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Biochemical and enzymatic alterations after application of fipronil, thiomethoxam and malathion to *Odontotermes obesus* (Isoptera: Termitidae)

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Abstract. In the present investigation toxic effects of synthetic termitisides, fipronill, thiomethoxam and malathion were determined in Indian white termite *Odontotermes obesus*. Thermites have shown very high toxicity to fipronil, thiamethoxam and malathion. It is proved by very low LD_{50} obtained i.e. 7.75, 9.0 and 11.50 μg/gm respectively. When termites were treated with sub-lethal doses (40% and 80% of LD_{50}) of each pesticide have significantly reduced (significant at $p < 0.05$) the glycogen level (40.2% and 50%) after 16 h in test insects in comparison to control. Similarly pesticides caused significant alterations in the level of certain molecules i.e. free amino acids, lipid protein DNA and RNA. In addition to it each pesticide significantly ($p < 0.05$) increased level of ALP, ACP, LDH, GPT and GOT enzymes while significantly ($p < 0.05$) decreased (72.75% and 73.45%) the level of acetylcholinesterase. It confirms neurotoxic effects of each pesticide.

Keywords: *Odontotermes obesus*, termiticides, fipronill, thiamethoxam, malathion, lipid, protein, glycogen, enzymes

1 Introduction

Termites are highly destructive polyphaguos insect pests, which largely damage plants, agricultural crops as well as stored household products. Both soldiers and workers of termites cause heavy damage to the agriculture crops, wood, fibers, cellulose sheets, clothes and food commodities. Termites heavily infest sugarcane, millet, barley, maize, paddy and vegetable crops. Therefore to control termite infestation in crop field various synthetic pesticides such as chlorodane [1, 2], borate [3], hexaflumuron [4] cypermethrin hydroquinone, fibronil and indoxacarb are used [5]. Fipronil is a highly toxic insecticide belongs to phylpyrazole class [6]. It shows neurotoxicity and disrupts central nervous system. However, toxic effects of synthetic pesticides are well known but very few reports are available on biochemical and enzymatic alterations caused by these termiticides. However, in the present investigation toxic effects of fipronil, thiomethoxam and malathion were determined on certain enzymes and biomolecules. More specifically, biochemical alterations in the level of DNA, RNA, Protein, Lipid, amino acid glycogen and various metabolic enzymes such as acid phospatase (ACP), alkaline phosphatase (ALP), lactic dehydrogenase(LDH), glutamate pyruvate transaminase(GPT), glutamate oxaloacetate transminase(GOT) and acetylcholinestrase (AChE) were measured after fixed time interval of treated and untreated termites.

2 Material and methods

Termites and plant material

Termite, *Odontotermes obesus* (Rambur) both soldier and workers were collected from the University garden and temporary culture was maintained in the laboratory at 37° C ± 2°C at 80% RH by providing green leaves as food material. Termite culture was protected from light illumination, by using black paper sheets wrapped around the glass containers (12 × 9 inch). Insects were provided fresh food material (green grass and cellulose pulp) and it was changed regularly after 24 h.

Toxicity bioassays

For determination of LD_{50} of fipronil, thiomethoxam and malathion, 5 gm of each pesticide was dissolved in 1 L of water separately. For evaluation of insecticidal efficacy of each pesticide serial concentrations i.e. 10 μg, 20 μg, 40

μg, 80 μg, 160 μg, 320 μg, and 640 μg was coated on cellulose paper strip of 1×1 cm^2 in size and air-dried. These pre-coated paper strips were placed in the center of Petri dishes (42 mm diameter) and 1 gm termite workers ($\sim$ 125 in number) were released in each Petri dish to observe the toxic effect of above extracts separately. Insects were exposed up to 24 hrs and number of living and dead termites was recorded after visualizing the their movements. Insects with no apparent mobility and external stimuli were counted as dead. Mortality was recorded after 4, 8, 12 and 16 of treatment and LD_{50} was calculated in μg/gm body weight. Three replicates were set for each control and test.

Determination of bio-molecular parameters

Termite workers (1gm.) were treated with 40% and 80% of LD_{50} of fipronil, thiomethoxam and malathion by spray. Changes in the level of various biomolecules were measured after 4, 8, 12 and 16h. For this purpose, termites were sacrificed, homogenized and centrifuged to prepare whole body extracts for biomolecular estimation. Few important biomolecules such as glycogen, total free amino acids, total lipid, nucleic acids (DNA and RNA) and total protein were determined.

Glycogen

Glycogen contents were measured according to method of Dubois et al. [7]. Glycogen content in unknown samples (supernatant) is calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50 ml of 5% TCA and 6 ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100gm of body weight of termites. Three replicates were set to obtain precision and accuracy.

Total free amino acids

Level of free amino acids was determined by using method of Spies et al. [8]. For calculation, standard curve was prepared by using known amount of glycine and is expressed in gm/100gm body weight of termites. Three replicates were used and data is statistically analyzed by ANOVA method.

Total Lipids

Level of total lipids in whole body extracts of termite was estimated according to method Floch et al. [9]. Total lipid contents were weighted at the end

and expressed in gm/100gm body weight of termites. Three replicates were set and data was statistically analyzed by ANOVA method.

Nucleic acids

Level of nucleic acids in whole body extracts of termites was estimated according to method Scheidner et al. [10]. For this purpose 1 gm of termite workers were fed with 40% and 80% of LD_{50} of synthetic pesticides separately. Insects were scarified and homogenized in 5% TCA with glass-glass homogenizer at 15,000 × g for 25 minutes.

DNA

For DNA estimated with diphenylamine reagent and blue colour developed in the solution, which is measured at 595 nm (O.D.).

RNA

For RNA estimated with orcinol reagent green colour was developed, which was measured at 660 nm.

Total protein

Total proteins of termites were estimated according to Lowry et al. [11] method with the help of Folin phenol Ciacalteu reagent. Blue colour, was measured at 600nm. Three replicates were set for each experiment. Standard curve was prepared by using various concentrations of Bovin serum albumin.

In vivo determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided 40% and 80% of LD_{50} of synthetic fractions. Insects were sacrificed at 4 h interval up to 16 h for the measurement of level of various enzymes. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged in cold for 25 minutes at 15,000 × g. Supernatant was isolated in a glass tube and used for the estimation. Three replicates were set for each bioassay.

Determination of acid and alkaline phosphatase

Acid and alkaline phophatase activity in termites was determined according to the method of Bergmeyer [12]. For the determination of acid phosphatase level p-nitrophenyl phosphate sodium salt were used. A yellow colour developed which was measured at 420 nm. Standard curve was prepared by using different concentrations of p-nitrophenol. Enzyme activity was expressed as the amount of p-nitrophenol formed/30 min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogease was measured according to the method of Annon [13]. For this purpose, 500 mg of termite workers were provided 40% and 80% of LD_{50} of each pesticides with diet and termites were homogenized in cold PBS and centrifuged to prepare whole body extract. Enzyme activity was expressed as μ moles of pyruvate reduced/ 30 min/mg protein.

Determination of glutamic-pyruvate transaminase (GPT) and glutamic-oxaolacetic transaminase (GOT)

GPT activity in whole body extract of termites was measured according to the method of Reitman and Frankel [14]. For this purpose, treated termites were homogenized in ice cold PBS buffer and estimation was done with the help of α-ketoglutaric acid, KH_2PO_4, 2–4 dinitrophenyl hydrazine solution. The optical density was noted at 505 nm and blank was set with water to make the background absorbance zero. Standard curve was prepared by using oxaloacetic acid as standard. The enzyme activity was expressed in units of glutamic-pyruvate transaminase activity/mg protein.

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al. [15]. For estimation of AchE level 0.050 ml of supernatant was mixed with (10 mm path length cuvette) 0.10 ml freshly prepared acetyl cholinethioiodide solution (5×10^{-4} M) and into it 0.05 ml DTNB (0.19818 gm/l) a chromogenic agent and 1.45 ml of PBS (pH 6.9) were added. The change in absorbance was recorded at 412 nm regularly for three minutes at 25°C. Enzyme activity was expressed in m moles 'SH' hydrolyzed per minute per mg protein.

Statistical analysis

The LD_{50} of each pesticide was determined in worker termites by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied by ANOVA program [16].

3 Results

Biochemical alterations

Fipronil, thiamethoxam and malathion showed 7.75, 9.0 and 11.50 μg/gm LD_{50} against *O. obesus*. 40% and 80% of LD_{50} of fipronil caused significant ($p < 0.05$) decrease in glycogen level at 4 h of treatment i.e. 50.61% and 50.92% in comparison to control. Fipronil also caused significant ($p < 0.05$) decrease in amino acid level 76.67% and 91.57% after 4 h of treatment. A similar dose of fipronil also caused a significant decrease in lipid level after 4 h of treatment i.e. 87.38% and 87.95% in comparison to control. In the same experiment both DNA (74.94% and 84.00%) and RNA (95.00% and 87.14%), levels were found to be decrease after 16 h of treatment. A similar dose of fipronil also caused significant ($p < 0.05$) decrease in protein level after 8 h of treatment in comparison to control i.e. 72.30% and 59.61% respectively (Table 1; Figure 1).

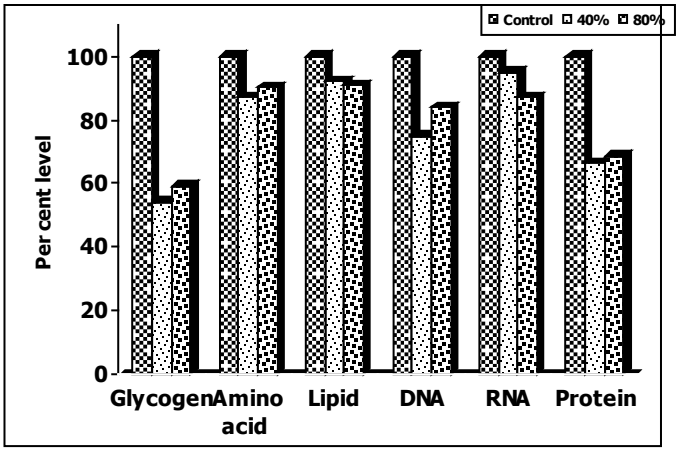

Figure 1: Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of fipronil at 16 hour

Table 1: Effect of 40% and 80% of LD_{50} of fipronil on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.011 (100)	1.64*±0.006 (50.61)	1.66*±0.045 (50.92)	1.36*±0.009 (41.71)	1.47*± 0.013 (45.09)	2.28*±.014 (69.93)	1.65*±.021 (50.61)	1.76*±0.010 (53.98)	1.93*±0.028 (59.20)
Amino acid	0.866±0.003 (100)	0.664*±0.005 (76.67)	0.793*±0.001 (91.57)	0.539*±0.025 (62.24)	0.757*±0.002 (87.41)	1.04*±0.002 (120.09)	0.835*±0.002 (96.42)	0.754*±0.006 (87.06)	0.783*±0.013 (90.41)
Lipid	1.046±0.001 (100)	0.914*±0.002 (87.38)	0.92*±0.001 (87.95)	0.872*±0.00 (83.36)	0.792*±0.008 (75.71)	0.975*±0.002 (93.21)	0.97*±0.014 (92.73)	0.963*±0.001 (92.06)	0.95*±0.003 (90.82)
D.N.A.	0.85±0.028 (100)	0.653*±0.003 (76.82)	0.642*±0.003 (75.52)	0.593*±0.003 (69.76)	0.575*±.0.004 (67.64)	0.685*±0.003 (80.58)	0.727*±0.002 (85.52)	0.637*±0.002 (74.94)	0.714*±0.002 (84.00)
R.N.A.	0.98±0.023 (100)	0.892*±0.003 (91.02)	0.845*±0.002 (86.22)	0.875*±0.006 (89.28)	0.718*±0.001 (73.26)	1.01*±0.001 (103.06)	0.884*±0.001 (90.20)	0.931*±.002 (95.00)	0.854*±.007 (87.14)
Protein	5.2±0.046 (100)	3.65*±0.023 (70.19)	3.38*±0.021 (65.00)	3.76*±0.014 (72.30)	3.10*±0.029 (59.61)	3.66*±0.012 (70.38)	3.52*±0.006 (67.69)	3.45*±0.029 (66.34)	3.56*±0.012 (68.46)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

In a similar experiment when termites were treated with 40% and 80% of LD_{50} of thiamethoxam glycogen level was found to be significantly ($p < 0.05$) decreased after 4 h of treatment i.e. 59.20% and 53.06%. Similar results were obtained in amino acid level. It was found to be decreased when termites treated with 40% and 80% of LD_{50} of thiamethoxam i.e. 62.93% and 86.60% after 4 h of treatment. Similarly, lipid level was also found to be de-creased after 4 h of treatment i.e. 68.16% and 87.66% later it was found to be increase was observed at 12 h of treatment i.e. 65.03% and 93.88% in comparison to control. A similar dose of thiamethoxam also caused significant decrease in DNA and RNA level i.e. 81.17% and 83.17% & 89.38% and 88.97% in comparison to control. Thiamethoxam also caused significant decrease in protein level i.e. 59.80% and 69.80% after 4 h of treatment in comparison to control (Table 2; Figure 2).

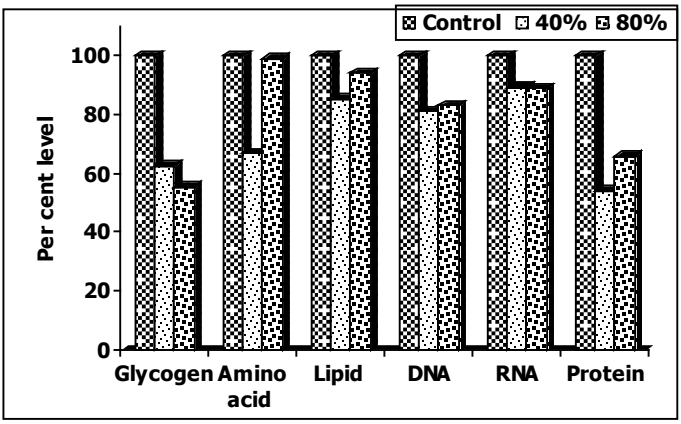

Figure 2: Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of thiamethoxam at 16 hour

When termites were treated with 40% of LD_{50} of malathion, it caused significant decrease in glycogen level i.e. 42.02% and 34.97% after 16 h of treatment in comparison to control (Table 3; Figure 3). While amino acid level was found to be slightly increased i.e. 100.23% after 4 h of treatment with 40% of LD_{50} of malathion. Similarly 80% of LD_{50} of malathion also caused significant ($p < 0.05$) decrease in amino acid level i.e. 87.29% after 16 h treatment in comparison to control. A similar dose of malathion also caused significant ($p < 0.05$) decrease in DNA and RNA level 80.58% and 81.76% & 72.75% and 93.57% after 16 h treatment in comparison to control respectively. Similarly at the same dose protein level was also found decreased up to 41.53% and 22.30% in comparison to control (Table 3; Figure 3).

Table 2: Effect of 40% and 80% of LD_{50} of thiamethoxam on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	1.93*±0.005 (59.20)	1.73*±0.027 (53.06)	1.67*±0.041 (51.23)	1.54*±0.02 (47.23)	2.12*±0.014 (65.03)	1.99*±0.043 (61.04)	2.04*±0.018 (62.57)	1.81*±0.034 (55.52)
Amino acid	0.866±0.003 (100)	0.545*±0.002 (62.93)	0.75*±0.0023 (86.60)	0.527*±0.002 (60.85)	0.735*±0.005 (84.87)	0.917*±0.003 (105.89)	0.868*±0.0058 (100.23)	0.578*±0.012 (66.74)	0.859*±0.0048 (99.19)
Lipid	1.046±0.001 (100)	0.713*±0.003 (68.16)	0.917*±0.003 (87.66)	0.592*±0.0028 (56.59)	0.822*±0.0058 (78.58)	0.975*±0.009 (93.21)	0.982*±0.0029 (93.88)	0.892*±0.0025 (85.27)	0.985*±0.0035 (94.16)
D.N.A.	0.85±0.028 (100)	0.672*±0.001 (79.06)	0.713*±0.002 (83.88)	0.547*±0.0057 (64.35)	0.624*±0.0037 (73.41)	0.723*±0.009 (85.06)	0.744*±0.003 (87.52)	0.69*±0.004 (81.17)	0.707*±0.0038 (83.17)
R.N.A.	0.98±0.023 (100)	0.79*±0.0042 (80.61)	0.818*±0.005 (83.46)	0.754*±0.0017 (76.93)	0.756*±0.0037 (77.14)	0.913*±0.0012 (93.16)	0.925*±0.0093 (94.38)	0.876*±0.0059 (89.38)	0.872*±0.0025 (88.97)
Protein	5.2±0.046 (100)	3.11*±0.018 (59.80)	3.63*±0.014 (69.80)	3.23*±0.022 (62.12)	3.54*±0.023 (68.07)	2.74*±0.017 (52.69)	3.5*±0.034 (67.30)	2.82*±0.009 (54.23)	3.43*±0.012 (65.96)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

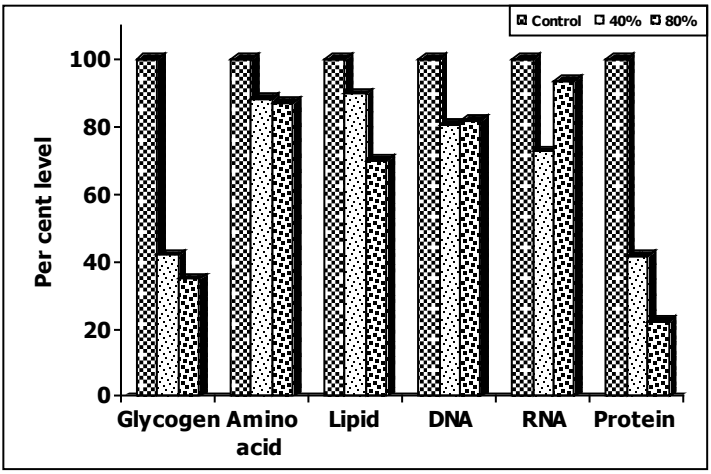

Figure 3: Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of malathion at 16 hour

Enzymatic alterations

In another experiment termites were treated with 40% and 80% of LD_{50} of fipronil, which caused a significant ($p < 0.05$) increase in acid phosphoatase (107.49% & 107.47%), alkaline phosphatase (105.60% & 105.06%), lactic dehydrogenase (103.24% & 102.65%) and glutamate pyruvate transaminase level (106.51% & 106.51%) after 4 h of treatment in comparison to control respectively. Later on decrease was recorded after 16 h of treatment of all the above enzymes. In a similar treatments fipronil ccaused a significant ($p < 0.05$) decrease in glutamate oxaloacetate transaminase (80.91% & 80.03%) and acetyl cholinesterase (60.60% & 61.12%) in termites body in comparison to control respectively (Table 4; Figure 4).

In another experiment termites were treated with 40% and 80% of LD_{50} of thiamethoxam. It caused increase in acid phosphoatase (114.63% & 113.55%), alkaline phosphatase (118.88% & 124.64%), lactic dehydrogenase (105.78% & 104.22%), glutamate pyruvate transaminase (110.83% & 112.62%) and glutamate oxaloacetate transaminase (110.83% & 108.59%) after 4 h of treatment in comparison to control respectively. Though the level of acetyl cholinesterase was also found to be increased at 4 h of treatment i.e. 110.45% and 101.72% respectively but at 16 h treatment a very high decrease was observed i.e. 72.75% and 73.45% in tested termites in comparison to control (Table 5; Figure 5).

Table 3: Effect of 40% and 80% of LD_{50} of malathion on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	3.26±0.0115 (100)	1.85*±0.009 (56.74)	1.76*±0.009 (53.98)	1.74*±0.005 (53.37)	1.38*±0.029 (42.33)	1.56*±0.009 (47.85)	1.66*±0.012 (50.92)	1.37*±0.02 (42.02)	1.14*±0.017 (34.97)
Amino acid	0.866±0.003 (100)	0.868*±0.0087 (100.23)	0.83*±0.002 (95.84)	0.74*±0.0018 (85.45)	0.79*±0.002 (91.22)	0.586*±0.010 (67.66)	0.756*±0.002 (87.29)	0.764*±0.0032 (88.22)	0.756*±0.0021 (87.29)
Lipid	1.046±0.001 (100)	1.12*±0.033 (107.07)	0.933*±0.006 (89.19)	1.09*±0.017 (104.21)	0.891*±0.0012 (85.18)	0.97*±0.003 (92.73)	0.765*±0.0015 (73.13)	0.94*±0.002 (89.86)	0.734*±0.0022 (70.17)
D.N.A.	0.85±0.028 (100)	0.842*±0.0087 (99.05)	0.725*±0.0012 (85.29)	0.779*±0.0026 (91.64)	0.715*±0.0018 (84.12)	0.738*±0.003 (86.82)	0.753*±0.0014 (88.58)	0.685*±0.0028 (80.58)	0.695*±0.0088 (81.76)
R.N.A.	0.98±0.023 (100)	0.92*±0.0027 (93.87)	0.925*±0.0026 (94.38)	0.881*±0.0072 (89.89)	0.927*±0.0058 (94.59)	0.84*±0.004 (85.61)	0.928*±0.0014 (94.69)	0.713*±0.003 (72.75)	0.917*±0.0036 (93.57)
Protein	5.2±0.046 (100)	3.29*±0.023 (63.26)	2.25*±0.017 (43.27)	2.91*±0.041 (55.96)	1.92*±0.041 (36.92)	2.54*±0.04 (48.84)	1.54*±0.04 (29.61)	2.16*±0.015 (41.53)	1.16*±0.015 (22.30)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant ($P < 0.05$, student t-test)

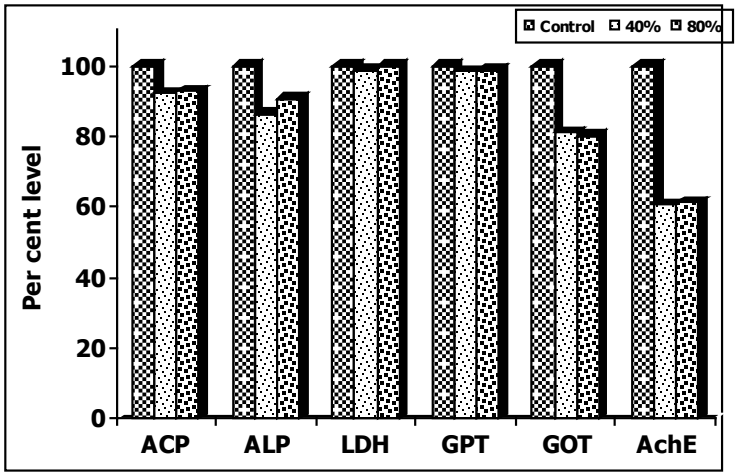

Figure 4: Comparison of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of fipronil at 16 h

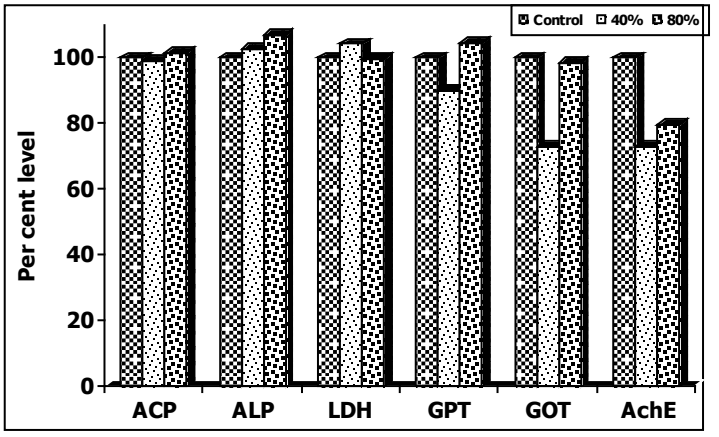

Figure 5: Comparison of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of thiamethoxam at 16 h

Table 4: Effect of 40% and 80% of LD_{50} of fipronil on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.21*±0.012 (107.49)	5.21*±0.0088 (107.49)	5.11*±0.014 (105.42)	5.11*±0.015 (105.36)	4.96*±0.0185 (102.19)	4.93*±0.0328 (101.71)	4.47*±0.0188 (92.21)	4.50*±0.0088 (92.71)
ALP	1.25±0.0057 (100.00)	1.32*±0.0058 (105.60)	1.31*±0.015 (105.06)	1.31*±0.0026 (105.14)	1.29*±0.002 (103.22)	1.17*±0.0115 (93.60)	1.28*±0.0017 (102.48)	1.08*±0.0173 (86.40)	1.13*±0.016 (90.40)
LDH	14.59±0.039 (100.00)	15.06*±0.0218 (103.24)	14.97*±0.02 (102.65)	14.96*±0.0058 (102.53)	14.89*±0.013 (102.10)	14.56*±0.0115 (99.79)	14.87*±0.006 (101.91)	14.37*±0.012 (98.51)	14.57*±0.02 (99.90)
GPT	6.55±0.015 (100.00)	6.98*±0.02 (106.51)	6.97*±0.0033 (106.51)	6.76*±0.012 (103.15)	6.88*±0.012 (105.13)	6.66*±0.012 (101.62)	6.73*±0.018 (102.79)	6.41*±0.02 (98.42)	6.46*±0.0088 (98.67)
GOT	1.64±0.023 (100.00)	1.62*±0.01 (98.78)	1.63*±0.0058 (99.39)	1.58*±0.042 (96.21)	1.56*±0.012 (95.32)	1.46*±0.0029 (88.73)	1.42*±0.012 (86.99)	1.33*±0.0065 (80.91)	1.311*±0.002 (80.03)
AchE	0.0116±0.009 (100.00)	0.0114*± 0.00079 (97.98)	0.014*±0.00028 (123.56)	0.0097*±0.00002 (83.64)	0.00893*±0.00012 (77.01)	0.00815*±0.0032 (70.25)	0.0081*±0.0034 (69.45)	0.007*±0.006 (60.60)	0.0071*±0.04 (61.12)

Values are mean ±SE of three replicates
Values are parantheses indicate percent level with control taken as 100%
*Significant at ($P < 0.05$, student t-test)

- acid phosphatase (ACP) and alkaline phosphatase (ALP)- µ moles of p-nitrophenol formed /30 minute/mg protein.
- lactic dehydrogenase (LDH): µ moles of pyruvate reduced/ 30min/mg/protein.
- glutamate-Pyruvate transaminase (GPT): Units of glutamate-pyurvate transaminase activity/hour/mg protein.
- glutamate oxalo acetate transaminase (GOT): Units of glutamate oxalo acetate transaminase activity/ hour/ mg protein.
- Acetylcholine esterase (AchE): µ moles 'SH' hydrolysed/min/mg/protein.

Table 5: Effect of 40% and 80% of LD_{50} of thiamethoxam on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.56*±0.0061 (114.63)	5.51*±0.0048 (113.55)	5.48*±0.0027 (113.09)	5.46*±0.0051 (112.69)	5.10*±0.0059 (105.13)	5.06*±0.026 (104.38)	4.82*±0.004 (99.27)	4.94*±0.017 (101.75)
ALP	1.25±0.0057 (100.00)	1.49*±0.0058 (118.88)	1.56*±0.012 (124.64)	1.45*±0.0152 (116.24)	1.49*±0.0033 (119.46)	1.386*±0.0042 (110.88)	1.42*±0.0047 (113.54)	1.28*±0.024 (102.61)	1.34*±0.0085 (106.96)
LDH	14.59±0.039 (100.00)	15.43*±0.029 (105.78)	15.21*±0.0044 (104.22)	15.23*±0.015 (104.38)	15.03*±0.012 (103.04)	15.19*±0.0088 (104.13)	14.81*±0.028 (101.53)	14.96*±0.0088 (102.58)	14.57*±0.0088 (99.91)
GPT	6.55±0.015 (100.00)	7.26*±0.015 (110.83)	7.37*±0.0088 (112.62)	7.08*±0.029 (108.16)	7.17*±0.012 (109.51)	6.92*±0.035 (105.60)	6.95*±0.01 (106.11)	6.83*±0.01 (104.27)	6.84*±0.02 (104.52)
GOT	1.64±0.023 (100.00)	1.80*±0.0058 (110.83)	1.78*±0.0017 (108.59)	1.70*±0.0012 (103.43)	1.75*±0.0015 (106.89)	1.59*±0.0039 (96.72)	1.66*±0.012 (101.31)	1.47*±0.014 (90.20)	1.61*±0.0018 (98.45)
AchE	0.0116±0.009 (100.00)	$0.0128*\pm7\cdot10^{-4}$ (110.45)	$0.0118*\pm3\cdot10^{-4}$ (101.72)	$0.016*\pm54\cdot10^{-4}$ (138.50)	$0.014*\pm15\cdot10^{-4}$ (124.13)	$0.0098*\pm3\cdot10^{-4}$ (84.83)	$0.0096*\pm2\cdot10^{-4}$ (83.10)	$0.0084*\pm2\cdot10^{-4}$ (72.75)	$0.0092*\pm6\cdot10^{-4}$ (79.45)

Table 6: Effect of 40% and 80% of LD_{50} of malathion on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	4.85±0.022 (100.00)	5.51*±0.0058 (113.60)	5.26*±0.012 (108.45)	5.18*±0.021 (106.80)	5.16*±0.0088 (106.39)	4.96*±0.037 (102.34)	4.99*±0.0088 (102.88)	4.76*±0.0088 (98.21)	4.56*±0.011 (94.02)
ALP	1.25±0.0057 (100.00)	1.42*±0.0047 (113.54)	1.36*±0.0058 (108.80)	1.35*±0.0088 (108.26)	1.34*±0.0037 (106.98)	1.26*±0.0145 (101.33)	1.33*±0.0058 (106.00)	1.13*±0.0088 (100.63)	1.15*±0.017 (92.26)
LDH	14.59±0.039 (100.00)	15.20*±0.031 (104.20)	15.19*±0.038 (104.16)	15.08*±0.043 (103.40)	15.04*±0.0176 (103.12)	14.93*±0.032 (102.33)	14.94*±0.0145 (102.42)	14.68*±0.038 (100.63)	14.5*±0.025 (99.38)
GPT	6.55±0.015 (100.00)	7.05*±0.017 (107.68)	7.04*±0.019 (107.53)	6.96*±0.012 (106.31)	6.94*±0.026 (105.90)	6.73*±0.012 (102.79)	6.73*±0.012 (102.69)	6.56*±0.015 (100.20)	6.53*±0.0218 (99.79)
GOT	1.64±0.023 (100.00)	1.66*±0.012 (101.42)	1.64*±0.0176 (100)	1.61*±0.0037 (98.45)	1.56*±0.0115 (95.12)	1.58*±0.0029 (96.62)	1.48*±0.0066 (90.45)	1.43*±0.015 (87.19)	1.38*±0.012 (82.72)
AchE	0.0116±0.009 (100.00)	0.01*±0.005 (87.06)	0.012*±0.001 (103.45)	0.0092*±0.008 (79.59)	0.00929*±0.000078 (80.08)	0.00826*±0.008 (71.26)	0.0085*±0.007 (73.56)	0.007*±0.001 (60.63)	0.0070*±0.001 (60.05)

In a similar experiment when termites were treated with 40% and 80% of LD_{50} of malathion, it showed a significant ($p < 0.05$) increase in acid phosphoatase (113.60% and 108.45%) and alkaline phosphatase (108.26% & 106.98%) glutamate pyruvate transaminase (107.68% & 101.42%) and glutamate oxaloacetate transaminase (107.53% & 100.00%) after 4 h of treatment in comparison to control (Table 6; Figure 6). Further, a similar dose of malathion caused slight variation in lactic dehydrogenase level i.e. 100.63% and 99.38% in comparison to control respectively (Table 6; Figure 6). Similar increase was also observed in acetyl cholinesterase level after 4 h treatment i.e. 103.45% and 97.98% respectively, but later on drastic decrease was found at 16 h of treatment i.e. 60.05% and 60.60% respectively in comparison to control (Table 6; Figure 6).

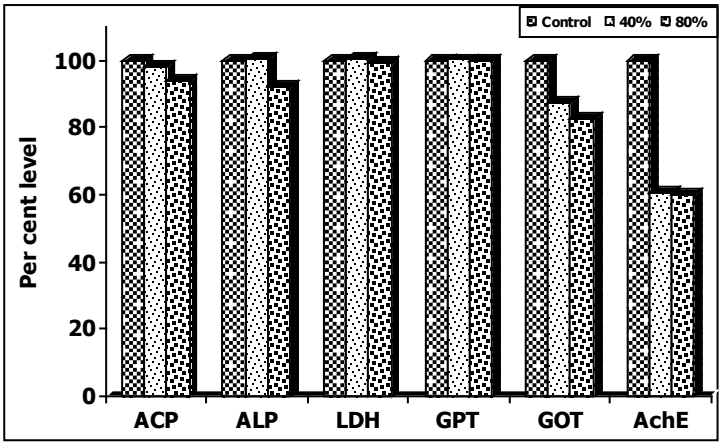

Figure 6: Comparison of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of malathion at 16 h

4 Discussion

For effective and fast control of termite population synthetic pesticides are used, which show vesry high lethality in different termite species. In the present study fipronil, thiamethoxam and malathion have shown very high lethality, which is proved by very low LD_{50} value. When termites were treated with 40% and 80% of LD_{50} of fipronil, thiamethoxam and malathion each of them

significantly reduced (significant at $p < 0.05$) the level of glycogen i.e. 40.02% and 50% after 16 h of treatment in comparison to control (Table 1-3; Figure 1-3). Similarly, cypermethrin affected the level of glycogen, protein and lipid in *Pimpla turionella* wasp's larvae, pupae and adult females [17]. It also increased the protein level in *Spodoptera litrua* larvae in comparison to control [18]. Similarly 40% and 80% of LD_{50} of malathion, fipronil and thiamethoxam significantly decreased the level of amino acid up to 62.93–87.29% in *O. obesus* after 16 h treatment in comparison to control.

A similar dose of above pesticides caused significant ($p < 0.05$) decrease in lipid, protein and amino acid levels after 4 hour of treatment in comparison to control (Table 1-3; Figure 1-3). These insecticides have also significantly cut down the level of both DNA and RNA levels were found to be decreased after 16 h of treatment. Similarly, amount of total lipids was also increased. It may be due to breakdown of glycerides and diglycerides. However, lipid reserves are built up during active feeding. On the other hand, mobilization of more lipids may induce hydrolysis of triglycerides, diglycerides by an enzyme lipase. Reduction in protein synthesis may lead to decrease in protein concentration which lead to physiological stress in insects.

Similarly, chloropyrifos, thiamethoxam, fipronil, and malathion caused significant depletion in total protein in haemolymph and fat body of silk worm *Bombyx mori* [19]. Malathion caused lipid depletion in haemolymph, fat body and oocytes of *Tenebrio molitor* [20]. It indicates that why more toxic stress in termites and more utilization of lipids occurs. To supplement the nutrients more carbohydrates are converted into lipids during development period in insects [21]. Similarly, glycogen depletion indicates more and more utilization of food reserve for production ATP to cope up the insecticide-induced stress [22, 23]. However, with in the body free glycogen floats in the haemolymph after its breakdown, which induce help to maintain glucose level. This, instant breakdown of glycogen may induce glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [24]. An increase in glycogenesis causes a significant decrease in free amino acid level [25]. Similarly, protein and nucleic acid synthesis may also block at cellular level and catabolism get increase, which results into low availability of proteins and nucleic acid. Hence, the level of amino acid increased after protein catabolism.

In a similar experiment when termites were treated with 40% and 80% of LD_{50} of malathion, fipronil and thiamethaxm showed a significant ($p < 0.05$) increase in acid phosphoatase (113.60 to 101.70), alkaline phosphatase (124.64 to 100.63), glutamate pyruvate transaminase (107.68 to 100.20) and glutamate

oxaloacetate transaminase (110.83 to 101.31) and lactic dehydrogenase (105.78 to 100.21) level after 4 h of treatment in comparison to control (Table 4-6; Figure 4-6). Contrary to this a significant decrease (60.60–6.12%) was observed in acetyl cholinesterase level after 16 h of treatment in comparison to control (Table 4-6; Figure 4-6).

In addition to it all three pesticides significantly ($p < 0.05$) cut down the level of acetylcholinesterase upto 60–61% after 4 hr that proves nuerotoxic effects of these pesticides in termites. Similarly, phenolic compounds such as phosphorus oxycholride showed acetylcholinesterase inhibition at sub lethal dose in subterranean termite C. formosanus [26]. Phosphorus oxycholride induce brain acetylcholinesterase in houseflies and selectively bind to acetylcholinesterase in comparison to other serine hydrolases. Contrary to this malathion also potentially inhibits acetylcholinesterase activity more than malaxon and isomalathion [27]. Similarly, dimethyl maleate inhibits acetylcholinesterase activity at concentration greater than 10 mM [28]. Besides this, malathion [S-(1, 2-dicarboethoxyethyl) O, O-dimethyl phosphorodithiote] shows genotoxicity in treated insects [29]. To fight against toxic insects show significant induction in hydrolytic activities with in the body tissues, which cut down the acid and alkaline phosphatase level [30, 31]. Moreover, few natural pesticides such as pyrethroids inhibit the phosphatase activity in insects [32]. Similarly, in presence of pesticides, transamination of amino acids is increased, which affect the level of glutamate pyruvate transaminase and glutamate oxalo acetate transaminase enzymes [33]. Similarly, increase in lactic dehydrogenase level induce tissue necrosis in insects while increase in alkaline phosphatase level induce lysosomal activities in cells, which leads to biochemical stress in insects [34]. Therefore, a decline in the level of above enzymes directly effect oxygen consumption in insects. More specifically, both fat body and heamolymph exhibit higher glutamate oxaloacetate transaminae activity than the glutamate pyruvate transaminase during successive developmental stages. However, the heamolymph aminotransferase is found significantly decreased [33]. In the present study elevation or reduction in enzyme level is associated with metabolic alterations in insects [35]. which lead to the death of insects after insecticide poisoning [36]. Present study proves termiticidal effects of malathion, fipronil and thiamethaxm on *Odontotermes obesus* which significantly altered the level of important metabolites and enzymes.

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References

[1] Jitunari, F., Asakawa, F., Takeda, N., Suna, S. and Manabe, Y. (1995), Chlordane compounds and metabolite residues in termite control workers' blood, *Bull. Environ. Contam. Toxicol.* 54(6), pp. 855-862.

[2] Sim, M., Forbes, A., McNeil, J. and Robert, G. (1998), Termite control and other determinants of high body burdens of cyclodiene insecticides, *Arch. of Environ. Health* 53(2), pp. 114-21.

[3] Peters, B. C. and Fiztgerland, C. J. (2006), Borate protection of softwood from *Coptotermes acinoaciformis* (Isoptera: Rhinotermitidae) damage variation in protection thresholds explained, *J. Econ. Entomol.* 99(5), pp. 1749-1756.

[4] Su, N. Y., Ban, P. M. and Scheffrahn, R. H. (2000), Control of *Coptotermes havilandi*(Isopetera: Rhinotermitidae) with hexaflumuron baits and a sensor incorporated into a monitoring and baiting program, *J. Econ. Entomol.* 93(2), pp. 415-21.

[5] Valles, S. M. and Woodson, W. D. (2002), Insecticide susceptibility and detoxication enzyme activities among *Coptotemes formosanus* Shiraki workers sampled from different location in New Orleans, *Comp. Biochem. Physiol.* 131(4), pp. 469-470.

[6] Hu, X. P. (2005), Valuation of efficacy and non-repellency of indoxcarb and fibronill treated soil at various concentration and thickness against two subterranean termites (Isoptera: Rhinotermitidea), *J. Econ. Entomol.* 98(2), pp. 509-517.

[7] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956), Colorimetric method for the determination of sugar and related substances, *Indian J. Biol.* 28, pp. 350-356.

[8] Spies, J.R. (1957), Colorimetric procedure for amino acids. In: *Methods in enzymology*, Colowich, S.P. and Kalplan, N.O. (eds), Acadmic Press.

[9] Floch, J., Lees, M. and Stanely, G. H. S. (1957), A simple method for the isolation and purification of total lipids from animal tissues, *J. Biol. Chem.* 226, pp. 497-509.

[10] Scheidner, W. C. (1957), Enzymology, Clowick, S. P., Kapalan, N. O. (eds), Academic Press, Pp. 680.

[11] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R. (1957), Protein measurement with phenol reagent, *J. Biochem.* 193, pp. 265-275.

[12] Bergmeyer, U. H. (1976), Determination of alkaline phophatase and acid phosphatase by using p-nitrophenyl phosphate, In: *Method of enzymatic analysis.* Acadmic Press, New York, Pp. 1129.

[13] Annon, T. M. (1984), Sigma diagnostic: Lactate dehydrogenase (Quantitative, Colorimeteric determination in serum, urine and cerebrospinal fluid) at 400-500nm. Procedure No. 500.

[14] Reitman, A. and Frankel, S. (1957), A colorimetric method for the determination of glutamate-oxaloacetate and serum glutamate-pyruvate transaminase, *Am. J. Clin. Pathol.* 28, pp. 56-63.

[15] Ellman, G. L., Courtney, K. D., Andres, V. and Featherstone, R. M. (1961), A new and rapid colorimeteric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* 7, pp. 88-95.

[16] Sokal, R. R. and Rohlf, F. J. (1973), Introduction to biostatistics, Freeman W.H. Co San Francisco.

[17] Sak, O., Uckan, F. and Ergin, E. (2006), Effects of cypermethrin on total body weight, glycogen, protein and lipid contents of *Pimpla turionellae* (L.) (Hymenptera: Ichneumonidae), *Belgian J. Zool.* 136(1), pp. 53-58.

[18] Vijayraghavan, C. and Chitra, K. C. (2002), Total protein and free amino acid content *Spodoptera litura* (Fabr.) due to botanicals and conventional insecticides, *Indian J. Entomol.* 64, pp. 92-95.

[19] Nath, B. S., Suresh, A., Varma, B. M. and Kumar, R. P. S. (1997), Changes in protein metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorous insecticides toxicity, *Ecotoxic. Environ. Safety* 36, pp. 169-173.

[20] Lohar, M. K. and Wright, D. J. (1993), Changes in the lipid content in hemolymph, fat body and oocytes of malathion treated *Tenebrio molitor* L. *Adult females*, *Pak. J. Zool.* 25, pp. 57-60.

[21] Candy, D. J. (1985), Intermediary metabolism. In: *Comprehensive Insect physiology*, Kerkut and Gilbert (eds), University Press, Oxford, Pp. 1-41.

[22] Sancho, E., Ferrando, M. D., Fernandez, C. and Andreu, E. (1998), Liver energy metabolism of *Anguilla anguilla* after exposure to fenitrothion, *Ecotoxic. Environ. Safety* 41, pp. 168-175.

[23] Rambabu, J. P. and Rao, M. B. (1994), Effect of organochlorine and three organophosphate pesticides on glucose, glycogen, lipid and protein contents in tissue of the freshwater snail *Bellamya dissimilis* (Muller), *Bull. Environ. Cont. Toxicol.* 53, pp. 142-148.

[24] Dezwann, A. and Zandee, D. I. (1972), The utilization of glycogen and accumulation of some intermediate during anaerobiosis in *Mytilus edulis* L., *Comp. Biochem. Physiol.* 43, pp. 47-54.

[25] Pant, R. and Gupta, D. K. (1979), The effect of exposure to low temperature on the metabolism of carbohydrates, lipids and protein in the larvae of *Philosamia ricini*, *J. Biosc.* 1, pp. 441-446.

[26] Kartal, S. N., Imamura, Y., Tsuchiya, F. and Ohsato, K. (2004), Preliminary evaluation of fungicidal and termiticidal activities of filtrates from biomass slurry fuel production, *Bioreso Techno.* 95(1), pp. 41-47.

[27] Quistad, G. B., Zhang, N., Sparks, S. E. and Casida, J. E. (2000), Phosphoacetylcholinesterase: Toxicity of phosphorous oxychloride to mammals and insects that can be attributed to selective phosphorylation of acetylcholinesterase by phosphorodichloridic acid, *Chem. Res. Toxicol.* 13(7), pp. 652-657.

[28] Kristic, D. Z., Colovic, M., Kralj, M. B., Franko, M., Krinulovic, K., Trebse, P. and Vasic, V. (2008), Inhibition of AChE by malathion and some structurally similar compounds, *J. Enz. Inhi. Med. Chem.* 7, pp. 1.

[29] Blasiak, J., Jaloszynski, P., Trzeciak, A. and Szyfter, K. (1999), *In vitro* studies on the genotoxicity of the organophosphorus insecticides malathion and its two analogues, *Mutant Res.* 445(2), pp. 275-283.

[30] Jaffrezic- Renault, N. (2001), New trends in biosensors for organophosphorus, *Pest. Sens.* 1, pp. 60-74.

[31] Luskova, V., Svoboda, M. and Kolaova, J. (2002), The effect of diazinon on blood plasma biochemistry in carp (*Cyprinus carpio* L.), *Acta. Vet. Brun.* 71, pp. 117-123.

[32] Ishaaya, I. and Casida, J. E. (1980), Properties and toxicological significance of esterase hydrolyzing permethrin and cypermethrin in *Trichoplusia ni* larval gut and integument, *Pest. Biochem. Physiol.* 14, pp. 178-184.

[33] Pant, R. and Morris, I. D. (1972), Variation in glycogen, total free sugars, protein, alkaline and acid phosphatases, citrate and inorganic phosphorus level in fat body of *Philosamia ricini* (Eri-Silkworm) during development, *J. Biochem.* 71(1), pp. 1-8.

[34] Chaidee, A., Wongchai, C. and Pfeiffer, W. (2008), Extracellular alkaline phosphatase is a sensitive marker for cellular stimulation and exocytosis in heterotroph cell cultures of *Chenopodium ruburm*, *J. Plant Physiol.* 165(16), pp. 1655-1666.

[35] Srinivas, R., Udikeri, S. S., Jayalakshmi, S. K. and Sreeramulu, K. (2004), Identification of factors responsible for insecticide resistance in Helicoverpa armigera (Hubner), *Comp. Biochem. Physiol.* 137, pp. 261-269.

[36] Abdel-Hafez, M. M., Abdel-Sattar, M. M. and El-Malla, M. A. (1984-85), Changes in the esterase, phosphatase and among amino transeferase of pink bollworm moths during the course of insecticide poisoning, *Bull. Entomol. Soc. Eco.* Ser 14, pp. 429-438.