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EFFECT OF THE MIXTURE OF PLANT EXTRACT OF BUXUS CHINENSIS, DIPEL 2X AND NUCLEAR POLYHEDROSIS VIRUS (NPV) ON SOME THE BIOLOGY OF THE COTTON LEAFWORM, SPODOPTERA LITTORALIS, (NOCTUIDAE: LEPIDOPTERA)

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ABSTRACT: *The effects of Buxus chinensis, extract B. thuringiensis and nuclear Polyhedrosis virus (NPV) on certain biological aspects of Spodoptera littoralis were evaluated .*

Both 2nd and 4th larvae instars of S. littoralis were for that purpose. The mixture of the all three compounds were the most toxic against the 2nd and 4th instars of S. littoralis larvae. Those caused considerably high percentage of mortality, while those mixed of B. thuringiensis (Dipel 2X) and nuclear Polyhedrosis virus (NPV) were slight effective against 2nd and 4th instars larvae of S. littoralis. Generally the ethanol extract of Buxus chinensis leaves was more effective than other B. thuringiensis (Dipel 2X) and nuclear Polyhedrosis virus (NPV) in the respect of pupal mortality and larval, pupal and adult duration. This plant extract also reduced the fecundity and fertility of a live females of the treated larvae of the 4th larval instar of S. littoralis.

Key Words: *Cotton Leafworm, Buxus chinensis, extract B. thuringiensis, nuclear Polyhedrosis virus (NPV), Biology*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis (*Boisd.*)* is considered as one of the most serious insect pests of many different Egyptian crops, it attack and damage in several parts of its host plants. Many researchers used different methods in order to control this pest Nakanishi, (1977); Radwan, *et al* (1986); Amer, (1989): Corbitt, *et al* (1989); Naqvi, (1990); Bermawy, *et al* (1992); Chanda, and Chakravorty (1993); Antonious and Rizk (1994); Abo El-Ghar, (1994); Sharaby et al (1994); Salem *et al* (1995); Ismail, *et al* (1996); Salama, and Ahmed (1997); Schmidt *et al* (1997); Ismail *et al* (1999) and Khalaf, (1999) .

The aim of this work is to assess the usage mixed of *Buxus chinensis, B. thuringiensis* and nuclear Polyhedrosis virus (NPV) mixture on certain biological aspects of *Spodoptera littoralis.* This can be attained by determining their possible latent effects on certain biological aspects.

MATERIALS AND METHODS

Insect culture:

Spodoptera littoralis (Boisd.) were obtained from Plant Protection Research Institute in Dokki, Giza, Egypt which reared on castor oils leaves according to the methods described by El-Defrawi *et el.*, 1964.The culture was maintained at 28° ± 2 & 55% RH.

Botanical extracts:

Preparation of plant extract:

Buxus chinensis leaves was left to dry at room temperature (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 freezer until bioassay.

Bioassay of compounds to *Spodoptera littoralis* larvae and data analysis:

Oral administration was under taken by feeding technique 2nd and 4th instars larvae of *S.littoralis* on castor leaves, by using different concentrations 4ppm, 300 g/400 L water and 5X 10^{12} /BIP mL for *Buxus chinensis, B.thuringiensis* and nuclear Polyhedrosis virus (NPV) respectively were used . The fresh castor-oil leaves were dipped in for five seconds in 10 ml of each used insecticide concentration. The treated castor oil leaves were used as a food for both 2nd and 4th larval 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 POLO-PC (Leora Software, 1994). Then data were subjected to the probit analysis Finney (1971). When it is necessary the control mortality was adjusted by the help of Abbott's formula (Abbott, 1925). The effects on the insect development by the treatment with the of 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 to larvae daily 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 efficiency of the different used compounds were calculated according to Vagras and Sehnal (1973) for calculating the developmental rates.

RESULTS AND DISCUSSION

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

Results represented in tables (1-2) were show the effect of ethanolic *Buxus chinensis* leaves extracts on 2nd and 4th larvae instar of *Spodoptrea. littoralis* fed on castor oil leaves. Where the ethanolic *N. oleander* leaves extracts were affected on 2nd and 4th larvae instar of *S. littoralis*. the mean percentage of larval mortality was decreased to 82, 60, 54, 45, 35 and 15 % for 2nd larvae instar but it were 27.5, 20, 16.5, 15.5 ,13 and 9.5 for 4th larvae instar at concentrations of 4, 2,1, 0.5, 0.25 and 0.125 ppm of ethanolic *N. oleander* leaves extracts respectively, compared to no mortality for control larvae. From the data in table (1-2) it was observed that a significant effect on the duration of 2nd and 4th larvae instar after treatment with ethanolic *N. oleander* leaves extracts. At concentrations of 4 ppm, it was mean 11.4 days for 2nd larvae instar 3.76 days for 4th larvae instar respectively, compared to 6 days for control larvae. The mean percentage pupal mortality was reduced to 3.5%, for 2nd larvae instar but it were 1% 4th larvae instar as compared to no mortality for the control.

According to data in tables (1-2) show a significant effect on the duration of the pupae produced from treated 2nd and 4th larvae instar with at concentrations of 4 ppm of ethanolic *Buxus chinensis* leaves extracts, the pupal duration was decreased to 6.59 days for 2nd larvae instar but it were 6.61 days for 4th larvae instar respectively, as compared to 9.8 days for the control larvae. In case of adult stage, resulted from treated 2nd and 4th larvae instar *of S. littorolis*, the mean percentage of emerged moths, fecundity and hatchability were greatly effected presented in tables (1-2). The mean percentage of emerged moths was decreased to 6 % 2nd larvae instar but it were 64 % for 4th larvae instar at the concentrations 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 extract the higher was the larval mortality and vice versa.

2- When 2nd and 4th larvae instar of *Spodoptera littoralis* were fed on castor oil leaves treated with mixture of *Buxus chinensis, B. thuringiensis* and nuclear Polyhedrosis virus (NPV) were affected on the insect developmental (tables. 3 and 4). From the data recorded in (tables. 3 and 4) it observed that the mean percentage of larval mortality was increased by increasing concentrations. 99% at the higher concentrations for 2nd larvae instar but it was 51% for 4th larvae instar with mixture of *Buxus chinensis, B. thuringiensis* and nuclear Polyhedrosis virus (NPV) respectively, compared to no mortality for control larvae.

Table (1): Effect of Ethanolic *Buxus chinensis* leaves extracts on 2nd instar of *S. littoralls* (Boisd) .

Treatments (ppm)	Experiment I						Experiment II					
	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.
4	86	12.21 (± 0.11)	3	7.14 (± 0.81)	8	140 (±37.42)	78	10.5 (± 1.5)	4	6.03 (± 0.8)	9	130 (± 21.6)
2	54	12.24 (± 0.21)	10	7.25 (± 0.82)	26	170 (±30.82)	66	11 (± 2)	14	7.15 (± 0.81)	10	220 (± 8.17)
1	48	12.27 (± 0.35)	10	7.37 (± 0.83)	28	220 (±28.22)	60	11.1 (± 2.6)	15	7.22 (± 0.83)	19	340 (± 29.37)
0.5	40	12.31 (± 0.66)	8	7.39 (± 0.86)	30	380 (±5616)	50	11.5 (± 2.25)	15	7.31 (± 0.86)	22	360 (± 52.27)
0.25	30	12.42 (± 0.72)	6	7.43 (± 0.88)	36	420 (± 56.57)	40	11.56 (± 2.59)	15	8.37 (± 0.87)	34	425 (± 62.01)
0.125	–	12.6 (± 0.84)	4	7.6 (± 0.92)	38	500 (±41.23)	30	11.59 (± 2.61)	20	7.45 (± 0.89)	46	500 (±42.25)
Treated with Ethanolic	–	12.61 (± 0.42)	–	8.11 (± 0.95)	99.8	658 (±88.73)	–	12 (± 2.9)	–	8.5 (± 0.88)	99	790 (±72.46)
Untreated	–	16.0 (± 0.21)	–	9.8 (± 0.22)	100	2499.1 (± 319.78)	78	10.5 (± 1.5)	4	6.03 (± 0.8)	9	130 (± 21.6)

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

Table (2): Effect of Ethanolic Buxus chinensis leaves extracts on 4th instar of S. littoralis (Boisd) .

Treatments (ppm)	Experiment I						Experiment II					
	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.
4	30	4 (± 2)	1	7.11 (± 0.42)	61	530 (± 21.6)	25	3.53 (± 1.07)	1	6.04 (± 0.21)	67	440 (± 35.63)
2	20	4.33 (±3.67)	2	7.3 (± 0.43)	69	620 (± 8.17)	20	3.55 (± 1.07)	–	6.31 (± 0.25)	72	590 (± 19.77)
1	18	4.66 (± 1.34)	3	7.5 (± 0.45)	71	740 (± 29.37)	15	3.6 (± 1.11)	–	6.37 (± 0.33)	75	670 (±48.06)
0.5	16	4.8 (± 1.5)	4	7.61 (± 0.47)	72	860 (± 52.27)	15	3.67 (± 1.16)	–	6.42 (± 0.39)	82	713.33 (± 37.58)
0.25	16	5 (± 3)	2	8.22 (± 0.56)	77	925 (± 62.01)	10	3.7 (± 1.6)	–	6.51 (± 0.45)	86	925 (± 68.92)
0.125	14	5.5 (± 4.5)	0	8.75 (± 0.58)	83	1000 (±42.25)	5	3.8 (± 1.14)	–	6.63 (± 0.48)	91	1090 (± 81.92)
Treated with Ethanolic	10	5.6 (± 1)	0	8.81 (± 0.59)	85	1090 (±72.46)	–	6 (± 2.16)	0.5	7 (± 0.4)	98.6	2530 (±76.16)
Untreated	–	12 (± 2.9)	–	8.5 (± 0.88)	99	2254.2 (± 319.78)	–	6 (± 0.15)	–	9.8 (± 0.22)	100	2688.5 (± 204.4)

F values = 404.806 ***L. S. D 0.05 = 3.096

According to the data in (tables. 3 and 4), from these results, 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), it was one day for 2nd larvae instar but it was 5 days for 4th larvae instar respectively, compared to 6 days for control larvae.

In case of pupal stage, which resulted from treated 4th larvae instar of *S. littoralis*, with mixture of *Buxus chinensis, B. thuringiensis* and nuclear Polyhedrosis virus (NPV) were affected on the mean percentage of pupation, mortality, deformed pupae and the pupal durtion.

3- When 2nd larvae instar 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 (tables 5 and 6). From the data recorded in tables (5 and 6) it were 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 larvae instar but it was while the higher mortality (54 %) for 4th larvae instar occurred at higher concentration, compared to no mortality for control larvae.

According to the data in tables (5 nd 6) it were noticed that a significant shortened of larval duration compared to 16 days for control larvae.

From tables (5 and 6), the mean percentage pupal mortality increased by increasing of concentrations the higher pupal mortality (6%) was obtained at higher concentration for 2nd larvae instar but it was (5.5%) for 4th larvae instar occurred at higher concentration, compared to no pupal mortality for control larvae.

In case of adult stage, resulted from treated 2nd and 4th larvae instar of *S. littoralis*, the mean percentage of emerged moths, deformed moths, fecundity and hatchability were greatly effected presented in tables (5 and 6). The mean percentage of emerged moths was decreased to 21.5 % for 2nd larvae instar but it was (33 %) for 4th larvae instar respectively, as compared to 100% emerged moths for control.

From the results tabulated in tables (1 to 6) 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 larvae instars of *S. littoralis* followed by mixed of *B. thuringiensis* and nuclear Polyhedrosis virus (NPV) while *Buxus chinensis* extract alone had a slightly effect. On the other hand the pupation percentages and adult emergence averages resulted from the 2nd and 4th larvae instars of *S. littoralis* treated with the mixture of *Buxus chinensis* extract, *B. thuringiensis* and nuclear Polyhedrosis virus (NPV) were greatly affected. These results are similar to those obtained by many authors.

Table (3): Effect of *mixd three compounds* on 2nd instar of *S. littoralis* (Boisd) .

Treatments	Experiment I						Experiment II					
	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.
A+b+c 100%	100	1 (± 0.01)	–	–	–	–	98	1 (± 0.01)	–	–	–	–
A+b+c 75%	90	7 (± 1)	3	5 (± 0.1)	–	–	88	7 (± 1)	5	5 (± 0.1)	–	–
A+b+c 50%	85	8 (± 0.2)	5	6 (± 0.1)	–	–	84	8 (± 0.2)	8	6 (± 0.1)	–	–
A+b+c 25%	80	9 (± 0.13)	6	7 (± 0.1)	–	–	78	9 (± 0.13)	10	7 (± 0.1)	–	–
A+b+c 12.5%	70	9.01 (± 0.15)	7	7.2 (± 0.5)	10	–	66	9.01 (± 0.15)	10	7.2 (± 0.5)	13	–
Untreated	–	16.0 (± 0.21)	–	9.8 (± 0.22)	100	2499.1 (± 319.78)	–	16 (± 0.21)	–	9.8 (± 0.22)	100	2254.2 (± 319.78)

NB:-
C = Buxus chinensis 4 PPM A = (npv) 5x 10^{12} PIB / larvae B = B.thuringiensis (Diple 2X) IU
F values = 2267 ***L. S. D 0.05 = 1.963

Table (4): Effect of *mixd three compounds* on 4th instar of *S. littoralis* (Boisd) .

Treatments	Experiment I						Experiment II					
	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.
A+b+c 100%	50	5 (± 1.87)	9	7 (± 0.01)	25	130 (± 21.6)	52	5 (± 1.87)	4	7 (± 0.01)	42	330 (± 111.6)
A+b+c 75%	45	5.08 (± 1.65)	8	7.3 (± 0.1)	35	420 (± 36.33)	43	5.08 (± 1.65)	10	7.3 (± 0.1)	35	420 (± 146.33)
A+b+c 50%	40	5.1 (± 1.83)	8	7.36 (± 0.3)	40	585 (±142.83)	39	5.1 (± 1.83)	10	7.36 (± 0.3)	40	685 (±202.83)
A+b+c 25%	35	5.28 (± 0.82)	7	7.41 (± 1.1)	50	725 (± 20)	32	5.28 (± 0.82)	10	7.41 (± 1.1)	50	725 (± 202)
A+b+c 12.5%	30	5.33 (± 1.63)	10	7.47 (± 2.9)	55	780 (±227.37)	29	5.33 (± 1.63)	11	7.47 (± 2.9)	55	980 (±227.37)
Untreated	–	16.0 (± 0.21)	–	9.8 (± 0.22)	100	23130 (± 21.6)	–	6 (± 0.15)	–	9.8 (± 0.22)	100	2688.5 (± 204.4)

NB:-

C = Buxus chinensis 4 PPM A = (npv) 5×10^{12} PIB / larvae B = B.thuringiensis (Diple 2X) IU

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

Table (5): Effect of *B.thuringiensis* (Diple 2X) and Nuclear Polyhedrosis Virus (SNPV) on 2nd instar of *S. littoralis* (Boisd).

Treatments	Experiment I						Experiment II					
	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.
A+b 100%	60	10.5 (± 0.3)	6	7.05 (± 0.81)	24	316 (± 33.74)	65	10.5 (± 0.3)	6	7.05 (± 0.81)	19	516 (± 33.74)
A+b 75%	40	10.55 (± 1.1)	6	7.25 (± 0.81)	30	426 (± 88.72)	43	10.55 (± 1.1)	6	7.25 (± 0.81)	42	626 (± 88.72)
A+b 50%	32	10.63 (± 2.3)	8	7.27 (± 0.83)	46	537 (± 121)	36	10.63 (± 2.3)	8	7.27 (± 0.83)	49	737 (± 121)
A+b 25%	22	10.67 (± 1.9)	10	7.36 (± 0.84)	56	675 (± 60.21)	25	10.67 (± 1.9)	10	7.36 (± 0.84)	52	875 (± 60.21)
A+b 12.5%	20	10.75 (± 3.1)	10	7.42 (± 0.86)	59	980 (± 46.21)	10	10.75 (± 3.1)	10	7.42 (± 0.86)	67	980 (± 46.21)
Untreated	–	16.0 (± 0.21)	–	9.8 (± 0.22)	100	2499.1 (± 319.78)	–	16 (± 0.21)	–	9.8 (± 0.22)	100	2254.2 (± 319.78)

NB:-
A = (NPV) 5x 10^{12} PIB / larvae B = B.thuringiensis (Diple 2X) IU
F values = 2267 ***L. S. D 0.05 = 1.963

Table (6): Effect of *B.thuringiensis* (Diple 2X) and Nuclear Polyhedrosis Virus (SNPV) on 4th instar of *S. littoralis* (Boisd).

Treatments	Experiment I						Experiment II					
	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.	* % Larval Mortality	Larval Duration (days) mean±S.E.	% Pupal Mortality	Pupal Duration (days) mean±S.E.	% Emerged Moths	Fecundity no. of egg Laid / female Mean±S.E.
A+b 100%	53	6.52 (± 0.3)	6	7.05 (± 0.81)	33	516 (± 33.74)	55	6.52 (± 0.3)	5	7.05 (± 0.81)	33	516 (± 33.74)
A+b 75%	40	6.54 (± 1.1)	6	7.25 (± 0.81)	45	626 (± 88.72)	47	6.54 (± 1.1)	4	7.25 (± 0.81)	40	626 (±1 88.72)
A+b 50%	32	6.63 (± 2.3)	8	7.27 (± 0.83)	54	737 (± 121)	38	6.63 (± 2.3)	3	7.27 (± 0.83)	51	737 (± 121)
A+b 25%	23	6.62 (± 1.9)	10	7.36 (± 0.84)	58	875 (± 60.21)	29	6.62 (± 1.9)	10	7.36 (± 0.84)	58	875 (±2 60.21)
A+b 12.5%	07	6.71 (± 3.1)	10	7.42 (± 0.86)	72	980 (± 46.21)	13	6.71 (± 3.1)	10	7.42 (± 0.86)	66	980 (±346.21)
Untreated	_	16 (± 0.21)	_	9.8 (± 0.22)	100	2254.2 (± 319.78)	_	16 (± 0.21)	_	9.8 (± 0.22)	100	2254.2 (± 319.78)

NB:-

A = (NPV) 5x 10^{12} PIB / larvae B = B.thuringiensis (Diple 2X) IU

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

Guirguis *et al.* (1991) 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) found that 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.

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تاثير مخلوط المستخلص النباتى الهوهوبا مع الدابيل 2X و الفيرس النووى NPV على بيولوجى دودة ورق القطن

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الملخص العربي

أجريت هذه الدراسة لدراسة تأثير كل من المستخلص النباتى الهوهوبا و مخلوط المستخلص النباتى الهوهوبا مع الدابيل 2X و الفيرس النووى NPV و مخلوط الدابيل 2X و الفيرس النووى NPV على بيولوجية دودة ورق لبقطن .

وقد أوضحت النتائج أنه يمكن تقسيم المواد تبعا لشدة تأثيرها على الحشرة الى ثلاث مجموعات كالتالى :

المجموعة الاولى وتشمل المخلوط مخلوط المستخلص النباتى الهوهوبا مع الدابيل 2X و الفيرس النووى NPV ذات التأثير القوى , المجموعه الثانية مخلوط الدابيل 2X و الفيرس النووى NPV ذات التأثير المتوسط و المجموعه الثالثة وتشمل المستخلص النباتى الهوهوبا الضعيفة التأثير.