Efficacy of Modified Bottom Boards to Control Varroa Mite (*Varroa destructor* Anderson & Trueman) in Honeybee Colonies

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Abstract: ***This study was designed to test whether hive bottom boards modified with polyvinyl chloride pipe or screen-mesh reduces number of Varroa mites in naturally infested honeybee colonies comparing to chemical control. Fifty-six colonies distributed equally between two locations each received one of four experimental treatments: Conventional solid board with/out Apistan, Screen bottom board and Tube bottom board. Results were inconsistent between apiaries. In apiary 1, colonies with Apistan and Tube bottom boards had fewer V. destructor than other treatments in spring only, but this benefit was not apparent in Apiary 2. There were no effects of modified bottom boards on honey yield, brood production, and stored pollen. We conclude that the efficacy of modified bottom boards in reducing varroa mite population in bee colonies remains uncertain due to observed differences of hygienic behavior.***

Keywords: integrated pest management, *Varroa destructor*, mesh bottom boards, tube bottom boards

1. Introduction

The ecto-parasitic varroa mite, *Varroa destructor*, dispersed world-wide and is currently considered the major threat for apiculture [1], [2]. *A. mellifera* colonies commonly die from *V. destructor* infestation within a few years if colonies are not treated [3]. Different strategies have been suggested to control *V. destructor* including chemical and physical methods [4]. Chemical acaricides are commonly used in attempts to control *V. destructor* because it is less effort and less time consuming than alternative methods. However, many of these pesticides cannot be used when hives are being used to produce honey. Also, resistant strains of varroa mites can develop [5]. Thus, the development of non-chemical, sustainable mite management methods is desirable to avoid pesticide resistance and prevent contamination of bee products.

Other strategies shown to be useful in varroa mite management are those which employ physical or mechanical methods. One of the most successful of these has been the use of modified hive bottom boards [6]. Screen bottom boards utilize metal mesh which aims to cause the varroa mites to fall out of the hive structure, directly reducing in-hive populations [7]-[10]. However, reported results of its efficacy have been inconsistent [11], [12], mainly because its success depends on the level of hygienic behavior of the treated colonies. Our objectives were to determine if modified bottom boards could have a measurable effect on bees and varroa mites during one-year field test. Also comparing Screen and Tube bottom boards against conventional wooden boards for their ability to increase in honey production, brood production and facilitate the in-hive storage of pollen.

2. Materials and Methods

The experiment was conducted in Research station of Agriculture collage, University of Tripoli (UOT) (32°54'N / 13°11'E), form Jan to Dec 2014. Fifty-six honeybee colonies housed in Dadant hives naturally infested with varroa mites (known as 4-years acaricides free); were equalized prior the commencement of the investigation. In two locations, Twenty-eight colonies (n=7) were arranged in a randomized complete design, and hives were equally assigned to one of four treatments, namely 1) Conventional solid board "control", 2) Apistan in conventional solid board, 3) Mesh bottom board and 4) Tube bottom board. The tube bottom boards comprise a wooden frame with similar dimensions to the inner surface of the hive body. Plastic tubes of 34 mm diameter and 450 mm length set 3.5 mm apart by three plastic spacer struts, and with an open space between the tubes. The screen-mesh bottom boards comprise a wooden attached with mesh (3 mm width). Both bottoms had a drawer underneath to facilitate the accounting of falling mites. Standard management practices were used throughout the year except medication for varroa. All parameters were assessed pre-treatment.

2.1 Efficacy of modified bottom boards in controlling varroa

2.1.1 Infestation rate adult worker bees

Adult bees for sampling mite infestation were taken directly from the combs of the hives into a jar (Approximately 300 bees /colony), the alcohol wash technique was used [13]. In the lab, containers (bees in 100 ml 70% alcohol) were vigorously placed for approx. 30 min on the shaker, to dislodge mites. Content was pour over sieve (mesh width 3 mm) to separate the mites from the bees and second sieve (mesh width 1 mm) placed below to collect the mites. Total number of mites and number of bees in each sample was recorded then percentage (number of mites per 100 bees) was calculated.

2.1.2 Infestation rate sealed brood

For sampling mites on worker brood cells, two frames with recently sealed brood were selected from each colony. Then one- hundred sealed brood cells were randomly selected. Each cell was uncapped, the pre-pupa or pupa inside it was carefully examined and any detected female mites were counted. The walls of the cells and removed caps were also examined as the mite frequently hides there [13]. Total number of inspected cells and number of adult mites was recorded and percentage of infestation of sealed brood was calculated. Data of brood and adult bees were taken on 8-weeks intervals basis for the entire investigation period.

2.2 Effects of modified bottom boards on colony performance

2.2.1 Honey Production

Each hive was weighed using a digital portable hive scale (Model FS-30KA, A & D Mercury Pty Ltd, The barton SA 5031, Australia) to monitor the honey flow into treated colonies. Hives were weighed late spring and at the end of the investigation period.

2.2.2 Brood production

The combs from each hive were removed individually and all bees were shaken off the frames. Each frame was then photographed on both sides with a digital camera (Canon C813). The images were then downloaded onto a computer and image analyzing software (Image Pro Plus version 3.0 Media Cybernetics Inc., Bethesda MD, USA) was used to estimate the area within a trace of the outline of the sealed brood in each image [14]. The sum of the sealed brood areas of all combs in a hive was, thus, the total area of sealed brood for that hive. Data were collected at four- weeks intervals.

2.2.3 Amount of Stored Pollen

Determination of the stored pollen area was made using the same images of combs used for assessment of the area of sealed brood. In this case, the area of stored pollen in each comb was determined by same technique, i.e. by tracing the outlines of the stored pollen on the image. The sum of the stored pollen areas of all the combs in a hive was considered as the area of stored pollen for that hive.

2.3 Statistical Analysis

Data for mean varroa infestation, area of stored pollen, area of sealed brood and honey production were compared between treatments using mixed model analysis of variance (ANOVA) SPSS® for Windows™ Version 14 [15]. Prior to analysis, each variable was visually tested for normality using P-P plot and Levene's test was used to test the assumption of equality of error variance [16]. If significant differences between treatments were detected, their means were separated using Duncan multiple rang test .In all cases, significance was accepted at the 0.05 level.

3. Results and Discussion

3.1 Efficacy of modified bottom boards in controlling varroa

The benefits of incorporating physical methods into honeybee colony management for *Varroa destructor* control were evaluated. In apiary 1, there were significantly less *V. destructor* on adult worker bees in colonies treated with Apistan only during spring ($P< 0.001$) compared to other treated colonies (Table 1). Colonies with Tube bottom boards were not different from Mesh bottom boards, but had significantly less *V. destructor* compared to untreated colonies (Control). Similarly, there were a significantly less *V. destructor* on sealed brood in colonies treated with Apistan only during spring ($P= 0.04$) compared to untreated colonies (Table 2), but both groups were not different from colonies with the two bottom board types, although the trend of varroa infestation on both bees and brood was similar on both apiaries.

Table 1: Infestation rate of *V. destructor* on adult worker bees in hives fitted with different bottom boards (Conventional, Mesh, Tube) at UOT, 2014

	Mean no. of *V. destructor* on 100 adult bees		
	Spring	Summer	Fall
	Apiary 1		
Apistan	0.25±0.6[a]	5.94±2.9	1.70±0.2
Mesh	3.64±0.5[bc]	12.97±2.9	1.48±0.3
Tube	2.90±0.5[b]	13.32±2.9	1.90±0.2
Control	5.15±0.4[c]	14.86±2.6	1.99±0.3
	Apiary 2		
Apistan	5.33±1.2	9.82±1.9	2.39±0.6
Mesh	3.42±1.3	5.77±2.5	0.65±0.9
Tube	5.59±1.2	9.86±1.9	0.93±0.7
Control	6.33±1.0	10.53±1.9	1.64±0.7

Colonies were fitted either a conventional solid bottom boards with/out Apistan or a modified bottom boards; mesh or tube. Values are Mean±SE. Means followed by the same letter are not different at the $p \leq 0.05$ level. Means were separated using Duncan's test.

In apiary 2, *V. destructor* numbers on both adult worker bees ($P= 0.51$) and sealed brood ($P= 0.33$) were not affected by the chemical (i.e. Apistan) or physical control (modified bottom boards) (Table 1). Although fewer numbers of *V. destructor* (but not significant) were obtain from colonies with Mesh bottom boards through the entire period. In contrary to apiary 1, Apistan treated colonies recorded the highest infestation level on both adult bees and sealed brood.

Table 2: Infestation rate of *V. destructor* on sealed brood in hives fitted with different bottom boards (Conventional, Mesh, Tube) at UOT, 2014

	Mean no. of *V. destructor* on sealed brood		
	Spring	Summer	Fall
	Apiary 1		
Apistan	0.75±1.2[a]	14.38±6.1	0.68±2.6
Mesh	3.69±1.0[ab]	18.94±6.1	0.68±2.6
Tube	2.59±1.2[ab]	20.32±6.1	4.08±2.2
Control	4.94±0.9[b]	16.27±5.4	2.30±2.3
	Apiary 2		
Apistan	3.75±1.7	10.96±3.5	3.95±2.1
Mesh	2.09±1.7	1.32±3.9	0.23±2.0
Tube	3.45±1.7	4.96±3.5	2.83±2.5
Control	6.13±1.5	3.41±3.5	2.05±2.2

Colonies were fitted either a conventional solid bottom boards with/out Apistan or a modified bottom boards; mesh or tube. Values are Mean±SE. Means followed by the same letter are not different at the $p \leq 0.05$ level. Means were separated using Duncan's test.

Theoretically, one of the most effective ways to reduce the threat of varroa mite to honeybees is to employ an integrated approach to minimize mite in-hive populations, thereby negatively influencing varroa's reproductive capacity. This approach his might be partially achieved by using colonies with hygienic behavior, but the use tube bottom boards, would be expected to provide additional control. When worker bees with hygienic behavior remove mites from an infested cell or adult bee during grooming those mites remaining undamaged are able to drop onto a conventional solid bottom board and walk in attempts to re-infest other bees. In contrast, if tube bottom boards (with gaps) are used, we expect that many mites will drop out of the hive and be unable to return.

The results for apiaries one and two suggest that other factors (such as varroa resistance and bee hygienic behaviour) may be crucial in finally establishing the efficacy of modified boards in controlling *V. destructor*. Indeed, factors such as varroa resistance to grooming and hygienic behavuoir may influence colony defence, which would directly contribute to the efficacy of modified floors in slowing varroa buildup population. In April, the hygienic behavuoir of the experimental colonies was assessed using freeze-killed brood method [17]. In both apiaries, all colonies detect, uncap and removed freeze-killed brood within 7 days and considered as hygienic colonies. More specifically, 29% of the colonies tested in Apiary 1 removed > 95% of the freeze-killed brood within 24h (highly hygienic), 36% of the colonies removed > 65% (medium hygienic), and 35% of the colonies removed < 65% (low hygienic). Furthermore, in all treated groups, at least two levels of hygienic behavuoir recorded. Unfortunately, we could not evaluate the correlation between hygienic level and infestation rate due to the limited number of replicates, thus statistical analysis could not be performed.

3.2 Effects of modified bottom boards on colony performance

In both apiaries, the net gain of honey supers was not affected by experimental treatments (Table 3), either on spring season (P= 0.175) or at the end of the study (P= 0.199). Similarly, Keshlaf and Spooner-Hart reported that using Tube and Mesh bottom boards at Sydney and Castlemaine did not affect honey gathering during spring [18]. Also, Harbo and Harris stated that screen floors did not effect on honey consumption [19]. Thus, modified bottom boards could be used in IPM strategy with no cost of colony productivity even during honey flow seasons.

Table 3: Mean weight of honey produced in hives fitted with different bottom boards (Conventional, Mesh, Tube) at UOT, 2014

	Mean weight of honey (kg)	
	Spring	Fall
	Apiary 1	
Apistan	14.78±1.3	12.77±1.3
Mesh	12.62±1.3	11.41±1.5
Tube	14.29±1.3	12.67±1.9
Control	17.30±1.1	13.65±1.5
	Apiary 2	
Apistan	16.14±1.2	17.50±2.0
Mesh	19.06±1.1	10.58±2.0
Tube	19.71±1.1	13.89±2.1
Control	19.70±0.9	13.25±1.7

Colonies were fitted either a conventional solid bottom boards with/out Apistan or a modified bottom boards; mesh or tube. Values are Mean±SE.

Concerning brood production, it reached peak at spring then dropped sharply during summer and fall. There was no significant effect of both designs of bottom boards (Table 4) on brood production for all seasons in both apiaries, although colonies treated with Apistan in Apiary 2 had significantly more brood compared to other colonies, however, this affect was only on spring (P= 0.05). These results are consistent with finding of Keshlaf and SpoonerHart [18], while Harbo and Harris reported that brood production was 17% greater in colonies with open screen floors in March in Louisiana [19] and 14% reported by Pettis and Shimanuki in June in Maryland [10]. Screen floors appeared to have no effect on brood production during the first brood cycle in either experiment [19].

Table 4: Mean area of produced brood in hives fitted with different bottom boards (Conventional, Mesh, Tube) at UOT, 2014

	Mean area of sealed brood (cm^2)		
	Spring	Summer	Fall
	Apiary 1		
Apistan	9392±1383	3140±836	4706±772
Mesh	12613±1383	3285±836	2154±772
Tube	11126±1383	3005±836	2727±772
Control	11296±1045	3010±836	3394±668
	Apiary 2		
Apistan	13036±1339^{a}	2259±306	3370±416
Mesh	7877±1093^{b}	1462±342	2574±465
Tube	9450±1198^{ab}	1644±306	3990±537
Control	9227±1093^{ab}	1432±306	3241±465

Colonies were fitted either a conventional solid bottom boards with/out Apistan or a modified bottom boards; mesh or tube. Values are Mean±SE. Means followed by the same letter are not different at the $p \leq 0.05$ level. Means were separated using Duncan's test.

In both apiaries, the amount of stored pollen was not significant affected by both designs of bottom boards or chemical treatment (Table 5). In apiary 1, a greater amount of pollen was stored during spring season, however, no significant differences (P= 0.10) was recorded. Generally, less pollen was stored at summer (P= 0.56) and autumn (P= 0.76) with no significant differences. Surprisingly, in apiary 2, lowest amount of stored pollen recorded at spring then increased gradually, with no significant differences in spring (*P*= 0.34), summer (*P*= 0.27), and autumn (*P*= 0.10).

Table 5: Mean area of stored pollen in hives fitted with different bottom boards (Conventional, Mesh, Tube) at UOT, 2014

	Mean area of stored pollen (cm^2)		
	Spring	Summer	Fall
	Apiary 1		
Apistan	1833±408	1942±403	1093±230
Mesh	1745 ±408	908 ±403	503±230
Tube	2194±408	1046±403	496±230
Control	2590±308	1641±403	1167±199
	Apiary 2		
Apistan	126±75	552±161	756±185
Mesh	412±82	283±180	635±207
Tube	183±82	582±161	966±239
Control	209±82	378±161	821±207

Colonies were fitted either a conventional solid bottom boards with/out Apistan or a modified bottom boards; mesh or tube. Values are mean±SE.

An integrated approach, utilizing bees with improved hygienic and grooming behavior in hives with modified bottom boards is likely to result in an increase in management of a number of honeybee diseases and parasites in addition to varroa mite, resulting in decreased bee losses and improved colony performance. Moreover, the strong demand for honey bees in this region can be fulfilled by using improved indigenous bee strains.

The data suggests that modified bottom boards were insufficient to reduce mite population under damaging level. However, results of other experiments used closed-screen design similar to ours [8]-[10] showed the same trend as our hives, with fewer mites than hives with traditional wood floor. Even if the predicted efficacy of the anti-varroa bottom boards is not confirmed [19], it is a valuable tool to evaluate the size of mite populations [13] and to monitor the efficacy of treatments [20]. Compared to other diagnostic methods like acaricidal treatments, alcohol or detergent washes of adult bee and brood sample [12] the measurement of the natural mite mortality is easy, cheap and fast.

In conclusion, Bottom board type did not significantly affect any hive production parameter (viz., area of stored pollen, area of sealed brood or honey production). Our results are in consistence with Keshlaf and SpoonerHart [18] and Ellis et al. [9], concluded that bottom board type did not affect any of the colony strength parameters except honey production (i.e. more honey was produced in colonies with conventional wooden bottom boards than screened ones). Also, On the other hand, our results were inconsistence to Pettis and Shimanuki [10] and Coffey [21] who reported that in their study that colonies with mesh bottom boards had significantly more sealed brood than colonies on normal bottom boards.

It is recommended that, at this stage, further trials with modified bottom boards need to be conducted to assess their benefits in the absence of Small Hive Beetle. It is also recommended that Libyan beekeepers are made more familiar with mesh and tube bottom boards and their use in varroa mite. In the event that SHB does establish in Libya, mesh and/or tube bottom boards may play a useful role in its integrated management. In such circumstances, the data from this project suggest that there will be no detrimental effect to hive development and production if modified bottom boards are used.

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