EFFECT OF *BUXUS CHINENSIS*, DIPEL 2X AND NUCLEAR POLYHEDROSIS VIRUS (NPV) MIXTURE ON SOME BIOLOGICAL ASPECTS OF COTTON LEAFWORM *SPODOPTERA LITTORALIS*

ABD-ELZAHER, S. MAHMOUD and ABEER. M. MOHAMMAD

Plant Protection Research Institute, ARC, Giza, Egypt

(Manuscript received 26 September 2013)

Abstract

Effects of *Buxus chinensis* extract, *Bacillus thuringiensis* and nuclear polyhedrosis virus (NPV) mixture on certain biological aspects of *Spodoptera littoralis* were studied against the 2nd and 4th larval instars of *S. littoralis*. The mixture of the three compounds was the most toxic against the 2nd and 4th instar larvae, where it caused high percentage of mortality, while *B. thuringiensis* (Dipel 2x) and nuclear polyhedrosis virus (NPV) mixture induced a slight effect against the 2nd and 4th instars. Regarding the ethanolic extract of *Buxus chinensis* leaves, it was more effective than *B. thuringiensis* (Dipel 2x) or nuclear polyhedrosis virus (NPV) in respect of pupal mortality and larval , pupal and adult duration. This plant extract also reduced the fecundity and fertility of females emerged from the treated larvae of *S. littoralis*.

Key words: *Spodoptera littoralis, Buxus chinensis, Dipel 2x , NPV*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd) is one of the most serious insect pests of many different Egyptian crops, it attacks and damages several parts of its host plants. Many researchers used different methods in order to control this pest (Chanda and Chakravorty 1993, Antonious and Rizk 1994, Abo El-Ghar , 1994, Schmidt *et al*., 1997, Ismail *et al*., 1999 , Marei *et al.,* 2009 and Abd El-Ghany *et al.,* 2012). Aim of the present work is to evaluate the effect of *Buxus chinensis*, *B. thuringiensis* (Dipel 2x) and nuclear polyhedrosis virus (NPV) mixture on certain biological aspects of *Spodoptera littoralis.*

MATERIALS AND METHODS

Insect culture.

Laboratory strains of *Spodoptera littoralis* (Boisd) were obtained from Plant Protection Res. Institute, ARC, Dokki, Giza, Egypt. It was reared on Castor bean leaves according to El-Defrawi *et al.,* (1964). The culture was maintained at 28°c ± 2 & 55 % RH, away from insecticides exposure for more than 10 generations.

Preparation of plant extracts .

Buxus chinensis leaves were left to dry at room temperature of 28 ± 2 °C for one week. The dried leaves were grounded to fine powder and extracted consecutively in a Soxhlet apparatus using ethanol solvent. Crude extracts were dried and filtered over anhydrous sodium sulphate and were subjected to remove the solvent used in the extraction. All the crude extracts obtained were kept in the refrigerator until use.

Bioassay of compounds on *Spodoptera littoralis*.

The tested compounds were used at concentrations of 4 ppm, 300 g/400 L water and $5x10^{12}$ /BIP mL for *Buxus chinensis*, *B. thuringiensis* (Dipel 2x) and nuclear polyhedrosis virus (NPV), respectively. For bioassay experiment, leaf dipping technique was used. Castor bean leaves were dipped in the different concentrations of tested compounds. Then treated leaves were left in air dry. After leaves drying they were offered for the 2nd and 4th instars to fed them for 24 hrs.

For control, the fresh castor-bean leaves were immersed for five seconds in 10 ml of each used insecticide concentration. The treated castor bean leaves were used as a food for both 2nd and 4th instars of *S. littoralis* according to the method of (Nakanishi, 1977) with modification. Two sets of experiments were carried out and the toxicity of each concentration was determined according to POLOPC (Leora Software, 1994). The effects on the insect development by the treatment with the mixture of *Buxus chinensis* extract, *B. thuringiensis* and nuclear polyhedrosis virus (NPV) at different concentrations (offered for 72-hrs to one hundred larvae of 2nd and 4th instars) were recorded.

After that untreated leaves were introduced daily to larvae until pupation 24-hrs after treatment the insects were examined daily and all biological parameters of the insect survivors (Mortalities as larvae, pupae and adult) were counted and recorded as percentage in relation to the total number of insects of the preceding stage. The biological efficacy of the different used compounds were calculated according to Vagras and Sehnal , 1973.

Data analysis.

Data were subjected to the probit analysis (Finney, 1971). When it is necessary the control mortality was adjusted by using Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

1- Effect of ethanolic *Buxus chinensis* leaf extracts on 2nd and 4th instar larvae.

Results presented in Table (1) show the effect of ethanolic *Buxus chinensis* leaf extracts on 2nd and 4th instar larvae of *Spodoptera littoralis* fed on castor bean leaves, The mean percentage of larval mortality was 82, 60, 54, 45, 35 and 15 %

for 2nd instars, while it was 27.5 , 20, 16.5, 15.5, 13 and 9.5 % for the 4th instars at concentrations of 4, 2, 1, 0.5, 0.25 and 0.125 ppm of ethanolic *Buxus chinensis* leaf extracts, respectively. Data in Table (1) showed a significant effect on the larval duration of 2nd and 4th instar larvae, was obtained after treatment with different concentrations of ethanolic *Buxus chinensis* leaf extract. At concentrations of 4 ppm, it averaged 12.32 and 4.72 days for the 2nd and the 4th ones, respectively. The mean percentage of pupal mortality was 15 and 10 % for the 2nd and the 4th instar as compared to zero mortality for the control treatment.

Regarding pupal duration, data recorded in Table (1), show that there was a significant effect on pupal duration resulted from treated 2nd and 4th instar larvae with ethanolic leaf extract at 4 ppm concentration of *Buxus chinensis* while, the pupal duration was 7.14 and 7.11 days for the 2nd and the 4th treated larvae, respectively. As it was 9.8 and 8.5 days for the two untreated larval instars,respectively.

Taking into considration adult stage, resulted from treated 2nd and 4th larvae of *Spodoptera littoralis*, the mean percentage of emerged moths, fecundity and hatchability were greatly affected as presented in Table (1). The mean percentage of emerged moths was decreased up to 15 % for 2nd instars while it was 69 % for the 4th instars at the concentration of 4 ppm, respectively, compared to 100 % emerged moths for control.

The concentration of the tested plant extracts played an important role in this respect. Concurrently, the higher the concentration of plant extracts the higher was the larval mortality and vice versa.

2- Effect of mixture of *Buxus chinensis* leaf extracts , *B. thuringiensis* and nuclear polyhedrosis virus (NPV)

When the 2nd and the 4th instars of *Spodoptera littoralis* were fed on castor oil leaves treated with the mixture of *Buxus chinensis*, *B. thuringiensis* and nuclear polyhedrosis virus (NPV), it was effective on the insect development (Table 2). From the data recorded in Table 2, it was observed that the mean percentage of larval mortality was increased by increasing concentrations. About 99 % mortality was obtained at the higher concentrations for the 2nd instars but it was 51% for the 4th instars with mixtures of *Buxus chinensis*, *B. thuringiensis* and nuclear polyhedrosis virus (NPV), compared to no mortality for control larvae.

Table 1. The effect of ethanolic *Buxus. Chinensis* leaf extract on 2nd and 4th instars of *S .littoralis.*

Treatment (ppm)	Larval stages	% Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of eggs/female Mean±S.E.
Control	2nd instar larvae	-	16 (± 0.21)	-	9.8 (± 0..22)	99.8	2499.1 (±319.78)
0.125		15	12.5 (± 0.84)	4	7.5 (± 0.92)	86	500 (± 41.23)
0.25		35	12.42 (± 0.72)	6	7.34 (± 0.88)	64	420 (± 56.57)
0.5		45	12.31 (± 0.66)	8	7. 39 (± 0.86)	52	380 (± 56.16)
1		54	12.27 (± 0.35)	10	7.37 (± 0.83)	42	220 (± 28.22)
2		60	12.24 (± 0.21)	10	7.25 (± 0.82)	36	170 (± 30.82)
4		82	12.32 (± 0.11)	15	7.14 (± 0.81)	15	140 (± 37.42)
Control	4th instar larvae	-	6	-	8.5 (± 0.88)	100	2254.2 (±319.78)
0.125		9.5	5.5 (± 4.5)	-	8.75 (± 0.58)	86	1000 (± 42.25)
0.25		13	5 (±3)	2	8.22 (± 0.56)	82	925 (± 62.1)
0.5		15.5	4.8 (± 1.5)	4	7.61 (± 0.47)	80	860 (± 52.27)
1		16.5	4.66 (± 1.34)	7	7.5 (± 0.45)	79	740 (± 29.37)
2		20	4.33 (± 3.67)	9	7.3 (± 0.43)	78	620 (± 8.17)
4		27.5	4.72 (± 2)	10	7.11 (± 0.42)	69	530 (± 21.6)

According to the data in Table 2, it appeared that a significant prolongation of larval duration was noticed at higher concentrations with mixture of *Buxus chinensis* , *B. thuringiensis* and nuclear polyhedrosis virus (NPV), where it was one day for 2nd instars and 5 days for te 4th instars, compared to 16 and 6 days for the control larvae. In case of pupal stage, which resulted from treated 4th instar larvae of *Spodoptera littoralis* with mixture of *Buxus chinensis*, *B. thuringiensis* and nuclear polyhedrosis virus (NPV), it was effective on the mean percentage of pupation , mortality, deformed pupae and the pupal duration.

3- Effect of mixture of *B. thuringiensis* and nuclear polyhedrosis virus (NPV).

When the 2nd instars of *Spodoptera littoralis* were fed on castor oil leaves treated with mixture of *B. thuringiensis* and nuclear polyhedrosis virus (NPV), the different developmental stages were affected (Table 3). It was observed that the mean percentage of larval mortality were increased by increasing concentrations, where the higher mortality (62.5%) was obtained at higher concentration for 2nd instars but it was 54% for the 4th instar larvae occurred at higher concentration, compared to no mortality for control larvae.

According to the data in Table (3) it was noticed a significant decrease in larval duration was achieved compared to 16 days for control larvae.

Table 2. The effect of *Buxus chinensis*, Dipel 2X and nuclear polyhedrosis virus (NPV) on *2nd and 4th instar of S. littoralis*.

Treatment (ppm)	Larval stages	% Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of eggs/female Mean±S.E.
Control	2nd instar larvae	0	16 (± 0.21)	-	9.8 (± 0..22)	100	2499.1 (±319.78)
A+B+C %100		99	1 (± 0.01)	1	-	-	-
A+B+C %75		90	7 (± 1)	10	5 (± 0.1)	-	-
A+B+C %50		85	8 (± 0.2)	15	6 (± 0.1)	-	-
A+B+C %25		80	9 (± 0.13)	20	7 (± 0.1)	-	-
A+B+C %12.5		70	9.01 (± 0.15)	20	7.2 (± 0.5)	10	-
Control	4th instar larvae	0	16 (±0.21)	-	9.8 (± 0.22)	100	2313 (± 21.6)
A+B+C %100		51	5 (± 1.87)	6	7 (± 0.1)	43	130 (± 21.6)
A+B+C %75		45	5.08 (±16.5)	8	7.3 (± 0.1)	47	420 (± 36.33)
A+B+C %50		40	5.1 (± 1.83)	8	7.36 (± 0.3)	52	585 (±142.83)
A+B+C %25		35	5.28 (± 0.82)	9	7.41 (± 1.1)	58	725 (± 20)
A+B+C %12.5		30	5.33 (± 1.63)	10	7.47 (± 2.9)	60	780 (± .37)

A: (NPV) 5×10^{12} Pib/larvae

B*: B.thuringiensis* (Diple 2X) IU

C: *Buxus chinensis*, 4 ppm

F values=2267 *** L.S.D 0.05=1.963

From table (3), the mean percentage pupal mortality was increased by increasing the concentrations. The higher pupal mortality (10%) was obtained at higher concentration for the 2nd instars but it was (9%) for the 4th instars occurred at higher concentration, compared to no pupal mortality for control larvae.

In case of adult stage, resulted from treated 2nd and 4th instars of *S. littoralis*, the mean percentage of emerged moths, fecundity and hatchability were greatly affected, as shown in Table 3. The mean percentage of emerged moths was decreased to 21.5% for 2nd instars but it was 33 % for the 4th instars, as compared to 100 % emerged moth for control.

From the results tabulated in tables (1-3) it could be concluded that the mixture of *Buxus chinensis* extract, *B. thuringiensis* and nuclear polyhedrosis virus (NPV) was the most active as natural pesticide against the 2nd and the 4th instar larvae of *S. littoralis* , followed by the mixture of *B. thuringiensis* and nuclear polyhedrosis virus (NPV), while *Buxus chinensis* extract alone had a slight effect in this respect. On the other hand, the pupation percentages and adult emergence averages were greatly affected. These results are similar to those obtained by many authors such Guirguis *et al.,* (1991b), who reported that citrus oils revealed varying toxicities against cotton leafworm eggs, while it could be used as insecticide synergists against *S. littoralis* larvae. Also, Guirguis *et al.,*(1991)[a] found that the larval treatments with sublethal doses of ethyl acetate extract of *S. fruticosa* induced serious effects on the biology and biotic potential of *S. littoralis*. Eid *et al.,* 1992 found that injection of *S. littoralis* larvae in the laboratory with sublethal doses of the *Lemina ninor* extract caused malformations in subsequent life stages.

Table 3. Effect of *B.thuringiensis* (Diple 2X) and nuclear polyhedrosis virus (NPV) on 2nd and 4th instar of *S. littoralis*.

Treatments	Larval stage	% Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of eggs/female Mean±S.E.
A+B (100%)	2nd instar larvae	62.5	10.5 (±0.3)	6	7.05 (±0.81)	68	316 (±33.74)
A+B (75%)		40	10.55 (±1.1)	6	7.25 (±0.81)	60	426 (±88.72)
A+B (50%)		32	10.63 (±2.3)	8	7.27 (±0.83)	54	537 (±121)
A+B (25%)		22	10.67 (±1.9)	10	7.36 (±0.84)	34	675 (±60.2)
A+B (12.5%)		20	10.75 (±3.1)	10	7.42 (±0.86)	21.5	980 (±46.2)
Control		0	16 (±0.21)	-	9.8 (±0.22)	100	2499 (±319.8)
A+B (100%)	4th instar larvae	54	6.52 (±0.3)	5	6.17 (±0.81)	71	516 (±30.77)
A+B (75%)		40	6.54 (±1.1)	6	6.02 (±0.81)	61	626 (±89.12)
A+B (50%)		32	6.63 (±2.3)	8	5.82 (±0.84)	54	737 (±123)
A+B (25%)		23	6.62 (±1.9)	8	5.80 (±0.86)	41	875 (±62.1)
A+B (12.5%)		7	6.71 (±3.1)	9	5.64 (±0.86)	33	980 (±48.23)
Control		0	16 (±0.21)	-	8.29 (±0.23)	100	2254 (±318.76)

A: (NPV) 5×10^{12} Pib/larvae

B*: B.thuringiensis* (Diple 2X) IU

F values=2267 *** L.S.D 0.05=1.963

REFERENCES

1. Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J.Econ.Entomol. 18, 265-267.
2. Abd El-Ghany, M. A., M. Farag, H. Yousef, M. H.M.Ahmed and A. H. Abd El-Rahman. 2012. Insecticidal activity of Melia azedarach, Triterpeniods against Spodoptera littoralis (Boisd). J. American Sci. , 8(3): 661-667.
3. Abo El-Ghar, E. S. 1994. Influence of abamection and juvenile hormone analogues on food utilization, ingestion and larval growth of *S. littoralis* (Boisd).Bull. Ent. Soc. Egypt Econ. Ser. 20: 173-184.

4. Antonious, A. G. and A. Rizk. 1994. Response of *S. littoralis* (Boisd) to neem *Azadirachta indica* (*A. juss*) seeds oil Egypt. J. Appl. Sci. 9 (10): 424-434.
5. Chanda, S. and S. Chakravory. 1993. Food with neem oil affects life and development of rice moth, Corycyra cephalonica (Stainton) (Lepidoptera:Pyralidae). Entomol. 23(2):153-156.
6. Eid, M.A. , M.A. Kandil, E. B. Mori and G. E. Sayed. 1992. Bioassay of duck weed vegetation extracts. Insect Science and its Application, 13(5), 741-748.
7. El-Defrawy, M. E., A. Toppozada ,N. A. Mansour and M.Zeid 1964. Toxocological studies on the Egyptian cotton leafworm, *Prodina litura* I. Susceptibility of different larval instars to insecticides. J.Econ. Entomol. 57: 591-593.
8. Finney, D. J. 1971. Probit analysis (3rd Cambridge University Press, New York. 333pp.).
9. Guirguis, M. W., K. M. Gouhar, W. M. Watson and R. M. Solim. 1991a. toxicity and latent effect of two plant extracts on the cotton leafworm *Spodoptera littoralis* (Boisd). Egypt J.Agric. Res. 69(1): 7-15.
10. Guirguis, M. W., K. M. Gouhar, W. M. Watson and R. M. Solim. 1991b. The toxic action of volatile oils extracted from Citruc fruit peels against different insect pests. Egypt J. Agric. Res., 69(1):15-21.
11. Ismail, L.A., N.A.Farag and M.A.Ragaei. 1999. Evaluation of certain insecticides and plant extracts on *Bemisia tabaci* Genn and their side effects on some natural enemies. J. Egypt Ger. Soc. Zool. Entomol. 2, 117-124.
12. Leora Software. 1994. POLO-PC Probit and Logit analysis. Leora Software, Barkley. CA.
13. Marei,S.S., E.M.Amr and N.Y.Salem. 2009. Effect of some plant oils on biological, physiological and biochemical aspects of *Spodoptera littoralis* (Boisd). Res. J. Agric. and Biol. Sci. 5(1): 103-107.
14. Nakanishi, K. 1977. Insect growth regulators from plants, natural products and the protection of the plants. (ed. Marini Bettolo, G.B.) Elsevier, Amsterdam.
15. Schmidt, G.H., A.A.Ahmed and M. Brewer. 1997. Effect of the Melia ozadarach extract on larval development and reproduction parameters of *S. littoralis* and *Agrotis ipsilon*. J.Econ. Entomol. 124,43-49.
16. Vagras,I. and F. Sehnal. 1973. USE OF jha's against *Hyphantris cunea*. Entomol. Exp. Appl., 16:115-122.

تأثير مخلوط مستخلص نبات الجوجوبا ***Buxus chinensis*** مع الدايبل **2x** و الفيروس النووي **NPV** على بعض المظاهر الحيوية لحشرة دودة ورق القطن

صبحي محمود عبد الظاهر و عبير محمود محمد

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة

أجريت هذه الدراسة على تأثير كل من المستخلص النباتي لأوراق نبات الجوجوبا مع الدايبل 2x و الفيروس النووي NPV و مخلوط الدايبل 2x و الفيروس النووي NPV على بيولوجية دودة ورق القطن . و قد أوضحت النتائج أنه يمكن تقسيم المواد تبعا لشدة تأثيرها على الحشرة الى ثلاث مجموعات كالتالي: المجموعة الاولى و تشمل مخلوط المستخلص النباتي مع لدايبل 2x و الفيروس النووي ذات تأثير قوي. المجموعة الثانية مخلوط الدايبل 2x و الفيروس النووي ذات تأثير متوسط و المجموعة الثالثة و تشمل المستخلص النباتي الضعيفة التأثير