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Controlling of some honeybee *Apis mellifera* (Hymenoptera: Apidae) colonies diseases by bee venom

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Abstract:

Controlling of some diseases in honeybee *Apis mellifera* L. (*Hymenoptera: Apidae*) colonies such as bacterium (*Paenibacillus larvae*), fungus (*Aspergillus flavus*), protozoa (*Nosema apis*), mite (*Varroa destructor*) and the monitoring activities of honeybee colonies after controlling by bee venom had performed. Feeding with bee venom solution gave the highest mean of infect reduction with American foul brood disease compared with other treatment. Spraying with bee venom solution gave the higher mean of infect reduction with the stone brood disease than feeding with bee venom. The reduction percentage of honeybee workers infected with *N. apis* that treated with bee venom were 41.0, 50.0, 50.8, 46.0 and 3.7 % for feeding, spraying for bee venom solution, positive control (Artemisia), (Septazole) and negative control, respectively. The bee venom solution gave highest values of fallen varroa mite followed by (Formic acid and negative control), respectively in sealed brood and adult. The worker brood rearing activity after the treating with bee venom solution had a major peak of rearing in next summer and spring (653.7 inch^2/col.) while the spring season (544.2 inch^2 /col.). The general adult population mean of workers recorded for the treated colonies were (25700 worker/colony), it is also appeared that the highest population of colonies recovered from *V. destructor* was recorded (28800 worker/colony) followed by colonies treated against *P. larvae*, *A. flavus*, *N. apis*, and untreated colonies were recorded (28400, 24900, 23200 and 23100 worker/colony), respectively.

Introduction

The apiculture industry plays an important role in generating employment and in increasing family income in the rural areas of the world. Many developing countries are trying to improve the quality of their honeybee products, but they frequently encounter the main obstacle in apiculture; The diseases and pests of honeybees. Therefore, it is very important to prevent and control them (Wahba *et al.*, 2020). The use of manufactured chemicals (Whether pesticides or antibiotics) which is used to control diseases and pests inside the honeybee colonies represents a risk to consumer health and reduces the efficiency of vital honeybee products. The use of natural materials secreted by honeybees such as bee venom and other products and use it to combat some diseases and

pests of honeybee colonies to obtain clean honeybee products free from any harmful residues, whether from pesticides or manufactured antibiotics, which increases their biological efficiency (Wang *et al*., 2014). The use of bee venom solution feeding or spraying on bees inside laboratory cages has increased the longevity of workers and improves some of the characteristics of honeybee colonies such as hoarding behavior, bee population, brood rearing, stored pollen, stored honey areas, hygienic behavior, and foraging activity inner honeybee colonies, the bee venom contains many proteins, lipids, some enzymes and vitamins, (Metwally, 2016; El-Ettreby, 2018 and Wahba *et al*., 2020). One of the most an important diseases is dangerous for honeybee colonies, varroa mite, *Varroa destructor*, is external parasite attacks three casts of the honeybee colony, *A. mellifera* ., at their different ages preferred to the drone broods, workers and queen, and the parasite destroys the honeybee colony if mite was not noted and early controlled (Al-Abbadi and Nazer, 2003 and Mabrouk *et al*., 2019),

American foulbrood is a disease of the larval stage of honey bees (Species of the genus *Apis*) caused by *Paenibacillus larvae* (*P. larvae*), which is widely distributed. *P. larvae* is a bacterium that can produce over one billion spores in each infected larva. The spores are very long living and extremely resistant to heat and chemical agents and only the spores can induce the disease. used concentrations of bee venom treatment succeed to inhibiting *P. larvae* bacterium except the last three concentrations that exhibited non-antibacterial activity against this bacterium (Wahba, 2020). Stone brood is a fungal disease associated with honeybee brood, caused by *Aspergillus flavus*, and is a common and widespread disease that can result in severe reduction of emerging worker bees and thus overall colony productivity, the highest three concentrations of bee venom that induced weak activity against the growth of *A. flavus* fungus. (Jensen *et al*., 2013 and Wahba *et al.* , 2020). *Nosema apis* and *cerana* are an obligate microsporidian intracellular parasite infectious to honey bees. *Nosema apis* and *N. cerana* both parasitize honeybees, *N. cerana* has geographically outcompeted *N. apis*. Severe *N. cerana* infections (Nosemosis) can cause bee mortality and have been correlated with colony losses (Li *et al*., 2018).

Therefore, this work aims to study the controlling with bee venom inside the honeybee colonies on the bacteria (*P. Larvae*), fungi (*A. flavus*), protozoa (*N. apis*) and mite (*V. destructor)* and the monitoring activities of honeybee colonies after controlling by bee venom inside honeybee colonies.

Materials and methods

The present investigation was carried out as a field trial to controlling the causes of American foul brood (AFB) , stone brood, nosema and varroa diseases at the apiary of the Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt, during years, 2019-2020.

1. Effect of bee venom on *Penibacillus larvae* bacterium:

The efficiency of the bee venom in controlling the causative agent of the bacterial disease was examined in private colonies placed away from the apiary; nine honeybee colonies of about equal strength (3 brood and 2 honey with pollen combs / hive) headed by open mated hybrid Carniolan queens, all colonies divided into 3 groups, each group treated with a different treatment and consisted of 3 replicates.

On July 2019, 100 ml spore suspension of pure bacterial isolate was prepared, completed to 450 ml with sugar syrup (w: v, 1:1), and its germ concentration was estimated by microscopy counting slide as 10^4 spore / ml , whereas each replicate of honeybee colonies was fed with 50 ml, and replicated three times by interval on week between each twice, then colonies were daily examined until the AFB symptoms appeared in all colonies after 21 days from the last feeding.

Whereas the AFB disease symptoms can be recognized by inserting a matchstick into the infected brood cell and drawing out, it is giving a threadlike longer than 2.5 cm (Morse and Nowogrodzki, 1990 and Hashish *et al.*, 2008 and 2016).

Hence, on the same day of disease symptoms appearance, all honeybee colonies treated three times by intervals one week with the following materials and manner:

Group (1): (Bee venom): Three infected colonies were treated with bee venom solution by feeding (5 mg/100 ml sugar syrup).

Group (2): (Positive control): Three infected colonies were treated by the veterinary antibiotic Tylosin as a positive control, since it received to each colony 200 mg of Tylosin tartrate antibiotic mixed with 20 g sugar powder on top of the brood frames (**Peng *et al*., 1996**).

Group (3): (Negative control): Three infected colonies were fed with sugar syrup only as a negative control.

Subsequently, the progress of disease was evaluated during and after treating by weekly inspection of all experimental groups. The diseased larvae were counted until the end of the experiment. The colonies with no visible signs of AFB disease were considered recovered.

2. Effect of bee venom on stone brood disease (*Aspargillus flavus*) :

Nine honeybee colonies appeared signs of stone brood disease in spring season 2019 were subjugated to honey bee venom according to the following plan; before the trial, both the stone brood infestation level and the size of all colonies were monitored to obtain three homogeneous experimental groups of bee colonies as the following:

Group (1): (Feeding treatment): 3 replicates of honeybee colonies were fed with mixture of bee venom with sugar syrup solution as 5 mg venom/ 100 ml sugar syrup 1:1.

Group (2): (Spraying treatment): 3 replicates of honeybee colonies were subjugated to bee venom solution (5 mg venom/ 100 ml distilled water) spraying on the workers and brood combs.

Group (3); (Positive control): 3 replicates of honeybee colonies were treated by human antibiotic Ultragriseofulvine and that used as a positive control to decrease a stone brood infestation by dose of 12.5 mg / Liter water spraying on the bees and wax combs.

Group (4: (Negative control): The honeybee workers and combs of 3 replicates were sprayed by only dilluted sugar syrup (1/2 : 1) as a negative control colonies.

3. Effect of bee venom on honeybee workers infected with *Nosema apis*:

Fifteen colonies were equalized to 8 combs covered with bees contain (5 brood combs + 3 honey combs) during late Autumn, 2019. One hundred honeybee workers infected by Nosema spores were collected from each colony, analyzed and checked by a microscope. Fresh Nosema spores from honeybee colonies were collected from each colony and processed separately according to (Botías *et al*., 2012 and Martín-Hernández *et al*., 2012).

Five groups of honey bee worker each group contain 3 colonies (Infected replicates with Nosema) were made as the following:

Group (1): (Feeding treatment): The colonies were fed with bee venom solution (5 mg/100 ml sugary solution).
Group (2): (Positive control of feeding): The colonies were fed on sugar syrup with Artemisia (100 ml boiled Artemisia/ litre of sugar surup).
Group (3): (Spraying treatment): The colonies were sprayed with bee venom solution (5 mg/100 ml distilled water).
Group (4): (Positive control of spraying): The colonies were were sprayed with septazole solution (2ml/ Liter of sugar syrup).
Group (5): (Negative control): Honeybee colonies fed on sugar syrup without any treatment.

Then, all colonies were treated once a week for six weeks to get rid a Nosema. One hundred bees were removed randomly from each colony and dissected to assess their degree of infection, the abdomens were introduced into sterile eppendorf microtubes filled with 200 µl of distilled water , and after crush of them, the spores were counted using a haemocytometer.

4. Efficacy of bee venom against *Varroa destructor* :

This application was performed from November to February 2019 in nine colonies infested with varroa mites, whereas before start of the treating, the infestation percentage was determined by immersion of 100 honeybee workers/ colony in soap solution, fallen varroa numbers observed and caculated on sheet paper per adult workers, the same matter was also performed for the brood cells. Then, The colonies were divided into three groups, each group contains on three replicates that subjugated along 8 weeks (56 days) to different treatment as the following;
Group (1): Spraying with bee venom solution (5 mg/100 ml water)/ replicate.
Group (2): Treated with Formic acid solution 65% as a positive control by vaporization using cardboard slices satisfied with this formic.
Group (3): Used as a negative control (Untreated colonies).

However, for along eight weeks that estimated the means of vival varroa mites on adults' workers and brood cells of all colonies before and after treating, and then the reduction percentages of infesting with Varroa mites were calculated by using of Henderson and Tilton equation (1955) which is:

% Reduction = (1- T_a X C_b / T_b X C_a) X 100

T_a = after Treatment T_b = before Treatment
C_a = after control C_b = before control

5. Biological activities of honeybee colonies:

Some population biomarkers were measured as indicators of the effect of the infection and treatments on the honeybees in comparison with the none-treated colonies. The biological activities of the honeybee workers; brood rearing and worker population were recorded at 21 days' intervals (Marzouk, 2009).

5.1. Activity of colonies in rearing worker brood:

The areas that occupied by unsealed and sealed worker brood were measured by using a frame divided into square inches. The measurements were taken at 21 day intervals included a complete generation of bee's worker.

5.2. Worker population:

This Parameter was determined one hour before sun set; combs of each colony were weighed with and without the adhesive workers, (workers covering them). On the other hand, sample of workers from each colony was taken, weighed and the average weight of a bee worker was estimated and recorded according to formula of (Abd Al-Hady, 2007 and Marzouk, 2009).

Average weight of a worker = Weight of worker's sample / No. of workers in this sample

5.3. The number of workers (in thousand) / colony =

Total weight of workers (in gm.) / Average weight of one worker (in gm.)

6. Statistical analysis

The results were described with means and standard diviation, data of all treatments were analyzed in a randomized complete block design (ANOVA) by MSTAT-C version 1.41 **(**Sendecor and Cochran, 1980). In addition, using graph pad prisma version 3.03 for windows, software. All means were compared by Duncan's multiple range test at level 0.05 (Duncan, 1955).

Results and discussion

1. **Effect of bee venom against *Penibacillus larvae* bacterium:**

Data in Table (1) showed the means of reduction% of infection with AFB disease inside honeybee colonies which treated with bee venom and Tylosin, whereas they were 66.7±19.1, 54.8±15.8, and 7.8±3.2 opposite feeding with bee venom solution, positive control and negative control, respectively. These results indicated that feeding with bee venom solution gave the highest mean of reduction of infection with American foul brood disease followed by the positive control treatment, then negative control.

Table (1): The mean of reduction (%) of infection with American foul brood disease inside honeybee colonies treated with bee venom during year, 2019.

Treat.	Rep	1st week		2nd week		3rd week		4th week		Mean/week		% Red
		Before	After	B	A	B	A	B	A	B	A	
Bee venom (Feeding)	R1	100	45	45	22	22	9	9	0	44	19.0	66.7 ±19.1 A
	R2	100	42	42	19	19	5	5	1	42	16.8	
	R3	100	39	39	16	16	3	3	0	40	14.5	
Mean		100	42.0	42.0	19.0	19.0	5.7	5.7	0.3	41.7	16.8	
% Red		53.2		52.3		68.2		93.2		66.7		
Positive control (Veterinary antibiotic Tylosin)	R1	100	50	50	38	38	13	13	3	50	26.0	54.8 ±15.8 B
	R2	100	43	43	20	20	10	10	4	43	19.3	
	R3	100	38	38	21	21	9	9	0	42	17.0	
Mean		100	43.7	43.7	26.3	26.3	10.7	10.7	2.3	45.2	20.8	
% Reduction		51.3		36.4		56.8		74.7		55		
Negative control (Sugar syrup)	R1	100	86	86	81	81	78	78	70	86	78.8	7.8 C ±3.2
	R2	100	90	90	79	79	81	81	72	88	80.5	
	R3	100	93	93	95	95	80	80	65	92	83.3	
Mean		100	89.7	89.7	85.0	85.0	79.7	79.7	69.0	88.6	80.8	
% Red		10.33		4.7		5.3		10.7		7.8		

The statistical analysis of data indicated that there were significant differences between feeding with bee venom solution compared with a negative control (Fed on sugar syrup only), also the positive control (Tylosin), the present investigations are supported by similar results were obtained by Metwally (2016) and El-Ettreby (2018), they mentioned that bee venom had inhibitory affect on viability and growth of *P. larvae* under field conditions, and the bee venom had a direct effect *in vivo* against the

vegetative cells of *P. larvae* bacterium and that very low concentrations of bee venom required to inhibit this bacterial growth, and this result also based on the bee venom component (milliten, 26 amino acids, phospholipase A2 and minerals) these compounds are responsible for the main parts of the biological activity of bee venom and these substances were that the reason to having of the bee venom on the antibacterial activity, On the other hand it's important to note that the concentration of experimental substances were significantly different, especially in regard to the active components.

2. Effect of bee venom against stone brood disease (*Aspargillus flavus*):

Data in Table (2) showed the mean percentage of reduction of infection with stone brood disease inside honeybee colonies which treated with bee venom and Ultragrizeofulvine as a positive control, it happened little of more reduction of the disease in the treated hives with bee venom spraying than feeding, whereas the mean reduction% of infection were 35.2±14.84 and 33.7±8.81 for spraying and feeding respectively, but not significant difference presented between both of them on bee, while the treating of the colonies with Ultra grizofulvine had the highest and significant effect against the fungus which resulted in 85.53±6.546 as a mean reduction% of infection, and all those compared by the negative control hives which recovered from disease by mean of reduction% equaled 7.01±1.993.

The statistical analysis of data indicated that there were significant differences between the treatment with bee venom whether feeding or spraying in a side and the positive control (Ultra grisevulvin) in other side, and these results were agreed by Zolfagharian *et al*. (2016) and Sang *et al*. (2016). They mentioned that the increasing the antimicrobial activity of bee venom could not be due to the antimicrobial effect of *A. flavus* fungus, because the *A. flavus* fungus has an inhibitory effect at high concentrations.

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Table (2): The mean of reduction (%) of infection with stone brood disease inside honeybee colonies treated with bee venom during year, 2019.

Treatment	Rep.	Number of mummies		Reduction %
		Before	After	
Bee venom (Feeding)	R1	45	30	29.7
	R2	38	25	27.6
	R3	42	22	43.8
Mean ± Sd		41.67 ±3.512	25.67 ±4.041	33.7±8.81^{B}
Bee venom (Spraying)	R1	42	19	52.3
	R2	48	35	27.6
	R3	39	27	25.7
Mean ± Sd		43.0 ±4.583	27.0 ±8.0	35.2±14.84^{B}
Positive control (Ultragriseovulvin)	R1	48	9	80.24
	R2	40	6	83.5
	R3	30	2	92.85
Mean ± Sd		39.33 ±9.018	5.67 ±3.512	85.53±6.546^{A}
Negative control	R1	39	37	5.13
	R2	44	40	9.1
	R3	59	55	6.8
Mean ± Sd		47.33 ±10.408	44.0 ±9.644	7.01±1.993^{C}

3. Effect of bee venom against *Nosema apis* :

Data in Table (3) showed that the percentages of adult honeybee workers infected with *N. apis* were 41.0, 50.0, 50.8, 46.0 and 3.7 % in the colonies which treated with bee venom feeding, and spraying, and with Artemisia as a natural positive control, septazole as antiseptic positive control and negative control respectively. These results indicated that spraying with bee venom solution gave the highest mean of reduction of infection with *N. apis* disease followed by feeding with bee venom solution. Statistical analysis from the data indicated that there were significant differences between feeding, spraying for bee venom solution compared with negative control (Fed on sugar syrup), while there were insignificant differences between the mean of reduction of infection with *N. apis* by effect of the spraying with bee venom solution and the natural positive control (Artemisia), these results are agreed with (Yemor, 2016).

Table (3): Effect of bee venom solution on the mean number of honeybee workers infected with *Nosema apis* during period from 1/11/2019 to 15/12/2019

Treatment		Feeding				Spray				Negative control	
		Bee venom		Positive control natural material (Artemisia)		Bee venom		Positive control antiseptic (Septazole)		Sugar syrup	
		Mean ±SD	% Red	Mean ±SD	% Red	Mean± SD	% Red	Mean± SD	% Red	Mean± SD	% Red
1st week	Before	100.0±0.0	43.4	100.0±0.0	29.8	100.0±0.0	45.5	100.0±0.0	35.9	100.0±0.0	2.7
	After	55.0±5.0		68.3±12.58		53.0±7.0		62.3±6.51		97.3±2.52	
2nd week	Before	55.0±5.0	30.3	68.3±12.58	51.1	53.0±7.0	25.1	62.3±6.51	22.4	97.3±2.52	3.4
	After	37.0±10.44		40.7±10.02		38.3±5.51		46.7±5.77		94.0±4.58	
3rd week	Before	37.0±10.44	39.6	40.7±10.02	46.0	38.3±5.51	51.5	46.7±5.77	37.8	94.0±4.58	0.0
	After	23.7±7.37		23.3±5.77		19.7±2.08		30.8±3.82		99.7±0.58	
4th week	Before	23.7±7.37	57.9	23.3±5.77	58.6	19.7±2.08	47.2	30.8±3.82	64.1	99.7±0.58	6.7
	After	9.3±1.53		9.0±2.0		9.7±0.58		10.3±2.52		93.0±2.65	
5th week	Before	9.3±1.53	55.0	9.0±2.0	53.5	9.7±0.58	70.9	10.3±2.52	52.3	93.0±2.65	4.3
	After	4.0±1.0		4.0±1.73		2.7±1.53		4.7±1.53		89.0±2.65	
6th week	Before	4.0±1.0	20.8	4.0±1.73	65.7	2.7±1.53	60.8	4.7±1.53	61.8	89.0±2.65	5.3
	After	0.3±0.58		1.3±1.53		1.0±1.0		1.7±1.53		84.3±5.13	
Mean/ week	Before	38.2±3.46	41.0	40.9±3.0	50.8	37.2±2.43	50.2	42.5±0.79	46.0	95.5±2.08	3.7
	After	21.6±3.59		24.4±2.61		20.7±2.41		26.1±0.66		92.9±2.87	
% Reduction		41.0 b		50.8 a		50.0 a		46.0 b		3.7 c	

4. Efficacy of bee venom against *Varroa destructor* :

Data in Table (4) showed that the mean number of fallen varroa mite on paper sheet during the experiment from the brood and adult bees that treated spraying with bee venom solution (5 mg venom /100 ml water) against the formic acid as a positive control and the negative control (Sugar syrup only), respectively. Whereas, these results indicated that treating with bee venom solution gave highest values of fallen varroa mite followed by formic acid treatment then the negative control respectively whether in sealed brood or adult. Statistical analysis from the data indicated that there were insignificant

differences between bee venom solution on fallen varroa, formic acid as positive control compared with control colonies in brood and adult. At the end of the treatment, more brood was presented in treated colonies with bee venom (81.8±2.13) and not significant differences between the mean brood on colonies treated with formic acid (79.1±1.44), whereas the extension of the sealed brood area of the treated hives of formic acid was significantly difference from that of the control colonies (57.4±4.88). In adult workers was presented in treated colonies with bee venom (83.6±1.31) and not significant differences between the mean of colonies treated with formic acid (81.9.1±1.95), whereas the extension of the adult workers by formic acid was significantly differences from that of the control colonies (57.5±2.57). The mean of percentage reduction of varroa mites on adult worker and brood cells during 8th week were recorded 100% inner colonies treated with bee venom compared with control colonies.

In addition, more adult bee infestation was recorded in treated hives. The percentage of reductions of the daily fallen mites increase in treated groups (Bee venom and positive control (Formic acid) than the control group), respectively, these results are agreed with (Rashid *et al*., 2011).

The results showed that the bee venom is a promising candidate for controlling *Varroa* mites. it has many advantages easy to use, safe for beekeepers, it also causes no honeybee toxicity, no loss of queen or brood, adult bee mortality, Furthermore, it can also be concluded from this study that, bee venom proved as effective against mites control, therefore they can be used safely without any side effects in controlling *Varroa* mites, it proved the effectiveness of bee venom as a natural compound in reducing the varroa parasite population in honey bee colonies, and this is consistent with Lodesani *et al.* (2008) and Mabrouk *et al*. (2019), they mentioned that the use of natural compounds against *V. jacobsoni* in honey bee colonies occured mortality reached 95% such as coumaphos (Preizin), fluvalinate (Apistan), flumethrin (Bayvarol), powder of thymol and formic acid.

Table (4): Weekly mean no. of the fallen varroa mites/col. after treating the honeybee colonies with bee venom solution during period from 1/11/2019 to 1/1/2020.

Treatment		Bee venom (Spraying)	Positive control (Formic acid)	Negative control
1st week	**Brood**	43.7±8.74	39.7±2.08	19.0±2.0
	Adult	48.3±3.06	43.0±6.24	19.0±4.58
2nd week	**Brood**	60.7±1.53	55.7±4.93	31.7±2.52
	Adult	61.7±4.73	55.3±6.51	35.0±6.56
3rd week	**Brood**	72.3±3.06	69.0±2.65	46.0±5.29
	Adult	78.0±3.0	74.0±6.24	44.7±6.66
4th week	**Brood**	86.0±4.0	81.3±3.06	55.0±6.56
	Adult	88.3±2.08	89.7±1.53	54.7±4.16
5th week	**Brood**	93.0±1.0	93.0±2.0	63.0±8.0
	Adult	94.7±0.58	95.3±2.08	67.7±2.52
6th week	**Brood**	99.0±1.0	96.0±1.0	77.0±6.08
	Adult	98.0±1.0	98.3±1.53	72.0±2.65
7th week	**Brood**	99.7±0.58	98.0±1.0	82.3±7.51
	Adult	99.7±0.58	99.7±0.58	79.7±1.53
8th week	**Brood**	100.0±0.0	99.7±0.58	85.0±5.0
	Adult	100.0±0.0	100.0±0.0	87.7±3.21
Mean/week	**Brood**	81.8±20.13 a	79.1± 1.44 a	57.4±4.88 b
	Adult	83.6± 1.31 A	81.9± 1.95 A	57.5±2.57 B

Table (5): Effect of bee venom on the mean of survival number and percentage of reduction of Varroa mites/100 adult worker and 100 brood cells during period from 1/11/2019 to 1/1/2020.

Treatment			Bee venom	% Red.	+ve control (Formic acid)	% Red.	-ve control	% Red.
1st Week	Brood	Before	100.0	30.4	100.0	25.5	100.0	19.0
		After	56.3		60.3		81.0	
	Adult	Before	100.0	36.1	100.0	29.6	100.0	19.0
		After	51.7		57.0		81.0	
2nd Week	Brood	Before	52.0	14.7	58.3	14.3	77.0	11.3
		After	39.3		44.3		68.3	
	Adult	Before	51.7	7.6	57.0	2.2	81.0	19.8
		After	38.3		44.7		65.0	
3rd Week	Brood	Before	39.3	10.8	44.3	11.4	68.3	20.9
		After	27.7		31.0		54.0	
	Adult	Before	38.3	32.4	44.7	41.0	65.0	14.9
		After	22.0		26.0		55.3	
4th Week	Brood	Before	27.7	39.3	31.0	27.6	54.0	16.7
		After	14.0		18.7		45.0	
	Adult	Before	22.0	35.0	26.0	51.6	55.3	18.0
		After	11.7		10.3		45.3	
5th Week	Brood	Before	14.0	39.1	18.7	54.4	45.0	17.8
		After	7.0		7.0		37.0	
	Adult	Before	11.7	36.4	10.3	36.0	45.3	28.7
		After	5.3		4.7		32.3	
6th Week	Brood	Before	7.0	77.0	7.0	8.0	37.0	37.8
		After	1.0		4.0		23.0	
	Adult	Before	5.3	56.4	4.7	58.2	32.3	13.3
		After	2.0		1.7		28.0	
7th Week	Brood	Before	1.0	61.0	4.0	35.0	23.0	23.0
		After	0.3		2.0		17.7	
	Adult	Before	2.0	79.3	1.7	75.6	28.0	27.5
		After	0.3		0.3		20.3	
8th Week	Brood	Before	0.3	100.0	2.0	82.3	17.7	15.3
		After	0.0		0.3		15.0	
	Adult	Before	0.3	100.0	0.3	100.0	20.3	39.4
		After	0.0		0.0		12.3	
Mean /week	Brood	Before	30.2	25.3	33.2	21.6	52.8	19.3
		After	18.2		21.0		42.6	
	Adult	Before	28.9	28.6	30.6	25.6	53.4	20.4
		After	16.4		18.1		42.5	

5. Biological activities of honeybee colonies:

The results obtained in Table (6) showed that the worker brood rearing activity after the treating with bee venom solution and other components inside honeybee colonies was continued on years-around during 2019-2020 years of study. This activity had a major peak of rearing in next spring and summer and reached the highest in May (881.4 inch^2 /col.). After that, the worker brood rearing curve was gradually declined until recorded the least brood quantity at the end of November (6.1 inch^2 /colony)

This activity had a major peak of rearing in the summer season, (Mean of June, July and August) was recorded (653.7 inch^2/colony) while spring season (544.2 inch^2 /colony). After that, the worker brood rearing curve was gradually declined until recorded the lowest brood quantity at Winter season (97.4 inch^2 /colony).

The numbers of adult workers population (in thousands) after treating with bee venom solution of honeybee colonies treated from some pathogens during different periods and seasons of the year study. The increase in colony population followed the brood trend. The general adult population mean of workers recorded for the treated colonies were (25700 worker/colony), It is also appeared that the highest population for colonies which recovered from *V. destructor* was recorded (28800 worker/colony) followed by colonies treated against *P. larvae*, *A. flavus*, *Nosema apis*, and control colonies were record (28400, 24900, 23200 and 23100 worker/colony) respectively, the colonies were significantly differed. The highest mean number of worker population was recorded during May, (46400 worker/colony) followed by July, (43200 worker/colony), but the lowest mean number of worker population was recorded in November, (8100 worker/colony). The recorded data showed the positive effect of using bee venom for treating certain pathogens in increasing the amount of worker brood areas and increasing of adult worker population, these results agreemented with (El-Ettreby, 2018 and Wahba *et al*., 2020).

Table (6): Mean of worker brood areas (inch^2) and mean no. of worker population (Inthousands) produced at 3 week intervals by honeybee colonies treated with bee venom solution during period from 1/9/2019- 25/8/2020.

Date	*P. larvae*		*A. flavus*		*Nosema apis*		*V. destructor*		Control		Mean	
	Brood	Population	Br.	Pop.	Br.	Pop.	Br.	Pop.	Br.	Pop.	Br.	Pop.
01\09\2019	200.8	13.4	199.5	13.3	193.7	13.9	183.5	13.4	8.1	13.1	191.1 F	13.4 g
21\09\2019	213.8	13.3	173.5	12.8	273.0	9.5	270.5	7.9	2.6	8.0	239.2 E	10.3 h
12\10\2019	210.1	7.9	113.8	7.9	196.1	9.3	131.5	7.9	2.6	8.3	155.5 F	8.3 k
02\11\2019	61.1	7.9	11.1	8.4	1.1	10.9	24.5	9.7	4.4	9.8	23.4 I	9.3 j
23\11\2019	9.8	7.1	16.1	7.1	4.5	9.1	0.0	9.1	0.0	8.3	6.1 J	8.1 k
14\12\2019	195.9	9.1	138.1	8.1	188.4	10.8	161.1	9.8	4.5	9.4	167.9 F	9.4 j
03\01\2020	116.8	9.1	46.8	7.1	44.8	13.5	23.5	12.0	6.7	11.6	50.0 H	10.7 i
24\01\2020	129.8	10.5	136.8	9.6	51.5	11.1	88.5	10.3	5.0	9.8	97.9 G	10.3 i
14\02\2020	123.3	12.0	91.8	10.6	48.2	24.3	56.0	25.1	19.8	23.0	73.9 G	19.01 f
07\03\2020	277.8	25.1	216.1	23.5	109.5	37.3	121.8	49.5	44.2	35.0	168.3 F	34.1 d
28\03\2020	481.1	47.2	294.5	33.5	167.8	38.1	167.5	51.8	46.5	38.2	254.6 E	41.8 b
18\04\2020	934.1	51.8	726.1	41.1	342.8	33.2	548.5	42.1	36.9	32.1	618.9 C	40.1 b
09\05\2020	1402.8	51.8	1004.5	41.1	699.5	35.6	652.8	54.1	48.8	40.2	881.4 A	44.5 a
30\05\2020	1053.1	52.7	870.1	54.1	822.8	35.5	624.5	51.3	46.0	38.3	797.9 B	46.4 a
21\06\2020	847.8	55.5	614.5	44.1	878.5	29.4	669.5	42.9	37.6	33.8	734.9 B	41.1 b
13\07\2020	732.8	51.3	728.5	44.1	887.8	33.5	662.1	49.4	44.2	37.4	733.6 B	43.2 b
04\08\2020	474.5	52.7	523.1	45.5	536.8	22.2	532.1	27.9	22.6	22.3	518.7 D	34.1 d
25\08\2020	810.1	33.1	581.1	37.0	716.8	40.0	518.1	43.8	38.5	37.1	627.7 C	38.2 c
Mean	459.8 a	28.4 A	360.3 b	24.9 B	342.42 c	23.2 B	301.9 d	28.8 A	297.0 d	23.1 B	352.3	25.7

From the previous results, it could be concluded that the diseases and pests of honey bee colonies are very dangerous that led to destroying bee colonies in a short time. On the other hand bee venom collected from honeybee colonies had the highest activities and inhibiting substances against the diseases. Since bee venom it could be a potential alternative natural antibiotic without any harmful for bees and having no chemical toxic residues in honey bee products and its improves the defense and immune system of the

worker body by increasing the defense cells such as plasmatocytes, granulocyte, and coagulocyte, which leads to increase the life of the worker bees (El-Ettreby, 2018 and Wahba *et al*., 2020).

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