BIOLOGICAL AND BIOCHEMICAL EFFECTS OF THE MICROBIAL INSECTICIDE MYCOTAL AND LINSEED OIL ON THE PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUND.)

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ABSTRACT

Entomopathogenic fungi *Verticillium lecanii* and linseed oil were used alone or in sequence to evaluate their direct and latent effect on newly hatched larvae of susceptible strain of the *Pectinophora gossypiella* (Saund.) under constant conditions 26±1C^{o} and 80-85 % RH. LC_{50} & LC_{25} values for these two materials were estimated which was 1.5 x 10^{7} & 0.5x10^{7} spores for *V. Lecanii* and 4ml & 2ml for linseed oil. Percentage of larval, pupal mortality and adult emergence were estimated. The latent effect of the survived larvae was considered and the obtained results could be summarized as follows: *V. lecanii*, linseed oil, or both produced mortality after 1 day, ranging between 1.8 and 37%. PBW neonate was more sensitive to linseed oil than to *V. lecanii*. Larval period was 15.35, 17.79 and 18.16 days, respectively, when neonate was fed on *V. lecanii*, linseed oil and the sequence treatment compared with 12.3 days for untreated control. Pupal duration increased significantly in all treatments. Fungi and linseed oil treatments prolonged larval and pupal durations and shortened the adult stages. Weights of 4th instar larvae resulted from the *V. Lecanii* /line seed oil treatment were greatly reduced to 0.009 mg, while increased to 0.034 mg for larvae treated with linseed oil. Also, pathogenic fungi, sequence treatment followed by linseed oil alone affected on some biological characters such as fecundity of emerged females, eggs hatchability percentage and longevity of males and females. In addition, carbohydrate hydrolyzing enzymes, tranamenase enzymes, total soluble protein & total lipid were affected after 10 days of treatment of the newly hatched larvae.

Key words: Entomopathogenic fungi *Veticillium lecanii*, Linseed oil, pink bollworm,*Pectinophora gossypiella* (Saund.), Biology, Biochemical.

INTRODUCTION

The Pink bollworm *Pectinophora gossypiella* (Saund.) is considered the most destructive cotton pest in Egypt. Recently, scientists directed their effect toward the control of important cotton pests by new trends such as using plant extracts, B.T. products and pheromones… etc., to avoid resistance development and the environmental pollution caused by conventional insecticides.

Lacey *et al* (2001) reported that the entomopathogens are safe for humans and other target organisms and help in reducing pesticide residues in preserved foods. The entomopathogenic fungus *Verticillum lecanii*, is commercially used control of the soft scales and aphids **(Hall1976&Spoer 1978)** some lepidopterous pests such as *Pieris rapae* L., (**Hong** *et al*.**, 2001)**. *Heliothes zea* (Boddie) and the larvae of the gypsy moth **(Hajek** *et al.,* **1997).**

Also, in the last decad many authors found that using natural oils, as neem oil, soy been oil, cotton seed oil, linseed oil, may serve as antifeedants or growth regulators to some insect pests **(Singh & Singh 1989; Broza *et al*.1989; Singh & Bhathal 1992; Lakhanpal *et.al* 1995 and Abo El-Ghar *et al.*** 1996**)**.

The present work aims to study the biological and biochemical effects of Mycotat®, linseed oil, as well as combined against the newly hatched PBW larvae. The biological impact of these materials the at LC_{50} & LC_{25} concentrations was also determined. Mycotat® is a microbial insecticide based on the spores of the entomopathogenic fungus *V. lecanii*, a special strain prepared as wettable powder.

MATERIALS & METHODS

The neonate larvae of pink bollworm, *P. gossypiella* used in the present study were taken from a laboratory culture reared for about 15 generations on an artificial diet previously described by **Rashad and Ammar (1985).** The materials used were prepared as follows:

1- A stock solution of Mycotal was prepared by dissolving 2 mg in 1 L of water. Serial dilutions were used from $2x10^7$ to $0.3x10^7$ for larvae treatments.
2- A stock solution of linseed oil was prepared by adding 8ml of oil and 10 drops of triton x100 to 1 L. of water. The solution was shaken till fused.

1- Toxicology tests:

a) Mycotal:

To estimate the LC_{25} and LC_{50} values for this microbial insecticide four concentrations namely $2X10^7$, $1X10^7$, $0.5X10^7$ and $0.25X10^7$ were tested in four replicates each. Petri dishes (9cm diameter) were used for this purpose. Each was sprayed with 2 ml of the chosen concentration, to which PBW larvae were transferred using a fine brush. A piece of moist cotton was placed in each dish to keep the R.H at 100%. Mortality percentages were determined after 1, 3 and 7 days (neonate were treated by different concentrations of Mycotal for 2 hours then transferred individually to untreated diet poured into (2x7.5 cm) glass tubes to estimate the LC_{50} & LC_{25} values.

To evaluate the effectiveness of this entomopathogenic fungi against PBW, 200 neonate larvae were exposed to the LC_{50} concentration for 2 hours only, then transferred individually to untreated diet. Similarly 100 untreated larvae were individually reared as control. Treated and untreated larvae were incubated under constant conditions; 26 ± 1^oC and 80-85 % RH. Mortality in larvae, pupae and adults, as well as other biological aspects were determined.

b) linseed oil:

Newly hatching larvae were individually transferred to 200 rearing diet glass tubes (50 x 4 replicates). One drop of linseed oil of the prepared concentrations 8, 4, 2 and 1ml/L was applied on top of the diet in each tube. Also,100 tubes were untreated as control. The LC50 & LC25 were estimated after 24 hrs. The same procedure was followed to study the effectiveness of both concentrations. The treated tubes were incubated until pupation. Pupae were transferred individually to clean tubes and incubated until moth emergence. The emerged moths (treated or untreated) were sexed and replicated in glass chimney cages (4 replicates,). A piece of cotton wool previously soaked in 10 % sugar

solution was hanged inside the cage for moths feeding and changed by new one every two days. Upper and lower opening of each cage were covered with muslin cloth followed by paper for egg – laying and tightly secured with rubber bands. Each cage was examined daily to separate the deposited eggs which were further observed to estimate % hatchability.

Larval mortality percentage, larval duration, % pupation, pupal duration and malformation, pre-oviposition, oviposition, post-oviposition periods, male and female malformation and longevity, number of eggs/ female and % hatchability were estimated.

c)- Mycotal in sequence with linseed:

Four Petri dishes (9 cm diameter) were sprayed with Mycotal at the LC_{25} rate to which 200 larvae (50 x 4 replicates) exposed as mentioned above. After 2 hours, the alive larvae from each treatment were individually transferred to diet tubes treated by linseed oil at the LC_{25} level. Treated and control (untreated) larvae were incubated under the same controlled conditions to estimate the mortality of larvae, pupae, adults & durations and malformations. Statistical analysis of data was carried out using Costat software.

2-Biochemical tests:

For the biochemical studies, 5 replicates of 25 tubes prepared by the previous manner with LC_{50} of linseed oil, *V. lecanii* & LC_{25} of *V. lecanii*/ linseed oil. Alive larvae were collected after 12 days for each treatment, placed in clean vials. These studies were undertaken in association with the Dept. of Physiology in Plant Prot. Res. Institute.

-Total soluble protein:

Colorimetric determination of total soluble protein in total homogenate of larvae PBW larvae was carried out as described by (**Gornalt *et al*. 1949**).

- Transaminase enzymes:

The activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvie transminase (GPT) enzymes were determined colorimetrically according to the method of **Reitman and Frankle (1957).**

-Carbohydrate hydrolyzing enzyme:

Activities of trehalase, amylase and digesting invertase, respectively were determined according to **Ishaaya and Swiriski (1976)**.

RESULTS AND DISCUSSION

- Larval mortality:

Data in Table (1) showed the effect of *V. lecanii*, linseed oil and both on newly hatched larvae of PBW. No mortality in PBW larvae was observed after 1day in control. On the other hand, *V. lecanii* and linseed oil and their sequence produced 1.8, 37 and 21.15% mortality, respectively. The larvae were more sensitive to linseed oil than *V. lecanii* after 1 day of treatment. Also, all treatments caused progressive mortality after 3&7 days. *V. lecanii* treatment caused progressive mortality also within 15 days similar result was reported by **Ramanujam *et al*. (2003)** for *V. lecanii* against *H. armigera* and *S. littura* under laboratory conditions, with maximum % mortality 54.44 & 76.66%, respectively. Also, **Hajek *et al.* (1997)** found that *V. lecanii* caused larval mortality for the gypsy moth in laboratory bioassays.

Table 1

- Larval duration:

Data in Table (1) also showed that all treatments gave highly significant influence on the developmental period of survived larvae. The larval period was significantly prolonged in all treatments than the untreated check which averaged 15.35, 17.79 and 18.16 days in *V. lecanii*, linseed oil & sequence treatments, respectively, compared with 12.3 days for control. Larvae treated with both materials were generally very small in size, colorless& inactive when compared with untreated ones.

- Pupation percentage and duration:

Data in Table (1) also showed that the highest percent of pupation (92.9%) was that for linseed oil, treated larvae and the lowest (73.8%) was that for larvae receiving both materials used.

The pupal duration increased significantly in all treatments compared with that for untreated larvae. Mean pupal duration increased from 7.9 days in control to 8.57, 9.13 and 11.9 days in *V. lecanii*, linseed oil and both treatments, respectively, i.e. increased for 1.1, 1.2 & 1.5 times, respectively.

-Duration of immature stage :

Table (1) clearly showed that the three tested treatments had no significant affect on the immature stage period compared with control. This period was 23.3, 26, 22.5 and 19.8 days for larvae treated with *V. lecanii*, linseed oil, both materials and untreated check, respectively.

Larvae treated with linseed oil after exposure to *V. lecanii* were remained active and continued feeding on the diet for 1-12 days then became inactive, slow feeding and list weight (0.009 mg/larvae). On the contrary, in case of linseed oil treatment, larvae became very active feeding from the 2^{nd} day of treatment until being full grown, weighting 0.034 mg /larva. Also, malformed full grown larva became large in size, black in color, (fig.1), whereas larvae treated with *V. lecanii* became inactive, slow feeding until death, and spores of fungi appeared externally on their bodies. Fig. (1).

-Adult emergence :

Data in Table (1) also showed that % adult emergence from larvae treated with *V. lecanii*, linseed oil, and both were lower than untreated control. However, female adults emerged from larvae treated with linseed oil, were 3 times more than males Table (2). In addition, % malformation in emerged adults reached 5.9 in *V. lecanii* treatment, increased to 10.8 in linseed oil treatment. Non was observed in check or combined treatment (Table 1).

Adult Longevity :

Data in Table (2) showed that longevity of females and males are highly affected in all treatments. That of females ranged 12.7-19.3days compared with 23.1 days in control, while that for males ranged 8.7-13.5days compared with 18.7days in control. The pre- oviposition periods were 2.1, 1.3, 2.2 and 2.5 days for larvae treated with *V. lecanii*, linseed oil & *V.* followed by linseed oil and control.

V. lecanii and linseed oil/*V. lecanii* treatments caused high reduction in oviposition period. This period was 7.8 and 10.3 days respectively, while for larvae treated with linseed oil, the oviposition period increased to 15.1 days compared with 17.3 days for control. From these data, the reduction in the oviposition period was observed in treatments used reaching to 1.63 to 2.22 times that in control.

Fig. 1

Also, the post – oviposition period was reduced. The lowest was 0.9 day with *V. lecanii* and line seed oil treatment and the highest was 2.5 days with linseed oil treatment.

Fecundity and fertility:

Data in Table (2) indicated that the mean number of eggs laid by females from neonate treated by *V. lecanii* and (*V.* and linseed oil) were lower than the check. Average no. of laid eggs was 80.3 &97.8 eggs / female, respectively. While, the mean no. of eggs were 145.1 eggs / female at linseed oil treatment.

Generally, all treatments caused reduction in total eggs laid / ♀ between 22.78-57.26% than control.

Also, all treatments reduced % hatchability than the check by 49.47-61.05%

Table (2).

Biochemical effect of Mycotal, seed oil and both in sequence on *P. gossypiella:*

Transamenase enzymes:

Glutamic oxalocetic transaminase (GOT):

Results in Table (3) showed that larvae of PBW fed on diet treated with *V. lecanii* caused decrease in GOT activity by 5.69 % than untreated, followed by *V. lecanii* and the treated with linseed oil increased the GOT activity by 3.64 and 4.83 times, respectively.

Glutamic Pyruvic transaminase (GPT):

The results presented in Table (3) show that all treatments caused decrease in GPT activity compared to control. The highest decrease in GPT activity recorded 71.9 % at linseed oil, and 54.63% at *V. lecanii*, while the least decrease recorded 50.9 %at *V. lecanii* followed by linseed oil treatment.

Total soluble protein.

The results (table, 3) showed that feeding treatment with *V. lecanii* and linseed oil and the combination resulted in an increase in total protein to 1.9, 1.24 and 1.42, respectively i. e., 41.1, 9.3 and 21.2 % more than the control (1.12mg/ml).

Total lipid:

The data in Table (3) showed also that treatment greatly increased the lipids than the untreated (check) by 2.77, 2.59 and 4.83 times for *V. lecanii*, linseed oil and both, respectively.

Carbohydrate hydrolyzing enzymes:

Amylase, Invertase and Trehalas:

The results presented in Table (3) show that the amylase activity seemed to be affected to some extent after larval feeding on diet treated with *V. lecanii* and linseed oil by 36.45% and 21.5% below that of the control treatment.

Also, invertase activity was greatly reduced in PBW that were fed diet treated with *V. lecanii* by about 76.9% below that of the control treatment (Table 3).

In addition, data in Table (3) demonstrated that larval feeding on diet treated with *V. lecinii*, linseed oil and (*V.* + linseed oil) resulted in significant increase in trehalase activity. It reached 1.58, 1.56 and 2.17 times of the control, respectively.

Table 2

Table 3

In conclusion, this work indicated that larvae of *P. gossypiella* were more susceptible to treatment of fungus & (fungus/oil) these treatment caused high larval and pupal mortality & malformation, reduction in body weight from initial number in tested larvae. Also treatment caused high significant reduction in oviposition, post oviposition and the total number of eggs laid by females treated with high reduction in hatchability.

In this respect, **Rashad& Aly (1994)** reported the susceptibility of *P. gossypiella* larvae to *M. anisopliae* fungi. **Aly (2002)** used the same entomopathogenic fungus *M. anisopliae* against *S. littoralis & A. ipsilon* which caused high mortality in larvae, pupa. **Maged (1999)** found that the oil extracted from lemongrass caused highly reduced developmental periods from neonate to adult emergence of *A. ipsilon*. Also, the *V. lecanii*, had high effect on larvae of some *lepidopterans; Pieris rapae, Heliothis Zea* and gypsy moth as reported by **Hong** *et. al* **(2001**) and **Hajek** *et . al* **(1997)**

The biochemical analyses of alive larvae of PBW treated with *V. lecanii*, linseed oil and *V. lecanii* followed by linseed oil indicates an obvious decrease in hamolymph nutrient content, i.e. total protein, lipids, and carbohydrate enzymes (Amylase & Trehalase and Invertase). In contrast an increase in GOT & GPT and invertase.

Similar results were obtained by **Klocke & Chan (1982),** who reported that feeding larvae of *Heliothis zea* on condenessd tannin- treated diet had lower total protein, carbohydrate enzymes invertase and trehalase than control.

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دراسات بيولوجية وبيوكيماوية للممرض الفطري فرتسليم وزيت بذرة الكتان ضد دودة اللوز القرنفلية(بكتينوفورا جوسيبييلا)

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تم دراسة نوعان من المركبات أحدهما فطري حشري (مركب الميكوتال المحتوي جراثيم الفطر فرتسليم ليكانيي) والأخر زيت بذرة الكتان على الفقس الحديث لسلالة حساسة من دودة اللوز القرنفلية (بيكتينوفيرا جوسيبلا) تحت ظروف ثابتة في المعمل (حرارة ٢٦ ± ١ درجة مئوية ورطوبة نسبية ٨٠- ٨٥%) .وتم تحديد التركيز القاتل لـ ٥٠% و٢٥% من الحشرات المختبرة بالأضافة لتأثير هذان التركيزان لكل مركب علي حدة وتأثير معاملة بالتتابع (معاملة بالفطر يتبعها الزيت) للتركيز٢٥% لكل منهما على بعض الصفات البيولجية وكذلك حدوث التشوة في الأطوار المختلفة بعد المعاملة للحشرة المختبرة مع دراسة التغيرات الحادثة في نشاط بعض الإنزيمات والدهون والبروتين. وأظهرت النتائج:

- إطالة فترات الطور اليرقى والعذراء كما ظهرت تأثيرات على الأطوار الكاملة.
- قلة أوزان كل من اليرقات للعمر الرابع لليرقات المعاملة بالفطر، الفطر والزيت معا عن وزنها الطبيعي
- أظهرت النتائج انخفاض أعداد البيض الموضوع لكل أنثى وكذلك نسبة الفقس كما أثرت علي طول حياة الحشرة الكاملة.
- أوضحت النتائج ان جميع المعاملات تسببت في قلة نشاط الإنزيمات الناقلة لمجموعة الأمين GOT&GPT فيما عدا معاملة الفقس الحديث بالفطر المتبوع بالزيت أو المعاملة بالزيت منفرد أدت إلي زيادة في نشاط GOT.
- سببت المعاملة بالتركيز النصفي للمواد المختبرة زيادة في البروتينات الكلية الذائبة والدهون في يرقات دودة اللوز القرنفلية بالمقارنة بالكنترول.
- أدت معاملة يرقات دودة اللوز القرنفلية بالفطر إلي قلة بعض الإنزيمات المحللة للكربوهيدرات (الأميليز، الأنفرتيز) وعلي العكس تسببت جميع المعاملات إلي زيادة في إنزيم التريهاليز.