TOXICOLOGICAL AND BIOLOGICAL EFFECTS OF NEEM AND JOJOBA OILS ON THE BLACK CUTWORM *AGROTIS IPSILON* (HÜFN) IN THE LABORATORY.

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Abstract

The present study aimed to investigate the toxicity and biological effects of (Neemix 4.5% azadirachtin) neem and (Nat-1 96%) jojoba oils on 4th instar *A. ipsilon* larvae. The two materials were tested as a bait (with wheat bran) at 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 liters / 25kg wheat bran under a constant temperature of 26±1°C. The study revealed that the Neemix was more toxic than Nat-1. LC_{50} and LC_{90} values were 0.84 and 1.85 for Neemix and 0.97 and 2.04 for Nat-1, respectively. In general the two plant oils caused an increase in larval and pupal durations and decrease in pupal weight. Also, Neemix was more effective than Nat-1, decreased pupation, pupal weight, adult emergence, fecundity and fertility. Malformed pupae were increased with the Neemix. While, Nat-1 was high in malformed adults.

INTRODUCTION

The black cutworm *Agrotis ipsilon* (Hüfn) is one of the most severe destructive pests in Egypt and many other countries of the world. It is a poly-phagous noctuid occurring throughout the year, and feeding on a great variety of crops. Neem oil is obtained from the seed kernel of neem trees (*Azadirachta indica* A Juss; family Meliaceae) (Rao, 1999). About 30 years ago, work on the neem tree ingredients concentrated on a substance which was called azadirachtin. Azadirachtin has a complex structure which was initially proposed by Zanno *et al.* (1975). Seeds of *A. indica* trees were found to contain up to 0.48% azadirachtin, Zhang *et al.* (1992). Jojoba oil is obtained from the seeds of the slow growing jojoba plant (*Simmodsia chinensis* (Clink); family Buxaceae), which is now widely grown in several countries, very little is known of the toxicological and health consequences of it to food, Ronhotra and Gelroth (1989). The range value of jojoba oil in the seeds is 45-60%, Haumann (1983). In Egypt, several laboratory studies were done on seed crude extracts of neem tree and jojoba plant about their pesticidal effect on various economic pests, especially order lepidoptera such as *Spodoptera littoralis,* El-Sayed

(1982-1983a) and Ghoneim *et al.* (2002), *Phthorimaea operculella* (Salem, 1991), *Helicoverpa armigera* (Ismail, 1994), *Pectinophora gossypiella* (Rofail *et al.,* 2000 and Abdel-Rahman *et al.,* 2002) and *Agrotis ipsilon* (Osman, 2003). The results indicated that neem and jojoba oils were affected as toxicants, antifeedants, growth and development inhibitors and oviposition inhibitors. So, the two oils of neem and jojoba were chosen to study their effects, as baits, against 4th instar larvae of *A. ipsilon.* This method demonstrates better properties, no effect on parasites and predators with no pollution to the environment.

MATERIALS AND METHODS

The cultural of *A. ipsilon* used in this study was originated from eggs obtained from a susceptible laboratory strain established in the Black Cutworm Department, Plant Protection Research Institute, ARC, Dokki, Giza. The culture was maintained and built-up under 26$\pm$ 1°C to gain 4th instar larvae were chosen randomly and individually left without feeding for two hours before treatment. All experiments were also conducted under the same temperature.

A- The following oils were tested as baits:

1- Neem seed oil, Neemix was used herein as an emulsifiable concentrate of 4.5% azadirachtin (Obtained from N.M Agro Egypt, Ltd. Co.).

2- Jojoba seed oil, Nat-1 was used herein as an emulsifiable concentrate of 96% (obtained from Egyptian Natural Oil Co.).

B- Preparation of the baits:

For each oil, seven concentrations of baits was prepared with mixing a limit volume with another quantity of wheat bran (WB) to gain the rates of 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 liters/25KgWB. Treacle solution (20%) was added to these rates (0.95 ml/1gm) with a complete hand mixing and left for 12 hours under room temperature to be used.

C- Treatment of the larvae:

Twenty 4th instar larvae, for each rate, 4 replicates were used; each replicate was of 5 larvae and each larva transferred into a small plastic pot (4 x 7cm) contained about 10 gm of the bait. Another 20 larvae were also reared by the same way on a bait free from the oils as a control. All larvae were allowed to feed for 2 days on the baits then complete their life cycle on castor oil leaves. Mortality was recorded 1, 2, 4, 6 and 8 days after treatment and LC_{50} and LC_{90} values were evaluated. Also, larval and pupal durations, percentages of pupation and adult emergence, pupal weight, sex

ratio, no. of eggs laying / female, no. of egg hatching, adult longevity and no. of both pupal and adult malformations were all recorded.

D- Calculation and data analysis:

The percentage of larval mortality were corrected according to Abbott's formula. The LC_{50} and LC_{90} calculations after 4 days from treatment were done by the method of Finney (1971), and statistical analysis was adopted according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

1- Toxicity effect of Neemix and Nat-1 on the larvae:

As shown in Table (1) and Fig. (1), while LC_{50} of Neemix and Nat-1 were 0.84 and 0.97 liter / 25Kg WB. LC_{90} recorded 1.85 and 2.04 liters /25KgWB, respectively. These results revealed that the Neemix was more effective than Nat-1. While the first oil gave 100% larval mortality after 2 days of treatemnt with the concentration of 2 liters/25KgWB, Nat-1 gave 89.47%. The results agreed with those obtained by El-Sayed (1982-1983a) who reported that the neem oil caused 100% mortality to 2^{nd} and 3^{rd} larval instar of *S. littoralis* when fed on treated leaves using 0.2 to 0.5% concentrations. All larvae of *Ostrinia furnacalis* developed malformations and died or became permanent larvae when treated with 500 ppm of neem oil Zhang *et al.*(1992). Neem seed kernel extract (1%) caused 90-95% larval mortality of *Helicoverpa armigera* (Natrajan and Shanthi, 1994); the highest concentration of neem leaf extract (*A. indica*) was tested as a bait at 5ml / 50 ml distilled water / 25gm crushed corn seeds resulted in 98% mortality for 5^{th} instar nymphs of *Gryllotalpa gryllotalpa* (Ibrahim, 2002). Also, this oil was more toxic than jojoba oil for 3^{rd} instar larvae of *A.ipsilon* (Osman, 2003).

2- Effect of Neemix and Nat-1 in some biological aspects:

The data in Tables (2 and 3) indicated that, the Neemix was more effective than Nat-1 one on the concerned biological aspects of *A. ipsilon.*

a- The larval stage:

As shown in Tables (2 and 3), the two oils prolonged the larval duration up to 31 days, which ranged from 25.50 to 31.00 days compared to 23.50 days in the control. This may be due to that, plant oils delaying the moulting process. This increase may be explained according to the assumption of Lu *et al.* (1978) that the accumulation of the toxic substances in any organism may be affect the longevity of insects. Also, Gupta and Rao (1990) studied the effect of azadirachtin injected into the

last instar larvae of *S. littoralis* on medial neurosecretory cells and corpora allata, they found a delayed accumulation of neurosecretory materials in these tissue. Also the percentage of pupation was greatly reduced comparing with the control. The percentage of pupation was 75, 50, 35, 30, 20, 15 and 0% at 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 liters of Neemix /25 Kg WB, respectively. The percentage in case of Nat-1 reached to 80, 65, 40, 35, 20, 20 and 5% at the same concentrations, respectively, compared to 90% in the control. These results are similar to those obtained by Atwal and Pajni (1964) when showed that the neem oil caused a growth inhibitory activity of the larvae of *Pieris rapae.* Also, it, prolonged development of larval growth of *S. littoralis* (Meisner *et al.,* 1983) and also Saxena *et al.* (1984) found that, neem oil inhibited growth and development of *Sesamia furcifera.* The percentage of pupation of *S. littoralis* was decreased with increasing the concentration of Egyptian neem (zanzalakht) (Emara *et al.,* 2002).

b- The pupal stage:

Data in Tables (2 and 3) showed that, the two oils prolonged the pupal duration up to 12 days, which range from 10 to 12 days compared to 9.94 days in the control. The increase in pupal duration may reflect a metamorphosis disruption. On the other hand, pupal weight decreased gradually as the concentrations of both oils were increased. In the same time, the weight was smaller if compared with those of the control. The pupal weight was 345.75, 315.12, 307.10, 295.37, 270.33 and 257.00 mg at 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 liters of Neemix/25Kg WB, respectively. The weight in case of Nat-1 reached to 357.56, 344.58, 325.98, 303.57, 286.30, 278.07 and 265.10mg at 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 liters / 25Kg WB, respectively, compared to 390.42mg in the control. This decrease in the pupal weight may be, partially attributed, to a decrease in total water content and/or a decrease in the intensity of protein synthesis needed for growth and development after treatment with the oils. Also, the percentage of adult emergence was greatly reduced comparing with the control. The percentage of adult emergence was 60.00, 60.00, 57.14, 50.00, 50.00 and 33.33% at 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 liters of Neemix /25Kg WB, respectively. The percentage in case of Nat-1 reached to 75, 61.54, 62.50, 57.14, 50.0, 50.0 and 0.0% at 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 liters / 25 Kg WB, respectively, compared to 94.44% in the control. These results agree with those obtained by Meisner *et al.* (1983) when showed that, the neem oil prolonged development of pupal growth of *S. littoralis* Aboel-Ghar *et al.* (1994) who showed that,

acetone and ethanol extracts of zanzalakht decreased significantly the pupal weight of *A. ipsilon.* Fouad and Abdel-Alim (1994) when revealed that, adult emergence of *Sitophilus granarius* was completely prevented in wheat grain treated with neem oil at 7-10 ml/Kg. In this respect, Emara *et al.* (2002) who said that, the pupal weight and percentage of adult emergence of *S. littoralis* were decreased with increasing the concentrations of zanzalakht and both levels (LC_{50} and LC_{90}) of the jojoba oil highly reduced the number of emerged adults of *Sitophilus oryzae* and *S. granarius* (Shemais *et al.,* 2002).

C- The adult stage:

The data given in Tables (2 and 3) indicate the number of eggs/female and percentage of hatching were 711.43 and 668.57 eggs/female and 35.70 and 21.43% at 0.50 and 0.75 liter/25 Kg WB for Neemix, respectively. Reached to 745.71, 691.43 and 384.28 eggs/female and 43.65, 28.15 and 11.67% at 0.50, 0.75 and 1.00 liter/25Kg WB for Nat-1, respectively, compared to 768.43 eggs/female and 81.87% in the control. This may be due to physiological disturbance in the hormonal system of adults. Also, Schmutterer (1990) found that, the component of azadirachtin isolated from *A. indica* had an effect on the balance of hormone system in the treated insects. The adult female lived longer than adult male. The treatment adult longevities were greatly reduced when compared with those of the control, may be due to the reduction in their weights and inhibition of proteins, lipids and carbohydrates. These results are similar to those obtained by El-Sayed (1982-1983b), more than 50% reduction in the number of eggs of *S. littoralis* deposited on *Nerium oleander* leaves treated with 2% neem seed suspension. Schmutterer (1990) who found that, the adult longevity of many insect spacies was shortened after application of higher concentrations of neem extracts or of azadirachtin. Also, Aboel-Ghar *et al.* (1994) found that, acetone and ethanol extracts of zanzalakht caused decrease in no. of eggs/female and percentage of hatching of *A. ipsilon.* Neem seed extract by ethanol was reached of oviposition deterrent indices of *H. armigera* to 72.67 and 83.49 with 1.0 and 2.0% concentrations, respectively, and the percentage of egg hatching decreased with increase of concentration (Ismail, 1994). Also, Emara *et al.* (2002) showed that, the adult longevity of *S. littoralis* decreased when the 2^{nd} instar larvae were fed on treated castor oil leaves by zanzalakht and also, both levels of LC_{50} and LC_{90} were reduced the no. of eggs of *S. oryzae* and *S. granarius* (Shemais *et al.,* 2002).

3- Malformations in the pupae and adults:

The morphological malformations appeared in the pupae and adults obtained from 4th instar larvae treated with 7 concentrations of Neemix and Nat-1 are shown in Tables (2 and 3) and Figs. (2 and 3). Malformed pupae increased with the Neemix. While, Nat-1 was high in malformed adults. These malformations may be due to hormonal disturbance caused by the plant oils. The malformations of pupae were; abnormal dwarfed pupa attached with larval exuvium, larval-pupal intermediate with larval head, thoracic legs and balloon shaped pupa from posterior, pupa failed in shedding off the larval exuvium and shrinked pupa. Also, the malformations of adults were; wings severely abnormal, wings severely short, adult failed to emerge from pupa, adult showing poorly developed with twisted wings, pupal skin covers all the adult body except thoracic legs, head and head appendages, adult with both fore and hind wings on one side slightly curled and the hind wings being shorter than normal. These results agree with those obtained by El-Sayed (1982-1983b) who found that, neem extract caused malformations in adults of *S. littoralis.* Also nymphs and adults of *Leptocorisa oritorius* were deformed when fed on neem oil treated food material (Saxena *et al.,* 1985); zanzalakht extract caused malformations in the different stages of *A. ipsilon* (Aboel-Ghar *et al.,* 1994) and *S. littoralis* (Emara *et al.,* 2002). Small formed nymph of *G. gryllotalpa* can molt after treatment with neem leaf extract and the resulted nymphs show an abnormal shape (Ibrahim, 2002).

Finally, it can be said that, (Neemix 4.5% azadirachtin) neem and (Nat-1 96%) jojoba oils as a bait (with wheat bran) were affected as toxicants, growth and development inhibitors on *A. ipsilon* larvae, pupae and adults, so it can be successfully used with the concentrations between 1.25 to 2.00 liters /25 KgWB.

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Table 1. Accumulative corrected% mortalities of 4th instar *A. ipsilon* larvae, fed on baits contained different concentrations of Neemix and Nat-1, after different post-treatment periods under a constant temperature of 26 ± 1°C.

Days after treatment	Neemix (liter / 25 Kg WB)							Nat-1 (liter / 25 Kg WB)						
	0.5	0.75	1	1.25	1.5	1.75	2	0.5	0.75	1	1.25	1.5	1.75	2
1	0	10.53	31.6	42.1	52.6	52.63	68.4	0	21.1	42	47.4	63.16	63.16	68.42
2	15.8	36.84	63.2	63.2	79	84.21	100	10.53	26.3	53	57.9	73.68	78.95	89.47
4	15.8	42.1	63.2	63.2	79	84.21	-	10.53	26.3	53	57.9	78.95	78.95	94.74
6	15.8	39.74	63.2	63.2	79	84.21	-	15.79	26.3	53	57.9	78.95	78.95	94.74
8	21	47.37	63.2	68.4	79	84.21	-	15.79	31.6	58	63.2	78.95	78.95	94.74
LC_{50}	0.84 Liter							0.97 liter						
LC_{90}	1.85 Liters							2.04 liters						
Slope ± S.E.	3.75± 0.29							3.98± 0.29						

Table 2. Effect of Neemix bait on some biological aspects of 4th instar *A. ipsilon* larvae under a constant temperature of 26 ± 1°C.

Conc. liters/25Kg WB	Larval duration (days)	Pupation (%)	Pupal wt. (mg)	Pupal duration (days)	% Adult emergence	Malformation (%)		No. of eggs /female	Egg hatching (%)	Adult longevity (days)	
						Pupae	Adults			Female	Male
0 (control)	23.50 ±1.25 g	90	390.42 ±1.91 a	9.94 ±0.75 efg	94.44	0	0	768.4 ±23.5 a	81.9	12.60 ±0.84 a	11.33 ±0.82 a
0.5	26.47 ±1.96 cdef	75	345.75 ±6.39 b	10.37 ±0.52 def	60	26.78	53.3	711.43]±27.34 b	35.7	11.80 ±1.30 b	9.75 ±1.50 b
0.75	26.90 ±2.08 cde	50	315.12 ±3.74 c	10.43 ±0.79 de	60	41.66	36.7	668.57 ±24.78 c	21.4	11.50 ±0.71 bc	9.60 ±1.14 bc
1	27.14 ±2.03 cd	35	307.10 ±8.51 cd	10.75 ±0.50 cd	57.14	75	25	-	-	9.70 ±0.95 d	-
1.25	27.83 ±2.14 c	30	295.37 ±10.12 e	11.00 ±0.0 bc	50	62.5	0	-	-	9.67 ±0.58 de	-
1.5	30.50 ±0.58 ab	20	270.33 ±5.72 f	11.50 ±0.71 ab	50	66.66	50	-	-	9.33 ±0.58 def	-
1.75	31.00 ±1.00 a	15	257.00 ±8.34 g	12.00 ±0.0 a	33.33	75	0	-	-	9.00 ±0.0 defg	-
2	-	0	-	-	-	-	-	-	-	-	-

Table 3. Effect of Nat-1 bait on some biological aspects of 4th instar *A. ipsilon* larvae under a constant temperature of 26 ± 1°C.

Conc. liters/25Kg WB	Larval duration (days)	Pupation (%)	Pupal wt. (mg)	Pupal duration (days)	Adult emergence (%)	Malformation (%)		No. of eggs /female	Egg hatching (%)	Adult longevity (days)	
						Pupae	Adults			Female	Male
0 (control)	23.50 ±1.25 h	90	390.42 ±1.91 a	9.94 ±0.75 efg	94.4	0	0	768.43 ±23.54 a	81.9	12.60 ±0.84 a	11.33 ±0.82 a
0.5	25.50 ±1.37 fg	80	357.56 ±7.12 b	10.00 ±0.45 ef	75	25	72.2	745.71 ±17.18 ab	43.7	12.29 ±1.50 ab	11.00 ±1.41 a
0.75	26.46 ±1.76 def	65	344.58 ±8.72 c	10.37 ±0.52 de	61.5	31	54.2	691.43 ±26.25 abc	28.2	11.75 ±0.50 abc	10.50 ±1.29 a
1	27.00 ±1.51 de	40	325.98 ±7.97 d	10.67 ±0.52 bcd	62.5	25	50	384.28 ±11.34 d	11.7	11.25 ±1.26 cd	9.75 ±0.96 a
1.25	27.43 ±1.40 d	35	303.57 ±11.49 e	11.00 ±0.0 abc	57.1	70	66.7	-	-	10.67 ±1.52 de	-
1.5	29.50 ±2.38 abc	20	286.30 ±8.98 f	11.00 ±0.0 ab	50	50	0	-	-	10.50 ±0.71 def	-
1.75	30.00 ±1.82 ab	20	278.07 ±13.99 fg	11.50 ±0.58 a	50	66.7	50	-	-	9.50 ±0.71 fg	-
2	31.00 ±0.0 a	5	265.10 ±0.0 h	-	0	100	-	-	-	-	-

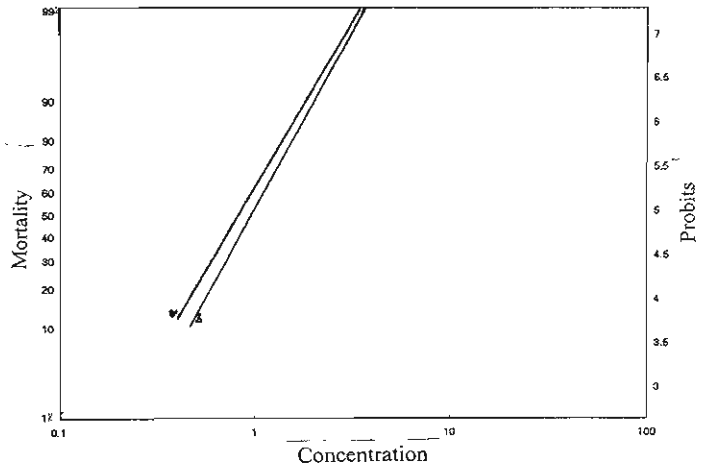

Fig. (1): Concentration – mortality regression lines of two plant oils against 4th instar larvae of *A. ipsilon.*

a- (Neemix) neem oil.
b- (Nat-1) jojoba oil.

Fig. (2): Malformed pupae resulted from 4th instar *A. ipsilon* larvae treated with (Neemix) neem and (Nat-1) jojoba oils.

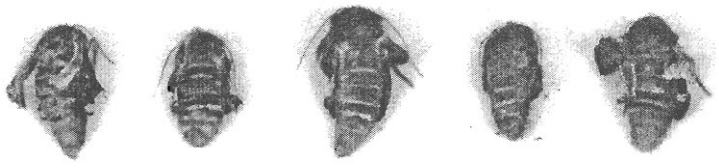

Fig. (3): Malformed adults resulted from 4th instar *A. ipsilon* larvae treated with (Neemix) neem and (Nat-1) jojoba oils.