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THE BIOLOGICAL EFFECTS AND TOXICITY OF TWO BIOINSECTICIDES AND FOUR BOTANICAL EXTRACTS AGAINST COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISDVAL) NOCTUIDAE: LEPIDOPTERA

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ABSTRACT: *The biological effects and toxicity of two microbial insecticides derived from Bacillus thuringiensis Berhiner (MVPa and MVPc) and four botanical extracts (Kochia indica, Curcuma zedoaria, Zygophyllum simplex and Sorghum bicolor) were studied on 6th instar larvae of Spodoptera littoralis. The bioinsecticide MVPc and the botanical extract Z. simplex were the most toxic agents of all tested materials. Larval mortality percentages and that of larvae which failed to pupate increased by increasing concentrations. In addition, pupation percentages and emerged adults increased by increasing concentrations of the tested treatments. Morphological deformities were increased by increasing concentrations, which was observed in the stages of pupae and adults produced from the survived treated larvae.*

Key words: *Bacillus thuringiensis, Kochia indica, Curcuma zedoaria, Zygophyllum simplex and Sorghum bicolor, biopesticides.*

INTRODUCTION

Microbial control agents are considered a good pesticides due to their usual narrow that range, absence of vertebrate toxicity or pathogenicity, compellability with beneficial organisms and biodegradability (Goettel, 1991). Several authors studied the susceptibility of different commercial bacterial formulations containing *B. thuringiensis* (El-Mandarawy,1992). Plant extracts were used as toxicants , repellents, synergists growth regulators or antifeedants of many insects (Ismail *et al.,*1996, Nassar *et al*.,1997 and 1999 and Salema, *et al*., 2003). The insecticidal activity of extracts of different plant species against many insects were studied by many authors (El-sayed, 1983, Salam, and Ahmed 1997, and Abdallah and Kandill, 1989).

The present study aims to study the toxicity of two bioinsecticides (MVPa and MVPc) and four botanical extracts (*Kochia indica, Curcuma zedoaria, Zygophyllum simplex* and *Sorghum bicolor*) on the 6th larval instar of *S. littoralis* and the biological effects on the developing stages.

MATERIALS AND METHODS

1- Rearing of insects:

The cotton leafworm, *Spodoptera littoralis* (Boisdval) was reared in the laboratory (Biological control Research Department at Plant Protection Research Institute) on castor bean leaves (*Ricinus communis L.*) according to the methods described by El-Defrawy *et al*., (1964) at 25 ± 2 ºC and 65 % R.H. and provide with the needed larvae for bioassay tests.

2- The Bioinsecticides:

Two commercial preparations derived from *Bacillus thuringiensis* and consisted of millions of bio0encapsulated flowable suspension were evaluated.

a- MVPa: contains delta endotoxin of *Bacillus thuringiensis* var Karstala as active ingredient of 20%.

b- MVPc: contains delta endotoxin of *Bacillus thuringiensis* var. Azawi as active ingredient of 20%.

Six concentrations of 5, 10, 15, 20, 25 and 30 ml / 100 ml water were prepared as a aqueous flowable suspensions , in addition to control.

3- Botanical extraction treatments:

Four botanical plants (Table 1) were collected to evaluate their extracts against cotton leaf worm, *Spodoptera littoralis*.

The plant leaves were grounded and soaked in distilled water for overnight, then filtered and the filtrations were dried using a Soxhlet apparatus. Crude extracts of different plants were dissolved in 100 ml water at six concentrations of 5, 10, 15, 20, 25 and 30 ml / 100 ml water.

4- Tests:

The castor bean leaves were dipped in each concentration, then the leaves after drying were offered to 6thinstar ***Spodoptera littoralis*** larvae.

The following procedures were followed in all treatments:

1-Three replicates of ten larvae, putted in 15 cm Petri dish were fed on castor bean leaves treated with bioinsecticides and botanical extracts for a period of 48 hours. After treatments, the surviving larvae were fed on clean untreated castor bean leaves till pupation.
Mortalities were recorded daily. Also, the number of pupated larvae and emerged adults were observed.

2- Larvae were starved for 60hours before introducing the treated food, in order to obtain rapid simultaneous ingestion of the offered food.

3- The control tests were conducted using castor bean leaves in water only and left to dry.

4- The experiments were carried out under laboratory conditions of 27± 2 ºC and 65± 5 % R.H.

Statistical analysis:

The LD_{50} was determined according to Leora POLO –PC (Leora Software, 1994) and the data subjected to the Probit analysis (Finney, 1971) when necessary, while mortality percentages were adjusted across concentrations within the probit procedure by Abbott's formula (Abbott,1925).

RESULTS AND DISCUSSION

The obtained results of the two pesticides derived from *Bacillus thuringiensis* and the different botanical extracts Tables (2 & 3) revealed a variation in their effectiveness against the 6th instar larvae of *Spodoptera littoralis*.

Percent of larval mortality:

The obtained results in (Tables 2 &3) show that the rates of larval mortality ranged between 35 to75 % and 29 to 56 % for concentrations of 5-30 ml /100 ml water of the bioinsecticides MVPa and MVPc, respectively. As for the botanical extracts of *Kochia indica, Curcuma zedoaria, Zygophyllum simplex* and *Sorghum bicolor* at concentrations of 5-30 ml/100 ml water, the larval mortality ranged between 2 - 65 %, 25 – 82 %, 30 – 68 % and 35 – 63 %, respectively.

From the obtained results, it appears that the percentage of larval mortality was directly proportional to the applied concentrations of the bioinsecticides and botanical extracts.

Results of bioinsecticides are similar to that observed by Pantanberkar *et al.,*(1997) who found that increasing concentrations of *B. thuringiensis* caused higher mortalities to neonate larvae of *P. operculalla* and *Spodoptera littoralis* to reach more than 85% at different concentrations. Moreover, Abou Bakr (1997) found that Fbrbae XL.V.EC.WP and EC was effective enough against *S. littoralis* even at higher concentrations.

As for the botanical extracts, Pandje *et al.,* (1993) studied the compounds analysed from *Curcuma zedoaria,* C.K.pandwrata for insecticidal constituents agents *S. littoralis* and reported pronounced toxicity against

neonate of *S. littoralis* in a contact residue bioassay irrespective of the larval stages. Also, Dadang and Ohsawa (1998) found that several Zingiberaceae species (*Curcuma zedoaria, C. heyneana, C. amada, C. aromatica* and *Alpinia golonga*) contained compounds that showed insecticidal affects against *P. xylostella* larvae and *Callosobruchus chinensis* adults.

Table (1): The tested plant extracts

English name	Scientific name	Family	Part used
Kochia	*Kochia indica*	Chenopodiaceae	leaves
Curcuma	*Curcuma zedoaria*	Zingiberaceae	leaves
Zygophyllum	*Zygophyllum simplex*	Zingiberaceae	leaves
Sorghum	*Sorghum bicolor*	Meliaceae	leaves

Table (2): The delayed effects of two bioinsecticides against the 6th instar larvae of *Spodoptera littoralis* and developmental stages

Concentrations ml/100m water	larval mortality %	pupation%	deformed pupae%	emerged adults %	deformed adults %
MVPa					
5	35	55	2	7	1
10	43	42	5	10	0
15	44	36	20	0	0
20	65	25	10	0	0
25	42	48	10	0	0
30	75	5	20	0	0
MVPc0					
5	36	61	5	7	1
10	29	67	3	1	0
15	33	49	16	2	0
20	45	33	22	0	0
25	53	21	26	0	0
30	65	10	25	0	0
control	0	98	0	98	0

Table (3): The delayed effects of four botanical extracts against the 6th instar larvae of *Spodoptera littoralis* and developmental stages

Concentrations ml/100m water	Larval mortality %	pupation%	deformed pupae%	emerged adults %	deformed adults %
botanical extracts *Kochia indica*					
5	2	63	25	9	1
10	25	35	30	8	2
15	31	43	26	0	0
20	39	35	26	0	0
25	61	9	30	0	0
30	65	2	33	0	0
Curcuma zedoaria					
5	27	8	9	9	1
10	25	44	12	7	1
15	39	35	21	5	0
20	45	35	20	0	0
25	56	36	8	0	0
30	82	18	0	0	0
Zygophyllum simplex					
5	30	57	5	8	1
10	38	54	14	7	1
15	44	31	25	0	0
20	55	45	0	0	0
25	61	39	0	0	0
30	68	32	0	0	0
Sorghum bicolor					
5	35	22	22	20	1
10	40	22	22	5	1
15	51	26	20	3	0
20	57	21	21	0	0
25	60	32	8	0	0
30	63	36	1	0	0
control	2	98	0	98	0

Pupation:

At concentrations between 5 to 30 ml, the pupation percentages were ranged between 5 – 25 %, 10 0 67 %, 2 – 63 %, 8 – 36 %, 31 – 57 % and 21 – 36 %, for MVPa, MVPc, *Kochia indica, Curcuma zedoaria, Zygophyllum simplex* and *Sorghum bicolor,* respectively (Tables 2 &3).

These results show that the pupation percentages decreased by increasing the concentrations of different treatments.

Deformation:

The deformed pupae are the individual which had cuticle containing pupal parts with parts of last larval skin or those which were completely covered with pupal exuvia but the external characters of larvae. The deformed pupae died before completed the pupal period. The deformity of emerged adults was manifested as bent or twisted wings of adults. The deformation increased by increasing the concentrations of the different treatments.

Deformed pupae percentages at (Tables 2 &3) as affected by the tested agents were ranged between 2 -20 %, 3-26 %, 25 – 33 %, 8- 21 %, 0-25 % and 1-22 % for MVPa, MVPc, *Kochia indica, Curcuma zedoaria, Zygophyllum simplex* and *Sorghum bicolor,* respectively at concentrations between 5 to 30ml.

From these results, it is evident that the percent of larvae which failed to pupate increased by increasing the concentrations of the bioinsecticides and botanical extracts.

Similar effects was produced by *B. thuringiensis* against flour moth, *Anagasta kuehniella* (1991), Salam (1991) who reported similar findings on *P. operculella* when pupae developing from larvae treated with 100 ppm neem oil. In addition, Chanda and Chakravorty (1993) found deformed adult with a prolonged period of development were obtained from larvae of *Corycyra cephalonica* maintained on (Azadirachtin, 0.03%) observed crushed jowar (Sorghum) grains.

These findings agree with those of Dulmage and Martinez (1973) who reported that when the tobacco budworm *Heliothis virescents* larvae were continuously subjected to sublethal levels of delta–endotoxin of *Bacillus thuringiensis* . Many larvae failed to pupate whereas some others pupated but failed to emerge. In addition, these results are in harmony with those conducted by El-Mandarawy (1992) who controlled *P. rapae* larvae by the pesticide, bactospine. In addition Schmidt *et al.,*(1997) noticed that the larvae of *S. littoralis* and *A. iposlion* failed to pupate at higher concentrations of *Melia azedarach* extract. Nassar *et al.,*(1997) reported the occurrence of malformed larvae which failed to pupate at high concentrations of *Nerium oleander* and *Sorghum bicolor* extracts.

Emerged adults:

Results in Tables (2 &3) show that the percent of emerged moths has been greatly affected and a gradual decrease has been achieved with bioinsecticides and botanical extracts treatments until adult emergence completely disappeared at high concentrations.

These findings are in agreement with those obtained by Salama *et al.,* (2003) who found that *Bacillus thuringiensis* reduced adult emergence of the greasy decreased has been achieved with bioinsecticides and botanical extracts treatments until adult emergence from the cutworm *A. ipsilon* pupae. Also, Abdel-Halim (1993) showed that the various doses of dipel 2x affected remarkably adult stages and the adult emergence was sharply decreased by the applied doses, as it ranged from 85% at 192 IU ml to 0% with the highest dose 6400 IU ml IU ml. Salem *et al.,* (1995) indicated that Melia leaves extract at high concentrations had a direct effect on emergence of adults and it was sufficient to increase mortality

rate to 100%. Moreover, Schmidt, *et al.,* (1997) found that no adult of *S. littoralis* emerged from pupae that developed from larvae treated with 5% *Melia azedarach* extract.

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التأثيرات البيولوجية والسمية لإثنان من المبيدات الحشرية الحيوية وأربعة مستخلصات نباتية على يرقات دودة ورق القطن أسبودبترا ليتورالس (الليليات : حرشفية الأجنحة)

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الملخص العربي

تم تقييم التأثير البيولوجى والسمية الابادية لمبيدين حشريين حيويين مشتقين من بكتريا الباسيلس ثوارنجسس (MVPa and MVPc) وأربعة مستخلصات نباتية وهى : كوخيا أندكيا (قضقاض) ، كوركوما زودايا (الكركم) و زيجوفيلم سيمبلكس(الزوار)، سورجام بيكلر (الذرة الرفيعة) على يرقات العمر السادس لدودة ورق القطن.

أوضحت النتائج ان المبيدين الحشريين الحيويين (MVPa and MVPc) ومستخلص الزيجوفيلم سيمبلكس (الزوار)، هى الأكثر فعالية في السمية مقارنة بفاعلية باقى المستخلصات النباتية .

أوضحت النتائج ان النسب المئوية لليرقات الميتة والتي فشلت في الوصول الي طور العذاري تزيد بزيادة التركيزات المعاملة. في حين أن النسب المئوية للتعذير وخروج الحشرات الكاملة تزيد بزيادة التركيزات المختلفة للمعاملات. وقد لوحظ التشوهات الخلقية في العذاري والحشرات الكاملة المنتجة من اليرقات المعاملة الحية .