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Effectiveness of some pesticides on endurance and emergence of the black cutworm, Agrotis ipsilon (Hufnagel) {Lepidoptera: Noctuidae}

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ABSTRACT : **Regular applications of synthetic insecticides might induce environmental pollution because of the high residue; rising insecticide resistance in many insect pests demands novel pest control methods. So, the study aimed to investigate the toxicity effect of three pesticide mixtures, extreme 36% SC (methoxyfenozide 30% + Spinetoram 6%), atifos-super 55% EC (chlorpyrifos 50%+cypermethrin5%), and tempo-xl 30% EC (chlorpyrifos 25% + lufenuron 5%) on the Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae), which considered as critical pests due to attacking diverse field crops and vegetables. These compounds are effectively against lepidopterous immature insect stages, with relatively slow but strong action. The data indicated that the highest mortality percent of treated 2nd and 4th instars caused by the highest concentration of pesticides recorded (92.73, 98.15&100), (96.36, 98.15&100), (96.43, 100&100), (82.76, 91.07& 98.21%), (91.38, 94.64 & 98.18%) and (86.21, 96.43& 98.18%) after 24, 48 and 72 hr. for extreme, atifos-super and tempo-xl pesticides, respectively. Tempo-xl recorded the highest efficacy against 2nd and 4th instar larvae with LC50 of 0.73and 4.09 ppm, followed by extreme with LC50 of 1.03 and 05.03 ppm and atifos-super with LC50 5.93 and 9.78ppm, respectively. The results showed that tempo-xl is the most toxic in the various toxic values of the 2nd and 4th A. ipsilon larvae followed by extreme and atifos-super. Also, a failure of ecdysis as a standard of the disruption of the development observed after treatment with these admixtures, especially tempo-xl. Tempo-xl could be recommended as an eco-friendly alternative to synthetic insecticides for the IPM program of A. ipsilon.**

Key words: mixture biocides; Agrotis ipsilon;toxic;deformation.

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I. INTRODUCTION

The black cutworm, A. ipsilon (Lepidoptera: Noctuidae) is a serious pest of corn and several agricultural crops in Egypt and many other countries of the world [1] This noctuid is almost polyphagous that attacks a large number of vegetable crops include beans, broccoli, cabbage, carrot, Chinese broccoli, Chinese cabbage, Chinese spinach, corn, eggplant, flowering white cabbage, green beans, head cabbage, lettuce, mustard cabbage, potato, spinach, sugarcane, sweet potato, tomato, turnip, as well as many other plants which causes extensive damage to a wide range of fiber and forage crops in agro-ecosystems of North Africa and the Middle East[2, 3] The damage is caused by the larvae which are usually hide in cracks and crevices in the soil or under the clods or debris around the plants during day time and come out of these places of hiding at night and cut the young plants at ground level and eat only the tender parts [4] The control of this pest has become a serious challenge facing applied entomologists nowadays regarding the widening circle of resistance and cross resistance to almost all available conventional insecticides, including organophosphorus and pyrethroids and insect growth regulators insecticides. Chemical control of multiple pests caused resistance to different classes of insecticides. Therefore, now it has become necessary to search for alternative means of pest control which can minimize the use of chemicals [5] the growers resort to the use of insecticide mixtures in an effort to obtain acceptable control of pests. Availability of cheaper, generic insecticides has further popularized the application of pre-and tank-mixed mixtures[6] In Egypt, as a result of unregulated and continued application of insecticides, insects began to develop high levels of resistance to insecticides and to overcome this problem and control the black cutworm damage involve using different groups of insecticides in rotation program which may be useful to delay the resistance problem [7]. Chemical interactions may occur when two or more toxic chemicals are given to a living organism simultaneously or sequentially. Such interactions may result in a toxicity that is additive of individual toxicities of the chemicals or that is greater or less than the additive toxicity of these chemicals if administrated separately. A relatively non-toxic chemical substance applied with an insecticide to increase the toxicity of the insecticide against insect pests is known as a synergist. They may inhibit the metabolic detoxification of the insecticide, increase the penetration of the insecticide through the insect cuticle, or act at the binding site(s) of the insecticide on the receptor proteins in target insects. Mixtures consisted of organophosphate, pyrethroid or carbamate insecticides have been found very effective in enhancing the toxicity of insecticides in different resistant insect pests worldwide like Helicoverpa armigera [8],and Spodoptera litura [6] This type of potentiation or synergism is explained by the inhibition of esterases [9] or monooxygenases activity [8] . Theoretically, mixing insecticides (with different modes of action) usually prove very effective in resistance management programs compared to mosaics or rotational use of insecticides[10] because, if a resistance mechanism to each insecticide in the mixture is independent and initially rare, the chances for the occurrence of resistance to both insecticides at the same time would be minimum [11] . Since pyrethroids and organophosphates have different modes of action, their mixtures have commonly been in practice against a variety of pests worldwide for the last many years [6] . Previously it has been assumed that organophosphates, when used in combination with pyrethroids, inhibit the enzymes responsible for metabolic detoxification in different insect pests [8, 9] .

The aim of the present study is to evaluate the toxicity of three admixtures; extreme 36% SC (methoxyfenozide 30% + Spinetoram 6%), atifos-super 55% EC (chlorpyrifos 50%+cypermethrin5%), and tempo-xl 30% EC (chlorpyrifos 25% + lufenuron 5%) and their disruptive effects against the 2nd and 4th instar larvae of the black cutworm, A. ipsilon as an applicable tool in programs of integrated pest management (IPM).

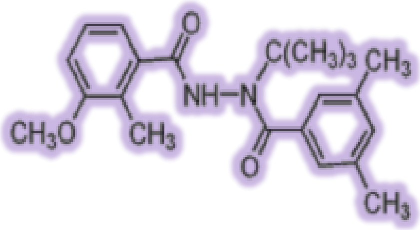
II. MATERIALS & METHODS

2.1. Insects rearing:

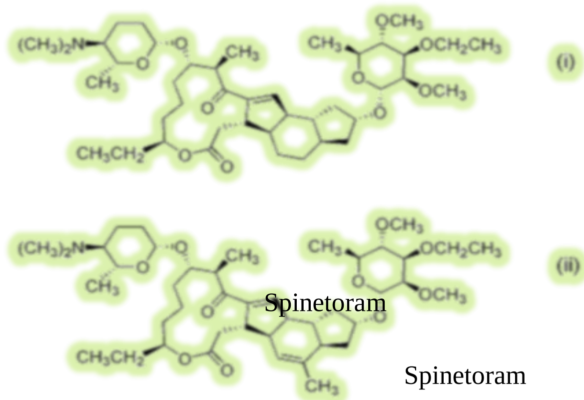
The colony of the black cutworm, A. ipsilon was maintained since 1982 in the Plant Protection Research Institute laboratories, Ministry of Agriculture Dokki, Giza, without any insecticidal pressure. According to [12] and [1] , the rearing technique was carried out under constant temperature of 25 ±2 ºC, 65± 5% relative humidity and 12:12 h. L: D (light: dark) photoperiod. Eggs were kept in a clean glass jar till hatching. The newly hatched larvae were held into new jars and fed on castor oil leaves, Ricinus communis. Larvae were reared individually in separate units of plastic cell trays (10.8 X 22.7 cm) to avoid cannibalism. Each tray contained 18 separated cells (5.2 x 3.2 x 3.7 cm), each cell having 5 small pores on its outer lateral walls for ventilation and its bottom was covered with glass sheets to prevent larval escape. The fresh castor oil leaves were offered daily till pupation. Pupae were then placed in glass jars until adult emergence. Couples of female and male moths were kept in a glass jar (9.5 cm diameter, 15 cm height) covered with muslin. In each jar, 5-7 stripes of porous filter paper were hanged as oviposition sites. Food was provided daily by using a cotton pad soaked in 10% honey solution, and then eggs were daily collected.

2.2. Tested Insecticides:

The current study used three commercial mixtures of pesticides representing three different insecticide groups: Extreme (36% SC): It is a product of DOW AgroSciences - UK and the common name consists of Methoxyfenozide 30% + 6% Spinetoram.

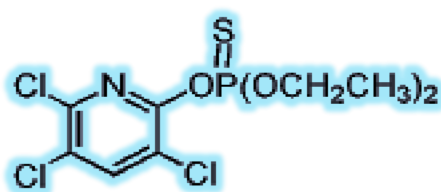


Methoxyfenozide

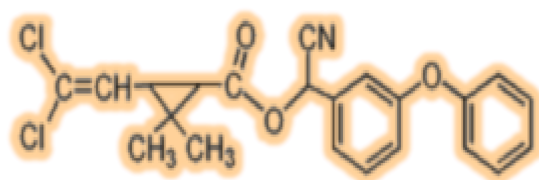


Spinetoram

B- Atifos- super (55% EC): It is a product of Jordan Insecticides &Agro-Treatment Manufacturing Co. and common name consists of Chlorpyrifos 50% + Cypermethrin 5%.

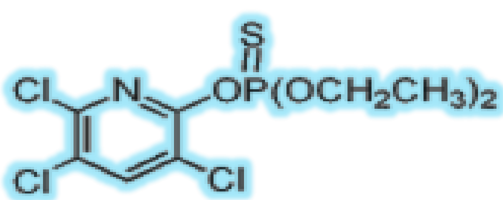


Chlorpyrifos

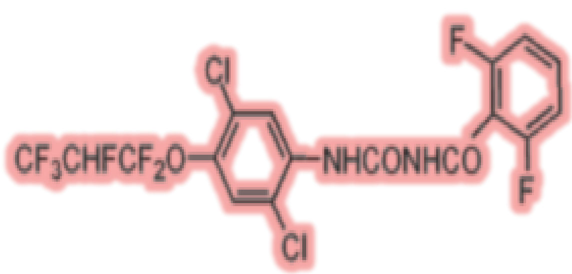


Cypermethrin

C- Tempo-xl (30% EC): It is the product of Starchem Industrial Chemicals - Egypt and the common name consists of Chlorpyrifos 25% + Lufenuron 5%.



Chlorpyrifos



Lufenuron

2.3. Toxicity study

Bioassay test: To study the toxicity of extreme, atifos-super and tempo-xl against the newly moulted 2nd and 4th instar larvae of laboratory strain, each compound was prepared as a series of aqueous concentration. The leaf-dipping technique was adopted according to[13], where fresh castor bean leaves were dipped for 10 seconds in one of the prepared concentrations. The treated leaves had dried under laboratory conditions before being offered to A. ipsilon larvae. Fifteen larvae distributed in four replicates (15 larvae/ replicate) were used for each concentration. Larvae were fed on leaves immersed in only water used as a control. Larvae were put individually in plastic cups and fed on the treated leaves with tested compounds in a glass jar covered with muslin for 24h. The treated leaves were replaced by another untreated one. Mortality percentages were calculated using the equation of [14] after 24, 48 and 72 hr. of treatment (Larval mortality %= A- B/A x 100, where (A) is the number of tested larvae and (B) is the number of tested pupae). The mortality was corrected using Abbott's formula [15] as follow:

Corrected mortality (%) = ((Observed mortality % - Control mortality %) / (100 - Control mortality %)) × 100

The LC25, LC50, LC90, and slope values of the tested compounds were calculated using the equation of [16] by software Program. The corresponding concentration probit line LC for tempo-xl and extreme were calculated as (40, 20, 10, 5, 2.5, 1.25& 0.625 ppm) and for atifos-super was calculated as (60.5, 30.25, 15.12, 7.56, 3.7, 1.84& 0.945 ppm) for 2nd and 4th instars. The control was evaluated for the three pesticides.

2.4. Statistical Analysis:

The statistical analysis of data on mortality was subjected to the Abbott formula [15] for correction wherever required. Probit analysis was determined to calculate LC50 [16] through a software computer program. Statistically significant differences between individual means were determined by one-way analysis of variance (ANOVA) analyzed by using COSTAT program. The difference between means was conducted by using [17] multiple range tests.

III. RESULTS AND DISCUSSION

The corrected mortality percentage of the 2nd & 4th instar larvae of A. ipsilon as affected by different concentrations of extreme, atifos-super, and tempo-xl was shown in figures (1, 2, 3 &4). The data obtained indicated that the highest mortality percent caused by the highest concentration of pesticides for the 2nd instar larvae (92.73, 98.15&100), (96.36, 98.15&100) and (96.43, 100&100); also, the 4th instar larvae recorded (82.76, 91.07& 98.21%), (91.38, 94.64 & 98.18%) and (86.21, 96.43& 98.18%) after 24, 48 and 72 h. for extreme, atifos-super and tempo-xl pesticides, respectively. Meanwhile, the lowest concentration of pesticides caused the lowest percentage of mortality for the 2nd instar larvae (58.18, 85.19& 90.57), (78.18, 87.04& 90.57) and (78.57, 88.68 &96.23); also, the 4th instar larvae recorded (53.45, 80.36&89.29%), (75.86, 85.71& 87.27%) and (72.41, 85.71& 90.91%) after 24, 48 and 72 h. for extreme, atifos-super and tempo-xl, respectively.

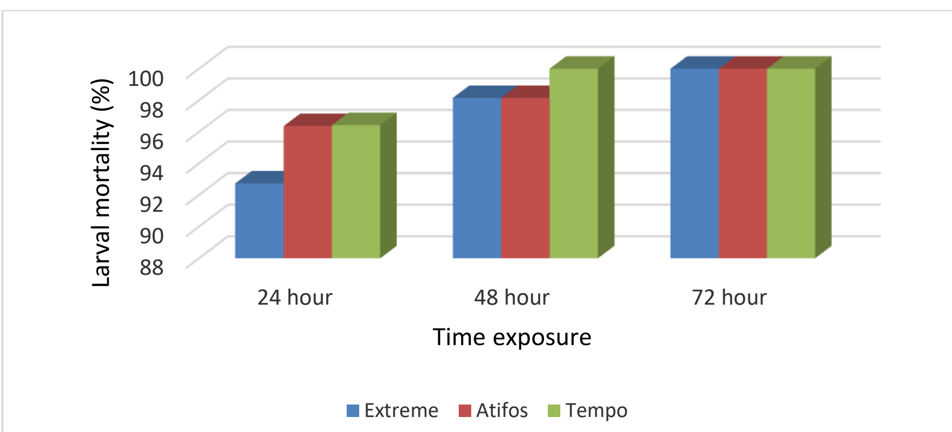


Fig. (1): Effect of high concentrations of extreme, atifos- super and tempo-xl on corrected mortality percentage of 2nd instar larvae of Agrotis ipsilon after 24, 48 & 72 hr. post treatment.

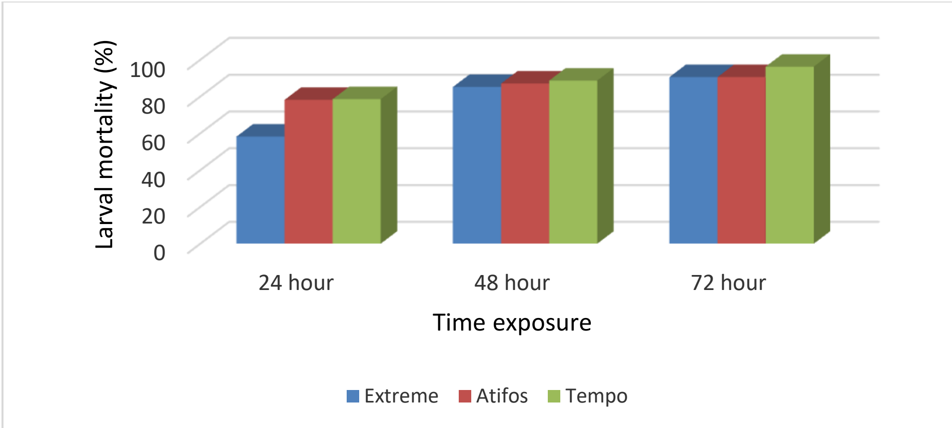


Fig. (2): Effect of low concentrations of extreme, atifos super and tempo-xl on corrected mortality percentage of 2nd instar larvae of Agrotis ipsilon after 24, 48 & 72 hr. post treatment.

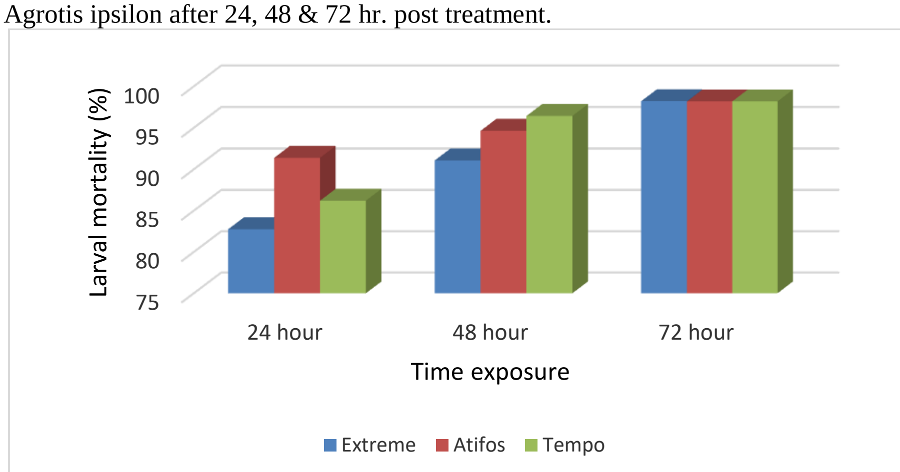


Fig. (3): Effect of high concentrations of extreme, atifos super and tempo-xl on corrected mortality percentage of 4th instar larvae of Agrotis ipsilon after 24, 48 & 72 hr. post treatment.

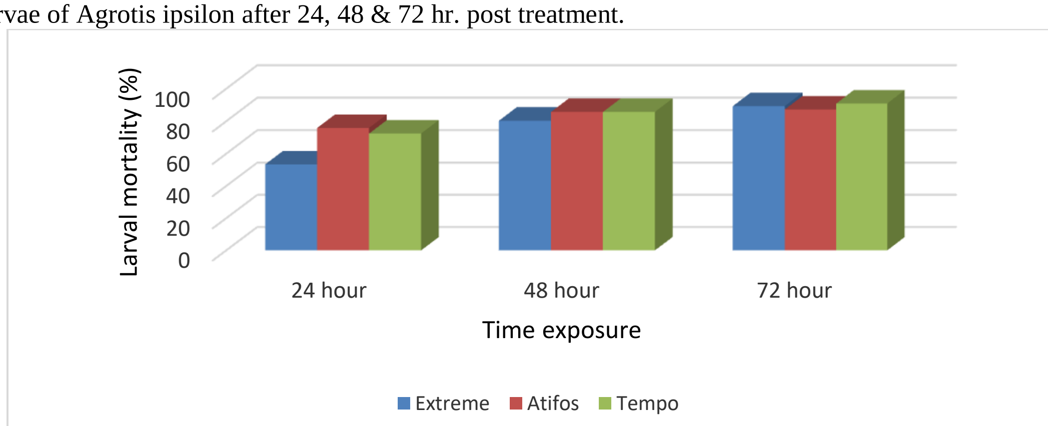


Fig. (4): Effect of low concentrations of extreme, atifos super and tempo-xl on corrected mortality percentage of 4th instar larvae of Agrotis ipsilon after 24, 48 & 72 hr. post treatment.

3. 1. **Toxicological studies:**

The registered results in table (1) showed that the lowest toxicity values of the sub-lethal concentrations (LC25 & LC50) against 2nd instar larvae displayed for atifos-super (1.9 & 5.93, respectively). Meanwhile, the highest toxicity values recorded for tempo-xl (0.07 & 0.73 ppm, respectively) at LC 25 & LC 50, whereas extreme recorded (14.38 ppm) at LC90. The results also showed that tempo-xl is the most toxic in the various toxic values followed by extreme and atifos-super.

Table (1): The toxicity of extreme, atifos- super and tempo-xl against 2nd instar larvae of Agrotis ipsilon

Pesticides	Lethal concentrations	Concentrations (ppm)	Lower limit (ppm)	Upper limit (ppm)	Slope ± SE (b)	Toxicity index
Extreme	LC25	0.37	0.13	0.67	1.227 ± 0.19	56.36
	LC50	1.3	0.72	1.94		
	LC90	14.38	8.8	31.93		
Atifos-super	LC25	1.9	1.1	2.84	1.39 ± 0.179	12.34
	LC50	5.93	4.22	8.18		
	LC90	49.91	30.39	107.75		
Tempo-XL	LC25	0.07	0.002	0.29	0.66 ± 0.16	100
	LC50	0.73	0.12	1.62		
	LC90	62.64	21.72	953.86		

SE= Standard error, LC25 (concentration that killing 25% of treated population), LC50 (concentration that killing 50% of treated population), LC90 (concentration that killing 90% of treated population).

Lethal concentrations of the three tested pesticides against the 4th instar larvae of A. ipsilon were recorded in table (2). The least toxicity values of the sub-lethal concentrations (LC25, LC50 & LC90) displayed for atifos-super (3.05, 9.78 & 89.82, respectively). Meanwhile, the highest toxicity values recorded for tempo-xl (1.81, 4.09 & 19.28 ppm, respectively) at LC25, LC50 & LC90.The current results also showed that tempo-xl is the most toxic in the various toxic values followed by extreme and atifos-super.

Table (2): The toxicity of extreme, atifos-super and tempo-xl against 4th instar larvae of Agrotis ipsilon

Pesticides	Lethal concentrations	Concentration (ppm)	Lower limit (ppm)	Upper limit (ppm)	Slope ± SE (b)	Toxicity index
Extreme	LC25	1.21	0.57	1.94		
	LC50	5.03	3.35	7.54	1.09 ± 0.17	81.34
	LC90	75.82	37.27	260.61		
Atifos-super	LC25	3.05	1.82	4.4		
	LC50	9.78	7.02	13.94	1.33 ± 0.1762	41.81
	LC90	89.82	50.7	224.1		
Tempo-XL	LC25	1.81	1.24	2.4		
	LC50	4.09	3.14	5.28	1.90 ± 0.22	100
	LC90	19.28	13.49	32.24		

SE= Standard error, LC25 (concentration that killing 25% of treated population), LC50 (concentration that killing 50% of treated population), LC90 (concentration that killing 90% of treated population).

3.2. Morphological aberration studies:

Figure (5) showed the normal life cycle of Agrotis ipsilon. The different forms of morphogenic aberrations resulted from 4th instar larvae treated with extreme, atifos-super and tempo-xl can be described as follows

3.2.1. Larval deformities:

Figure (6) showed that the effect of sub-lethal dose (LC50) of extreme, atifos-super and tempo-xl on the 4th instar larvae appeared as several deformities after the next moult. Malformed larvae turned translucent and then black (Fig. 6A). They shed off the old cuticle but failed to exuviate. Cuticle malformation resulted from treatment with atifos –super and tempo-xl were clear as pale regions of larval cuticle with dark strips and stains. Some larvae looked even totally black as if they were burned (Fig. 6 B & C).

3.2.2. Pupal deformities:

Figure (7) demonstrated that all the three pesticides caused high deformities in the developed pupae at antennal and wing regions and some destructions in the last abdominal regions. Moulting integument remained with pupae and colored black. Also, moulting inability at last instar larvae and emaciation of head region were observed in figure (7B). Among the treated larvae some individuals succeeded to pupate with different degrees of malformation. Characteristic phenomena of larval intermediate were detected with extreme treatment, in which pupal appendages were still there, as well as hard brownish and deformed pupal skin (Fig. 7A). Tempo-xl caused malformed pupae as stretching of the abdomen with attached exuviate which failed to be shed (Fig. 7C).

3.2.3. Adult deformities:

Figure (8) illustrated that the three tested pesticides caused incomplete adult emergence and sever shrinkage of the adult bodies which were accompanied by shortened, curly and deformed wings (Fig. 8A, B & C).

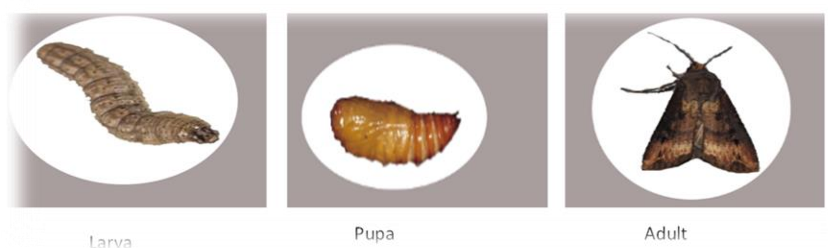


Fig. (5): Normal 4th instar larva, pupa and adult of Agrotis ipsilon (Hufn.).

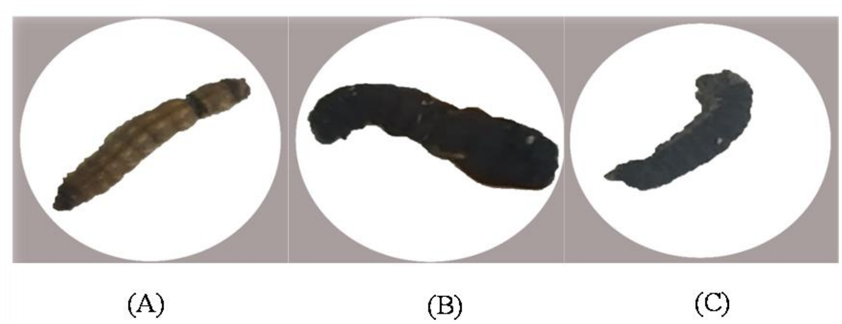


Fig. (6): Malformation of the 4th instar larva of Agrotis ipsilon due to treatment with extreme (A), atifos-super (B) and tempo-xl (C).

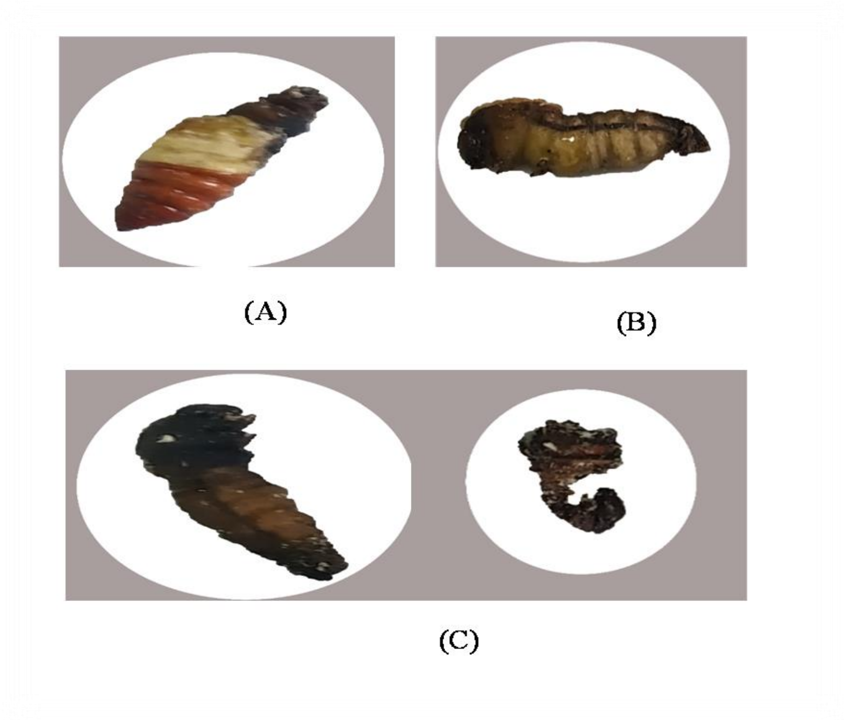


Fig. (7): Malformation of the pupal stages of A Agrotis ipsilon due to treatment with extreme (A), atifos-super (B) and tempo-xl (C).

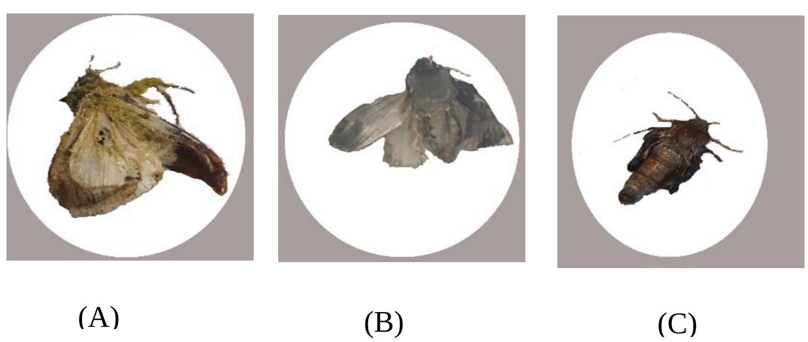


Fig. (8): Malformation of the adult stage of Agrotis ipsilon due to treatment with extreme (A), atifos-super (B) and tempo-xl(C).

3.3. Discussion:

The black cutworm, A. ipsilon (Hufnagel) is a prominent pest attack seedling of many economic plants. It's one of the most important insect pests of vegetables and several field crops in Egypt and the world [1]; also, it induces significant economic damage to a broad range of crops via root damage, as it consumes cotton, corn, wheat, and numerous vegetables [18]. One of the most critical problems of this pest is its resistance to almost all chemical groups used against it. Consequently, it has sparked much interest in avoiding or overcoming this problem. As stated by[19], mixing pesticides with different modes of action may delay the development of resistance within pest populations; this is because the resistance mechanisms required for each pesticide in the mixture may not be widely distributed or exists in insect populations [20] .

[21] demonstrated that applying synergists is an effective strategy for playing a critical role in agriculture and insecticide resistance management, increasing the pesticide's effectiveness, slowing or stopping the breakdown process, making the insecticides more toxic to the pest, and reducing the amount of pesticide usage quantity. The reasons for employing mixtures of insecticides of various chemical types in agriculture are as follows: the mix may give the best control of a complex of pests with varying susceptibilities to the different components of the assortment; insects that are resistant to one or more insecticides may be susceptible to a combination of toxicants; or synergism may be exhibited by the combatants [22].

The current data indicated that the highest mortality percent caused by the highest concentration (40ppm) of extreme 36% containing (methoyfenozide 30% & spinetoram 6%), 60.5ppm for atifos-super 55% containing (chlorpyrifos 50% & cypermethrin 5%), and 40ppm for tempo-xl 30% containing (chlorpyrifos 25% & lufenuron 5%), respectively (after 24, 48, and 72 hr) and vice versa for the 2nd and 4th instar larvae of A. ipsilon. Insecticidal activity of the three mixtures extreme, atifos-super and tempo-xl against 2nd and 4th instars larvae of A. ipsilon revealed that organophosphorous, chlorpyrifos (cholinesterase inhibitor) 25% in combination with insect growth inhibitor, lufenuron (insect growth inhibitor) 5% (tempo-xl) was the most effective compound with LC50 0.73 & 4.09 followed by insect growth regulator (IGR), methoxyfenozide (ecdyson agonist) in combination with bio-seed-spinosad, spinetoram (activator for the nicotinic acetylcholine receptors) 36% (extreme) with LC50 1.3 & 5.03 and finally organophosphorous, chlorpyrifos 50% in combination with synthetic pyrethroid, cypermethrin (sodium channel modulator) 5% (atifos-super) with LC50 5.93 & 9.78 in both tested 2nd and 4th instars, respectively. The efficiency of different tested compounds in the control of the 2nd and 4th instar larvae of the black cutworm, A. ipsilon (Hufn.) varied tremendously according to the instar of larvae and the chemical structure of the tested mixtures. Generally, the 2nd instar larvae were more sensitive to the three tested compounds with lesser LC50 values than the 4th instar larvae of A. ipsilon. [22] reported that the 2nd instar larvae of Spodoptera littoralis were more sensitive to the five tested compounds (chorosan, feroban, cygron, engeo and kingbo) than the 4th instar larvae.

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Moreover,[23] found that the mixture of diflubenzuron with chloropyrifos was more effective (initial kill) than diflubenzuron alone. Pyrethroids are always used in combination with organophosphates (OPs) to control the broad mite, the sucking pests Aphis gossypii Glover and Bemisia tabaci Gennadius, and the leafworm [8].

Insecticide mixtures belonging to different groups are usually applied in the field to enhance the spectrum of the control when multiple pests are attacking simultaneously. They are also recommended to increase the efficacy of the control of a single pest to delay the development of insecticide resistance or to combat current resistance in a pest species. Using mixtures as a countermeasure for resistance management in insect pests has been supported by several researchers[22, 24].[25] declared that the use of insecticide mixtures with synergistic effects have been used in order to decrease the insecticide dose, and thus delay the selection of resistance-strains, and limit their negative impact.

There was a positive correlation between the pesticides concentration and its corresponding mortality i.e. as the pesticides concentration increased; the corresponding larval mortality percent was also increased. [26] evaluated the toxicity of three insecticides contains two effective substances, chlorpyrifos & lufenuron (tempo-xl), lufenuron & emamectin benzoate (heater) and thiamethoxam & chlorantraniliprole (folliam felixi) against the 2nd and 4th instar larvae of S. littoralis. It could be shown that heater achieved superior toxic efficacy compared to other tested compounds, where, it had the highest efficacy against 2nd and 4th instar larvae, with LC50 of 0.041 and 0.135 ppm, respectively, followed by folliam felixi with LC50 of 0.076 and 0.233 ppm and finally tempo xl with LC50 of 0.097 and 0.411 ppm for 2nd and 4th instar larvae, respectively. Also[27], and [28] reported that emamectin-benzoate was the most effective compound against 4th instar larvae of S. littoralis.

Also, [22] proved that feroban was the most effective (initial kill) of the tested mixtures on S. littoralis larvae because it contains a double concentration of chloropyrifos (47.5%) compared with chlorosan (it contains 24% of chloropyrifos). Also, feroban contains lufenuron (2.5%), which has a much slower mode of action (residual toxicity) and inhibits the production of chitin; therefore, the larvae are unable to successfully moult into the next stage. Moreover, they stated that among the insect growth inhibitors tested, lufenuron required a shorter time at a lower concentration as compared with the other insecticides of this group tested. Although a high level of resistance was observed against lufenuron[29], yet it was proved as an effective insecticide against S. littoralis (Boisd.). [30] observed that organophosphates in combination with pyrethroids exhibited an enhanced toxicity showing the high probability of synergistic effects in Helicoverpa armigera (Hub.) treated with different insecticide mixtures.

Moreover, [31] evaluated six insecticides (bifenthrin, clothianidin, flubendamide, indoxacarb, thiamethoxam, and thiodocarb) against the cutworm. Amongst these, bifenthrin was found most effective with LC50 values of 0.009 and 0.089 ppm for third and sixth instar larvae, respectively. Toxicity of six insecticides was evaluated against black cutworm, A. ipsilon using leaf-dip method by [32] and the results showed that the toxicity of chlorfenapyr, indoxacarb and emamectin benzoate were significantly higher than that of chlorpyrifos, phoxim and lambda cyhalothrin. Likewise, chitin synthesis inhibitors, chlorfluazuron and triflumuron, exhibited remarkable toxicities against 4th instar larvae of A. ipsilon[33]. [34] elucidated that indoxicarb was the most effective followed by imidacloprid, pyridalyl and lufenuron against the 2nd and 4th larval instars of S. littoralis (Boisduval) (Lepidoptera: Noctuidae) and the highest potentiation effect was observed, when lufenuron mixed with indoxicarb followed imidacloprid and pyridalyl. Also, all tested combinations have positive effect, this effect depending upon their different modes of action for these insecticides which are mixed on the assumption that they would complement the action of each other for killing the target pest. Furthermore, these mixtures are potentiating, it is a useful tool in enhancing control efficacy and combating insecticide resistance, in this case, there may be potential for reducing the application rate of one or both components of the mixture, so, favorable to mix lufenuron with tested insecticides. These results were compatible with the results obtained by[35, 36]. They reported that insect growth regulator mixtures with the insecticides resulted in additive effect; this positive effect may due to the insecticides from different chemical groups with different mode of action to act on S. littoralis.

The chitin synthesis inhibitors CSIs cause an inhibition of facilitated diffusion and active transport of nucleosides and amino acids across cell membranes that lead to insect morphogenesis [37]. The present study cleared a morphogenic abnormalities after treatment the 4th instar larvae of the black cutworm with LC50 of extreme, atifos-super and tempo-xl, malformed larvae turned translucent and then black due to treatment with extreme, they shed off the old cuticle but failed to exuviate. Moreover, larvae treated with atifos-super and tempo-xl looked even totally black as if they were burned and showed clear pale regions of larval cuticle with dark strips and stains, this may be attributed to the disorganization of light and dark bands of the larvae muscles or the inhibition in melanin synthesis [38]. Moreover, [39] detected the molting disorders of methoxyfenozide on the thorax of Aedes aegypti such as dangling larval exuvium, head capsule slippage disappointment and more exhaustive sclerotization and melanization. Additionally, [40] indicated that the treatment of S. exigua larvae with methoxyfenozide directed to induce the premature, larval molt, existence of a paired head capsule and presence of larval-pupal intermediate. Pre-pupal failure to win a complete metamorphosis program of pauperism, because the CSIs lead to inhibit the creation of the new cuticle through the apolysis leading to the production of moulting abnormalities, or the molt stimulus for the achievement of larval-pupal transformation. The formation of endocuticle was prohibited, so the exocuticle and epicuticle did not properly attach to the epidermis.[41] cleared that the malformation in a pre-pupal stage that appeared as larval-pupal intermediate may be deemed to the inability of the treated larvae to liberate themselves from their old cuticle.

The current study revealed that the pupal morphogenic abnormalities appear as darkening pupa and compressed skinniness of head region owing to treatment with atifos-super, meanwhile, dry c-shaped pupa with a small body size and stretching of the abdomen with attached exuviate which failed to be shed due to treatment with tempo-xl. Moreover, the larval intermediate was detected with extreme treatment, in which pupal appendages were still there, as well as hard brownish and deformed pupal skin compared to the control pupa. Almost of abnormal pupae that collapsed to complete their metamorphosis then died inside the puparium, failed to emerge and changed to incomplete adult.

Moreover, the present study illustrated the emerged adults with curled wings, small body size (dwarfism) and malformed legs; this is maybe due to the alteration of the ecdysteroid that resulted in modifications in the activity of the lysosomal enzyme causing morphological abnormalities [42]. These results coincided with [40], who indicated that methoxyfenozide had an efficacy effect when were applied to S. exigua larvae that lead to malformation in wings, the emerging adults often had a problem in discarding the pupal exuvium. Also, the current results concurred with[43], who recorded different morphological deformation that included retardation of development of larvae, failure to emerge from the pupal stage, and incomplete development of wings in adults that died 12 h after emergence.

V –CONCLUION

The present study concluded that, tempo-xl showed a significantly toxic effect on diverse development stages of A. ipsilon followed by atifos-super and extreme. Also, it remarkably recorded different morphological deformation. Therefore, tempo-xl could be recommended as an eco-friendly alternative to synthetic insecticides for the management of this dangerous insect. Additional investigations are still required to regulate their effects on the environment according to IPM program of the black cutworm.

REFERENCES

1. Abdou, L. and A. Abdel-Hakim, *Some biological and biochemical aspects of Agrotis ipsilon (Lepidoptera: Noctuidae) larvae as Influenced by Methoprene (JHA).* Current Science. Volume: 06 (3): 631, 2017. **639**.
2. Sadek, M.M., B.S. Hansson, and P. Anderson, *Does risk of egg parasitism affect choice of oviposition sites by a moth? A field and laboratory study.* Basic and applied ecology, 2010. **11**(2): p. 135-143.
3. Specht, A., et al., *Life cycle of Agrotis malefida (Lepidoptera: Noctuidae): a diapausing cutworm.* Zoologia (curitiba), 2013. **30**(4): p. 371-378.
4. Bhattacharyya, B., et al., *Management of potato cutworm, Agrotis ipsilon (Hufnagel) in Assam.* Pesticide Research Journal, 2014. **26**(1): p. 82-85.
5. El-Aswad, A., *Efficiency of certain insecticides and insect growth regulators alone or in mixture with chlorpyrifos for the integrated control of the Egyptian cotton leafworm.* Journal of Pest Control and Environmental Sciences, 2007. **15**(2): p. 29-48.
6. Ahmad, M., *Observed potentiation between pyrethroid and organophosphorus insecticides for the management of Spodoptera litura (Lepidoptera: Noctuidae).* Crop protection, 2009. **28**(3): p. 264-268.
7. El-Mageed, A.A. and S.E.J.P.P.S. Shalaby, *Toxicity and biochemical impacts of some new insecticide mixtures on cotton leafworm Spodoptera littoralis (Boisd.).* 2011. **47**(4): p. 166-175.

8. Martin, T., et al., *Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, Helicoverpa armigera (Hübner)(Lepidoptera: Noctuidae) from West Africa.* Journal of economic entomology, 2003. **96**(2): p. 468-474.
9. Montella, I.R., R. Schama, and D. Valle, *The classification of esterases: an important gene family involved in insecticide resistance-A review.* Memorias do Instituto Oswaldo Cruz, 2012. **107**(4): p. 437-449.
10. Roush, R.T., Hopper, Keith R, *Mate finding, dispersal, number released, and the success of biological control introductions.* Ecological entomology, 1993. **18**(4): p. 321-331.
11. Curtis, C., *Theoretical models of the use of insecticide mixtures for the management of resistance.* Bulletin of entomological research, 1985. **75**(2): p. 259-266.
12. Ahmed, A., et al., *Impact of Spodoptera littoralis (Boisd.) and Agrotis ipsilon (Hufn.) larval frass on oviposition of conspecific insects.* Archives of Phytopathology and Plant Protection, 2013. **46**(5): p. 575-592.
13. Abo El-Ghar, G., et al., *Histopathological effects of abamectin, thuringiensis and diflubenzuron on the midgut of Spodoptera littoralis (Lepidoptera: Noctuidae) larvae.* Bull. Ent. Soc. Egypt, 1994. **21**: p. 41-52.
14. Briggs, J.D., *Reduction of adult house-fly emergence by the effects of Bacillus spp. on the development of immature forms.* Journal of Insect pathology, 1960. **2**(4): p. 418-32.
15. Abbott, W.S., *A method of computing the effectiveness of an insecticide.* J. econ. Entomol, 1925. **18**(2): p. 265-267.
16. Finney, D.J., *Probit analysis, Cambridge University Press.* Cambridge, UK, 1971.
17. Duncan, D.B., *Multiple range and multiple F tests.* Biometrics, 1955. **11**(1): p. 1-42.
18. Wang, Y., et al., *The genome of the black cutworm Agrotis ipsilon.* Insect Biochemistry and Molecular Biology, 2021. **139**: p. 103665.
19. Ahmad. *A comparative analysis of crop water productivity of rice-wheat and cotton-wheat rotations in Rechna Doab, Punjab, Pakistan*. in *" New Directions for a Diverse Planet", 4th International Crop Science Congress in conjunction with the 12th Australian Agronomy Conference and the 5th Asian Crop Science Conference, 26 September-1 October 2004, Brisbane, Australia*. 2004. Australian Society of Agronomy Inc.
20. Yu, S.J., *Detoxification Mechanisms in Insects.* In Encyclopedia of Entomology (ed. Capinera, J. L.) 1187–1201 (Springer, Netherlands, 2008.
21. Yadav, S., Y. Kumar, and Lalita, *Effectiveness of Different Plant Extracts against Galleria Mellonella Larvae in Laboratory.* Archives of Agriculture and Environmental Science, 2018. **3**(1): p. 64-67.
22. Abd El-Mageed, A.E. and S.E. Shalaby, *Toxicity and biochemical impacts of some new insecticide mixtures on cotton leafworm Spodoptera littoralis (Boisd.).* Plant Protection Science, 2011. **47**(4): p. 166-175.
23. Kassem, S.M., et al., *Studies on toxic effect of endotoxin, chitin synthesis inhibitor and mixtures of diflubenzuron with different insecticides on Egyptian cotton leafworm Spodoptera littoralis (Boisd).* Alexandria Journal of Agricultural Research (Egypt), 1986.
24. Ahmed, S., et al., *Comparative Efficacy of Some Insecticides against Helicoverpa armigera Hub., Spodoptera spp. on Tobacco.* Int. J. Agri. Biol., 6: 93, 2004. **95**.
25. Taillebois, E. and S.H. Thany, *The use of insecticide mixtures containing neonicotinoids as a strategy to limit insect pests: Efficiency and mode of action.* Pesticide Biochemistry and Physiology, 2022: p. 105126.
26. Abdel Aziz, *Effects of some Insecticide Mixtures on Toxicity and Some Biochemical Parameters of Cotton Leafworm, Spodoptera littoralis (Boisd.).* Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control, 2019. **11**(3): p. 139-148.

27. Ezz El-Din, H., et al., *Toxicity of spinosad and abamectin compared with some conventional insecticides against parent field strain of cotton leafworm, Spodoptera littoralis (Boisd.).* J. Agric. Sci. Mansoura Univ, 2009. **34**(5): p. 5221-5229.
28. Abdu-Allah, G., *Laboratory and field evaluation of emamectin benzoate and spinetoram on cotton leafworm larvae.* Resistant pest management newsletter, 2010. **20**(1): p. 12-16.
29. Sudhakaran, R., *Efficacy of lufenuron (Match 5% EC) against Spodoptera litura (F.) under in vitro condition.* Insect Environment, 2002. **8**(1): p. 47-48.
30. Hamed, M., R. Khan, and F. Jamil, *Toxicity of different insecticide mixtures against cotton bollworm, Helicoverpa armigera (Hub.)(Lepidoptera: Noctuidae).* Pakistan Journal of Zoology, 2006. **38**(1): p. 39.
31. Sharma and Verma, *Laboratory evaluation of some novel insecticides against Agrotis segetum (Denis & Schiffermuller) fed on potato leaves.* Potato Journal, 2013. **40**(2): p. 109-113.
32. Yu, W., et al., *Toxicity of six insecticides to black cutworm Agrotis ypsilon (Rottemberg) and safety evaluation to oil organisms.* Acta phytophylacica sinica, 2012. **39**: p. 277-282.
33. Fahmy, A.R., *Toxicological, biological and biochemical impact of some chitin synthesis inhibitors on the black cutwom, Agrotis ipsilon (Lepidoptera: noctuidae)(Hufn.).* Egyptian Academic Journal of Biological Sciences. A, Entomology, 2014. **7**(2): p. 119-128.
34. Ismail, S.M., et al., *INFLUENCES OF SUBLETHAL DOSE OF CERTAIN INSECTICIDES ON BIOLOGICAL AND BIOCHEMICAL AGAINST SPODOPTERA LITTORALIS (BOISDUVAL).* J. Plant Prot. Res, 2018. **6**: p. 1-16.
35. El-Helaly, A. and H. El-Bendary, *Field study to evaluate the joint action of certain insecticides, IGR's and baculoviruses against Spodoptera littoralis (Bosid.).* Journal of Entomology and Zoology Studies, 2015. **3**(6): p. 289-293.
36. Bushra, S., et al., *Assessing the combined toxicity of conventional and newer insecticides on the cotton mealybug Phenacoccus solenopsis.* Ecotoxicology, 2017. **26**: p. 1240-1249.
37. Mayer, R., et al., *Evidence that chitin synthesis inhibitors affect cell membrane transport.* Endocrinological Frontiers in Physiological Insect Ecology".(Sehnal, F), 1988.
38. Gelbič, I. and V. Němec, *Developmental changes caused by metyrapone and azadirachtin in Spodoptera littoralis (Boisd.)(Lep., Noctuidae) and Galleria mellonella (L.)(Lep., Pyralidae).* Journal of Applied Entomology, 2001. **125**(7): p. 417-422.
39. Darvas, B. and L. Polgar, *Novel-type insecticides: specificity and effects on non-target organisms*, in *Insecticides with novel modes of action*. 1998, Springer. p. 188-259.
40. Carton, et al., *Effects of RH-2485 on larvae and pupae of Spodoptera exigua (Hübner).* Mededelingen-Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent (Belgium), 1998.
41. El-Hadek, *Phytochemical and toxicological effect of plant extractives againstSpodoptera littoralis (Biosd.) and Agrotis ipsilon (Hufnagel). M.Sc. Thesis, Dept. Econ. Entom.& Pesticide, Fac. Agric., Cairo Univ.* 2002.
42. Josephrajkumar, A., B. Subrahmanyam, and S. Srinivasan, *Plumbagin and azadirachtin deplete haemolymph ecdysteroid levels and alter the activity profiles of two lysosomal enzymes in the fat body of Helicoverpa armigera (Lepidoptera, Noctuidae).* Eur. J. Entomol, 1999. **96**(4): p. 347-353.
43. Mansour, S., et al., *Larvicidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, MuscaDomestica L.* Seeds, 2011. **10**(100.0): p. 100.0.