INFLUENCE OF HONEY BEE DISEASES ON BEE VENOM EFFICIENCY

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Abstract

Many factors can be affected or neutralizes inhibits the toxin activity of the bee venom as exposure honey bee colonies to different diseases or parasites affects on venom active proteins and differentiation in the amino acid concentrations led to harmful of the venom components as the ailment by Varroa mite or Nosema diseases. Bee venom extracted from infested nurse bee workers with Varroa mites showed decreases in amino acids concentration particularly with Glutamic acid, Methionine and Phenyl Alanine certain amino acids. While Asparagine,Q-Aminobutaric acid, Aniline, Tyrosine, Methionine, Cystine, Isoleucine, Leucine, Phenyl Alanine, Tryptophan and Lysine represented momentum concentration decreased with the nurse bees infected with the Nosema disease which consider one of the most affects diseases to honey bee products.

INTRODUCTION

Bee venom is consider one of the most important bee colonies products, take care in the last decade for its pharmacological active peptide which consists of a complex mixture of protein, polypeptides and lower molecular weights of aromatic and aliphatic constituents in variable amounts. Venom excretion of the synthesis glands of the healthy honeybees is an aqueous secretions contains significant quantities of enzymes and peptides such as phospholipase A, hyaluronidase and amino acid sequence Raghuraman, and Chattopadhyay, 2007. The whole bee venom of many chemical agents were polypeptides (Melittin, apamin, mast cell degranulating peptide); enzymes, amines (histamine, dopamine) and others (Valentin *et al.*, 2000). Melittin is the major components of bee venom composed of more than twenty amino acids , it is known – water soluble toxic peptide (Terra *et al.*, 2007). The productivity of honey bee colonies depends upon many factors including race of bees, good preparation bee colony to new season and controlling bees from diseases. Exposure honey bee colonies to different diseases or the parasites can be affects on venom efficiency. One of the series pests is Varroa mite caused severer damages to different bee organs. It is an obligate mite reproduce in capped bee brood cells feeds by sucking the haemolymph from different bee members affects on bee gland characteristics *Nosema apis* is a microsporidian pathogen to honey bees colony not less risk than other bee diseases. It develops in the gut tissue of adult bees and

has been significantly shorten bee life span and consider to be a disease of the colony rather than one of the individual bee (Malone *et. al.*, 1995). The aim of this work is to correlate between the effect the infested bee colonies by the most sprayed diseases and bee venom product composition, that may be affected on the bee venom pharmacological active peptides.

MATERIALS AND METHODS

The present study was carried out in the apiary of the Plant Protection Research Institute, Agriculture Research Center during spring season, 2009. The spring season consider the best period for collected higher quantities of bee venom than autumn one which regards as dearth period for the venom collection according to EL Shaarawy *et al.*, (2007). Further than it suppose time to exposure honey bee colonies to higher Nosema infection level and beginning of emergence drone bees the most attractive to Varroa mites.

1- Varroa infestation level determination

Five honey bee colonies of Carniolian hybrids of high infested with Varroa mites were tested for this study. Other five bee colonies nevi of the parasites were served as control.

One hundred of sealed worker brood cells and another twenty of sealed drone brood cells were tested to determine the Varroa infestation level through 4 brood combs / bee colony. Other one hundred of adult nurse bees collected of each bee colonies were also tested according to method described by De Jong *et al.*, (1982).

$$\text{Level of Varroa infestation level (\%)} = \frac{\text{No. of Varroa mites}}{\text{No. of tested bees}} \times 100$$

The mean Varroa percentage of brood nurse bees was about 20% and 5% on adult bees.

2- Nosema infection concentration

Five healthy honey bee colonies of Carniolian hybrids were fed on sugar syrup (2 w. sugar/1 v. water) containing *Nosema* spores. The suspension of the *Nosema spores* was prepared according to method of Malone and Gatehouse, (1998) with a final concentration (6×10^{6}) spores per / 1 µl and stored at ($-4\ ^{0}C$) till used.

3 – Samples

Collected bee venom of healthy individuals and diseased bee workers by Varroa mites and Nosema disease based on the method of Pence, (1981) modified by Dawoud and Zakaria, (2007). Five hundred individuals of alive nurse worker bees of unknown ages were collected from bee brood combs of different tested bee colonies

and anesthetized by quick freezing at (– 20 ^{0} C) for 20 min. The venom sacs were dissected out by removing and disruption the stinging apparatus using thin two glasses in presence of one ml of distilled water helping of Binocular apparatus to extracted whole venoms. The samples solution were centrifuged at 10,000rp. for 5 min. to discarded out the remainders. The supernatant was scraped off. The venom was completely dried at 40° for 6 hrs., weighed, freezing and stored at (– 50 ^{0}C) till analyzed. This technique was carried out on the following bee samples;

1- Nurse bees non infested and infested with Varroa mites and Nosema disease.

2- Forager worker bees of non infected and infected by Nosema disease.

4- SDS Polyacrylamide Gel Electrophoresis

The Electrophoretic bee venom analysis was carried out to identify how long the changes may be occurred in the venom protein structures resulted the infestation with Varroa and Nosema diseases. Differentiation of the bee venom proteins were made by the computerized Gel, Using Gel Pro. Analyser V.300report program (Mas. comp., Cairo, Egypt) according to the method of Lammelli, (1970).

5-Amino acids Analysis

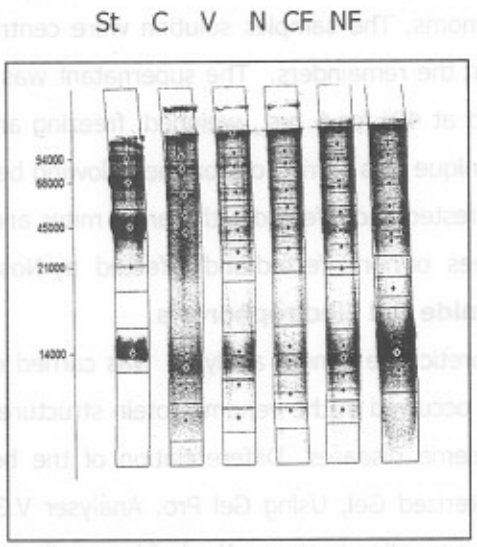
The amino acids analysis determination was carried out according to method described by Gmachl and Kreil, (1993) in the chemical laboratory of National Research Center
Dokki -Egypt.

RESULTS AND DISCUSSION

I- Electrophoretic Venom Differentiation

The pixel intensity of the crude bee venom proteins be shown in Table & Fig.(1). The profiles of the bee venom fractionations revealed presence 12 protein bands with molecular weights ranged between (6.345 to 101.629 kDa.,) for healthy bee workers. While diseased nurse bee with Nosema and Varroa disease revealed presence 9-10 protein bands with molecular weights ranged between (6.238-83.269 kDa.,) and (6.329–82.487 kDa.,) respectively. The venom fractions of infected forager worker bees with Nosema spores showed presence 10 protein bands in comparison with healthy one who recorded 13 differentiation proteins. All bee venom collected from diseased nurse and forager worker bees had proteins less than those recorded with healthy one. The venom collected of infested bee workers with Varroa disease had proteins with molecular weights not increased than (83.269 kDa.,), in comparison with healthy one who reached to (101.629 kDa.,), while with Nosema disease it was not increased than (82.487 kDa.,). Collected bee venom from forager bee workers infected with Nosema disease had

molecular weights reached to (82.879 kDa.,) on more, less than those recorded with the control one (85.042 kDa.,).



10% Polyacrylamide gel (SDS)

Fig.1 Electrophoretic venom proteins extracted of healthy and diseased bee workers. St: Protein standard. C: Venom of healthy nurse bees as control. V: Venom of infested nurse bees by Varroa mites. N: Venom of infected nurse bees by Nosema spores. CF: Venom of healthy forager bee workers. NF: Venom of infected forager bee workers by Nosema spores.

Table 1. Electrophoretic venom proteins collected of healthy and diseased honey bee colonies.

		Nurse bees						Forager bees			
		Control		Varroa infestation		Nosema infection		Control		Nosema infection	
MW (kDa.,)	Band %	MW	Band %	MW	Band %	MW	Band %	MW	Band %	MW	Band %
94.000	13.57	101.629	12.82								
66.000	22.17	84.126	4.89	83.269	5.73	82.487	8.40	85.042	4.81	82.879	5.2
		74.384	8.28	74.225	8.71	73.446	7.52	73.845	6.16	75.571	7.01
								60.017	5.18		
		57.392	6.53	57.179	9.49	56.436	12.39	56.672	6.68	57.040	3.29
		46.475	5.52	46.861	7.75	47.416	6.75			45.585	6.59
		39.326	10.03	39.437	14.02	38.785	11.39	43.877	5.41	39.085	8.06
								36.779	14.65	34.711	3.51
21.000	9	31.986	5.57			31.443	12.62	31.517	7.65	32.496	7.89
14.000	26.27	27.789	5.99					25.502	2.58		
										20.573	6.67
		12.108	11.86	12.056	9.99	11.986	15.54	12.047	13.14		
		10.848	11.96	10.511	20.29	10.390	12.26	10.600	14.54	10.973	38.54
		8.746	9.56	8.539	10.87			8.885	7.51	9.204	13.22
						7.466	4.98	7.715	4.14		
		6.345	7	6.238	13.15	6329	8.15	6.382	7.54		
No. of Protein bands		12		9		10		13		10	

MW(kDa.,): Molecular weight (kilo-Dalton)

II- Venom Amino Acid Analysis

Extracted bee venom of healthy honey bee workers revealed presence 17 free amino acids. Sharp decrease in most of amino acids concentration were recorded with Varroa and Nosema diseases with significant differences. The acute decrease was recoded with the Nosema diseased nurse bees. The following amino acids were recorded in healthy bee venoms of the honey bee workers; Asparatic acid, Glutamic acid, Serine, Asparagine, Glysine, Taurine, Histidine, Q-Aminobutaric acid ,

Aniline, Tyrosine, Methionine, Cystine, Isoleucine, Leucine, Phenyl Alanine, Tryptophan & Lysine (Table 2). Collected bee venom from infested bee workers with Varroa disease showed disturbances in the amino acids concentration. Decreases in some amino acids concentration was detected particularly with Glutamic acid, Asparatic acid, Methionine and Phenyl Alanine and increases fluctuation in the following amino acids concentration; Asparagine, Q-Aminobutaric acid, Aniline, Cystine, Isoleucine, Tryptophan and Lysine.

Nine amino acids were completely disappeared of the bee venom collected of infected nurse bees with the Nosema disease; Aniline, Tyrosine, Methionine, Cystine, Isoleucine, Leucine, Phenyl Alanine, Tryptophan & Lysine. Seven types of the amino acids concentration were decreased in the bee venom of infected forager honey bee workers with Nosema disease; Asparatic acid , Serine, Tyrosine, Cystine, Phenyl Alanine, Tryptophan and Lysine. In the other side there were sharp increases in portion of the amino acids concentration of the bee venom collected from diseased worker bees.

Table 2. Relative differences of the free amino acids concentration of bee venom collected of worker bees during spring season.

Free amino acids	**Amino acids concentration (mg./g. dry wt) of bee venom**						
	Nurse worker bees				Forager worker bees		
	Control	Varroa infestation	Nosema infection	LSD $_{0.05}$	Control	Nosema infection	LSD $_{0.05}$
Asparatic acid	43.4b	0.438c	1831.970a	0.1159	0.978a	0.0544b	0.016
Glutamic acid	219.5b	0.033c	259.7a	0.1631	9.470a	8.167b	0.0481
Serine	10.6a	9.680b	3.337c	0.1176	46.044a	17.861b	3.205
Asparagine	8.1b	74.50a	0.0614c	0.9855	4.176b	128.53a	2.266
Glysine	23.8a	14.41b	25.3102a	3.8256	9.417b	23.353a	5.7796
Taurine	40.6b	23.98c	48.450a	1.6353	12.611b	54.999a	6.411
Histidine	11a	9.91b	1.537c	0.0117	5.287b	19.106a	1.602
Q-Aminobutari acid	14.9b	18.25a	0.20c	0.2883	8.990b	21.777a	2.266
Aniline	17.2b	50.88a	0.0c	1.1763	10.887b	75.082a	8.0149
Tyrosine	444.1a	285.88b	0.0c	0.1176	209.4a	135.68b	8.0165
Methionine	2164.6a	22.407b	0.0c	0.1153	434.30b	2190.31a	1.1609
Cystine	1799.8b	6786.588a	0.0c	0.1153	1569.8a	1186.02b	0.0802
Isoleucine	0.70b	25.655a	0.0c	0.0577	0.7150b	10.777a	0.0032
Leucine	1.0b	16.54a	0.0b	3.4604	1.637b	6.0330a	0.0033
Phenyl Alanine	6.3a	0.631b	0.0c	0.2306	8.701a	0.8135b	1.6109
Tryptophan	1.0b	5.32a	0.0c	0.023	8.533	6.698	F=5.5
Lysine	0.2b	2.446a	0.0c	0.1154	3.1898a	0.0215b	1.602

From the result obtained it could be concluded that exposure honey bee workers to Varroa mites and Nosema spores caused higher disturbances in the amino acids concentration and bee venom qualitative peculiarity with Nosema disease led to decreasing of the venom efficiency. The amino acids concentration showed higher disturbances with the forager honey bees infected with the Nosema disease different than those recorded with nurse worker bees which showed the sharp decreased, that may be attributed to the disease recycle which be more frequently inside bee colony by feces of contagion bees. It could be established that diseased bee workers can be produce venom less qualitative can be affected on the medication industry. That actuate the operators to exert augmented for obtained the higher quality of the venom from free bee colonies of diseases or parasites. Blondelle &Houghten ,(1991) found 26 individual amino acids making up melittin's sequence of the bee venom. 17 free amino acids were detected in the ant, wasp and bee venoms with aspartic acid, glutamic acid and proline together making up 72% of the total mass of amino acids. Glycerol was present at a concentration of 3.1% of the dry venom weight and the venom was devoid of lipids. The mast cell degranulating peptide (MCDP) is a basic of 33 amino acid residue components of honey bee venom with immunological and pharmacological activities (Ziai, *et al.*, 1990). Otis and Robinson (1996), suggested that honey flow, nectar sources and venom collection methods may affect the composition of bee venom. *Nosema apis* could affected on the amino acid contents, especially when spore present in the haemolymph. Bee venom is composed of a variety of proteins; peptides ,active amines and other compounds which possess a variety of activities. Schmidt, (1995), found that the amount of the bee venom in the sting apparatus of honey bee genus; *A. dorsata, A. cerana* , *A. florae* and three population of *Apis mellifera* varied between 27 to 187 µ/ bee. Kato, (1994), found that the toxicity and variability of worker venom increased from emergence until the last test at 35 days. The highest hyaluronidase content was 33% higher in *Apis mellifera* L. than *Apis mellifera adansonii*. Dawoud and Zakaria, (2007), suggested that honey flow, nectar sources and venom collection methods may affect the composition of bee venom. *Nosema apis* could affected on the amino acid contents, especially when spore present in the haemolymph. Bee venom is composed of a variety of proteins; peptides ,active amines and other compounds which possess a variety of activities. De Graagf *et. al.*,(1994) found that the microsporidian pathogen Nosema protozoa attack the epithelial cells of the midgut of the adult honey bees and caused significant lower levels of the enzymes secretion. Nosema infection reduces digestive enzymes in infected worker bees that may be reflects on the secretion glands (Malone *et. al.*, 1995). There were else some factors affecting on the bee venom qualitative and quantities such as; race and age of bees, season of the year, type of feeding and the defense behavior (Marz *et al.*, 1981 and Nour *et al.*, 2004).

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تأثير أمراض النحل على كفاءة سم النحل

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أجرى هذا البحث خلال موسم الربيع فى منحل قسم بحوث النحل بالدقى– مصر وذلك لبيان أثر إصابة طوائف النحل بمرضّ النوزيما وطفيل الفاروا على تركيب وكفاءة سم النحل وقد أظهرت النتائج أن سم النحل المستخرج من شغالات نحل العسل الحاضنة المصابة بطفيل الفاروا يظهر بها نقص معنوى فى تركيز الأحماض الأمينية ال جلوتاميك– مثيونين – فينايل الآنين . بينما الأحماض الأمينية الأسبراجين– أمينو بيوتريك اسيد–الأنيلين– التيروسين– المثيونين –السيستين – الأيزوليوسين – الليوسين– الفينايل الآنين– التربتوفان والليسين أظهرت نقص حاد فى مكونات سم النحل عند الإصابة بمرض النوزيما والتى تعتبر من الأمراض الهامة التى تؤثر على منتجات نحل العسل .