Effect of different temperature on the cotton leafworm *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae) toxicity by insecticides and the biochemical responses measurements

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Abstract

The influence of post-treatment temperature on the toxicities of insecticides against Spodoptera littoralis (Boisd.) larvae was evaluated in laboratory bioassays under different temperatures sets and enzymes contents like beta glucosidase, mixed function oxidase, lipase and the free amino acids. The thiamethoxam, imidacloprid, profenofos and malathion LC_{50} values in ppm decreased by increasing temperatures degrees but deltamethrin LC_{50} was slightly increased when temperature increased from 15 °C to 35 °C by 1.4 fold. Remarkably the toxicity increased with the temperature increase, except for deltamethrin. All the tested insecticides were significantly have positive correlation with temperature. Temperature coefficient were calculated for both one and total increments.

Biochemical investigation of enzymes amount changes for thiamethoxam treatments at two sublethal concentrations and different temperature established were measured. MFO were generally increased at higher temperature and with increased concentrations. But the total amino acids decreased with concentration increase at both temperatures respectively and increased with temperature increase. The enzyme β-glucosidases and lipases recorded highest activity at higher temperature and lower activity at lower temperature of both thiamethoxam concentration respectively.

Keywords: Beta glucosidase, mixed function oxidase, lipase, free amino acids, *Spodoptera littoralis* and insecticide exposure temperature.

Introduction

The developmental rates of insect life stages are dependent on ecological temperature that strongly affects insect body physiological processes and induces morphological changes[9]. Temperature is one of the environmental factors that strongly affects pest biological trait as population growth rate[6,35]. Changes of temperatures daily and seasonal fluctuation affect insect occurrence in crop field, subsequently change the pest survival, sex ratio, generation per year, migration attitude, invasion of new areas, alter environment adaptations, overwinter or offspring capacity changes, altering hosts and modified plant insect interactions[8]. Formerly every change in environmental temperature will face shifts of insect pest habitats features and change in its geographical dwellings suitable to build higher population size. Temperature changes can impact insect pest predators, parasites and diseases.

Most insects have their growth period during the warmer part of the year because of which the niche is defined by climatic regime response. Temperature increases already have caused changes in species diversity and distribution of many species in different taxa[4,39] nThe effective chemical control of *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) anciently depends on chemical insecticides including organophosphates (Op), carbamates, pyrethroids (Py), neonicotinoids (Ni) and insect growth regulators. Temperature influencing on insect biology affect insecticides toxicities[46].

Relationships of field temperature and insecticide toxicity to many pest species must be manipulated properly at most of searches to point out shifts on toxicity due to temperature changes, to avoid increase in crop losses due to insect attacking crops and reduced reproductive fitness[48]. Positive or negative change of toxicity due to temperature is depends on the differences in the mode of action, exposure routes of insecticide and target insects[28].

The effective management here properly depends on avoiding insecticides due to negative changes in chemical toxicity and making decisions about which compound is effective under different environmental conditions[20]. The significance of measuring insect enzymes activities in relation to insecticide toxicities practically cited by Rajangam et al[37] and many others.

Mixed Function Oxidase (MFO) is a large diverse superfamily of enzymes found in all insect tissues, structure and function diverse and various activities like hydroxylation, epoxidation, N-, O-, S-dealkylation, deamination, sulfoxidation, desulfuration and oxidative dehalogenation involved in bioactivation of OP insecticides.

They are involved in the metabolism of pesticides and plant toxins besides ecdysteroids and juvenile hormones[7]. β-glucosidases (β-g) is an enzyme that can remove glucopyranosyl residues from the non-reducing end of (β-g) by catalysing hydrolysis of the glycosidic bond[29].

Material and Methods

Source and rearing of *S. littoralis* (L.) culture: A reared strain of *S.littoralis* for many generations in the Egyptian Central Agricultural Laboratory by methods described by Gaaboub and Halawaa[11] and Khedr et al[21] is highly susceptible to insecticides, was used in laboratory bioassay. Egg masses were placed in a big glass jars, then the larvae emerged fed on the Castor Bean leaves, *Ricinus communis* (Euphorbiaceae) to complete their life cycle. Pupae were kept individually in separate and cleaned jars till adult emergence. Newly emerged adults were placed in cylindrical transparent plastic jars. Rearing conditions were 25±2ºC, 60± 5% relative humidity (RH), with a photoperiod of 16:8 (L: D) h. Adults were fed on 10% honey solution and allowed to oviposit on the Defla leaves (*Nerium oleander*) (Family Apocynaceae).

Insecticides: Commercial formulations of several insecticides were thiamethoxam (Agita 10% WG), imidacloprid (Chinook 35% SC), profenofos (Profy 10% EC), malathion (Chemnova 57% EC) and deltamethrin (Deltamethrin 10% EC) used for the bioassay.

Bioassay under different temperature procedure: Toxicological test was completed in contact bioassay according to Jung et al[17] by using Whatmann filter paper saturated with insecticides. Commercial formulations of several insecticides were dissolved in acetone to give a sequence of seven serial concentrations of each insecticide. 5 ml of each concentration were applied on the filter paper and then left to dry. Every filter paper was placed on Petri dish provided with ten 3rd instar larvae for insecticide exposure. Four replicate were performed for each insecticide concentration and control was treated with acetone only. Dishes were maintained under laboratory conditioned temperature was 15, 20, 30 and 35 ±2ºC, 60± 5% relative humidity (RH), with a photoperiod of 16:8 (L: D). For biochemical analysis insect specimen preparation was as follows: a big bulk of 4th instar larvae of *S.littoiralis* was treated with two thiamethoxam concentrations. LC_{50} and LC_{25} values were placed in separate containers and the containers placed in separate room temperature at 35±2ºC and 20±2ºC at 60± 5% relative humidity. Then after 24h, both survived larvae were collected and kept in deep freezer (-20 ºC) a waiting for biochemical investigations.

Biochemical analysis Methods

Insects tissue preparations for biochemical analysis: The insects were homogenized in distilled water (50 mg /1 ml), in a chilled glass teflon tissue homogenizer (ST – 2 Mechanic-Preczyina, Poland). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 5ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer at -20ºC until usage for biochemical assays. Double beam ultraviolet / visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

Determination of detoxification enzyme activities

Lipase activity: It was determined calorimetrically by Spectrum diagnostic kit. A synthetic substrate (DGMRE) is split by lipase to yield the colored final product methylresorufin. The increasing absorbance of the red methylresorufin is measured photometrically at 578 against air.

β-glucosidase activity: It was measured by assaying glucose liberated by enzymatic hydrolysis of salicin as described by Lindorth[24]. One ml of the reaction mixture consisted of 200 µl enzyme solution, 0.1 M phosphate buffer (pH 6) and 50 u mole salicin. Mixtures were incubated at 35 C° for 30 min, then boiled for 2 min to stop the reaction. Glucose liberated by salicin hydrolysis and measured enzymatically by a glucose kit (Sigma kit, Sigma Co.). Optical densities were measured against blank and activities were expressed as µg glucose liberated / min / mg protein.

Total free amino acids: They were assayed according to method of Lee and Takabashi[23]. The reaction mixture consists of 1 ml sample and 1.9 ml ninhydrin-citrate buffer-glycerol mixture that consists of 0.5 ml of 1% ninhydrin solution in 0.5 M citrate buffer (pH 5.5), 0.2 ml of 0.5 M citrate buffer (pH 5.5) and 1.2 ml glycerol.

The mixture was heated in a boiling water bath about 10 min and cooled in a tap water bath. The developed color was read at 570 nm. The amino acids were expressed as µg alanine per gm body weight.

Mixed Function oxidase activity: P-nitroanisole o-demthylation was assayed to determine the mixed function oxidase activity according to the method of Hansen and Hodgson[13] with slight modification. The standard incubation mixture contained 1 ml sodium phosphate buffer (0.1 M, pH 7.6), 1.5 ml enzyme solution, 0.2 ml NADPH, (Final concentration 1 mM), 0.2 ml glucose-6-phosphate (G.6 P, final concentration, 1 mM) and 50µg glucose-6-phosphate dehydrogenase. (G-6PD).

Reaction was initiated by the addition of p-nitroanisole in 10 µl of acetone to give a final concentration of 0.8 mM and incubation for 30 min at 37ºC. Incubation period was terminated by addition of 1 ml HCl (1N). P-nitrophenol was extracted with $CHCl_3$ and 0.5 N NaOH and absorbance of NaOH solution was measured at 405 nm. An extinction coefficient of 14.28 mM cm^{-1} was used to calculate 4-nitrophenol concentration.

Statistical analysis: Mortality data of *S.littoralis* 4th instar larvae exposed to each insecticide concentrations at different temperature conditions was analyzed by Probit analysis using Polo software for median lethal concentrations determination. Temperature coefficients of each insecticide were calculated as the ratio of higher to lower LC_{50} and called negative when the lower LC_{50} was at the lower temperature[20,30].

Results and Discussion

Insecticide toxicity results: Toxicity response results (Slop, intercept, LC_{50}, LC_{90} and chi-squared) of the insecticide treatments (OP, Py and Ni) at different room temperature (15, 20, 30, 35°C) against the 4th instar of the cotton leaf worm *S. littoralis* larvae susceptible reared strain were found in table 1. The tested temperature was 15 to 35, about 5 or 10 degrees increments between one condition to another, furthermore the thiamethoxam toxicity values decreased from 2.4 ppm to 1.1 ppm and changes equal to 2.4 fold and imidacloprid LC_{50} decreased from 8.4 to 5.4 and changes equal to 1.55 fold. Profenofos and malathion LC_{50} decreased from 1.29 to 0.66 and 11.8 to 5.1 ppm respectively and changes equal to 1.95 and 2.31 for respectively deltamethrin LC_{50} slightly increased from 1 to 1.4 ppm when temperature increased from 15 °C to 35 °C by 1.4 fold. Remarkably the LC_{50} for each insecticide increased for every 5 degrees of temperature decrease, this mean that the toxicity increases with the temperature increase, except for deltamethrin.

The second increment (20) of temperatures establishment give 0.458, 0.643, 0.51, 0.43 and -1.4 coefficients of temperature for thiamethoxam, imidacloprid, profenofos, malathion and deltamethrin respectively. In the green peach aphid, *Myzus persicae* (Sulzer), acephate, methvomyl, imidacloprid had positive temperature coefficients but lambda-cyhalothrin showed a negative temperature coefficient[10].

Nilaparvata lugens results showed that sensitivity to cycloxaprid, nitenpyram, triflumezopyrim, chlorpyrifos and etofenprox LC_{50} increased when the temperature changed from 18 to 36 °C according to Mao et al[15]. More developed information was provided by Jaleel et al[28]. Toxicity of emamectin benzoate, chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide at four different temperature levels against diamondback moth, *Plutella xylostella*, was found to be positive correlation with temperature increase. And overall positive temperature coefficients were 1.50, 2.35, 1.58, 2.45 and 2.17 fold respectively by Teja et al[42]. Temperature coefficients were evaluated at 20 to 40°C, for toxicity of lambda cyhalothrin and spinosad that decreased by 2.15- and changes was 1.87-fold while toxicity of acetamiprid and chlorpyrifos against green lacewing *Chrysoperla carnea* increased by 2.00 represent changes about 1.79-fold respectively.

Different enzymes activity results: Temperature effect on thiamethoxam toxicity that metabolized by enzymes biotransformation including MFO, β-glucosidase, free amino acids and lipase was determined. Through measuring amount of enzyme at 20 °C and 35 °C of temperature and at two thiamethoxam concentration (LC_{50}=1.78 ppm and LC_{25} =1.1ppm defined from ordinary bioassay at the common laboratory temperature) (Table 2). Results revealed that insecticide exposure at different laboratory temperature induced relative enzyme amount changes.

Table 1
Toxicity of some insecticides against *S.littoralis* larvae at low and high temperature.

Pesticide	Temp.	Slope	LC_{50} (95	LC_{90}	Intercept	χ2	RT	TC	TC20°
Thiamethoxam	15	1.76±0.1	2.4(1.5-	12.9(8-	4.3	0.99	2.4		
	20	2.0±0.09	1.78(1.1-	7.8(5-	4.56	0.99	1.78	0.74	0.458
	30	1.2±0.158	1.0(0.5-	12.3(6-	4.9	0.99	1	0.56	
	35	1.47±0.134	1.1(0.62-	8.7(4.7-	4.9	0.96	1.1	1.10	
Imidacloprid	15	1.17±0.157	8.4(4-17)	104(51-	3.99	0.93	1.55		
	20	1.2±0.145	6.2(3.1-	71(35-	4.0	0.99	1.148	0.74	0.643
	30	1.4± 0.137	5.7(3-	48(26-	3.9	0.99	1.056	0.92	
	35	2.0±0.1	5.4(3.4-	23.6(15-	3.5	0.97	1	0.947	
Profenofos	15	1.55±0.126	1.29(0.73-	8.6(4.9-	4.8	0.95	1.95		
	20	1.65±0.124	1.0(0.58-	6.2(3.5-	4.9	0.97	1.5	0.77	0.51
	30	1.33±0.147	0.96(0.49-	8.8(4.5-	5.0	0.87	1.4	0.96	
	35	1.6 ±0.133	0.66(0.36-	4.0(2.2-	5.28	0.96	1	0.68	
Malathion	15	1.3±0.14	11.8(5.8-	103(55-	3.6	0.97	2.31		
	20	1.54±0.126	8.4(4.7-	57(32.4-	3.57	0.87	1.64	0.71	0.43
	30	1.98±0.105	6.8(4.2-	30(19-	3.34	0.94	1.33	0.81	
	35	1.66±0.125	5.1(2.9-	30(17.2-	2.8	0.94	1	0.75	
Deltamethrin	15	1.7±0.12	1(0.6-	5.7(3.3-	4.98	0.97	1		
	20	1.84±0.11	1.15(0.69-	5.8(3.5-	4.88	0.96	1.15	-1.15	-1.40
	30	2.1±0.10	1.5(0.97-	6.5(4-	4.6	0.93	1.5	-1.3	
	35	1.79±0.11	1.4(0.86-	7.4(4.5-	4.7	0.93	1.4	-0.93	

RT= relative toxicity= the higher LC_{50} / the lower LC_{50} of the same insecticide

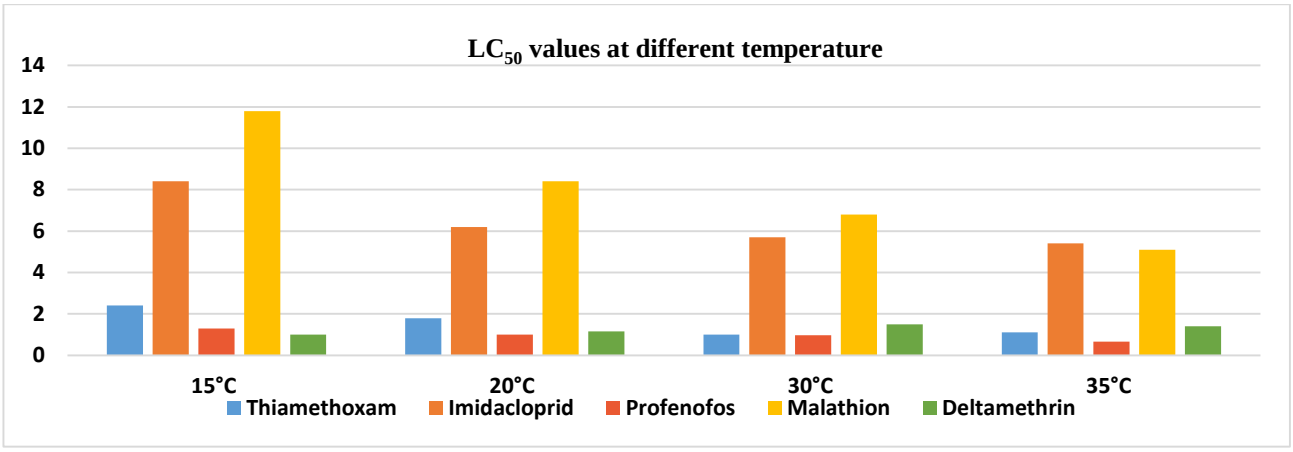


Table 2
Enzymes activity of *S.littoralis* larvae treated at low and high temperature and two thiamethoxam insecticide concentration

	High temperature 35			Low Temperature 20 °C		
	Concentration			Concentration		
Enzyme	1.78 ppm	1.1 ppm	Control	1.78 ppm	1.1 ppm	Control
MFO	717.67±1.5	541.3±5.9	503.67±7.4	419.67±10	296.3±3.8	231.67±5.46
β -glucosidase	29.67±1.2	27.27±0.64	29.67±1.18	13.0±0.21	18.2±0.4	17.1±0.46
Lipase	278.0±6.2	189.0±2.1	286.3±5.3	125.0±2.9	118.3±3.5	176.3±2.0
Total free amino acids	1789.3±11.7	2368.0±49.8	2340.67±13.6	976.0±14.7	1950.3±32.4	1960.3±46.6

Values represented by mean± S.E.

Table 3
Percentages of enzyme activities compared with control of *S.littoralis* larvae treatments at different temperature and by two thiamethoxam insecticide concentration.

Enzyme Difference percentage (%)	High temperature 35		Low Temperature 20°C	
	Concentration	Concentration	Concentration	Concentratio
	1.78 ppm	1.1 ppm	1.78 ppm	1.1 ppm
MFO	1.424	1.074	1.811	1.279
β -glucosidase	1	0.919	0.760	1.066
Lipase	0.970	0.660	0.708	0.671
Total free amino acids	0.764	1.011	0.497	0.994

Percentage of control= (mean of treatment – mean of control/ mean of control) x 100
If result is minus values, it mean decrease and if plus values, means increase.

Noticeably MFO was generally activated at higher temperature and increased from 419.67 to 717.67 (mmol sub. oxidized/min / g.b.wt) of 1.78 ppm treatment where activity increases with increasing temperatures in the range from 20 °C to 35 °C. Also activity of enzymes increased with increased concentrations where MFO increased from 541.33 to 717.67 (mmol sub. oxidized/min/g.b.wt) at 1.1 ppm treatment and 1.78 ppm treatment respectively.

The total amino acids decreased with concentration increase from 1950.33 to 976.0 and from 2368.0 to 1789.33 µg alanine/g.b.wt. The enzyme β-glucosidases recorded highest activity at higher temperature and lower activity at lower temperature of both thiamethoxam concentration respectively. Similarly lipases enzyme highest amount recorded was 278.0, 189.0 at higher temperature and 125.0, 118.3 (mU / g.b.wt) at lower temperature respectively. The fluctuations in activities detected because the activity of detoxification enzymes at higher temperature was activated and increase in its metabolic rate may be related to the weakened antioxidant defense capacity at high temperature animal[2]. The increase of MFO at high temperature indicates availability of non-enzymatic antioxidants such as GSH or through hydrolysis of carboxylates/ phosphates, resulting in the formation of metabolites at lower toxicity[31].

Similar results were found by Abdel-Salam et al[1] where significant decrease in the amount of total protein and total lipid in the 4th instars larvae of *S. littoralis* treated with cascade and atabron.

Results of the 4th instar larvae of *S. littoralis* exposure indicated decrease in total proteins, lipids content and alkaline phosphates in all insecticides except spinetoram

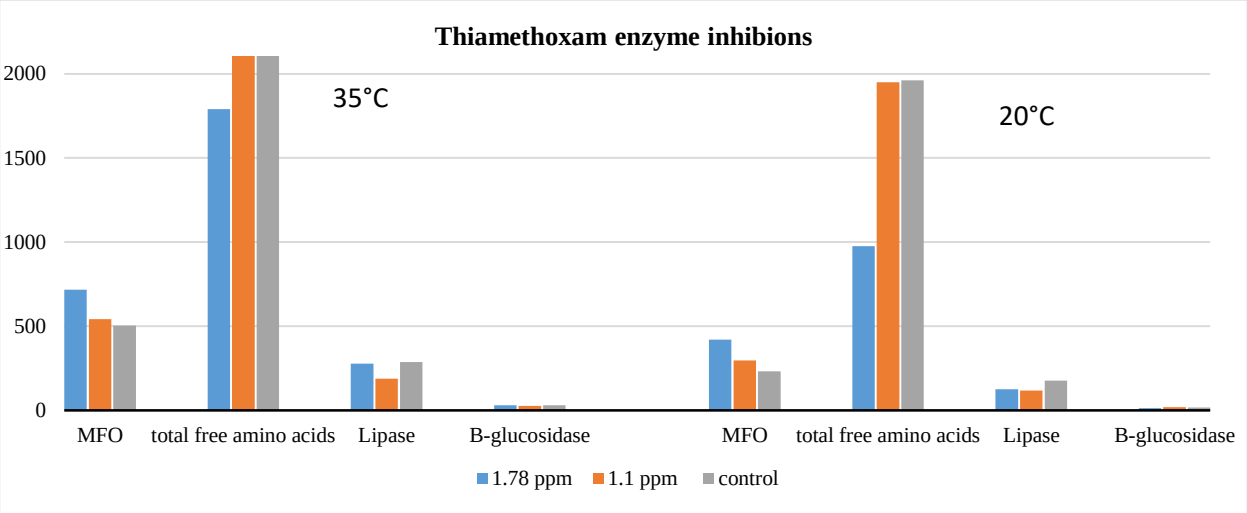
according to Assar et al[3]. Teflubenzuron and spinetoram showed increase in alpha esterases and decrease with hexaflumuron and emamectin benzoate.

Free amino acids of *S. littoralis* larvae increased by raising temperature to reach their maximum activities at 30ºC. The total amount of lipids was decreased by raising temperatures. Enzymes inhibition values may differ between populations as Fonseca-González et al[10]. The absorbance averages for MFO enzymes were different between mosquitoe populations. The increase in MFO levels may be correlated with insecticide resistance as factors affect enzymes inhibition. Activity of MFO enzymes is not affected by the synergistic in combinations of some insecticides, but metabolic activity of *Amsacta albistriga* with synergist was inefficient due to the absence of suitable active binding sites on MFO enzymes for synergist[31].

The enzyme reached its maximum activity in larvae and adult stages, then declined during pupation. Both haemolymph and gut represent about 87.22 % of the larval β-glucosidases and were affected severely by starvation[8]. Moreover, glucosidase in larvae of the fall armyworm, *S. frugiperda* was found in various tissues, with the midgut exhibiting the highest activity. Constant rate was 0.63 raM. It hydrolyzed a variety of glucosides including toxic plant alleloehemicals. Substrate specificity was different in the fall armyworm, cabbage looper and corn earworm, suggesting a qualitative difference in the enzyme[47].

Generally, in insects, β-glycosidases play a role in terminal digestion of cellulose and hemicelluloses, in the cleavage of the carbohydrate moieties of glycoproteins and may also hydrolyze glycolipids. Some insects have three or four digestive β -glycosidases with different substrate specificity[25].

Finally, β-glucosidase from *S. frugiperda* by Gel filtration chromatography separation described how the rate constant of monomerization is affected by temperature[34]. The sub lethal dose effect of dichlorvos on the lipid peroxidation of juvenile *Clarias gariepinus* at 15 days exposure showed increase from 6 to 19, suggesting that dichlorvos may induce oxidative stress in catfish especially at higher concentrations with long exposure[14]. Nie et al[33] showed that protein content, antioxidant enzyme activity, in *T. urticae* were induced by heat stress and play an important role in reducing the oxidative damage.



Conclusion

Lower temperatures lead to slower chemical reactions. A ten-degree centigrade rise in temperature will increase the activity of most enzymes by 50% to 100%. Most of them become denatured at temperatures above 40°C. But storage of enzymes at 5°C or below is the most suitable.

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