AGRICULTURAL SCIENCES

Development of the Mite (*Varroa jacobsoni*) in two-Honeybee Races *Apis mellifera jemenitica* (indigenous) and *Apis mellifera carnica* (Imported) in Riyadh.

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Abstract

Two honeybee races; *Apis mellifera jemenitica* (Indigenous) and *Apis mellifera carnica* (Imported) were used to study and compare the development of *Varroa jacobsoni* under Riyadh's conditions. Five-colonies from each race were inoculated with 20-mites and the development of the mites infestation was monitored every other month from April until September 2001. Estimation of different mites population was based on daily mites downfall and the mites on adults and brood itself. The results of the study based on Adult bees and broodhe showed high mites development in carnica bees where the population increased to 96.05, 124.3, 181.2, and 81.35 folds, whereas in Indigenous colonies the increase was very low 15.29, 14.56, 31.24 and 22.86 during their respective months of inspection. Based on daily mites downfall the population estimation revealed 6.08, 9.42, 78.85 and 32.57 fold increase in Carnica colonies whereas in Indigenous colonies the mite population was increased to 3.34, 5.4, 29.14 and 12.5 folds. The mite population estimated directly from live bees and broods was higher than mite estimates obtained from sticky board. The sticky board method was found better than adult bees and brood samples for the detection of mite population at low infestation level.

Key words: *Varroa jacobsoni* , *Apis mellifera jemenitica* , *Apis mellifera carnica* , Riyadh

Introduction

The ecotoparasitic mite *Varroa destructor* Anderson and Trueman (3) (formerly called *Varroa jacobsoni* Oudemans) was discovered by Jacobson in 1904 on *Apis cerana* the natural host. The mite later switched host to the western honeybee *Apis mellifera* and now has become a serious pest of that bee worldwide (3,23,36) They are threatening the survival of managed and feral honeybees, the beekeeping industry and, due to the role of bees in pollination, the future of many agricultural crops.

There are several factors that contribute to the population growth rate of *Varroa jacobsoni,* such as duration of brood rearing season, presence of drone brood, host specific effects of the bee (4,19,27,35), geographic and climatic factors (6,25) and, possibly Varroa genotypes (7,8).

Kraus and Page (18) found that, in the Mediterranean climate of California, the initial population increased 300-fold during one year. Al Ghamdi (1) reported that over the period of one summer, the mite population increased to 81, 188 and 193-fold for the groups that were inoculated with 5, 10 and 25-mites, respectively, from the sticky board estimate, whereas the mite population estimates were recorded 2032, 1880 and 968 mites for the three groups, respectively. In temperate climates, the average increase in mite population is about 10-fold per year (11,17,31), while it increases up to 100-fold within one summer (12). In tropical climates, the parasites seems to be less virulent (32) whereas in sub-tropical climates the infestation rate is lower than in temperate climates (26). Khanhash (16) in Yemen, in *Apis mellifera yemenitica,* found different rate of Varroa infestation from one place to another. Thus the increasing mite population can wipe out the colonies in three to four years if not controlled (31).

Lange and Natskii (20) determined the Varroa infestation level by using the method of counting mites fall down on the hive floor during winter and flying season, and reported maximum mite's mortality in spring and autumn. By using the same method, Flories (10) investigated that the number of brood cells decreased in population by the end of spring flowering season when the average number of fallen mites increased from 0.4 to about 15 mites /day in two weeks. The number of fallen mites in late July and in August was 132.7 and 50.3 mites /day, respectively.

Allam (2) observed the highest number of naturally dead mites which was fallen down on the sticky cardboard during autumn (294 mites /colony every two weeks) followed by spring 114.1 mites /colony every two weeks, then winter where it reached to 64.2 mites /colony. However, the lowest numbers recorded were 3.7 mites /colony during summer. Romaniuk and Duk (34) found that the number of live *Varroa jacobsoni* females per 100 bees was low in the period from April to July (3.2:7.7). This number increased to 15.9 then to 23 in August, and September, respectively. The number of female per 100 worker larvae was 7.5 in May, 8.1 in June, 14.9 in July, 29.7 in August and 134.7 in September.

Rademacher (29) found that the natural death rate of mites varies with season; it increases slowly from May to July, andreaches at peak in September then falls in October. He also added that there are considerable variations between different years and locations. Matthes *et al.* (24) reported that, during first year, mites were present in 10.9% of worker brood, and 85.8% of drone brood increased in the second year to 31.5% then to 96.8 %, respectively. While studying the reproduction of female mites throughout autumn and spring in temperate climate, Marcangeli *et al.* (22) recorded higher reproduction rate in springtime. A large proportion of non- reproductive females was observed in autumn. Such variations in reproduction levels could produce differential growth rhythms in mite population during different seasons.

Detection of mite attack in a colony at initial stage is imperative for effective control of *Varroa jacobsoni*. Ritter (30) and De Jong (6) suggested the following diagnostic method: count mites that fall from the bee due to natural causes, examine bee samples for phoretic mites, check capped brood samples for mites while they are reproducing, and treat the colony with acaricide. For low infestation levels (below ten mites), the use of acaricide may be the only effective method for detecting the mites with acceptable level of precision in broodless colonies (31). If mite population is between 10 and 100, then the examination of hive debris should allow for detection (31). In fact, Ritter reported that adult bee or brood examination is insufficient for population levels below 100 mites per colony.

Fries *et al.* (11) compared different diagnostic methods for detection of Varroa mite at low infestation levels. They concluded that debris was more effective than examining the brood itself for low infestation rates. They said it was preferable to other methods because of its simplicity and efficacy.

Garza and Wilson (14) found highest correlation coefficient between the total number of mites and the adult bee sub-sample infestation ($r = 0.97$) followed by the sticky board ($r = 0.83$), while determining the infestation level of *Varroa jacobsoni* by sticky board and adult bee sub-sample wash. Liebig *et al.* (21) reported a close correlation between mites collected in hive debris and the size of the Varroa mite population.

The main aim of this research project was to investigate the population growth of *Varroa jacobsoni* in *Apis mellifera yemenitica* and *Apis mellifera carnica* under Saudi conditions, and investigate methods of detecting mite infestations at low rates.

Materials and Methods:

Ten honeybee colonies, five *Apis mellifera yemenitica* (Indigenous) and five *Apis mellifera carnica* (Carniolan) were used in the research program which was carried out in the Bee Research Units of King Saud University, Saudi Arabia.

The Carniolan bees were imported from Egypt, whereas the indigenous bees were brought from the south of the country in February (2001). In the end of February, the indigenous bees were transferred from log hives to the modern hives where the frames were provided with one-inch foundation strip to give them the chance to build the comb with small cells. After the transfer, the bees were treated with 'Perizin' to make sure that the colonies were free of mites. Then the bees were left till 13-April to get adaptation and were given intensive feeding during that period. The mites were removed from adult bees (host) by shaking the bee's combs in a polyethylene bag and after filling the bag with CO_2 the bees were shacked to separate the mites from the bees. Then the mites were collected in small tubes and, thereafter, 30-mites were introduced directly upon the bees in each colony on 13-April, 2001. The sticky boards were examined from each hive 2-hours and 24-hours after inoculation to record the number of mite fall down. After 2-hours in Carnica colonies, an average of 9mites fall down and in Indigenous colonies12-mites. After 24-hours, the average number of mites fall down was recorded as 14mites in Carnica and 12-mites in Indigenous colonies. Then after 24-hours, more mites were added to make mite population up to 30. In the first two weeks after inoculation, an average of 10mites fall down on sticky board, so we considered 20-mites instead of 30-mites. The groups were separated in different locations, and robbing screens were installed on the colonies to prevent robbing and drifting (15). Colonies were provided with sugar and pollen during the study period.

The mite population was estimated using bee and brood samples and hive debris. Between 200 and 300 live adult bees were taken from brood combs and stored in a deep freezer until examination. The mites were separated from the bees by vigorously shaking the bees in 70% ethanol for 3 to 4 minutes and this was repeated 3-times. The mites were washed from the bees using a hand – shower over a double wire screen. The number of bees and mites in the sample were counted to determine the level of infestation on adult bees. Data was adjusted to the number of mites/100 bees.

Samples of 200-sealed worker brood cells were examined as we could not find the drone brood because few cells were scattered in the colony while others were opened. So we only used worker brood cells, with a month interval. The cells were opened and the numbers of adult females in the cells and on the bees were counted. The total amount of brood in the colonies was estimated in each colony using the double –sampling technique described by Roger *et al.* (33). The adult bee population was estimated as described by Burgett and Burikam (5). The collection of samples was started 60-days after inoculation to give a chance to mite population for development.

The hive debris was collected weekly from April until September on cooking oil coated paper sheet placed on the bottom of the hive to estimate the natural mortality. A wire screen prevented bees from gaining access to debris or mites (30). The adult females were counted directly on the paper.

The total number of mites in each colony with brood was estimated, using the following two methods: (1) average daily down fall *120 (21); and (2) mites on bees and brood: the infestation rate of sampled bees * number of bees + infestation rate of sampled brood * number of brood cells (13).

Results

The experiment was started with ten colonies; five colonies of *Apis mellifera yemenitica* (Indigenous), and five colonies of *Apis mellifera carnica* (Imported). Each colony was inoculated with 20-mites and the colonies were maintained by feeding them on sugar and pollen. The data was recorded with one-month interval starting from 13th June, 2001 – 13th September 2001 to study and compare the mite development.

The mites population estimate based on average daily mites downfall on sticky board in indigenous bee colonies on inspection days were recorded 66.85, 108, 582.8 and 250 (3.34, 5.4, 29.14 and 12.5 folds increase, respectively) every 13th of June, July, August and September respectively. While the mite estimates in Carniolan bee colonies were higher in number as 121.7, 188.5, 1577 and 651.4 (6.08, 9.42, 78.85 and 32.57 folds increase) on their respective dates of inspection (Fig. 1, Tab. 1). But the analysis of variance showed a non-significant difference between the mites population estimates in Indigenous and Imported bees colonies based on sticky board method (F =1.16, P =0.323) but both populations presented a strong correlation (r = 0.999). The figure showed an increase in mites from June to August, then a decrease in September. In both honeybee races the highest peaks in mite population were observed in August, but the increase was more drastic in Carnica colonies.

Table: 1. Estimation of the number of Varroa mites in Indigenous and Carnica honeybee colonies. Calculation is based on average daily mites downfall * 120.

	Indigenous bees			Carnica bees		
Date	No. of mites fall/ week	Average no. of mites fall /day	Estimated No. of mites	No. of mites Fall /week	Average no. of mites/day	Estimated No. of mites
13-6-2001	3.9	0.55	66.85	7.1	1.01	121.7
13-7-2001	6.3	0.9	108	11	1.57	188.5
13-8-2001	34	4.85	582.8	92	13.14	1577
13-9-2001	14.6	2.08	250	38	5.42	651.4

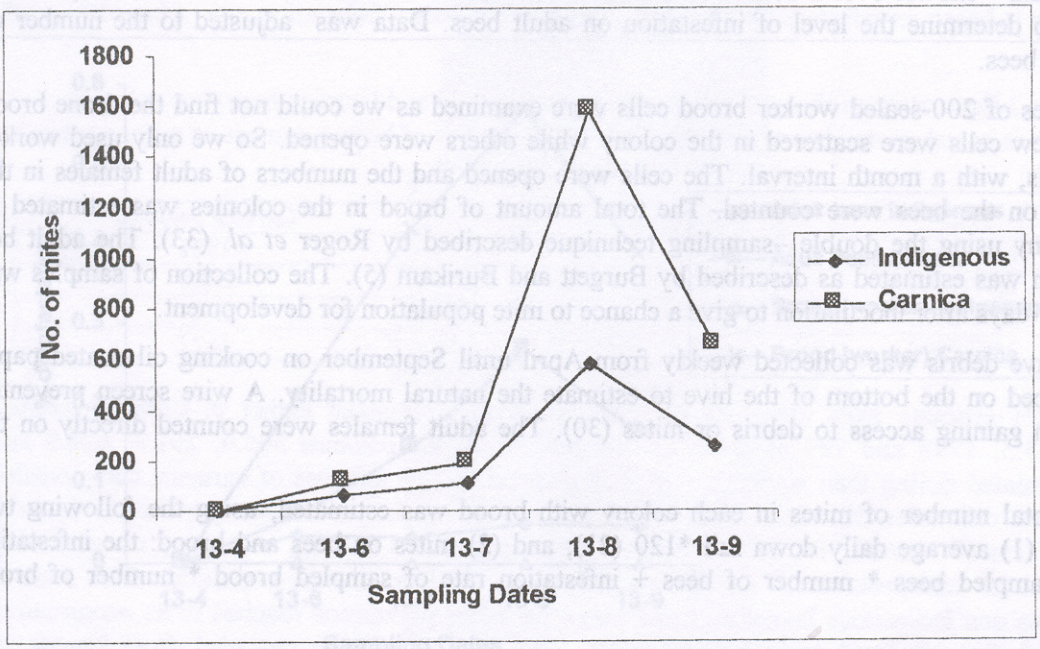

Figure 1. Estimation of the number of Varroa mites in Indigenous and Carnica honeybee colonies, calculation based on average daily mites downfall * 120.

The calculation is based on infestation rate of live bees * no. of bees + infestation rate of brood * no. of brood cells, in Carnica bee colonies presented higher mites population 1921,2486, 3624 and 1627 as compared to Indigenous colonies as 305.9, 291.3, 624.9 and 457.2 calculated during their respective dates of inspection (Fig. 2, Tab. 2). The graph depicted high mite population in the Carnica honeybee colonies where the increase in mites population reached to 96.05, 124.3 and 81.35 folds in June, July, and August, respectively, and then it declined towards September where the population remained 81.35 folds. The mite population in indigenous colonies increased 15.29, 14.56, 31.24 and 22.86 folds in June, July, August and September.

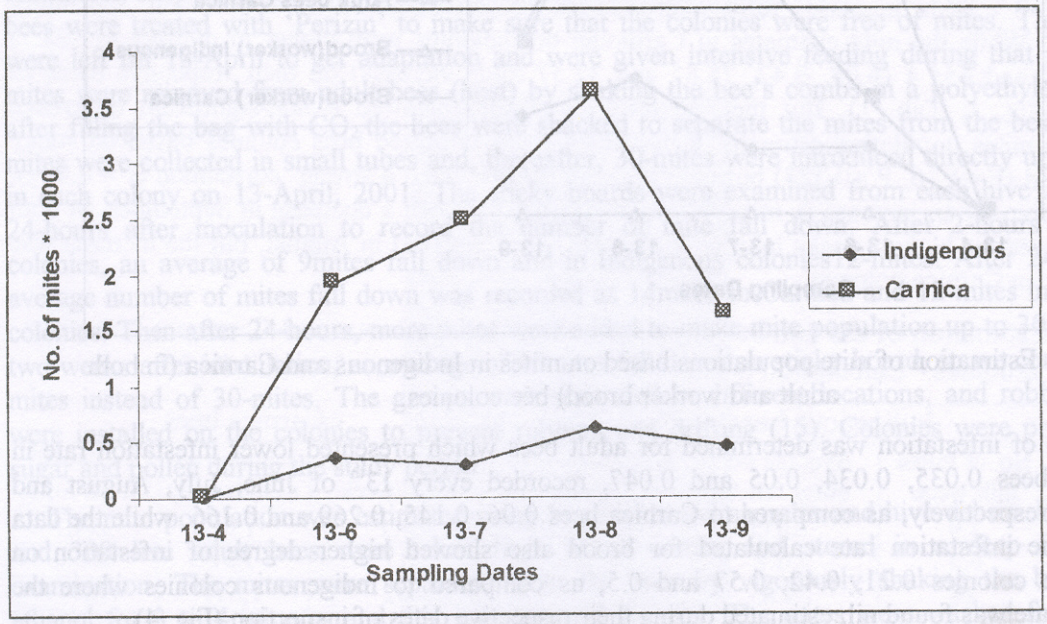

Figure 2. Estimation of the number of Varroa mites in Indigenous and Carnica honeybee races. Calculation is based on infestation rate of live bees * no. of bees + infestation rate of brood * no. of brood cells.

Table: 2 Estimation of the number of mites in Indigenous and Carnica honeybee colonies. Calculation is based on number of mites per live bee * number of bees + number of mites per brood cell * number of brood cells.

Date	Indigenous colonies	Carnica colonies
13-6-2001	305.9	1921
13-7-2001	291.3	2486
13-8-2001	624.9	3624
13-9-2001	457.2	1627

The mites estimate from the adult bee population also remained higher in Carnica colonies 524.4, 1135.5, 1793 and 789 as compared to indigenous bee colonies 305.9, 291.3, 624.9 and 457.2, calculated during their respective dates of inspection. The analysis of variance also revealed a significant difference between mite population based on the adult bee population in both honeybee races ($F = 19.86$, $P = 0.004$), but the mites population showed a positive correlation ($r = 0.644$) in both honeybee races. The Graph showed an increase in mite population from June in both Carnica and Indigenous honeybee races where the mites population reached to its maximum in August, and then declined towards September. The mite population recorded from broods of Carnica bee colonies was 1396.5, 1350.3, 1831 and 838.5 in June, July, August and September, respectively in comparison with indigenous colonies where the mite population remained nil during their respective dates of inspection (Fig. 3).

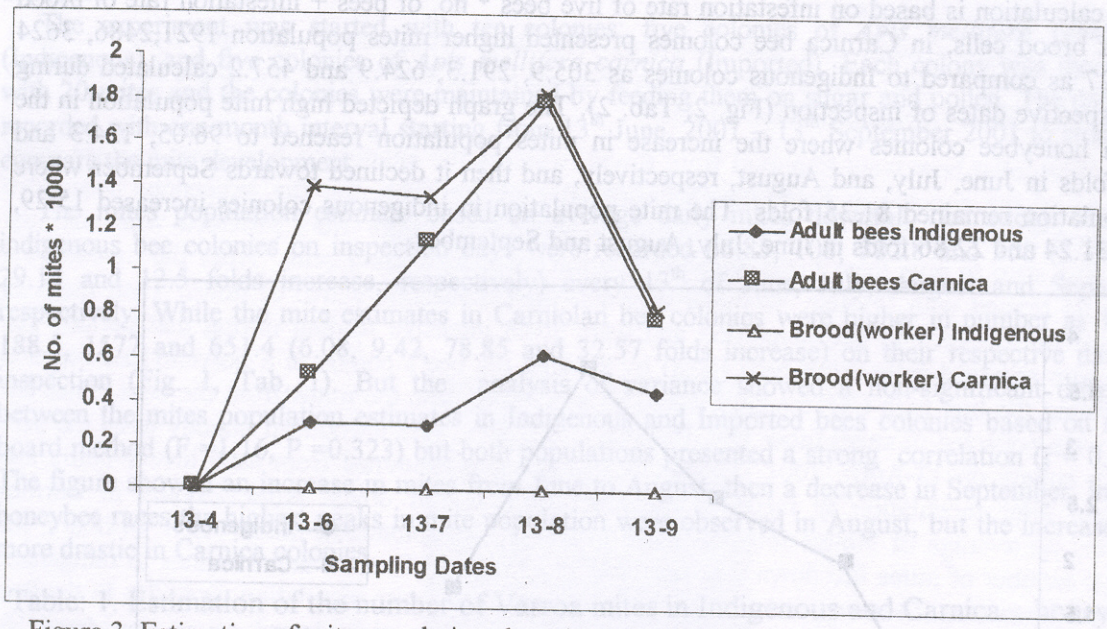

Figure 3. Estimation of mite populations based on mites in Indigenous and Carnica (in both adult and worker brood) bee colonies.

The rate of infestation was determined for adult bees which presented lower infestation rate in indigenous bees 0.035, 0.034, 0.05 and 0.047, recorded every 13th of June, July, August and September, respectively, as compared to Carnica bees 0.06, 0.145, 0.269 and 0.166, while the data regarding the infestation rate calculated for brood also showed higher degree of infestation on Carnica bee colonies 0.21, 0.42, 0.57 and 0.5, as compared to indigenous colonies where the infestation rate was found nil, estimated during their respective dates of inspection (Fig. 4).

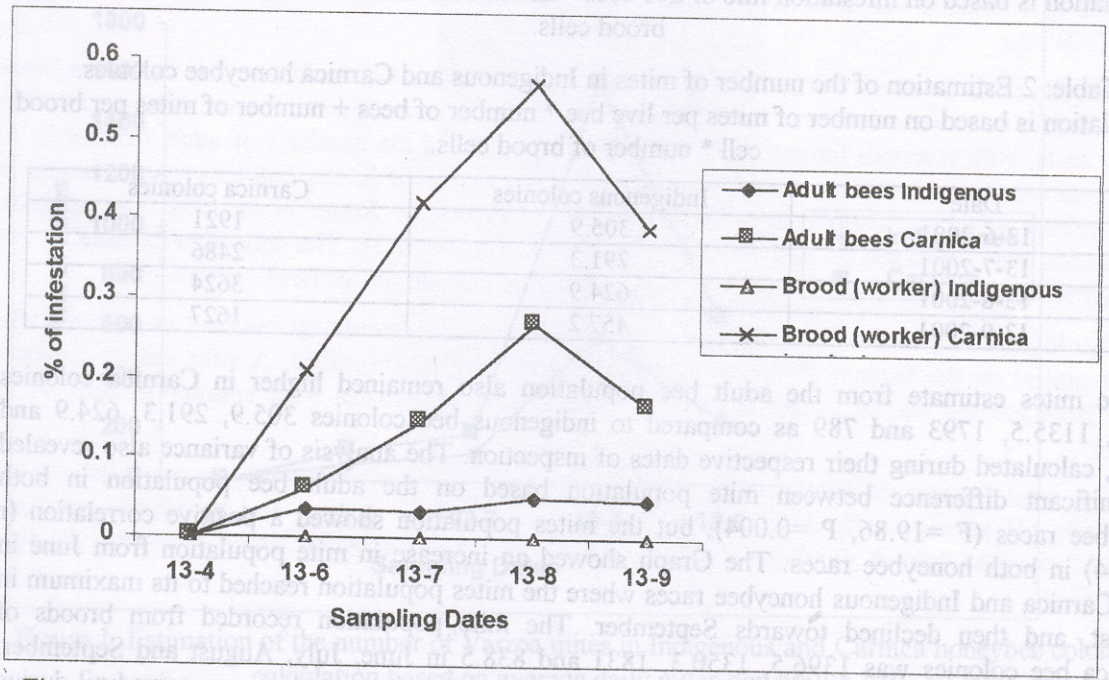

Figure 4. The percentage of mites infestation based on mites in Indigenous and Carnica (in both adult and worker brood) bee colonies.

One of the objectives of this study was also to compare the two methods used for the estimation of mite population (Tab. 3). The found sticky board method was better for the initial detection of mite population at low infestation levels. As in the case of present study, the mites were detected in all colonies, where as the adult and brood population detection method did not show mites in all colonies. Therefore, the sticky board method has the advantage over the adult and brood method, and sticky board detects the mites at initial infestation level and moreover, it is simple in use that can be used by the beekeepers.

Table: 3 Mites populations based on different sampling methods for the detection and estimating of *Varroa jacobsoni* at different infestation levels.

	Detection of mites from adult and worker brood		Sticky board method	
Date	Indigenous colonies	Carnica colonies	Indigenous colonies	Carnica colonies
13-6-2001	305.9	1921	66.85	121.7
13-7-2001	291.3	2486	108	188.5
13-8-2001	624.9	3624	582.8	1577
13-9-2001	457.2	1627	250	651.4

Discussion

The results based on natural mites downfall on sticky boards (Fig. 1) revealed that Carnica bees were more susceptible to *Varroa jacobsoni* compared to Indigenous bees, and presented a continuous increase in mites population till August, which is in agreement with Lange and Natskii (20) and Flories (10).

The estimate based on average number of mites on adults and brood presented a similar trend where the population of mites was more abundant among the Carnica bee colonies which showed more susceptibility to *Varroa jacobsoni* but yielded a higher population level of mites than those obtained from the natural mite fall on sticky board, which was in agreement with Fries *et al.* (11), and ALGhamdi (1). The results also showed an increase in mite population from June to August in both Carnica and Indigenous bee colonies, then a decrease in mites population towards September, which was in line with those of Romaniuk (34), Rademacher (29) who recorded the same trend in mite population growth rate. The mite population remained low in Indigenous bee colonies, which could be attributed to its more efficient defensive behavior.

A variation was observed in the rate of infestation both in adult and brood inside the colonies and between the honeybee colonies in the same treatment in both honeybee races, which were in agreement with Rosenkranz *et al.* (35); Fuchus (13); Khabash (16) and Al Ghamdi (1). The infestation of the adult bees varies from one comb to another (28). Liebig reported that adult bee estimates are more likely to be affected by the part of the hive from which the sample was taken. Ellis and Baxendale (9) found that the distribution of mites among adult bees and brood were affecting the results of the sampling method.

The sticky board is the most reliable method of detecting mite infestation when the population is low. In the case of the present study, the mites were detected in all colonies with sticky board method whereas the adult and brood population detection method did not show mites in all colonies which is in agreement with Fries *et al.* (11) who compared different diagnostic methods for detection of Varroa mite at low infestation levels. They concluded that sticky board method was more effective than examining the adult and brood itself for low infestation rates. They also declared it preferable to other methods because of its simplicity and efficacy.

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تطور أعداد طفيل حلم الفاروا *Varroa jacobsoni* على سلالتي نحل العسل المحلي (*Apis mellifera jemenitica*) والمستورد (*Apis mellifera carnica*) في منطقة الرياض

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كلية الزراعة – جامعة الملك سعود - ص.ب: 2460 الرياض 11451 - المملكة العربية السعودية

الملخص

أخذت سلالتين من سلالات نحل العسل وهما المحلي *Apis mellifera jemenitica* والمستورد *Apis mellifera carnica* لدراسة ومقارنة نمو طفيل حلم الفاروا على هاتين السلالتين تحت ظروف منطقة الرياض حيث تم إعداد خمسة طوائف من كل سلالة بعدد 20 حلم في منتصف شهر إبريل وبدأت متابعة تطور الإصابة بهذا الحلم شهرياً حتى شهر سبتمبر من نفس العام 2001م.

تم تقدير الأعداد المختلفة للحلم بطريقتين الأولى على أساس التساقط اليومي الطبيعي للحلم، والثانية على أساس الحلم المتواجد على الحشرات البالغة والحضنة ، وأوضحت نتائج الدراسة أن الزيادة في أعداد الحلم في النحل المستورد (الكارنيولي) عند استخدام الطريقة الثانية "بناء على الحلم الموجود على النحل البالغ والحضنه" كانت كبيرة حيث وصلت في المتوسط إلى 96 ، 124 ، 181 ، 81 ضعفا بينما في النحل المحلي وصلت إلى 15 ، 4، 31، 22 ضعفاً فقط وذلك في الأشهر يونيو ويوليو وأغسطس وسبتمبر على التوالي.

أما عند تقدير تعداد الحلم على أساس التساقط الطبيعي اليومي فقد أظهرت النتائج بأن الزيادة في أعداد الحلم كانت 08,9،68,32 ضعفاً بالنسبة للسلالة المستوردة (كارنيولي) بينما كانت 3 ، 5، 29 ، 12 ضعفاً في السلالة المحلية.

من النتائج السابقة يلاحظ أن التقديرات بناءً على الحلم الموجود على النحل الحي والحضنة كان أعلى من تلك التي قدرت بناء على موت الحلم الطبيعي على الألواح اللاصقة وهذا يتفق مع الدراسات التي أجريت بواسطة باحثين آخرين في دول أخرى كما وجد أيضا أن طريقه جمع الحلم المتساقط على الألواح كانت أفضل من الطريقة الأخرى في الكشف عن الإصابة في بداية العدوى.

الكلمات المفتاحية : حلم الفاروا ، سلالة النحل اليمني ، سلالة النحل الكرنيولي ، الرياض .