

## CONTROL OF STONE BROOD DISEASE IN HONEYBEE COLONIES USING SOME NATURAL MATERIALS AT NEW RECLAMID LANDS OF NOUBARIA REGION, BEHIERA GOVERNORATE, EGYPT

Amany S. M. Abou-Lila

Honey Bee Res, Dep. Plant. Protection Res. institute ARC. Dokki, Giza, Egypt

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**ABSTRACT:** Clove oil, Thyme oil at three concentrations (5 cm<sup>3</sup>, 2.5 cm<sup>3</sup> and 1.5 cm<sup>3</sup> / colony) and Ascorbic acid at 3 concentrations (5%, 2.5% and 1.5% /colony) an natural were applied against stone brood disease infesting honeybee colonies which caused by *Aspergillus flavus* at new lands of Noubaria region, Behiera Governorate, Egypt. The infested honeybee colonies were treated with the tested materials at three concentrations during the summer season, of 2019. The obtained results indicated that all tested materials were effective against the stone brood disease under field conditions. The average reduction of infection reached 84.40, 78,46 and 63.76% respectively after using clove oil at the rate (5,2.5 and 3 cm<sup>3</sup> / colony), respectively. Ascorbic acid treatments registered 83.26, 75.16 and 68.73% reduction in stone brood disease at the concentration (5, 2.5 and 1.5%) respectively. Thyme oil treatments recorded 77.36, 69.1 and 60.86% reduction in stone brood disease at the concentrations (5,2.5 and 1.5 cm<sup>3</sup> / colony) respectively.

**Key words:** *Aspergillus flavus*, safe control, honeybee, plant oils, stone brood.

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### INTRODUCTION

Stone brood disease of honeybee, *Apis mellifera* L., is usually caused by the fungus, *Aspergillus flavus*, and sometimes other *Aspergillus* species. These fungi are common soil inhabitants that are pathogenic to adult bees, other insects, mammals and birds. The disease is difficult to identify in its early stages of infection. The fungus grows rapidly and form a characteristic whitish, yellow spores. When a bee larva takes in spores they may hatch in the gut, growing rapidly to form a collarlike ring near the head After death the larvae turn black and become difficult to crush, therefore called the name stone brood. In this stage the larvae are covered with powdery fungal spores. A wet mount prepared from the larva shows mycelia penetrating throughout the insect. Eventually, the fungus erupts from the integument of the insect and forms a false skin. At this stage, the larva may be

covered with green powdery fungal spores. The spores of *A. flavus* are yellow green, Contwell (1974).

This spores can become so mummeries that they fill the comb cells which contain the affected larvae. *Aspergillus* spp. can be grown on potato dextrose or Sabouroud dextrose agars Bailey (1981), Al-tahawi (2009), Shimaanuki and David (1991), Shimanuki, *et al.* (1992). Several studies have shown essential oils to be effective in controlling bee diseases such as fungal diseases (Higes *et al.*, 1998) and reported two reasons to the fungal infection: the first is the highly infestation of varroa mite without controlling, and the second is the high humidity of most apiaries through summer season. Published research works in controlling this disease are few except some primary tests were done by (Zambonelli and Innocent 1989) using some chemicals

such sodium propionate, sorbic acid (as natural compounds).

The aim of the present study is to evaluate some natural products for controlling stone brood disease in Behiera Gov. during summer, season 2019.

## MATERIALS AND METHODS

### I-Materials:

- 1- Clove oil
- 2- Ascorbic acid
- 3- Thyme oil

Stock solution of Ascorbic acid was prepared (20% w/v) by dissolving 20 g in distilled water and completing total volume to 100 ml after primary screening of different concentrations. Ascorbic acid was applied at 3 concentrations (5%, 2.5% and 1.5% /colony), while Clove oil and Thyme oil were applied at three concentrations (5 cm<sup>3</sup>, 2.5 cm<sup>3</sup> and 1.5 cm<sup>3</sup> / colony).

### II-Experimental technique:

The experiments were conducted in Noubaria, Region, Behiera, Governorate during summer season, 2019 where thirty honeybee colonies nearly of equal strength were used in apiary. Every colony was provided with first hybrid Carniolan sister queen of about one year old.

The infested colonies was divided into 3 groups, each material was applied at 3 colonies (replicates) as follows:

Group (1) Clove oil, *Syzygium aromaticum* at (5, 2.5, 1.5 cm<sup>3</sup>) /colony.

Group (2) Ascorbic acid (5,2.5,1.5%) / colony by rate 3 cm<sup>3</sup>/ brood comb.

Group (3) Thyme oil, *Thymus vulgaris* (5, 2.5, 1.5 cm<sup>3</sup>) / colony.

and 3 colonies were served as untreated (control).

The tested materials were applied using plastic hand sprayer.

As for ascorbic acid treatment, every frame required 3 cm<sup>3</sup> of the tested

solution/ brood comb colony at the two sides. Clove oil and Thyme oil were applied at the rate of 1.5, 2.5 and 3 cm<sup>3</sup>/ colony (put on a piece of cotton between frames). These applications were repeated 4 times, at one week interval.

### III- Reduction percentages of stone brood disease:

Mummies number/ colony were counted before and after treatment (28 days). The reduction percentage of stonebrood mummies was calculated according to Henderson and Tilton (1955).

Statistical analysis was computed according to SAS institute computer program (1998).

Reduction % =

$$\left[ 1 - \left( \frac{\text{Treatment after}}{\text{Treatment before}} \times \frac{\text{Control before}}{\text{Control after}} \right) \right] \times 100$$

## RESULTS AND DISCUSSION

Three natural products, namely (clove oil, Ascorbic acid and thyme oil) were evaluated against *Aspergillus flavus* under field conditions.

Data in Tables (1,2,3,4) and graphically illustrated in Fig. (1) show that the average reduction of infection reached 84.40; 78.46 and 63.76% after using clove oil at the rates (5, 2.5 and 1.5 cm<sup>3</sup> / colony) respectively.

As for the treatments of Ascorbic acid at the concentrations (5, 2.5 and 1.5%), the reduction percentages were 83.26, 75.16 and 68.73 %, respectively.

Thyme oil treatments recorded 77.36, 69.10 and 60.86 % at (5,2.5,1.5 cm<sup>3</sup> / colony) respectively.

The obtained data clearly show that clove oil and Ascorbic acid were more effective than thyme oil for controlling stone brood disease in infested honeybee colonies.

**Control of stone brood disease in honeybee colonies using some natural .....**

The obtained data are in harmony with those of Aboulila and El-sisi (1998) who tested sodium benzoate, formic acid, ascorbic acid and citric acid as natural

compounds at different concentrations against *Ascaptharea apis*, and recorded good results in reduction.

**Table (1): Reduction percentages of infection with stone brood disease in *Apis mellifera* colonies treated with clove oil at Noubaria, Beheira Governorate**

Clove oil (rate cm <sup>3</sup> ) / colony	No. of colony	No. of mummies / colony before treatment ±SD	No. of mummies/ colony after treatment + SD	Reduction (%)
5 cm <sup>3</sup>	(1)	32.0	9.0	85.4
	(2)	35.0	8.0	83.5
	(3)	39.0	10.0	84.3
Mean		35.33±3.51	9.0±1.00	84.40±.95
2.5 cm <sup>3</sup>	(1)	37.0	13.0	81.8
	(2)	41.0	15.0	73.6
	(3)	43.0	14.0	80.0
Mean		40.33±3.06	14.0±1.00	78.46±4.36
1.5 cm <sup>3</sup>	(1)	29.0	17.0	69.6
	(2)	30.0	18.0	56.5
	(3)	30.0	17.0	65.2
Mean		29.66±0.58	17.33±0.58	63.76±6.66
Untreated (control)	(1)	25	48	
	(2)	29	40	
	(3)	37	60	
mean		30.33±6.11	49.33±10.07	

**Table (2): Reduction percentages of infection with stone brood disease in *Apis mellifera* colonies treated with ascorbic acid at Noubaria, Beheira Governorate**

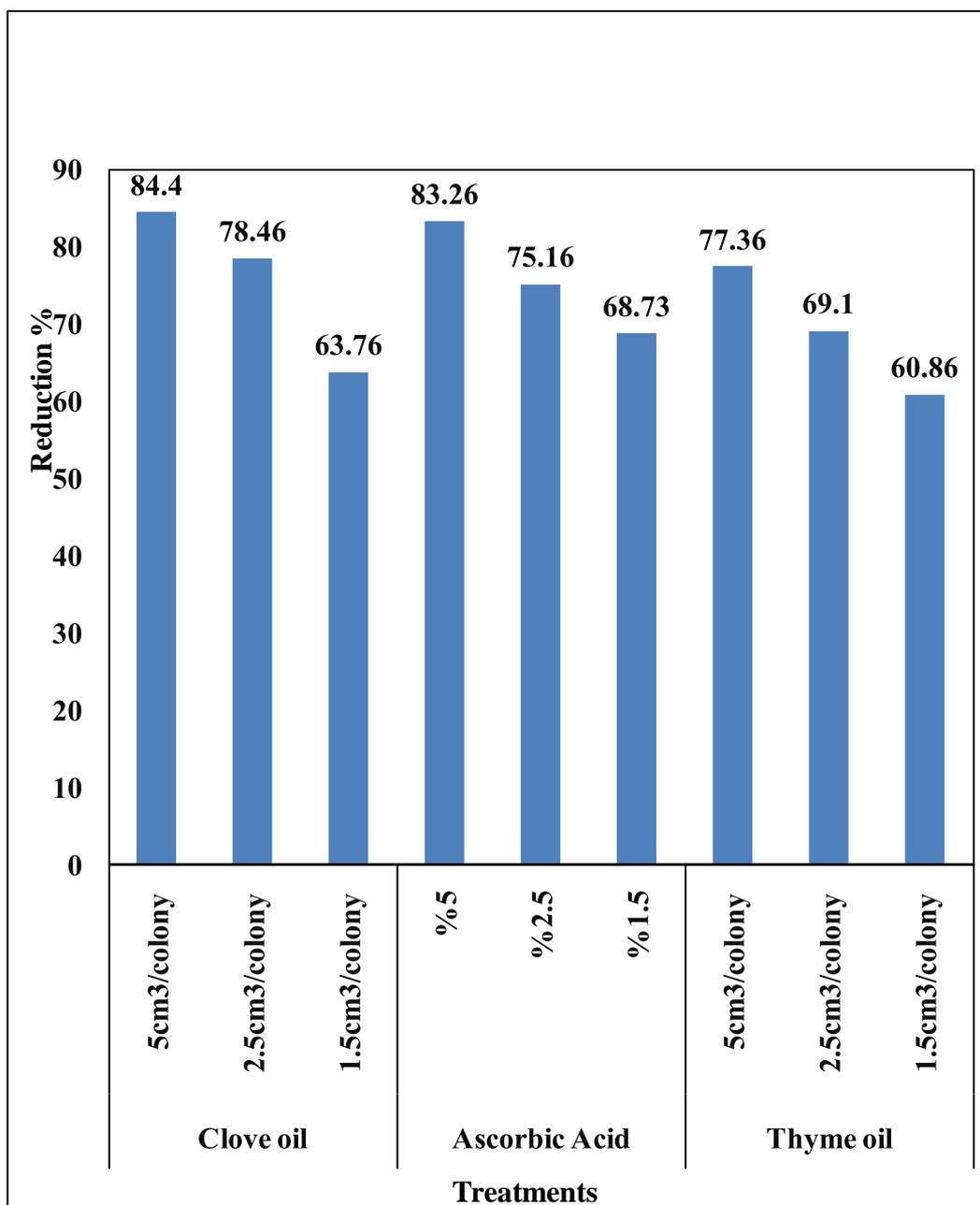
Ascorbic acid concentration	No. of colony	No. of mummies / colony before treatment±SD	No. of mummies/ colony after treatment + SD	Reduction (%)
5%	(1)	35.0	8.0	88.2%
	(2)	30.0	9.0	78.3%
	(3)	34.0	9.0	83.3%
Mean		33.0±2.56	8.66±0.58	83.26±5.0
2.5 %	(1)	40.0	15.0	80.5%
	(2)	31.0	12.0	72.0%
	(3)	37.0	10.0	73.0%
Mean		36.0±4.58	2.33±2.52	75.16±4.65
1.5 %	(1)	28.0	17.0	68.5%
	(2)	25.0	13.0	62.3%
	(3)	35.0	14.0	75.4%
Mean		29.33±5.13	14.66±2.08	68.73±6.55
Untreated (control)	(1)	25	48	
	(2)	29	40	
	(3)	37	60	
mean		30.33±6.11	49.33±10.07	

Table (3): Reduction percentages of infection with stone brood disease in *Apis mellifera* colonies treated with thyme oil at Noubaria, Beheira Governorate

Thyme oil (rate cm <sup>3</sup> ) /colony	No. of colony	No. of mummies before treatment SD±	No. of mummies/ colony after treatment SD±	Reduction (%)
5 cm <sup>3</sup>	(1)	41.0	14.0	82.3%
	(2)	39.0	16.0	70.3%
	(3)	42.0	14.0	79.5%
Mean		40.66±1.53	14.66±1.15	77.36±6.28%
2.5 cm <sup>3</sup>	(1)	31.0	17.0	71.6
	(2)	29.0	15.0	62.6
	(3)	32.0	14.0	73.1
Mean		30.66±1.53	15.33±1.53	69.10±5.68%
1.5 cm <sup>3</sup>	(1)	25.0	18.0	62.6
	(2)	28.0	16.0	58.7
	(3)	27.0	17.0	61.3
Mean		26.66±1.53	17.0±1.00	60.86±1.99%
Untreated (control)	(1)	25	48	
	(2)	29	40	
	(3)	37	60	
mean		30.33±6.11	49.33±10.07	

Table (4): Reduction % of infection with stone brood disease in *Apis mellifera* L. treated with some natural materials at Noubaria, Beheira Governorate

Treatments / colony		Mean no. of mummies ±SD	mean no. of mummies after treating ± SD	Reduction (%) ± SD
Clove oil	5 cm <sup>3</sup>	35.33 ± 3.51	9.0 ± 1.00	84.40 % ± 0.95
	2.5 cm <sup>3</sup>	40.33 ± 3.06	14.0 ± 1.00	78.46 % ± 4.36
	1.5 cm <sup>3</sup>	29.66 ± 0.58	17.33 ± 0.58	63.76 % ± 6.66
Ascorbic acid	5%	33.0 ± 2.65	8.66 ± 0.58	83.26 % ± 5.00
	2.5%	36.0 ± 4.58	12.33 ± 2.52	75.16 % ± 4.65
	1.5%	29.33 ± 5.13	14.66 ± 2.08	68.73 % ± 6.55
Thyme oil	5 cm <sup>3</sup>	40.66 ± 1.53	14.66 ± 1.15	77.36 % ± 6.28
	2.5 cm <sup>3</sup>	30.66 ± 1.53	15.33 ± 1.53	69.10 % ± 5.68
	1.5 cm <sup>3</sup>	26.66 ± 1.53	17.0 ± 1.00	60.86 % ± 1.99
Treated (control)		30.33 ± 6.11	49.33 ± 10.1	---
LSD at 0.05 = 4.76				



**Fig. 1. Mean reduction (%) of infection with stone broad disease in *Apis mellifera* colonies treated with some natural materials at Noubaria, Beheira Governorate**

Furthermore, Abdel fatah (1999, 2007) and Abdel hammed (2007) found that the highest percentage of reduction with chalk brood was obtained by formic acid, varrozal, sugar powder. Also, Abou lila (2012) tested some natural materials

against Chalk brood disease in Beheira governorate during 2009 – 2010 and recording good results. In addition Matter (2001) recorded that formic acid 85% had the highest efficiency (100%) against varroa and chalk brood diseases.

The disease affects only the bee larvae but it highly infectious and deadly to the brood, infected larvae darken and die Mabrouk (2008).

Generally the statistical analysis reported significant differences among compounds and concentrations for treatments.

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مكافحة مرض تحجر الحضنة في طوائف نحل العسل باستخدام بعض المواد الطبيعية في  
الأراضي الجديدة - منطقة النوبارية - البحيرة - مصر

أماني سعد مصطفى محمد أبو ليلة

قسم بحوث النحل - معهد بحوث وقاية النباتات - مركز البحوث الزراعية - دقي - جيزة - مصر

الملخص العربي

يهدف البحث إلى دراسة مدى فاعلية بعض المواد الطبيعية وهي زيت القرنفل وحمض الاسكوربيك (فيتامين C) وزيت الثيمول ( زيت الزعتر) على فطر *Aspergillus flavus* الذي يسبب مرض تحجر الحضنة في طوائف نحل العسل بالاراضي المستصلحة حديثا بمنطقة النوبارية.

تم إستعمال حامض الاسكوربيك بتركيزات ٥٪، ٢.٥٪، ١.٥٪ / طائفة رشاً على صورة حبيبات على أقراص الحضنة الصمابة بواسطة بخاخة بمعدل ٣ سم لكل قرص حضنة من الجهتين، وإستعمال زيوت القرنفل والثيمول بمعدلات ٥، ٢.٥ سم<sup>٢</sup> لكل طائفة نحل حيث تم وضع الزيت على قطعة قطن بين الإطارات في الخلايا وتم إجراء مكافحة لجميع المواد بعدد ٤ مرات كل أسبوع مرة في فصل الصيف ٢٠١٩ .

وقد أوضحت النتائج وجود فروق معنوية بين المركبات والتركيزات لجميع المعاملات وكانت كما يلي:

- ١- بلغت متوسطات النسب المئوية للخفض في الإصابة ٨٤.٤٠، ٧٨.٤٦، ٦٣.٧٦٪ بإستخدام زيت القرنفل
  - ٢- بلغت متوسطات النسب المئوية للخفض في الإصابة ٨٣.٢٦، ٧٥.١٦، ٦٨.٧٣٪ من حامض الاسكوربيك.
  - ٣- بلغت متوسطات النسب المئوية للخفض في الإصابة ٧٧.٣٦، ٦٩.١، ٦٠.٨٦٪ من زيت الثيمول
- بمعدلات وتركيزات ٥، ٢.٥، ١.٥ سم<sup>٢</sup>، ٥، ٢.٥، ١.٥٪ على التوالي ومن النتائج وجد أن معدل ٥ سم<sup>٢</sup> وتركيز ٥٪ طائفة أعلى أفضل النتائج في خفض النسب المئوية للإصابة بمرض تحجر الحضنة يليه معدل ٢.٥ سم<sup>٢</sup> وتركيز ٢.٥٪ طائفة نحل لجميع المواد المستخدمة.

طبقاً للنتائج المتحصل عليها يمكن التوصية باستخدام المواد الطبيعية في مكافحة مرض تحجر الحضنة في طوائف نحل العسل حيث أثبتت فاعليتها. كما ان هذه المواد سهلة الإستخدام ورخيصة الثمن وغير ضارة بالنحل ومنتجاته المختلفة عند مقارنتها باستخدام المواد الكيماوية في مكافحة المرض.

السادة المحكمين

أ.د. رضا عليوه سند إبراهيم مركز البحوث الزراعية - الجيزة

أ.د. مكرم ياسيلي عطيه كلية الزراعة - جامعة المنوفية