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Toxicological Effects of Profenofos and Carbosulfan on AAT (E.C. 2.6.1.1) and ALAT (E.C. 2.6.1.2) Activity Levels of Freshwater Fish Labeo rohita

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Abstract: The freshwater fish *Labeo rohita* were exposed to lethal and sublethal concentrations of profenofos and carbosulfan 50%, 25 %(EC) to study the impacts on AAT and ALAT enzymes. The increased AAT activity seems to result from enhanced enzyme turnover under profenofos and carbosulfan stress. Increase or decrease ALAT activity can also be interpreted as a shift of the tissue emphasis on energy breakdown pathway, involved in the inter-conversion of important compounds such as pyruvate, oxaloacetate, α-ketoglutarate and amino acids thus bringing the protein and carbohydrate metabolism on one hand and alanine, aspartic acid and glutamic acid. The activity levels of AAT and ALAT in general, were decreased in all the tissues of experimental fish in 24h and 8 days sublethal and lethal exposures.
Key words: Freshwater, Profenofos and Energy.

Introduction

Increasing the productivity in the tropics and resulting human activities have caused serious damage to tropical ecosystems. The degration of terrestrial and aquatic ecosystems due to xenobiotics is a major concern and is a direct result of the increased use of the synthetic chemicals such as pesticides in the productivity [1].Organophosphorus and Carbamate pesticides are widely used in tropical agriculture. The toxicity of these pesticides is based on inhibition of the enzyme acetylcholine esterase (AchE) and other enzymes like AAT and ALAT. When used in the vicinity of aquatic ecosystems, these pesticides may enter the water bodies as result of spray drift,leaching from the soils and surface runoff during precipitation in concentrations which may exert adverse effects on the non-target organisms inhibiting the area[2].Aminotransferases mobilise the amino acids into carbohydrate and lipid metabolism. There exists a rapid turnover of free amino acids from cell to cell, tissue to tissue through the circulating fluid and utilize for various purposes through interconversions. Transaminases form an important group of enzymes mediating carbohydrates, protein and lipid metabolism. Transamination represents the mechanism causing eventual deposition of nitrogenous waste products like ammonia and urea resulting in the production of carbon compounds, which contribute towards gluconeogenesis and fatty acid formation. AAT and ALAT are two important enzymes mainly involved in the inter-conversion

of important compounds such as pyruvate, oxaloacetate, α-ketoglutarate and amino acids thus bringing the protein and carbohydrate metabolism on one hand and alanine, aspartic acid and glutamic acid. Considering the number of potential organophosphates and Carbamate pesticides which contaminate the aquatic environment and difficulty in detecting low levels of these individual chemicals, measuring the AAT,ALAT in non-target organisms is probably the best indicator of serious organophosphate and Carbamate Pollution. The activity of aspartate and alanine amino transferases (AAT and AlAT), which serve as strategic links between protein and carbohydrate metabolisms, which is known to alter under several physiological and pathological conditions [3].

Materials And Methods

The Freshwater fish *Labeo rohita* measuring 6 to 8 cm in length and 6.5 to 7.5 gm in weight irrespective of the sex were used in the experiment. Fish were washed with 0.1% $KMnO_4$ solution to avoid dermal infection. All the precautions laid down by [4], are followed, for maintaining the fish. The fish were exposed to organophosphorus pesticide profenofos 50% EC and Carbamate pesticide 25% EC (Emulsifiable Concentration) to 96 h LC_{50} Sublethal lethal ($10\mu g/L^{-1}$, $1.8\ mg/L^{-1}$) concentrations for 24 hrs and 8 days. If mortality occurred during the experimental period, dead fish were removed immediately to avoid depletion of dissolved oxygen (DO) level which adversely affects other fish. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of AAT and ALAT.

Estimation of aminotransferase activity

The activity of AAT and ALAT were determined by the method of [5].The selected tissues were homogenized in 5% ice-cold 0.25 M sucrose solution. The supernatants were used for the analysis of the enzyme activities.

Estimation of AAT activity

The reaction mixture of 1.5 ml contains: 1 ml of phosphate buffer (pH 7.4), 0.1 ml of L- aspartate (L-Aspartic acid), 0.1 ml of α-ketoglutaric acid and 0.3 ml of supernatant as enzyme source. The reaction mixture was incubated at 37^0 C for 30 minutes. The reaction was stopped by adding 1 ml of 2, 4-dinitrophenyl hydrazine solution prepared in 0.1 N HCl and was allowed to stand for 20 minutes at room temperature. The rest of the details were the same as for alanine aminotransferase. The activity levels were expressed as µ moles of pyruvate formed/mg protein/hr.

Estimation of ALAT activity

The reaction mixture of 1.5 ml contains 1 ml phosphate buffer (pH 7.4), 0.1 ml of L- alanine, and 0.1 ml of α-ketoglutarate and 0.3 ml of supernatant as enzyme source. The contents were incubated at 37^0 C for 30 minutes. The reaction was stopped by the addition of 1 ml of 2, 4- dinitrophenyl hydrazine solutions. After 20 minutes, 10 ml of 0.4 N sodium hydroxide was added and the colour developed was read at 545 nm in a spectrophotometer (ELICO Model SL171) against a reagent blank. The enzyme activity was expressed as µ moles of pyruvate formed/mg protein/hr.

Results And Discussion

The calculated values of Amino transferases and percent change over control along with standard deviations were given shown in Table 1-4, are graphically represented in Fig 1-4.The changes in the levels of aspartate aminotransferases (AAT) and alanine aminotransferases (ALAT) were studied in different vital organs like brain, liver, muscle, gill and kidney in the Experimental fish *Labeo rohita* under lethal and sublethal concentrations of Profenophos and Carbosulfan after 24h and 8 days of exposure. The values are expressed as micro moles of pyruvate formed/mg protein /h.

AA T Activity

The calculated values and percent change over control along with standard deviation and the changes for AAT activity are given in Table 1-4 and Fig1-4. The AAT activity in the control fish is in the order of:

Kidney > Gill > Liver > Muscle > Brain. On exposure to sub lethal and lethal concentrations of profenofos, carbosulfan, the lyotropic gradation series in terms of percent increase in AAT activity is in the order of Profenofos: Liver > Kidney > Gill >Brain > Muscle. Carbosulfan: Gill > Liver> Kidney >Muscle >Brain

In profenofos 24h sublethal exposure maximum percentage of elevation in AAT activity was (65.38%) in muscle and minimum elevation was (47.12%) in Gill. But in lethal exposure maximum percentage of elevation was (87.19%) in muscle and minimum percentage of elevation was (55.49%) in liver. In Carbosulfan sublethal exposure maximum percentage of elevation in AAT activity was (61.85%) in muscle and minimum elevation was (4.02%) in brain. But in lethal exposure maximum percentage of elevation was (78.84%) in muscle and minimum percentage of elevation was (50.78%) in liver.

In profenofos 8days sublethal exposure maximum percentage of elevation in AAT activity was (58.10%) in muscle and minimum elevation was (27.46%) in kidney. But in sub lethal exposure of carbosulfan maximum percentage of elevation was (47.29%) in muscle and minimum percentage of elevation was (31.25%) in liver.

Table 1:Change in the specific activity levels of Aspartate amino transferase (AAT) (µ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of fish *Labeo rohita* exposed to sublethal and lethal concentrations of Profenofos and Carbosulfan

Organs	Control	profenofos/24 hrs				carbosulfan/24hrs			
		Sub – Lethal	% Change	Lethal	% Change	Sub-Lethal	% Change	Lethal	% Change
Gill	3.48 ±0.16	5.13 ±0.73	+47.12	5.59 ±1.54	+60.63	4.99 ±0.19	+43.40	5.37 ±1.94	+54.32
Liver	3.82 ±1.24	1.92 ±1.61	+50.26	5.95 ±0.91	+55.49	1.87 ±1.33	+51.04	5.82 ±1.97	+50.78
Kidney	5.29 ±0.98	2.62 ±0.82	+50.47	8.52 ±1.32	+61.86	2.72 ±1.81	+48.59	8.21 ±0.20	+55.19
Brain	2.58 ±1.54	3.87 ±1.94	+49.61	4.10 ±1.61	+58.91	3.69. ±0.81	+43.02	4.06 ±0.54	+57.36
Muscle	3.12 ±1.94	1.08 ±1.51	+65.38	5.84 ±1.54	+87.17	1.19 ±0.78	+61.85	5.58 ±1.31	+78.84

Values are the mean of five observations Standard Deviation is indicated as (±),Values are significant at $p < 0.05$.

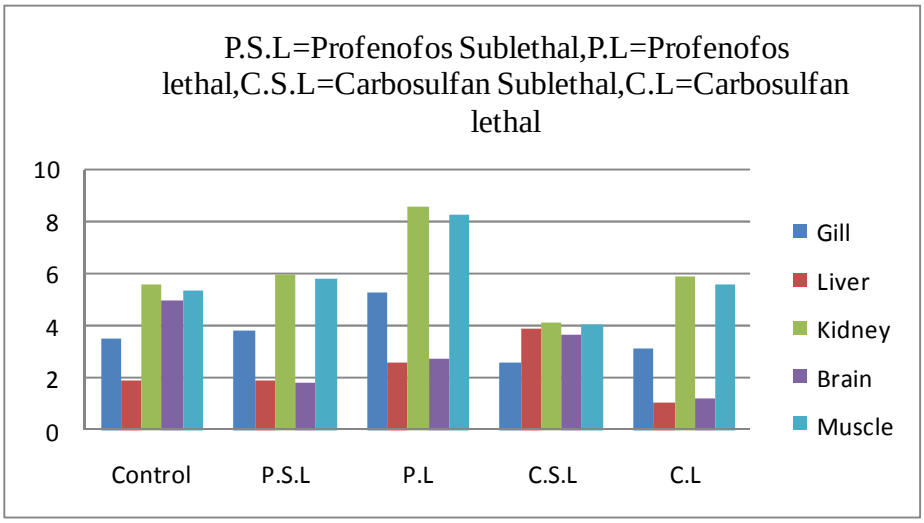

Figure 1: Change in the specific activity levels of Aspartate aminotransferase (µ moles of pyruvate formed/mg protein/hr) in different tissues of fish exposed to sublethal and lethal concentrations of Profenofos and Carbosulfan

Table 2: Change in the specific activity levels of Aspartate amino transferase (µ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of fish *Labeo rohita* exposed to sublethal concentrations of Profenofos and Carbosulfan

Organs	Control	Profenofos/8days		Carbosulfan/8days	
		Sub –Lethal	% Change	Sub-Lethal	% Change
Gill	1.66 ±0.26	0.86** ±0.19	+48.12	0.94** ±0.73	+43.37
Liver	1.92 ±1.34	1.27 ±1.33	+33.85	1.98 ±1.61	+31.25
Kidney	2.04 ±0.88	1.48 ±1.81	+27.46	1.95 ±0.82	+44.11
Brain	1.82 ±1.54	0.79** ±0.78	+56.59	0.84** ±1.51	+53.84
Muscle	1.48 ±1.64	0.62** ±0.81	+58.10	0.78** ±1.94	+47.29

Values are the mean of five observations Standard Deviation is indicated as (±),
Values are significant at $p < 0.05$,**$P < 0.001$.

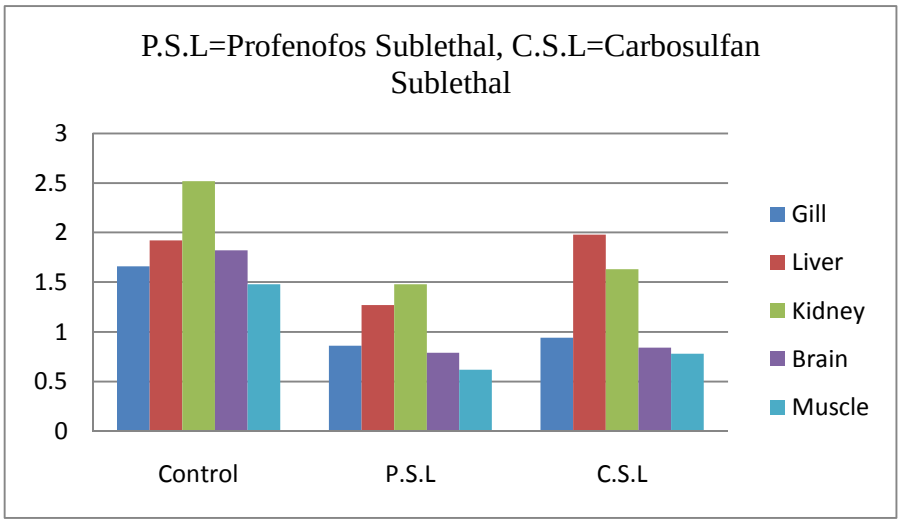

Figure 2: Change in the specific activity levels of Aspartate aminotransferase (µ moles of pyruvate formed/mg protein/hr) in different tissues of fish exposed to sublethal concentrations of Profenofos and Carbosulfan

Increase in aminotransferases activity was reported in *Tilapia mossambica*, under different pesticides exposure[6 - 9] reported an elevation of AAT and ALAT in fish *Tilapia mossambica* following fenvalerate intoxication. [10] Reported an increase of AAT and ALAT activities in fish *Cyprinus carpio* under fenvalerate intoxication. The GOT and GPT activities increased under aldicarb, phosphamidon and endosulfan toxicity on fish tissues[11]. An elevation in AAT and ALAT activity levels was reported by[12], when crab *Barytelphusa guerins* exposed to endosulfan 35% EC.

GDH catalyses the reversible deamination of glutamate to α-ketoglutarate and ammonia. AAT catalyses reversible transamination of glutamate and oxaloacetate to α-ketoglutarate and asparte, while ALAT catalyses the reversible transamination of glutamate and pyruvate to α- ketoglutarate and alanine. Thus, the aminotransferases along with GDH contribute some strategic substances such as α-ketogluterate, pyruvate, oxaloacetate, glutamate etc., to oxidative metabolism.

The elevation of AAT activity provides the oxaloacetate required for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of AAT and ALAT in different tissues of brain, liver, muscle, gill and kidney of the Freshwater fish *Labeo rohita* can be considered as a response to the stress induced by

profenofos and carbosulfan to generate ketoacids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestations.

The depletion of proteins under the stress of profenofos and carbosulfan toxicity observed in different tissues of *Labeo rohita* indicates the proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. The alterations in the levels of activity of aminotransferases induced by the pesticide profenofos and carbosulfan clearly indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems. The increase in activities of aminotransferases as observed in the present study were in agreement with earlier reports, demonstrating a consistent increase in the activities of these enzymes under conditions of enhanced gluconeogenesis. The alterations in the levels of activity of aminotransferases induced by the organophosphate and Carbamate pesticides clearly indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems.

Table 3: Change in the specific activity levels of Alanine amino transferase (μ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of fish *Labeo rohita* exposed to Sublethal and Lethal Concentrations of Profenofos and Carbosulfan

Organs	Control	profenofos/24 hrs				carbosulfan/24hrs			
		Sub – Lethal	% Change	Lethal	% Change	Sub-Lethal	% Change	Lethal	% Change
Gill	5.56 ±0.15	7.80 ±0.82	+40.28	7.32 ±1.56	+58.36	7.67 ±0.21	+37.05	8.35 ±1.74	+50.18
Liver	4.17 ±1.34	5.95 ±1.81	+42.68	6.10 ±0.81	+46.28	5.62 ±1.23	+34.77	5.84 ±1.98	+40.04
Kidney	6.28 ±0.88	9.63 ±0.72	+54.34	10.19 ±1.42	+62.26	9.28 ±1.64	+47.73	9.54 ±0.21	+51.92
Brain	3.15 ±1.64	4.29 ±1.89	+36.19	4.86 ±1.72	+54.28	4.11 ±0.96	+30.47	4.72 ±0.62	+49.84
Muscle	3.98 ±1.36	6.87 ±1.61	+72.61	7.68 ±1.44	+92.96	6.59 ±0.54	+65.52	7.55 ±1.51	+89.62

Values are the mean of five observations Standard Deviation is indicated as (±),
Values are significant at $p < 0.05$.

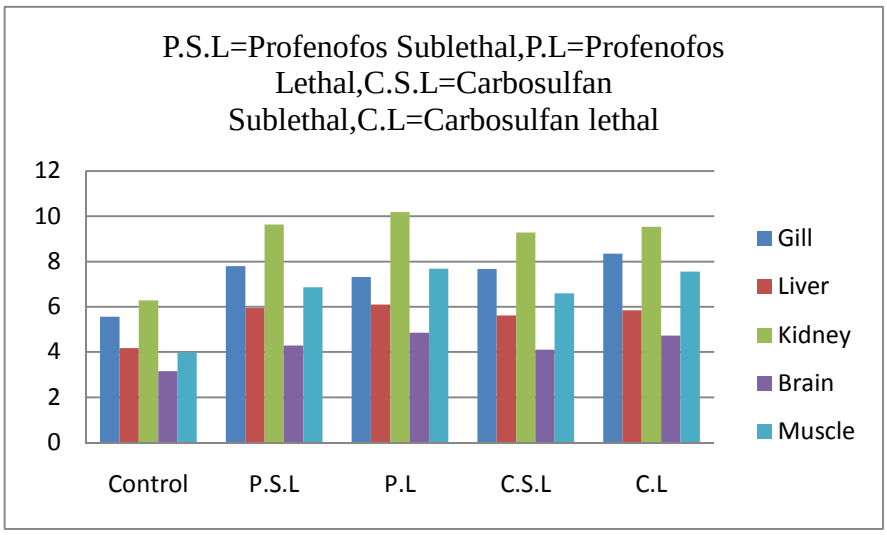

Figure 3: Change in the specific activity levels of Alanine aminotransferase (μ moles of pyruvate formed/mg protein/hr) in different tissues of fish exposed to sublethal and lethal concentrations of Profenofos and Carbosulfan

Table 4: Change in the specific activity levels of Alanine amino transferase (µ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of fish *Labeo rohita exposed* to Sublethal Concentrations of Profenofos and Carbosulfan

Organs	Control	Profenofos/8 days		Carbosulfan/8 days	
		Sub-Lethal	% Change	Sub-Lethal	% Change
Gill	3.26 ±1.47	1.58 ±1.52	+51.53	1.64 ±0.54	+49.69
Liver	2.10 ±1.84	1.52 ±1.34	+27.61	1.59 ±1.59	+24.28
Kidney	1.89 ±0.45	1.16** ±0.34	+38.62	1.20 ±0.44	+36.54
Brain	0.84** ±0.6 7	0.63** ±0.74	+25.95	0.68** ±0.97	+19.05
Muscle	1.01** ±0.34	0.47** ±1.67	+53.47	0.54** ±1.47	+52.46

Values are the mean of five observations Standard Deviation is indicated as (±), Values are significant at $p < 0.05$, **P<0.001.

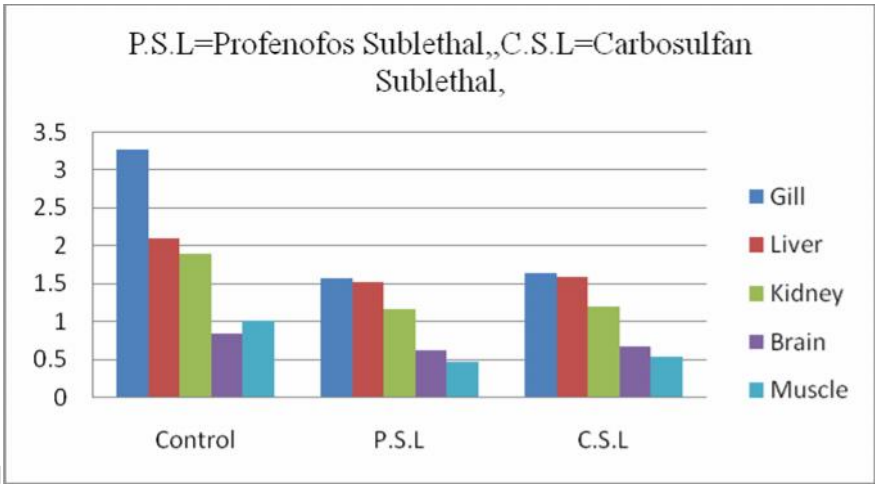

Figure 4: Change in the specific activity levels of Alanine aminotransferase (µ moles of pyruvate formed/mg protein/hr) in different tissues of fish *Labeo rohita* exposed to sublethal and lethal concentrations of Profenofos and Carbosulfan

ALAT Activity

The calculated values and percent change over control along with standard deviation and the changes for ALAT activity are given in Table .3 &4 and Fig 3&4. The ALAT activity in different tissues of control fish was in the order of: Gill > Liver > Brain > Muscle > Kidney. On exposure to sublethal and lethal concentrations of Profenofos and carbosulfan , the lyotropic gradation series in terms of percent increase in ALAT activity is in the order of: Profenofos sublethal: Kidney > Muscle >Gill> Brain > Liver, Profenofos lethal : Kidney > Muscle > Gill> Liver>Brain.

Under exposure to sublethal concentrations of Carbosulfan the lyotropic gradation series in terms of percent increase in ALAT activity is in the order of: Carbosulfan sublethal: Kidney > Gill >Muscle > Brain > Liver, Carbosulfan lethal : Kidney > Gill >Muscle > Liver > Brain>Muscle. The ALAT specific activity levels increased significantly during the 8 days exposure period compare with controls,24h exposure period. In Profenofos 24h sublethal exposure maximum percentage of elevation in ALAT activity was (72.61%) in Muscle and minimum elevation was (40.28%) in Gill. In lethal exposure of Profenofos maximum percentage of elevation was (92.96%) in Muscle and minimum percentage of elevation was (46.28%) in liver.

In Carbosulfan sublethal exposure maximum percentage of elevation in ALAT activity was (65.52%) in Muscle and minimum elevation was (34.77%) in liver. But in Carbosulfan lethal exposure maximum percentage of elevation was (89.62%) in kidney and minimum percentage of elevation was (40.04%) in Liver.

In profenofos 8days sublethal exposure maximum percentage of elevation in AAT activity was (53.47%) in muscle and minimum elevation was (25.95%) in Brain. But in sub lethal exposure of carbosulfan maximum percentage of elevation was (52.46%) in muscle and minimum percentage of elevation was (19.05%) in Brain.

Since the pesticide stress was known to induce significant change in protein metabolism, it is likely that the aminotransferases were also considerably affected. Increased activities of AAT and ALAT in different tissues of fish suggest either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or fatty acids during profenofos and Carbosulfan intoxication. The ALAT and AAT are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histophathalogic changes and can be assessed within a shorter time[13]. The increase in ALAT and AAT indicate the tissue damages in liver, kidney and gill[14-15].

Aminotransferases are important as they convert amino acids into keto acids and incorporate them in to TCA Cycle. Both ALAT and AAT level increased in tissues of fish suggesting the conversion of amino acids released by the proteolysis into keto acids for energy production. The increase in ALAT and AAT activities in our study supports earlier findings and serves as indicator of tissue damage[16-18].

AAT and ALAT are located in both mitochondrial and cytosol fractions of the cell. A close relation appears to exist between the mitochondrial integrity and transaminase levels[19] and any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it. The alteration in the activities of AAT and ALAT as observed in the present study may also be due to the mitochondrial disruption and damage as a result of Profenofos and Carbosulfan induced stress.[20] reported elevation in the levels of AAT and ALAT in different tissues of brain, liver, muscle, gill and kidney of the fishes *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* exposed to chlorpyrifos. [21]also reported increase in activities of AAT and ALAT in different tissues of fish *Catla catla* and *Channa punctatus exposed* to fenvalerate.[22]reported that the free amino acid content of the liver tissue decreased after dimethoate treatment in *C.batrachus* and the increased amino acids might have been converted to ketoacid by transaminases which in turn fed into TCA cycle. So there was an increase in the activity of transaminases. Similar increase in aspartate and alanine aminotransferease activity was observed in exposed fish [23]. The elevation in transaminases suggests the existence of heavy drain on metabolites during dimethoate stress, since stress is known to induce elevation of aminotransferease [24].

Conclusions

Activities of aminotransferases as observed in the present study , demonstrating a consistent increase in the activities of these enzymes under conditions of enhanced gluconeogenesis. The alterations in the levels of activity of aminotransferases induced by the organophosphate and Carbamate pesticides clearly indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems.

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