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CONTROLLING THE GREATER AND LESSER WAX MOTHS USING NATURAL AND CHEMICAL PRODUCTS

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ABSTRACT

The efficacy of natural and chemical compounds were investigated against wax moth greater, *Galleria mellonella* L.and lesser, *Acheroia gresilla* Fab. . Spinosad and *Bacillus thuringiensis* (Agerin) as natural compounds , while Formic acid, oxalic acid, Phostoxin, and Bara-dichlorobenzen were used as chemical compounds . The mortality percentage of wax moths dead larvae were 99.33, 90, 64 and 56.66 by using Phostoxin, PDB, Formic acid and Oxalic acid , respectively , while these mortality was 79.67 and 74.33 % when using Spinosad and Agiren .

Results obtained in this study indicated that, the highly and lowest effecte was Phostoxin and Oxalic acid respectively against larvae of wax moths

INTRODUCTION

Wax moths are serious pests of beeswax worldwide. It can be considered an extremely destructive pest that can destroy empty combs in a very short time (Burges, 1978 and Watkins, 2005) and it can cause huge problems for beekeepers by decimating storage wax combs. The moths neither cause a disease nor they parasites the individual honey bees, but they are responsible for tremendous destruction to the colony (Jedrusk *et al.*, 1994). Larvae of Greater wax moth, *Galleria mellonella* L., and lesser wax moth *Acheroia gresilla* fab. are by far the most danger pest, especially to comp both in the weak hives and in storage. Weakened colonies by disease or other causes are particularly susceptible to invasion and takeover by the larvae which cause damaging or destroying combs. These destructive comb become value less, the spread of the moths is due mainly to the interchange of comps and other equipments between infested and non infested colonies, causing significant economic losses to apiarists (Morse, 1978). In addition, pollute the combs with faces, which may contain pathogenic bacteria and a mass of webbing, rendering what is left of the wax comb useless. (Atallah *et al.*, 1983 and Tucker, 2001).

Possibilities and various methods have been proposed for controlling wax moth both for stored equipment and other treatments in the bee colonies i.e. irradiation of gamma rays, will kill all development stages, but costs are high (Hornitzky, 1986) and technical, physical, biological, microbial and chemical methods e.g. sulphur fumigation, acetic and formic acids evaporation and applying Bara-dichlorobenzene are practiced (Calderone, 2000). Although the use of these chemicals is somewhat easy and effective, some precautions of safety and contamination of bee products are considered. Also, some of these materials seemed to be ineffective against eggs of the wax moth (Fraser, 1997).

From this stand point, we attempted to control the wax moths in this study with some materials i. e. 1). biological control by a new natural product, spinosad and biological compound content B.T, Agerin. 2) Chemical

materials i.e. organic acids, (Formic acid 85% and Oxalic acid 5%) and insecticides (Phostoxin and Bara- dichlorobenzene against the wax moth, the greater, *Galleria mellonella* L. and the lesser, *Acheroia gresilla* fab. under laboratory and storage conditions.

MATERIALS AND METHODS

This experiment was achieved at the apiary of Beekeeping Research Department at Dakhlia Oasis. New Valley, Governorate during 10 successive weeks, from 15 of Sept. 2008 to the end of Nov. 2008.

1- Culturing of wax moths:-

Naturally infested wax comps with greater and lesser wax moths were obtained form the apiary of Beekeeping Research department at Dakhla Oasis and were taken to a rearing in the empty hive boxes and kept in the apiary under the weather condition of Dakhla Oasis (24±5^{o}C temp. and 60±5 R.H.) till the adult emergence.

Emerged moths (males and females) were taken and transferred to clean big glass Jars fortified with un infested old waxes (1X1 cm.) and left to copulate and oviposition, pieces of wax blocks having eggs were transferred into Petri dishes until hatching. The newly hatching larvae were fed on old bee wax until pupation and provided with additional amounts of sterilized bee wax when needed.

2- Tested material:-

a. Laboratory trials:

2.1. A new natural product contain *Saccaropolyspora spinosa*, Spinosad, Tracer 24% (natural secration of bacteria fermentation).

Spinosad is the first active ingredient proposed for a new class of insect control products. It has been shown to be highly active on insects including species from the orders Lepidoptera, Diptera, Hymenoptera, Thysanoptera and a few of Coleoptera (Crouse and Sparks, 1998 and Sparks *et al.*, 1998).

Spinosad has been found to be highly active on almost all life stages of moths including egg, larva and adult, egg must be sprayed directly but larvae and adults can be effectively dosed through contact and feed on the treated wax comb (Sparks *et al.*, 1998).

Under laboratory condition, honeybees are highly sensitive to Spinosad via both oral and contact routes of exposure. The acute oral $L.D_{50}$ value is 0.060 Ug / bee, and the acute contact $L.D_{50}$ between 0.0025 and 0.045 Ug / bee, however dry residues of spinosad on plant flowers or on the treated wax comb appear no significant mortality of the all life stages when the treated combs were introduced to the colonies (Crouse and Sparks, 1998), the lack of toxicity of dry residues of spinosad indicates that the risk to bees will bee negligible if applications of spinosad are made when bee activity is low or the bees are allowed to visit treated areas only after spray deposits have dried , night applications or after the first three hours post-application demonstrated no adverse effects of honeybee .

Spinosad has been classified by EPA as a reduced risk insecticide product because of it's:- low acute mammalian – low toxicity to fish and wild life – safely to beneficial and compatibility with integrated pest management

2.2. Biological control:

Agerin is biological compound which contain of *Bacillus thuringiensis*, sup-sp. *aegyptiaca* spores, this bacteria has been successfully aced for plant protection for several years. The bacterial strain of the product B-401Bacillus thuringiensis sup-sp aizawai was selected in particular for its activity against the wax moth, the bacterium produces spores containing a toxin, when the larvae ingest the spores, the toxin is freed and damages the intestinal walls. This results caused the death of the larvae, this bacteria is harmless for vertebrates, (man, livestock) and bees and leaves no residues in wax or honey.

In the first of study, the Spinosad and Agerin compounds were maintained in the laboratory in order to determined the best concentration for controlling wax moths, 30 old combs were selected from the apiary, and kept in household freezer at -7 ^{o}C for 4.5 hrs (Shimanuki and Knox, 1997) to eliminating any previous infestation with either wax moth. These combs divided into six groups, five combs each, and every group was enclosed into nucleus box, 50 larvae of greater and lesser wax moth were introduced in the boxes, wax combs of three groups were sprayed one time with Spinosad concentration 0.01, 0.15 and 0.02 % and the other three groups were also sprayed with Agerin with concentration 0.1, 0.2 and 0.3 %.

All boxes were kept in the apiary; the all combs were examined 10, 20 and 30 days after treatment, the numbers of dead larvae were counted and recorded and the mortality percentages were calculated.

b. Field experiment:

189 of the old combs were selected from the apiary and kept in an storage container attached with a refrigeration unit at -7 ^{o}C for 4.5 hrs. After that, the combs were divided into seven groups, 27 combs each, these combs were divided also to three replicates for each treatment, 9 combs each, every group was located into one bee breeding box, to accomplish artificial infestation, 100 larvae of 5 th instar of two wax moth species were put into the wax combs harbored in the breeding box, three hours later, all wax combs in the groups were treated except control treatment.

All breeding boxes were kept in the store of the apiary, treated and untreated combs were examined 15, 30 and 45 days after treatment, and the number of dead wax moth larvae were counted and recorded

Spinosad used at a rate of 0.02 % concentration(0.2 Cm / one liter distilled water), while Agerin (B.T) was sed with 0.2% concentration with 20 ml/ comb sprayed directly on the two faces in the wax comb. The procedure was done to every rearing box three times at 15 days, intervals.

2.3. Chemical methods:-

a) Organic acid:

a.1. Formic acid:-

Formic acid, 85% concentration at a rate 10 ml/comb was applied on absorbent cardboard plates a volume of 90 ml of formic acid used per one

box having 9 wax combs. The treated cardboard plates were placed over the top bars of the frames for three times at 15 intervals.

a.2. Oxalic acid:-

Oxalic acid 5% concentration, 50 g of solid oxalic acid was dissolved in one liter of distilled water for obtained to oxalic acid 5% at a rate 20 ml/ comb which sprayed directly on the two faces of the wax comb.

This application was used per one rearing box for three times at 15 days intervals.

b. Insecticide:-

b.1. Phostoxin (Aluminum phosphide 57% + inters 43%):

Phosphine was used as an indoor fumigation purposes is usually produced by the reaction of atmospheric moisture with slow release formulations containing Aluminum phosphide , the chemical equation for the liberation of phosphine is : 2 Alp + $6H_2O$ =2Al (OH)$_3$ + 2 pH_3. Aluminum phosphide is an inorganic phosphide used to control insects and rodents in a variety of setting at crop transport, storage are processing facilities (or in shipholds, railcars, ect.) for both food and non-food crops. Phosphine has a low degree of absorption by food stuffs and penetrates well into the stored product. It has high toxically with no knowing medical antidote and it is also the most offenders in suicidal poisoning. Human tolerance is 0.11 ppm , aluminum phosphide 57% tablets (3 g) were used by introduced the 1.0 g per one rearing box contenting 9 wax combs for three times at 15 days intervals (Guidelines on tablets:3 tables per CBM).

Each treatment was represented by 3 breeding boxes (9 combs each). In addition, three breeding boxes (3 times) each of 9 wax combs were untreated to be used as a check.

Number of larvae and pupae of greater and lesser wax moth were recorded 15 days, intervals, after each treated from 15 Oct. to the last of Nov.

b.2. Bara-dichlorobenzene (PDB) was tested as a commercial chemical compound commonly used to kill and repel the greater and lesser wax moth. PDB crystals provide good control of wax moth, they were sprinkled on the tops of the combs at a rate of 3 ounces /5 breeding box (Shimanuki and Knox, 1997).

When chemical methods are used the combs must be well aired out for several days after storage before using them in the colonies and particularly if you are intend to hive swarms on them , then the wax combs treated with using PDB without airing heavy damage may occur and can result in the death of the colony.

3. Chemical analysis of wax samples:

After storage of the wax comb, samples were taken and analysis the elements of the wax sample were extracted by ethanol. The extraction was treated by three methods:-

a: Silver nitrate test according to method of Bansal *et al.* (2006).

b: Gas chromatography separation according to Wallner (1992) and Yadav *et al.* (2007)

c: Amonium molybdate test according to method of Bruce *et al* (1962).

These analysis was done in the laboratory of molasis and honey export and technical support united (founded by the Candian International Development Agency (C I D A), Minia Governorate.

4:- Statistical analysis:-

The resulted data were subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using Duncan multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSIONS

1) Activity of tested material against wax moth larvae in laboratory:

In laboratory trials, data in table (1) declared that the percentage of mortality of greater and lesser wax moths were 80,88 % and 94 % after 30 days from treatment with concentration 0.01 , 0.015 and 0.02 %, respectively by using Spinosad, while they were 60,74 and 76 % when using Agerin (B.T) with concentration 0.1, 0.2 and 0.3 % respectively. These high values of mortality in wax moths larvae should encourage additional experiments to confirm the efficient application of Spinosad (tracer 24 %) as a new natural product against wax moths with 0.02 % effective concentration The obtained results clearly indicated that Spinosad and Agiren gave a best results for controlling wax mothe under the laboratory condition which encourage additional experiment to be done for storage bee combs..

Table (1): Effect of natural products on greater and lesser wax moths larvae:

Compound	Concentration %	% Mortality at days after treatments				
		No. of dead larvae			Total	% Mortality
		after 10 days	After 20 days	After 30 days		
Spinosad	0.010	10	13	17	40	80
	0.015	13	18	13	44	88
	0.020	17	20	10	47	94
Agerin	0.10	7	12	11	30	60
	0.20	9	13	15	37	74
	0.30	10	14	14	38	76

On the other hand, the efficiency of these materials tracer 24% (Spinosad) and *Bacillus thurengenses* (Agerin) for treating the moths gave the best results after 20 days of spraying the wax combs.

2) Efficiency of Spinosad and Agerin in storage bee combs :

Data in table (2) declared that treating infested combs by Spinosad achieved the best results for controlling wax moth larvae therefore the mortality percentage was 79.67 % after three application at ,15, 30 and 45 days intervals for a total period of 45 days. While this mortality was 74.33 % using Agerin after three treatments at the same periods of days .

On the other hand , it could be noticed that, the highly effective on larvae mortality appeared after 30 days of treatment, the percentage were 33.33 and 31 % using Spinosad and Agerin respectively, with non significant differences between them through the periods of treatments.

Table (2): Efficiency of tested materials on mortality percentage of wax moth larvae:

Biological and chemical compound			Treatments			Total	% Mortality
			After15 days	After30 days	After45 days		
Biological control	Natural products	Spinosad	22.33	33.33	24.00	79.66	79.67 %
	Biological compounds	Agerin	18.00	31.00	25.33	74.33	74.33 %
Chemical treatments	Organic acids	Formic acid	29.66	17.66	16.67	63.99	64.00 %
		Oxalic acid	25.66	16.00	15.00	56.66	56.66 %
	Insecticide	Phostoxin	63.33	34.33	1.66	99.33	99.33 %
		PDB	46.00	34.00	10.00	90.00	90.00 %
Untreated combsl			41.33	78.00	138.00	257.33	-
L.S.D (0.5%)			5.83	10.04	14.98	16.75	-

3) Efficiency of chemical methods toward wax moth larvae:

As shown in table (2) there were markedly differences in the mean numbers of death wax moth larvae when using phostoxin (Aluminium phosphide), PDB (Bara-dichlorobenzin) , Formic acid and oxalic acid , the percentages of larvae mortality were 99.33, 90, 64 ,and 56.66 % respectively. From the above data it could be seen that the all wax moths larvae attacking wax combs were killed, however a few percent of larvae (0.66 and 10 % larvae) were a live after 45 days of treatment , when using Phostoxin and PDB, respectively, but it is very important to maintain that , when using chemical materials , the treated combs must be airing before introduced to the bee colonies.

From the preliminary experiment, the tested materials exhibited variable efficacies against wax moths, the least effective material was oxalic acid, the mortality percentage of wax moth larvae was 56.66%, while the highest effecte was Phostoxin, the mortality was 99.33 %, while, in the chick treatment the population density increased progressively and reached to 138 at the end of experiments 45 days after, thus it could be concluded that these insect pest activity reproduce on old bee wax when no control measure were applied.

4) Chemical analyses of wax samples:

According to method of (Bansal *et al.*, 2006), Aluminium phosphide was detected by 0.011 ppm. while Phosphine is oxidized to phosphate with bromine and then determined calorimetrically with the ammonium molybdate (Bruce *et al.*, 1962), the concentration was 0.008 ppm. While separation of gas was done according to (Wallner, 1982 and Yadav *et al.*, 2007), in which the concentration was 0.009 ppm. , but the human tolerance is 0.11 ppm.

From the above mentioned results, it is suggested that,

a : Using biological method toward controlling wax moth larvae was of grate effect the safety and effectiveness then, Spinosad has been found to be highly active on the two species of wax moths (Sparks *et al.* 1998) , who found that Spinosad has contact activity on all life stages of the pest including eggs , larvae, and adults , but the eggs must be spraying directly, while larvae and adults can be effectively dosed through contact with treated surfaces and it is most effective when ingested. Agerin (B.T)

is easily applied as a preventative measure by spraying a diluted amount directly on to combs and it has many advantages over other treatments such as PDB and phostoxin, which were toxic to the humans , honeybees and leave residues in wax and honey.

b: Control of wax moth should be doing in September, October and November months, then the degree of temperature of these periods (25-35 ^{o}C) was favorable to developmental activity of moths but warm and cool temperature will influence on each stage of life cycle and there is no developmental activity of moths below 18 ^{o}C and above 38 ^{o}C, the eggs are laid not be able to hatch.

c: After fumigated and treated the wax combs should be placed in a plastic sealed container till needed , they should be airing before introduced to the colonies .

d: The moths should be controlled again by repeating the treatment when the live larvae were exist.

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مكافحة ديدان الشمع الكبيرة والصغيرة باستخدام بعض المنتجات الطبيعية والكيميائية

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اجريت هذه الدراسة بقسم بحوث النحل بالوادي الجديد خلال ثلاث اشهر متتالية من بداية سبتمبر الي نهاية نوفمبر ٢٠٠٨ .

درست كفاءة استخدام بعض المركبات الطبيعية والكميائية لمقاومة دودة الشمع الكبيرة والصغيرة وكان من المركبات الطبيعة التي تم استخدامها في المقاومة، مركب التريسر ٢٤% (سبينوساد) وهو عبارة عن افراز طبيعي ناتج عن تخمر بكتريا سكاروبوليسيورا سبينوسا بتركيز ٠,٠٢ % وكذلك المركب الحيوي الاجيرين الذي يحتوي علي بكتريا باسيلس ثيورنجنسيس (BT) بتركيز ٠,٢% حيث اظهرت النتائج ان النسبة المئوية لموت يرقات دودة الشمع عند استخدام المركب الطبيعي سبينوساد ٧٩,٧٦% بينما كان ٧٤,٣٣% في حالة استخدام مركب الاجيرين مع عدم وجود فروق معنوية بينهما. كما استخدمت بعض المواد الكيمائية مثل الاحماض العضوية (حمض الفورميك بتركيز ٨٥% ، وحمض الاوكساليك بتركيز ٥%) وكذلك بعض المبيدات الحشرية مثل الفوستوكسين (فوسفيد الالومنيوم) بمعدل ١,٥ جم (نصف قرص) لكل صندوق تربية يحتوي ٩ اقراص و البارادكس (البارادای كلوربنزين) بمعدل ١٧ جم لكل صندوق تربية يحتوي ٩ اقراص شمع ، واظهرت النتائج ان هناك فروق عالية المعنوية ما بين المركبات المستخدمة حيث ثبت ان هناك تأثير ايجابي مع وجود اختلافات واضحة بينهما فكانت النسبة المئوية لموت اليرقات ٩٩,٣٣، ٩٠، ٦٤ و ٥٦,٦٦% عند استخدام الفوستوكسين ،البارادكس ، حمض الفورميك وحمض الاوكساليك علي التوالي حيث كان حمض الاوكساليك اقل تأثيرا من المركبات الاخري.

ومن جانب اخر اظهرت معاملة المقارنة والتي لم يتم معاملتها باي من المركبات السابقة زيادة كبيرة في اعداد اليرقات الحية في كل من دودة الشمع الصغيرة والكبيرة حيث كان متوسط اعداد اليرقات ٤١,٣٣ ، ٧٨,٠٠ و ١٣٨ يرقة وذلك بعد ١٥ ،٣٠ و ٤٥ يوم علي التوالي من بداية التجربة باجمالي ٢٣٧,٦٦ يرقة حية بعد ٤٥ يوم .