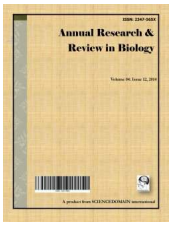

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Effects of Cyfluthrin Insecticide on *Agrotis ipsilon* Immature Stages Development with Respect to Different Temperatures

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Authors' contributions

This work was carried out in collaboration between all authors. Author ARE designed the study. Author MAG performed the statistical analysis and wrote the protocol. Authors ARE and WLA wrote the first draft of the manuscript and managed the literature searches. Authors ARE, MAG and WLA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Toxicity effects of Cyfluthrin insecticide on *Agrotis ipsilon* (Lepidoptera: Noctuidae) immature stages were studied with respect to temperature thresholds and thermal requirements for each stage. The bioassays were carried out by applying the recommended concentration, its half and its quarter at four constant temperatures (15, 20, 25 and 30℃). The LC-values of Cyfluthrin were estimated, and the LC_{25} values were applied for each tested temperature as sub-lethal concentration. Results revealed that, the LC_{50} values at 30℃ were highly toxic to all treated indiv iduals than other tested temperatures, followed by that at 20℃ then at 25℃ , while the least LC_{50} value was recorded at 15℃. The mean estimated thermal units and Zero of development were 37.66, 106.39, 31.88,

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151.70 & 238.33 degree days (DD), and 13.30, 12.53, 8.30, 8.04 and 11.37^{o}C for eggs, larvae, prepupae, pupae and total immature stage, respectively. The developmental period of the examined stages was decreased with increasing temperature.

Keywords: Agrotis ipsilon; Cyfluthrin; temperatures; temperature thresholds; thermal requirements.

1. INTRODUCTION

Black cutworm*, Agrotis ipsilon* (Hüfnagel), is recorder as a very important seedling pest of several economic plants, resulting in a great damage for many crops. Starting from 3rd larval instar, the larvae spend the rest of their life under the soil surface attacking the basal parts of stems of the plant. *Agrotis* have a wide host range, where it feed on a wide variety of plants [1], it feeds nearly on all field crops including clover, wheat, barley, potato, corn and sugar-beets and many vegetables. The plants were cut-off 2-3cm below soil surface, this phenomenon severing as the principal symptom of *A. ipsilon* infestation. Presently, the heavy use of insecticides to control the black cutworm causing pollutions for the environment and/or pose a threat to public health or ground water [2].

The side effects of insecticides and toxic material on insects depend on the percent of mortality evaluation, especially when the recommended doses used [3].

The combined effects of various environmental parameters as heat, cold, drought, oxygen reduction, pathogens and toxicants show the real side effects of pesticides on target organisms [4-5].

Variation of temperature is the most essential factor that has a great effect on the developmental rate of insects that exposed to insecticide [6-7]. The effect of the pesticides at various temperatures on insect susceptibility was studied with respect to different pests by many authors [8] on *Tropinota squalida* and [9] on *Aedes albopictus* and *Culex pipiens*. Insects require a definite value of heat units (degree days) for development from one stage to the other next one [10]. There is a minor threshold temperature degree for each insect species where no growth occurs lower than this degree. These degrees could be helpful for predicting insect activity and its appearance during the plant-growing season [11].

These thermal units are necessary to complete the growth of different stages and one generation, serving the plan of development and used for determining the time needed for these stages to develop under different temperatures in the field [12].

This study aims to estimate thermal thresholds (T_0) and degree days (DD) by linear regression analysis and to test the side effects of sub-lethal concentration of Cyfluthrin insecticide with respect to the temperature and thermal requirements on some biological parameters of *Agrotis ipsilon*.

2. MATERIALS AND METHODS

2.1 Incubators and Temperature

Incubators with four constant temperatures of 15, 20, 25 and 30℃ used to select the most suitable temperature to control *Agrotis ipsilon*. The experiments were conducted at 70±5% RH to estimate the duration of each stage. Thermal requirements as the lower temperature threshold, *i.e.* zero of development (T_0) and thermal constant (DD) were estimated for immature stages. The developmental rate (1/development days) was regressed against temperature using the linear regression equation (Y=a+bx) to calculate the lower temperature threshold (T_0= - a/b) and the thermal constant [6,13,11].

2.2 Treated Insect

Agrotis ipsilon was obtained from NRC Permanent Rearing Lab. maintained for several generations on castor bean leaves at constant conditions (25±2℃ & 75±5% RH).

2.3 Insecticide Used

Cyfluthrin: Cyfluthrin is a Pyrethroid insecticide, the Chemical formula is: $C_{22}H_{18}Cl_2FNO_3$. The recommended concentration is (250 ml/200 L).

2.3.1 IUPAC name

[(R)-cyano-[4-fluoro-3-(phenoxy)phenyl]methyl] (1R,3R)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate.

2.4 Bioassay and Technique of the Treatment

In a preliminary experiment, three concentrations of Cyfluthrin were prepared (the recommended, it's half and it's quarter). These concentrations were applied as spray to the egg stage directly, and to the castor bean leaves for feeding the newly hatched larvae under the four tested temperatures to determine the LC-values. The LC_{25} concentration was used as a sub-lethal concentration for our experiments.

Eggs of *A. ipsilon* were transferred into plastic cups (5 cm in diameter and 5 cm height) then covered with muslin. These cups were divided into 4 groups each was marked for one of the tested temperatures. Each group comprised 5 sub-groups each has 30 individuals; 4 sub-groups of them were marked for treatments (each sub-group represented one of the tested temperature), while the 5^{th} sub-group was marked for check (control) one.

The abovementioned steps were repeated by substitution the eggs with the newly hatched larvae. Larval groups were feed on treated castor bean leaves. After three days of feeding on treated leaves, the individual's mortality of each tested temperature was recorded.

Exposure assay was carried out at four constant temperatures 15, 20, 25 and 30℃. Ten replicates were applied for treated and untreated groups per each temperature. The number of survived and dead larvae was recorded every day.

2.5 Statistical Analysis

Percentage of mortality was corrected according to Abbott's formula [14]. Analysis of variances (ANOVA) F-test was carried out through the SPSS Computer program to discriminate between treatments and/or concentrations used. Differences between mean were compared using Duncan's Multiple Range test [15]. The LC-value was calculated according to Finney equation [16].

3. RESULTS AND DISCUSSION

3.1 Effects of Cyfluthrin

Pesticide and temperature are considered the main factors significantly affected the survival of the insect pest, *i.e., A. ipsilon*. The toxicity effects of Cyfluthrin insecticide were studied on the activity of *A. ipsilon*.

Toxicity bioassay revealed that Cyfluthrin treatment was highly toxic at 30℃, where the LC_{50} was 3.77 µl/ml followed by the treatment at 20ºC then the treatment at 25℃. The treatments at 15℃ exhibit the lowest toxicity, where the LC_{50} value was 13.05 µl/ml. It was noticed also that the LDP-line takes the normal shape, with a slope ranging between 1.06 to 2.24 (Table 1). The optimum rate of survival was recorded at 25℃ and an increasing percent in mortality was observed with a decrease and/or increase in temperature, this finding was matched with Sato and Kishino [17]. The durations of egg, larva, prepupa and pupa of Cyfluthrin-treated *A. ipsilon* were significantly reduced at 30^{o}C temperature compared to lower temperatures (Table 2 a & b).

Table 1. The LC-values of Cyfluthrin applied to *A. ipsilon* at different temperatures

Temperature	LC_{25} (µl/ml)	LC_{50} (µl/ml)	Slope
15ºC	3.004	13.05	1.06±0.19
20ºC	1.932	5.90	1.39±0.22
25ºC	3.076	6.16	2.24±0.28
30ºC	1.615	3.77	1.83±0.28

3.2 Duration and Developmental Rate of *Agrotis* at Four Applied Temperatures

The decrease in the duration time of different stages of *A. ipsilon* because of the increase of temperature leads to increase of developmental rate (Tables 2 & 3 and Fig. 1).

3.2.1 Egg stage

The mean incubation period of the eggs exposed to toxic effect decreased from 9.76 days at 15℃ to 2.00 days at 30℃; being significantly different between each other (F=122.705**, df=3, 12, *P*=0.000) (Table 2a), while the control recorded higher incubation period ranging from 16.00 (at 15℃) to 2.60 (at 30℃); being significantly different with the treated on at each tested temperature (Table 2b). Estimation of lower threshold, temperatures and average thermal unit for egg stage of *Agrotis* from linear regression revealed lower threshold temperature (T_0) were 13.30℃ and 37.66 degree days (DD) and 12.8℃ and 44.28 DD for treated and control, respectively (Table 3).

Table 2a. Duration of immature stages under different tested temperatures

	Temp. (℃)	Mean±SE				
		Eggs (days)	Larvae (days)	Prepupae (days)	Pupae (days)	Total immature (days)
Duration at	**15°C**	9.76±0.58a	34.48±1.67a	5.33±0.04a	20.00±0.52a	69.48±0.57a
	20℃	8.00±0.09b	15.25±0.43b	2.50±0.16b	15.00±0.40b	40.75±0.57b
	25℃	4.33±0.33c	10.20±0.39c	1.80±0.09c	7.78±0.15c	24.60±0.28c
	30℃	2.00±0.00d	5.80±0.09d	1.60±0.14c	7.10±0.20c	16.46±0.18d
F-value		122.705**	203.009**	215.457**	304.506**	290.233**

Table 2b. Comparison between treated and untreated immature stages durations under tested temperatures

	Eggs (days) Mean±SE			
Temperature	**15°C**	**20°C**	**25°C**	**30°C**
Treated	9.76±0.58b	8.00±0.09a	4.33±0.23a	2.00±0.00 b
Control	16.00±0.11a	7.40±0.17b	3.60±0.15b	2.60±0.19 a
T-value	10.962**	3.159*	2.677*	3.085*
	Larvae (days) Mean±SE			
Temp.	**15°C**	**20°C**	**25°C**	**30°C**
Treated	34.48±1.67b	15.25±0.43b	10.20±0.39b	5.80±0 .09b
Control	55.60±2.42a	34.00±0.47a	22.00±0.69a	10.80±0 .16a
T-value	7.177**	29.327**	14.892**	27.014**
	Prepupae (days) Mean±SE			
Temp.	**15°C**	**20°C**	**25°C**	**30°C**
Treated	5.33±0.04b	2.50±0.16b	1.80±0.09a	1.60±0.14 a
Control	7.00±0.32a	4.75±0.12a	2.00±0.11a	1.50±0.10a
T-value	5.172**	11.199**	1.427^{NS}	0.578^{NS}
	Pupae (days) Mean±SE			
Temp.	**15°C**	**20°C**	**25°C**	**30°C**
Treated	20.00±0.52b	15.00±0.40b	7.78±0.15b	7.10±0. 20a
Control	43.47±0.05a	18.20±0.28a	12.20±0.33a	6.60±0. 09a
T-value	20.017**	6.514**	12.289**	2.273^{NS}
	Total immature (days) Mean±SE			
Temp.	**15°C**	**20°C**	**25°C**	**30°C**
Treated	69.48±0.57a	40.75±0.57b	24.60±0.28b	16.46± 0.18b
Control	68.48±0.60a	61.80±1.31a	38.20±067a	19.60±0. 13a
T-value	1.213^{NS}	32.480**	18.810**	13.909**

*** Denote highly significant * Denote significant ; NS not significant*
In a vertical column, means with the same letter(s) are not significantly different (P>5%).

3.2.2 Larval stage

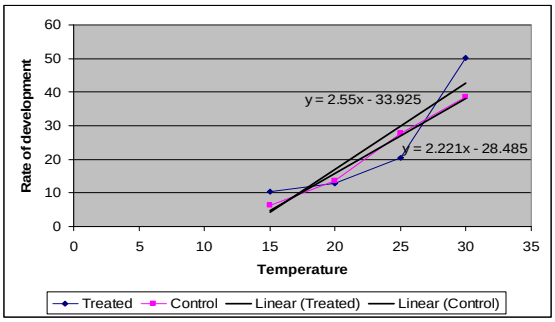
Duration of the treated larval stage decreased with increasing temperature from 34.48 days at lower temperature (15℃) to 5.80 days at higher temperature (30℃); being significantly different between each other (F=203.009**, df=3, 12, *P*=0.000)(Table 2a), while the control recorded longer period ranging from 55.60 (at 15℃) to 10.80 (at 30℃); being significantly different with the treated on at each tested temperature (Table 2b). The lower threshold temperatures (T_0) and thermal units for larval stage were 12.56℃ and 106.39 DD as compared with that of the control (13.10℃ and 196.14 DD) (Table 3).

3.2.3 Prepupal stage

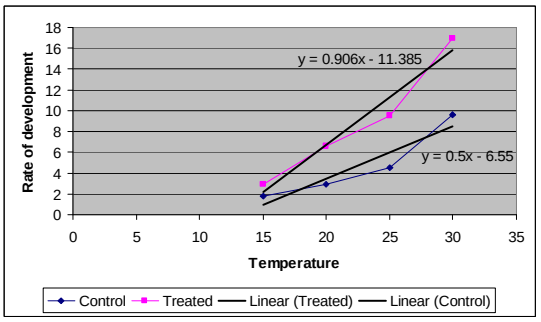
The decreased values of duration as temperature increased in case of the resulted prepupae lowered than that of the control at all examined temperature except at 20 and 30℃ where the time elapsed by the prepupae increased little more than the control; being insignificantly different (Table 2b). This leading to the increase of (T_0), and thermal units for treated prepupal stage were 8.30℃ and 31.88 DD as compared with that of the control (12.29℃ and 26.89 DD) (Table 3).

Table 3. Development of immature stage of *Agrotis ipsilon* under different constant temperatures and its thermal requirements

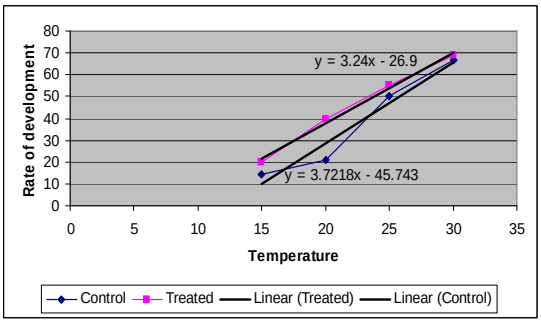
	Temperature (°C)	Eggs (days)		Larvae (days)		Prepupae (days)		Pupae (days)		Total immature (days)	
		Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**
Rate of development at	15°C	10.25	6.20	2.90	1.8	20.00	14.28	5.00	2.30	1.40	0.80
	20°C	12.50	13.51	6.55	2.9	40.00	21.05	6.60	5.49	2.40	1.62
	25°C	20.40	27.78	9.55	4.6	55.00	50.00	12.80	8.19	4.06	2.62
	30°C	50.25	38.46	17.00	9.6	69.00	66.66	14.00	15.2	6.06	5.00
Zero of development (t_0)		**13.30**	**12.80**	**12.56**	**13.10**	**8.30**	**12.29**	**8.04**	**13.08**	**11.37**	**13.42**
Thermal unit (DD)	15°C	17.00	35.20	84.13	105.64	33.49	18.97	139.20	83.46	117.91	107.80
	20°C	53.60	53.28	113.46	234.60	29.25	36.62	179.40	125.94	272.90	406.83
	25°C	46.80	43.92	126.83	261.80	30.06	25.42	132.29	145.42	287.69	442.47
	30°C	33.23	44.72	101.15	182.52	34.73	26.57	155.92	111.67	274.83	325.03
Average of thermal unit		37.66	44.28	106.39	196.14	31.88	26.89	151.70	116.63	238.33	320.53



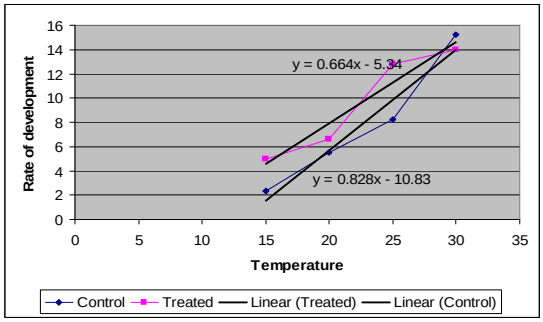
(a) Egg



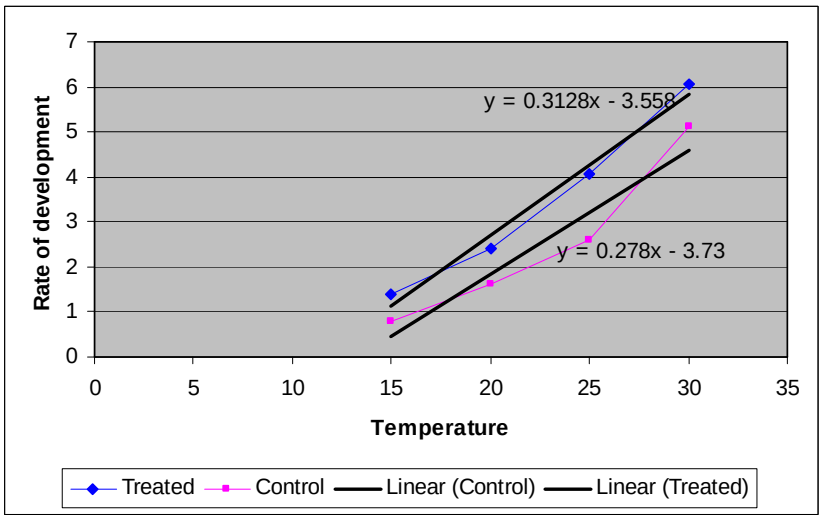
(b) Larvae



(c) Prepupae



(d) Pupae



(e) Total immature

Fig. 1. Relationship between different constant temperatures and developmental rate of *Agrotis ipsilon* immature stage (zero of development)

3.2.4 Pupal stage

Nearly, the same trend was observed for the pupal stage, where the duration decreased in case of the treated larvae than that of the control during 15, 20, 25℃; being significantly different, but the opposite was occurred at 30℃ where there was insignificant different between treated and control individuals (Table 2b).

The lower threshold of temperature and the thermal constant for pupal stage were 8.04℃ and 151.70 DD, as compared with that of the control (13.08℃ and 116.63 DD) (Table 2b).

3.2.5 Total immature stage

The total immature stage duration decreased with increasing the temperature. This decrease leading to the increase of the developmental rate and the average thermal unit to reach 238.33 DD, compared with the control (320.53 DD) (Table 3).

The relation between temperature and the developmental rate is important, as it affect insect biology, distribution, and abundance [11,18]. Temperature affects the immature development and durability of insects, due to the exposure to different temperature in their environment and may show tolerance effect to extreme temperatures [19,20].

Eggs, larvae, prepupae, pupae and the total developmental period's variations were investigated by many authors, as [8,21]. The mentioned data clarify decrease in the developmental period with increasing the temperatures, this finding was in accordance with Rahman and Khalequzzaman [22] on some rice stem borers, *e.g., Chilo suppressalis, C. partellus, C. polychrysa, Scirpophaga innotata*, and *S. incertulas*.

These results showed that the thermal constant is influenced by temperature were accordance with [23] on *Chilo medinalis*. Related insect species shared in similar thermal requirements of temperature and duration of development by several phenology models was reported by [24]. The estimated temperature thresholds and thermal constants are useful in the prediction of population peaks, for identify optimal time of insecticide application, to develop a forecasting system to monitor the adult emergence and flight activity were mentioned by [25,26], and to develop phenology models by [23]. The estimation of threshold temperatures are useful to study the impact of climate change on the distribution of different species [27]. The lower threshold value obtained in our study was similar to that report by [12] on *Agrotis*. For these reasons, the observed variations in lower threshold and thermal constant among different insect pest populations from different geographical areas could be attributed to more than one environmental factor and/or the method of estimation [28].

4. CONCLUSION

It could be concluded that, insecticide and temperature are considered the main factors significantly affected the survival of any insect pest. The toxicity effects of Cyfluthrin insecticide were studied on the survival and the activity of *A. ipsilon* under our prevailing climatic thermal conditions ranging from about 10 to 40℃ through out winter-summer seasons. Toxicity bioassay revealed that, Cyfluthrin treatment was highly toxic at the higher tested temperature (30℃), while the lowest toxicity was observed at 15℃ treatment. An increase percentage of mortality was observed with a decrease and/or increase in temperature. The activity of *Agrotis* larvae reached the highest value at 30℃, followed by 25℃. In addition, it was found that the optimum insecticide application could be achieved within a range of temperature ranging between 25-30℃.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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