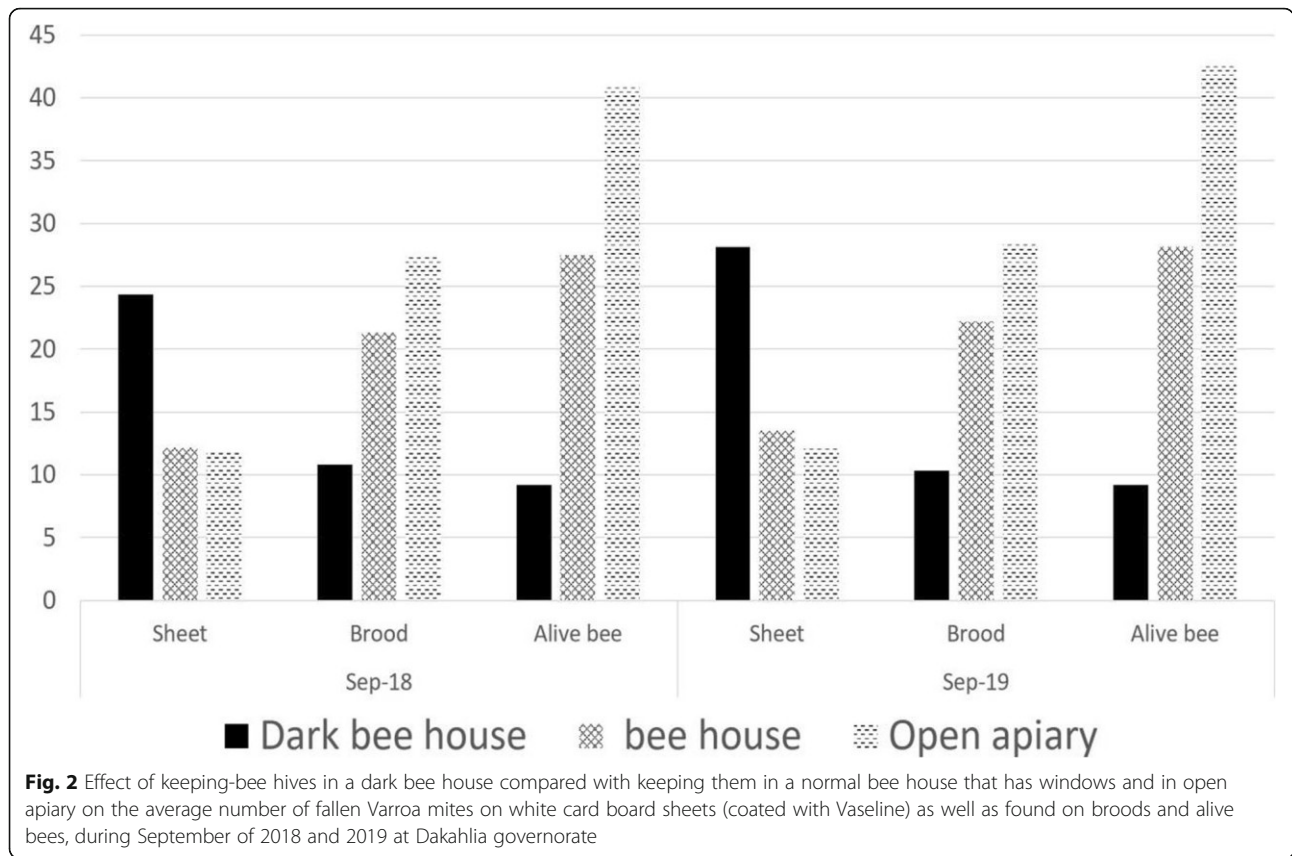


Fig. 2 Effect of keeping-bee hives in a dark bee house compared with keeping them in a normal bee house that has windows and in open apiary on the average number of fallen Varroa mites on white card board sheets (coated with Vaseline) as well as found on broods and alive bees, during September of 2018 and 2019 at Dakahlia governorate

Table 3 Average numbers of Varroa mite fallen on adhesive sheets during September 21 to December 21 of 2019 under bee house conditions at Dakahlia governorate, Egypt

Date of weekly inspection	Normal mice trap	Modified mice trap ^a	Mean
21 Sep	13.3	15.7	14.5
28 Sep	14.7	16.3	15.5
05 Oct	15.3	17.0	16.2
12 Oct	14.7	17.7	16.2
19 Oct	14.7	16.7	15.7
26 Oct	14.3	18.0	16.2
02 Nov	15.0	18.7	16.9
09 Nov	15.0	18.0	16.5
16 Nov	15.0	18.7	16.9
23 Nov	16.3	18.7	17.5
30 Nov	16.7	18.7	17.7
07 Dec	17.7	19.7	18.7
14 Dec	17.7	19.0	18.4
21 Dec	17.3	19.7	18.5
Mean	15.6	18.0	----

^aJuice of five broods + royal jelly (1 g) were spread on each sheet to attract Varroa mite to the sheet
 L.S.D. at 5% for date (D) = 1.2, type of mite trap (K) = 1.4, and D × K = 1.9

Honeybees can initiate allo-grooming via a “grooming invitation signal” a whole body vibrational dance lasting several seconds, which stimulates other workers to groom the dancer. Grooming workers use their mandibles and forelegs to forcefully remove the mites from adult bees, leading to mite injury or death (Hamiduzzaman et al., 2017). In the USA, scientists have produced a strain of bees, now commercially available, that exhibit elevated grooming and mite biting (Arechavaleta-Velasco et al., 2012).

It has been found that the modified mite trap was more efficient than the normal one for sticking Varroa mites. The number of the conjoined Varroa mites was gradually increased by prolonging the period of the experiment. The increase of the number of the conjoined Varroa mites on the modified mite trap may be due to the volatile substances from brood juice and royal jelly, which attracts Varroa mite to the adhesive sheets and stick them on the sheet. The use of both kinds provides the effort to change the regular sheets every week, as the effectiveness of these sheets last for up to 6 months.

Boecking and Spivak (1999) mentioned that it is commonly thought that bees that vigorously shake and bite the mites are the most efficient groomers. However, this disturbance by the bees may cause the mites to invade brood cells more readily, but this possibility remains to be tested. Likewise, it is thought that colonies that rapidly uncap and remove diseased and parasitized brood are more resistant to diseases and mites.

A study conducted in Mexico identified grooming behaviour as the most important factor that reduces mite population growth in a genetically diverse set of colonies. Colonies that had the lowest mite population growth during an 8-month period exhibited higher grooming behaviour, had higher proportions of chewed mites falling from bees in the colonies, and reduced infestation levels of adult bees (Arechavaleta-Velasco and Guzman-Novoa, 2001).

Tahmasbi (2009) reported that grooming behaviour is considered one of the important mechanisms of honey bee defence against parasitic mites, and affected with genetic characteristics as well as environmental conditions. In addition, Arechavaleta-Velasco and Guzman-Novoa (2001) mentioned that there were two types of grooming behaviour: (1) the removal of foreign objects and pathogens from oneself (auto-grooming) and (2) from another adult in the nest (allo-grooming). Moreover, there is a kind of grooming, so-called mite-grooming behaviour associated with higher proportions of mutilated *V. destructor* falling from bees in colonies.

The ability of worker bees to groom other bees may contribute to drone hygiene and health (Hrassnigg and

Crailsheim, 2005), and post-mating changes of the queen (Abdelkader et al., 2014).

Conclusion

This is the first time dark bee houses were used for self-management or grooming (biologically and/or mechanically) of Varroa mites. Also, replacing the normal adhesive white sheets by adhesive sheets for mite traps kept their efficiency in sticking Varroa mites for more than 4 months instead of 1 week for the normal adhesive white sheets.

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Competing interests

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