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Effect of *e*CO_2 and *e*Temp on the nutritional composition and enzymatic activities of *Spodoptera litura*

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Abstract
Experiment was conducted to study the effect of *e*CO_2 and *e*Temp on the carbohydrate, mid gut protein content and Carboxylesterae activity of *S. litura* after exposed to sublethal concentrations of insecticides (Spinosad, emamectin benzoate, thiodicarb, monocrotophos and fenvalerate). The results revealed that *S. litura* larvae treated with insecticides (emamectin, thiodicarb and monocrotophos) showed increased midgut protein and carbohydrate content with increase in CO_2 and temperatures and opposite trend was noted with spinosad and fenvalerate treated larva.

Keywords: Climate change, carboxylesterae, carbondioxide, temperature

1. Introduction
The leaf eating caterpillar, *Spodoptera litura* is the major polyphagous pest distributed widely and its severe infestation resulted into crop losses. This pest challenged the agricultural production throughout the world by causing damage to important crops like castor, sunflower, tobacco, soybean, cotton and groundnut. It is reported that it is a major pest of sunflower, causes total losses in crop yields (Bilapate and Chakravarthy, 1999) [1]. This larvae initially was gregarious and cause skeletonization of leaves later they cause severe defoliation which lead to reduced supply of assimilates to the capitulum, affecting the production of floret and seeds (Sujatha and Lakshminarayana, 2005) [2]. Among all the control methods chemical control is the standard measure for managing the menance. Neverthless, this pest has acquired resistance to several insecticides due to promiscuous usage. Insecticides shows two types of effects on the insects *viz.*, direct toxic effects and sublethal effects where the former one causes the mortality of insects. Sublethal effects are expressed as physiological or behavioural impacts on individuals that survives exposure to pesticides (Desneux *et al*., 2007) [3]. These sublethal doses of insecticides also effects the nutritional composition and enzymatic activities of *S. litura*.
The increased concentration of CO_2 and temperature has lot of implications in agricultural sector, influencing crops and herbivore insect pests. These two dimensions of climate change also influences the biochemical constituents (Carbohydrate and midgut protein) and enzymatic activity (carboxylesterase) of the insect (Barkat *et al*., 2016) [4]. However, not much work was done on the combined effect of elevated Carbondioxide (*e*CO_2), temperature (*e*Temp) and insecticides on physiological chemistry of the insects. Hence an effort was made to study the combined effect of sublethal concentrations of insecticides (Spinosad, emamectin benzoate, thiodicarb, monocrotophos and fenvalerate) under two CO_2 levels (380 ± 25 and 550 ± 25 ppm) at five different temperatures *viz*., 28, 29, 31, 33 and 35 ± 0.5 ºC.

2. Material and Methods
2.1 Rearing of *S. litura* larvae
The egg masses of *S. litura* were collected from field and initially maintained in the entomology laboratory at Central Research Institute for Dryland Agriculture (CRIDA) to build up the population. Later the insects were maintained under at respective set conditions (*e*CO_2 and *e*Temp conditions *viz.,* 550 and 380 ± 25 ppm and 28, 29, 31, 33 and 35 ± 0.5 ºC) inside the growth chambers.

2.2 Preparation of Sublethal Concentrations of insecticides
Bioassays were conducted on third instar (six day old, 30 mg) larvae of *S. litura* (Balasubramanian, 1982) [5] under different set conditions (550 and 380 ± 25 ppm and 28, 29, 31, 33 and 35 ± 0.5 ºC) using leaf dip method (Method No. 7 of IRAC, 2014) [6]. Mortality data recorded after 72 HAT was subjected to probit analysis (Finney, 1971) [7] by using Statistical Packages for Social Sciences (SPSS) to calculate LC_{10} and LC_{30} were considered as sublethal concentrations (Table 1). The leaves of sunflower were treated with LC_{10} and LC_{30} concentrations of insecticides (Spinosad, emamectin benzoate, thiodicarb, monocrotophos and fenvalerate) and were fed to fifth instar larvae for 24 hrs at respective set conditions. These larvae were starved overnight prior to exposure to the treated leaves. Later the treated larvae were again starved for 3 hrs, rinsed with distilled water, dried with filter paper and weighed. They were three replications for each treatment and 5 larvae per each replication.

Table 1: Sublethal concentrations used for determinig carbohydrate, protein and enzymatic activity in *S. litura*

Insecticide	Set condition	LC_{10} (%)		LC_{30} (%)	
		aCO_2	*e*CO_2	*a*CO_2	*e*CO_2
Spinosad	28	0.0005	0.0010	0.0013	0.0018
	29	0.0007	0.0011	0.0018	0.0020
	30	0.0008	0.0015	0.0020	0.0028
	33	0.0012	0.0027	0.0030	0.0037
	35	0.0022	0.0034	0.0058	0.0066
Emamectin benzoate	28	0.0005	0.0002	0.0017	0.0012
	29	0.0003	0.0002	0.0015	0.0011
	30	0.0002	0.0001	0.0012	0.0009
	33	0.0001	0.00008	0.0009	0.0007
	35	0.00005	0.00003	0.0008	0.0005
Thiodicarb	28	0.0008	0.00062	0.0028	0.0020
	29	0.0006	0.00048	0.0020	0.0014
	30	0.0004	0.00025	0.0014	0.0010
	33	0.0002	0.00015	0.0010	0.0008
	35	0.0001	0.00008	0.0009	0.0003
Monocrotophos	28	0.0156	0.0110	0.0270	0.0200
	29	0.0122	0.0108	0.0220	0.0190
	30	0.0072	0.0068	0.0140	0.0130
	33	0.0058	0.0049	0.0110	0.0090
	35	0.0048	0.0032	0.0090	0.0070
Fenvalerate	28	0.0030	0.0022	0.013	0.011
	29	0.0080	0.005	0.025	0.018
	30	0.011	0.008	0.037	0.026
	33	0.016	0.011	0.058	0.04
	35	0.029	0.021	0.096	0.07

2.3 Isolation of Midgut extract
The larval midguts of fifth instar larvae were dissected and homogenized in 0.1 M glycine, NaOH buffer, pH 10.0. The homogenate was centrifuged at 8000 g for 15 min, and the supernatants were collected and used for assay. Amount of protein in the extracts were determined by Bradford, 1976[8]

2.4 Carbohydrate content
The carbohydrate content was estimated by Anthrone method (Yemm and Willis, 1954) [9] by using glucose as standard.

2.5 Assay of carboxylesterase
Carboxylesterase activity was determined according to the method of Van Asperen, 1962 [10]. Larval midguts were dissected in 1.15 per cent potassium chloride (KCL) solution and later rinsed with 0.04 M sodium phosphate. Later the rinsed tissue was homogenized in one ml ice cold buffer in motor driven homogenizer. The homogenate thus obtained was centrifuged at 4 °C in 10000 rpm for 10 minutes. Different aliquots of 10, 20, 30, 40 and 50 µl was taken in each test tube. 0.05 ml of substrate solution (α - Naphtyl acetate + acetone + phosphate buffer) was added to each test tube containing homogenate. Incubated at 27 °C for 30 minutes and later one ml of Fast Blue B salt were added to each test tube. A red colour immediately developed and that changed into fairly stable blue colour. Read the intensity at 600 nm.

2.6 Statistical analysis
The Nutritional status and enzymatic activities of *S.litura* under different set conditions were quantified by two way analysis of variance (ANOVA). Treatment means were compared and separated using LSD at $p<0.05$.

3. Results and discussion
3.1 Carbohydrate content: The carbohydrate content in larvae of *S. litura* differed significantly after exposed to sublethal concentrations of insecticides at *e*CO_2 and *e*Temp conditions. In general carbohydrates content of *S. litura* declined with increase in sublethal concentrations and higher carbohydrate content was found at increased temperatures (28 to 35 ºC) under both *a*CO_2 and *e*CO_2. But after exposure to LC_{10} and LC_{30} concentrations of spinosad and fenvalerate, the carbohydrate content in the larvae decreased with increase in temperature from 28 to 35 ºC under *a*CO_2 and *e*CO_2 and were higher at *e*CO_2 than at *a*CO_2. Contrastingly, after exposure to sublethal concentrations of emamectin benzoate, thiodicarb and monocrotophos the carbohydrate content in the larvae increased with decrease in temperature from 28 to 35 ºC under *a*CO_2 and *e*CO_2 (Table 2a & 2b).

Table 2a: Effect of sublethal concentrations of spinosad and fenvalerate on the carbohydrate content of *S. litura* under *e*CO_2 and *e*Temp conditions

Interaction (CO_2 × Temp)	Spinosad (mg g^{-1})						Fenvalerate (mg g^{-1})			
	Control		LC_{10}		LC_{30}		LC_{10}		LC_{30}	
	aCO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2
28	3.85±0.04	4.03±0.03	3.52±0.06	3.74±0.07	3.46± 0.04	3.58±0.06	3.78±0.06	3.89±0.05	3.56 ± 0.12	3.72±0.06
29	3.62±0.02	3.88±0.02	3.48±0.04	3.62±0.06	3.32±0.01	3.46±0.04	3.58±0.04	3.62±0.03	3.32±0.07	3.62±0.08
31	3.52±0.01	3.74±0.04	3.32±0.06	3.51±0.01	3.26±0.03	3.32±0.02	3.42±0.02	3.54± 0.05	3.21±0.06	3.42±0.05
33	3.44±0.07	3.68±0.05	3.26±0.05	3.39±0.03	3.14±0.06	3.28±0.11	3.32± 0.03	3.49±0.06	3.18±0.07	3.31±0.04
35	3.28±0.02	3.54±0.04	3.18±0.04	3.21±0.06	3.02±0.06	3.17±0.06	3.26±0.06	3.39±0.08	3.13±0.02	3.26±0.06
F.test	2.78*		37.14*		2.73*		3.33*		3.81*	
S.Em±	0.02		0.02		0.015		0.02		0.03	
CD(p=0.05)	0.04		0.06		0.042		0.06		0.07	
aCO_2	3.54		3.39		3.24		3.47		3.28	
eCO_2	3.78		3.45		3.36		3.59		3.47	
F. test	378.33*		135.97*		243.91*		215.59*		107.77*	
S.Em	0.01		0.009		0.01		0.009		0.01	
CD (p= 0.05)	0.03		0.027		0.03		0.024		0.03	
28	3.94		3.63		3.52		3.84		3.64	
29	3.75		3.55		3.39		3.60		3.47	
31	3.63		3.42		3.29		3.48		3.32	
33	3.56		3.22		3.21		3.41		3.25	
35	3.41		3.30		3.10		3.33		3.20	
F. test	649.63*		19.82*		139.88*		86.63*		133.98*	
S.Em±	0.006		0.02		0.007		0.014		0.02	
CD (p =0.05)	0.018		0.04		0.019		0.038		0.05	
CV (%)	1.05		1.59		1.19		1.44		2.00	

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant

Table 2b: Effect of sublethal concentrations of emamectin benzoate, thiodicarb and monocrotophos on the carbohydrate content of *S.litura* under *e*CO_2 and *e*Temp conditions

Interaction (CO_2 × Temp)	Emamectin benzoate						Thiodicarb				Monocrotophos			
	Carbohydrate (mg g^{-1})						Carbohydrate (mg g^{-1})				Carbohydrate (mg g^{-1})			
	Control		LC_{10}		LC_{30}		LC_{10}		LC_{30}		LC_{10}		LC_{30}	
	aCO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2
28 ± 1 ºC	3.85±0.04	4.03±0.03	1.26 ±0.04	1.68±0.02	1.06±0.06	1.47±0.04	2.62±0.06	2.74±0.02	2.28 ±0.07	2.32±0.06	3.02±0.03	3.26±0.01	2.79±0.03	2.92±0.05
29 ± 1 ºC	3.62±0.02	3.88±0.02	1.48±0.06	1.85±0.03	1.21±0.02	1.52±0.02	2.75±0.02	2.86±0.04	2.34±0.04	2.46±0.05	3.25±0.05	3.48±0.02	2.91±0.05	3.08±0.02
31 ± 1 ºC	3.52±0.01	3.74±0.04	1.64±0.03	1.92±0.03	1.36±0.04	1.74±0.02	2.88±0.04	2.86±0.01	2.42±0.01	2.52±0.03	3.52±0.07	3.73±0.04	3.03±0.03	3.24±0.04
33 ± 1 ºC	3.44±0.07	3.68±0.05	1.78±0.04	2.06±0.03	1.48±0.05	1.81±0.04	2.92±0.02	3.08±0.06	2.59±0.08	2.62±0.03	3.68±0.06	3.85±0.03	3.25±0.08	3.56±0.06
35 ± 1 ºC	3.28±0.02	3.54±0.04	1.85±0.03	2.12±0.06	1.58±0.02	1.95±0.03	3.14±0.02	3.28±0.05	2.72±0.04	2.89±0.05	3.74±0.02	3.91±0.06	3.42±0.07	3.67±0.07
F.test	2.78*		10.07*		4.51*		2.65*		4.99*		2127.54*		3.54*	
S.Em±	0.02		0.015		0.013		0.013		0.018		0.017		0.021	
CD (p=0.05)	0.04		0.042		0.038		0.036		0.052		0.048		0.059	
CO_2														
aCO_2	3.54		1.60		1.34		2.98		2.47		3.44		3.07	
eCO_2	3.78		1.93		1.70		2.86		2.56		3.65		3.29	
F. test	378.33*		377.44*		467.36*		491.44*		225.09*		NS		404.31*	
S.Em	0.01		0.007		0.006		0.006		0.008		0.008		0.009	
CD (p= 0.05)	0.03		0.019		0.017		0.016		0.023		NS		0.026	
Temperatures (ºC)														
28 ± 1 ºC	3.94		1.47		1.27		2.68		2.30		3.14		2.82	
29 ± 1 ºC	3.75		1.67		1.37		2.80		2.40		3.37		3.00	
31 ± 1 ºC	3.63		1.78		1.55		2.90		2.47		3.62		3.14	
33 ± 1 ºC	3.56		1.92		1.65		3.00		2.61		3.77		3.41	
35 ± 1 ºC	3.41		1.99		1.77		3.21		2.81		3.83		3.54	
F. test	649.63*		1168.63*		1829.03*		231.96*		61.55*		59.71*		298.32*	
S.Em±	0.006		0.01		0.009		0.009		0.013		0.012		0.015	
CD (p =0.05)	0.018		0.03		0.027		0.026		0.037		0.034		0.041	
CV (%)	1.05		2.25		2.32		1.17		1.94		1.28		1.72	

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm
*Significant @ 5 per cent level of significance
NS – Non significant

3.2 Midgut protein: The protein content in midgut of *S. litura* differed significantly after exposed to sublethal concentrations of insecticides at *e*CO_2 and *e*Temp conditions. In general protein content decreased with increase in sublethal concentrations and with increase in temperature. The protein content in the larvae after exposed to LC_{10} and LC_{30} of spinosad and fenvalerate were decreased with increase in temperatures from 28 to 35 ºC under *a*CO_2 and *e*CO_2. Contrastingly, the midgut protein content of *S. litura* under *e*CO_2 and *e*Temp after exposed to sublethal concentrations of emamectin benzoate, thiodicarb and monocrotophos increased with decrease in sublethal concentrations under *a*CO_2 and *e*CO_2 at temperatures (28, 29, 31, 33 and 35 ºC) (Table 3a & 3b).

Insecticides emamectin benzoate, thiodicarb and monocrotophos were positively correlated with temperature and recorded lesser lethal concentrations at higher temperatures (Lesser the lethal concentrations, higher is the toxicity) which resulted in increased carbohydrate and protein content at higher temperatures. On the other hand, spinosad and fenvalerate showed negative correlation with temperature and recorded higher lethal concentrations with increase in temperatures (Higher the lethal concentrations, less is the toxicity) resulted in decreased carbohydrate and decreased protein content.

These two biochemical constituents *viz.*, carbohydrates and proteint content was closely linked with several metabolic processes in the insect body are major biochemical constituents required by an organism for its vital activities. The changes in nutritional composition such as carbohydrates and proteins with increase in subethal concentrations of test insecticides indicated the susceptibility of insects to insecticides. The insecticides reduces the feeding efficiency as a result the biochemical components of the body was reduced. Reduction in protein content was attributed to decreased feeding, extended larval durations and decreased pupation rates. The reduced protein content might be due to inhibition of ATP synthesis, DNA and RNA synthesis. Under toxicant stress, proteins are degraded into amino acids (keto acids) and supply compensatory energy to the insects (Nath *et al*., 1997) [11]. The carbohydrate and protein content decreased compared to control at all tested temperatures under both *a*CO_2 and *e*CO_2. The protein content in the third instar larvae of *S. littoralis* was reduced after exposure to spinosad and cypermethrin (Osman *et al*., 2014) [12]. Megahed *et al.*, 2013[13] reported that total protein and carbohydrate content in *S. littoralis* was reduced after treated with emamectin benzoate and spinosad. Similarly, the findings of Piri *et al*. (2014) [14] reported that sublethal concentrations of spinosad decreased the protein and carbohydrate content in the *Glyphodes pyloalis* and was in conformity with present study. Kalita *et al*. (2016) [15] reported organophosphorous insecticides under toxicant stress decreased the carbohydrate and protein content of *Philosamia ricini*. The findings of Elbarky *et al*. (2008) [16] indicated the reduction in carbohydrate content by 65.06 per cent in the fourth instar of *S. littoralis* after treatment with spinosad over untreated control.

3.3 Carboxylesterase activity: Carboxylesterase activity in *S. litura* varied significantly after exposure to sublethal concentrations of test insecticides under *e*CO_2 and *e*Temp conditions. The esterase activity increased after exposed to sublethal concentrations of spinosad compared to untreated control (Fig. 1).

The esterase activity increased with increase in temperatures after exposure to sublethal concentrations of spinosad under both *a*CO_2 and *e*CO_2. The enzymatic activity increased from 186.28 to 262.53 nmol/min/ml (at LC_{10}) and 201.33 to 282.92 nmol/min/ml (at LC_{30}) concentrations with increase in temperatures from 28 to 35 ℃ under *a*CO_2. Similarly even under *e*CO_2 with increase in temperature the esterase activity increased after exposure to sublethal concentrations *viz.*,LC_{10} (238.33,247.56, 255.58, 268.48 and 295.92 nmol/min/ml, respectively) and LC_{30} (252.23, 266.31, 284.98, 301.23 and 331.33 nmol/min/ml, respectively) compared to untreated control (215.26, 225.58, 245.26, 258.23 and 274.23 nmol/min/ml, respectively). Similar trend of increased esterase activity was observed in *S.litura* after exposure to sublethal concentrations of fenvalerate with increase in temperatures under both *a*CO_2 and *e*CO_2.

Highest esterase activity was recorded at LC_{30} concentrations of emamectin benzoate (218.25, 186.25, 165.12, 138.56 and 108.25 nmol/min/ml, respectively) than at LC_{10} (188.30, 152.65, 125.00, 114.25 and 98.56 nmol/min/ml, respectively) compared to untreated control under aCO_2 at 28, 29, 31, 33 and 35℃, respectively. Similarly under *e*CO_2 with increase in temperature, highest esterase activity was observed at LC_{30} (238.56, 224.56, 205.32, 168.56 and 145.23 nmol/min/ml, respectively) than at LC_{10} (226.32, 206.85, 185.23, 152.56 and 125.36 nmol/min/ml, respectively) compared to untreated control. However the esterase activity in *S.litura* decreased across temperatures under both *a*CO_2 and *e*CO_2. Similar trend of decreased esterase activity with increase in temperature and increased activity with increase in sublethal concentrations was noticed in the insect after exposure to sublethal concentrations of thiodicarb and monocrotophos. The most common resistance mechanisms in insects were increased levels or activities of esterase detoxification enzymes that metabolize a wide range of insecticides. In the present study the esterase activity varied significantly across all temperatures at two levels of CO_2. Esteractivity decreased in *S litura* larva after exposure to emamectin benzoate, thiodicarb and monocrotophos whereas opposite trend was noticed for spinosad and fenvalerate. The decrease in the activity of detoxification enzyme suggests increased mortality at higher temperatures and shows susceptibility against insecticides. Higher esterase activity of spinosad at higher temperatures indicates resistance against insecticides (Barkat *et al*., 2016) [4]. The esterase activity increased with increase in sublethal concentrations of insecticides and slight increase in esterase activity at high concentrations of insecticides, this enzyme might be effective in detoxification (Ali and Iman, 2015) [17].

Table 3a: Effect of sublethal concentrations of spinosad and fenvalerate on the midgut protein of *S. litura* under *e*CO_2 and *e*Temp conditions

Interaction (CO_2 X eTemp)	Spinosad (Protein mg g^{-1})						Fenvalerate (Protein mg g^{-1})			
	Control		LC_{10}		LC_{30}		LC_{10}		LC_{30}	
	aCO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2
28	6.75±0.06	7.02±0.08	2.14±0.02	2.04±0.02	2.02 ± 0.01	1.94±0.02	5.37±0.02	4.49±0.05	4.63±0.02	3.99±0.02
29	6.52±0.11	6.95±0.02	1.98±0.02	1.92±0.02	1.85±0.02	1.78±0.01	4.68±0.04	3.86±0.02	4.11±0.06	3.32±0.03
31	6.38±0.05	6.56± 0.04	1.74±0.008	1.68±0.01	1.62±0.01	1.58±0.02	4.22±0.02	3.08±0.02	3.91±0.13	2.79±0.02
33	6.20±0.06	6.35±0.08	1.62±0.01	1.54±0.02	1.52±0.02	1.38±0.02	3.56±0.03	2.60±0.21	3.23±0.02	2.15±0.01
35	6.15±0.06	6.23±0.11	1.58±0.02	1.42±0.01	1.42±0.01	1.29±0.02	3.13±0.01	3.11±0.03	2.74±0.03	1.95±0.01
F.test	13.88*		18.42*		22.12*		9.66*		65.35*	
S.Em±	0.03		0.007		0.006		0.03		0.02	
CD(p=0.05)	0.07		0.019		0.018		0.08		0.06	
CO_2										
aCO_2	3.54		1.81		1.69		4.19		3.72	
eCO_2	3.78		1.72		1.59		3.23		2.84	
F. test	258.24*		2649.85*		3314.37*		2146.82*		3318.35*	
S.Em	0.012		0.003		0.003		0.012		0.009	
CD(p= 0.05)	0.033		0.009		0.008		0.034		0.025	
Temperatures										
28	3.94		2.09		1.98		4.93		4.31	
29	3.75		1.95		1.82		4.27		3.71	
31	3.63		1.71		1.60		3.65		3.35	
33	3.56		1.58		1.45		3.08		2.69	
35	3.41		1.50		1.36		2.63		2.35	
F. test	181.57*		453.43*		529.00*		2939.25*		4847.20*	
S.Em±	0.018		0.005		0.004		0.02		0.014	
CD(p =0.05)	0.052		0.014		0.013		0.05		0.039	
CV (%)	1.06		1.02		1.02		1.92		1.49	

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm

*Significant @ 5 per cent level of significance NS – Non significant

Table 3b: Effect of sublethal concentrations of emamectin benzoate, thiodicarb and monocrotophos on the midgut protein of *S. litura* under *e*CO_2 and *e*Temp conditions

Interaction (CO_2 × Temp)	Emamectin benzoate Protein (mg g^{-1})						Thiodicarb Protein (mg g^{-1})				Monocrotophos Protein (mg g^{-1})			
	Control		LC_{10}		LC_{30}		LC_{10}		LC_{30}		LC_{10}		LC_{30}	
	aCO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2	*a*CO_2	*e*CO_2
28	6.75±0.06	7.02±0.08	0.060±0.002	0.054±0.003	0.052±0.002	0.048±0.001	3.32±0.02	3.02±0.02	3.17±0.02	2.96±0.02	5.98±0.02	4.42±0.02	5.06±0.03	4.18±0.06
29	6.52±0.11	6.95±0.02	0.082±0.002	0.068±0.001	0.060±0.01	0.054±0.002	3.54±0.03	3.16±0.01	3.28±0.01	3.08±0.02	6.06±0.03	4.89±0.03	5.48±0.02	4.68±0.04
31	6.38±0.05	6.56±0.04	0.092±0.001	0.074±0.002	0.064±0.002	0.062±0.001	3.68±0.02	3.28±0.01	3.45±0.02	3.16±0.02	6.54±0.02	5.21±0.03	5.95±0.03	4.85±0.04
33	6.20±0.06	6.35±0.08	0.101±0.001	0.082±0.001	0.072±0.001	0.078±0.001	3.74±0.02	3.41±0.03	3.58±0.03	3.28±0.03	6.86±0.03	5.56±0.03	6.26±0.05	5.21±0.03
35	6.15±0.06	6.23±0.11	0.126±0.002	0.099±0.001	0.084±0.002	0.082±0.001	3.86±0.02	3.58±0.07	3.62±0.02	3.46±0.07	7.02±0.03	6.02±0.15	6.85±0.04	5.58±0.06
F.test	13.88*		4.12*		3.18*		9.65*		13.15*		60.68*		57.51*	
S.Em±	0.03		0.003		0.002		0.012		0.02		0.02		0.07	
CD (p=0.05)	0.07		0.008		0.005		0.033		0.05		0.06		0.20	
CO_2														
aCO_2	3.54		0.092		0.066		3.63		3.42		6.49		5.92	
eCO_2	3.78		0.075		0.065		3.29		3.19		5.22		4.90	
F. test	258.24*		118.51*		NS		653.65*		516.29*		1510.60*		2481.85*	
S.Em	0.012		0.001		0.001		0.008		0.007		0.009		0.03	
CD (p= 0.05)	0.033		0.003		0.002		0.023		0.020		0.025		0.09	
Temperatures														
28	3.94		0.057		0.050		3.17		3.07		5.20		4.62	
29	3.75		0.075		0.057		3.35		3.18		5.47		5.08	
31	3.63		0.083		0.063		3.48		3.31		5.88		5.40	
33	3.56		0.092		0.075		3.58		3.43		6.21		5.74	
35	3.41		0.113		0.083		3.72		3.54		6.52		6.22	
F. test	181.57*		100.41*		109.47*		2104.49*		964.42*		10429.06*		8683.78*	
S.Em±	0.018		0.001		0.001		0.005		0.01		0.014		0.012	
CD (p =0.05)	0.052		0.003		0.004		0.015		0.03		0.039		0.035	
CV (%)	1.06		8.39		7.29		5.26		1.26		3.14		3.36	

aCO_2 – 380 ± 25 ppm; *e*CO_2 – 550 ± 25 ppm *Significant @ 5 per cent level of significance NS – Non significant

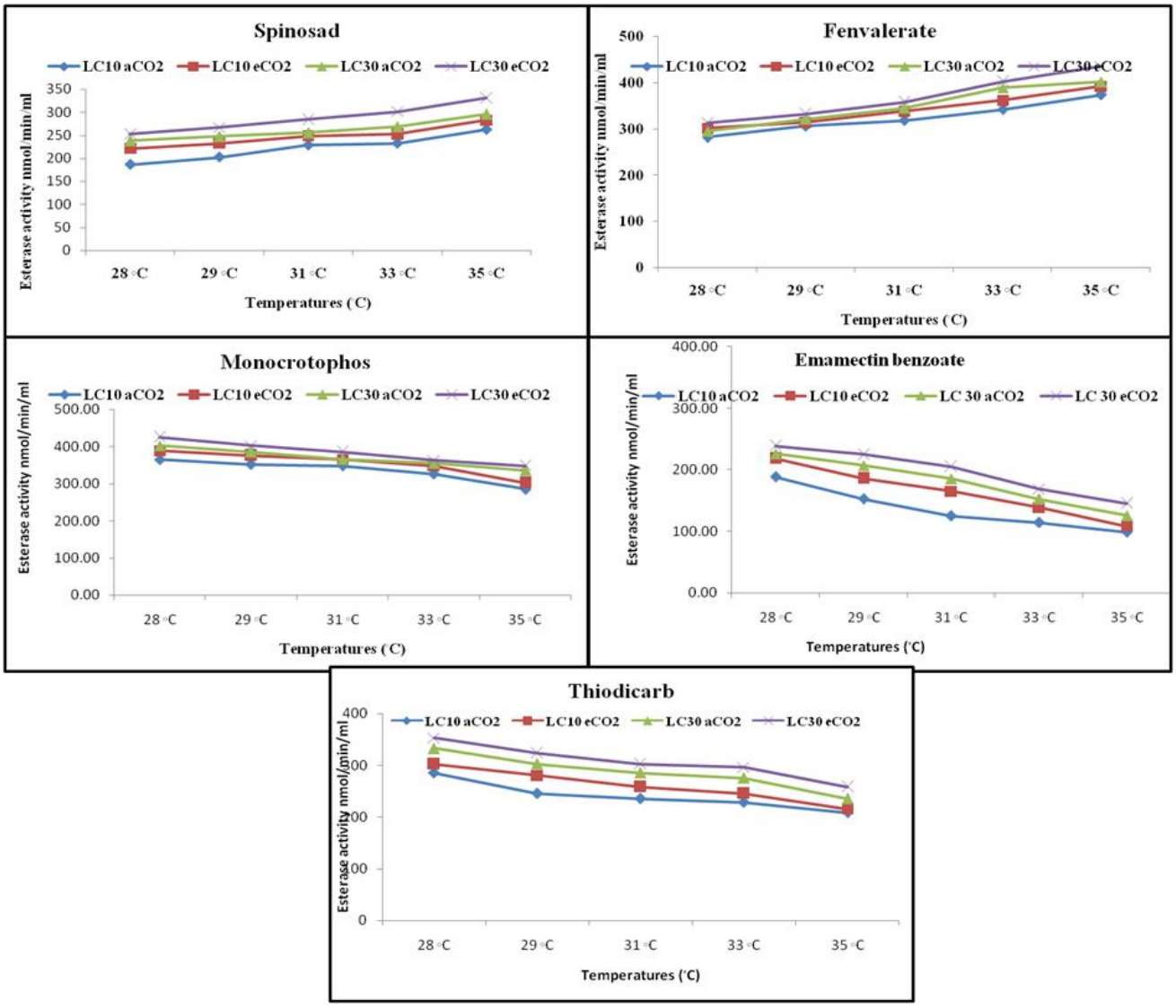

Fig 1: Carboxyl esterase activity in third instar larvae of *S. litura* after exposure to sublethal concentrations of insecticides under *e*CO_2 and *e*Temp conditions

4. Conclusions

In this study it was cocluded that based on enzymatic activity among the five insecticides the toxicity of emamectin benzoate, thiodicarb and monocrotophos found to be effective at higher temperatures, whereas spinosad and fenvalerate decreased their toxicity at higher temperatures. The results revealed that *S. litura* larvae treated with insecticides (emamectin, thiodicarb and monocrotophos) showed increased midgut protein and carbohydrate content with increase in CO_2 and temperatures and opposite trend was noted with spinosad and fenvalerate treated larva.

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