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Possibility of Using Latex Extracts of *Nerium indicum* Plant for Control of Predatory Fish *Channa punctatus*

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Abstract

Laboratory evaluation was made to develop an environmentally safe and effective plant-origin piscicide. Piscicidal activity of different solvent extracts of *Nerium indicum* latex against the freshwater predatory snakehead, *Channa punctatus* was both time and dose-dependent, in terms of the lethal concentrations for 50% of the fish (LC_{50}) always. Methanolic extract of *N. indicum* latex had LC_{50} of 17 mgL^{-1} at 24 h and 10 mgL^{-1} at 96 hour. Acetone, chloroform and diethyl ether extracts of *N. indicum* latex had LC_{50s} of 18-23 mgL^{-1} at 24 h and 13-17 mgL^{-1} at 96 h. Exposure to sublethal doses (40% and 80% of LC_{50}) of methanolic extract of *N. indicum* latex (which had maximum piscicidal activity) for 24 h or 96 h caused significant time and dose-dependent alterations in the concentrations of total protein, total free amino acid, nucleic acid, glycogen, pyruvate and lactate, and in the activity of the enzymes protease, alanine aminotransferase, aspartate aminotransferase, acid phosphates, alkaline phosphatase and acetylcholinesterase of snakehead. Thus, *N. indicum* latex shifts fish respiration towards the anaerobic pathway. The toxicity of the latex extract was reversible and 7 days after withdrawal, all biochemical parameters were back to normal. Thus, solvent extract of *N. indicum* latex can be used in aquatic environment for controlling unwanted populations of the snakehead *Channa punctatus*.

Introduction

Aquaculture as an enterprise has some innate advantages, such as high returns, high productivity, high feed conversion ratio, utilization of agriculture and animal wastes, high employment generation etc (Katiha

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2000). However, the presence of predatory fishes in fish culture ponds is a serious problem in India, and elsewhere. Due to their faster growth rate, predatory fishes share and better utilize cultured carp habitats and their food (Jhingran 1983). Most of the predatory and weed fishes breed in ponds a little earlier than the spawning time of cultured carps and their fry feed vigorously on available food in the pond. When the spawn of cultured carps are released, the young predatory fish are sufficiently large to feed upon finger lings of cultured carps (Chakraboraty et al. 1972) and by this, predatory fishes adversely effect the aquaculture production. Air breathing predatory fish species such as *Channa punctatus, Channa marulius*; etc. cause special problems because they may survive in moist burrows, even when ponds are drained (Jhingran 1983). So, removal of these predatory fish population from the ponds is necessary before the seed of cultured carps is added. For controlling these fishes, fish farmers often use synthetic piscicides e. g. chlorinated hydrocarbons such as dialdrin, aldrin, endrin, and organophosphates such as phosphamidon, dimethyl dichlorovinyl phosphate (Hickling 1962), PCP-Na (Terazaki et al. 1980), malachite green (Gribgratok 1981) and sodium cyanide (Marking 1992).

Synthetic piscicides are often very slowly-degradable nature, are persistent, have toxic effects, to non-target organisms by accumulating in their body tissues (Arasta et al. 1996) and thus adversely effect the fish quality and their status (Cullen and Connell 1992).

In recent years, the use of medicinal plants as effective alternatives of synthetic pesticides and fertilizers has gained more importance (Dahiya et. al. 2000), because they are highly toxic to the target pests (Singh et al. 1998). Botanical piscicides are suggested as best alternatives to synthetic materials because of their easy availability, easy biodegradability, greater effectiveness, less expensive, lower toxicity against nontarget organisms, and their comparative safety toward the environment and human beings (Marston and Hostettmann 1985).

At present, a large number of plant products are commonly used for controlling these unwanted fish population, such as the powdered seed of *Croton tiglium* and *Barringtonia acutangula* (Kulakkattolickal 1989), tea-seed cake and mahua oil cake (Bhatia 1970). Chiayvareesajja et al. (1997) reported the toxicity of 221 plants in Thailand and found five plant species that are effective in killing predatory fishes. Shigueno (1975) reported that plant materials such as derris powder or tea seed cake, are commonly used in Japan to kill the fish selectively in shrimp culture ponds. The toxic plant products of tea seed cake and derris root powder are degraded within 7-12 days (Chakroff 1976), but derris root powder is not readily available through out the world and is also expensive (Chakraborty et. al. 1972; Jhingran 1983). The price of tea seed cake

is also expensive (in 1995 at Hat yai market was US$ 1.4/Kg). So the use of derris root powder and tea seed cake is very limited.

Nerium indicum (Family-Apocynaceae) is a common medicinal plant of India, useful in the treatment of inflammation of gums, dysentery, bronchitis, asthma, and menorrhagia (Frohne and Fander 1983). The toxicity of aqueous latex extract of *N. indicum* to the snails *Lymnaea acuminata* and *Indoplanorbis excustus* and fish *C. punctatus* has been established (Singh et al. 1993; Singh and Singh 2000), but the doses of aqueous extract were so high. So their further purification is necessary to develop a environmentally safe and effective plant origin piscicides.

In the present study, piscicidal activity of different solvent extracts of *N. indicum* latex and their ultimate mode of action i.e. short-term as well as long-term biochemical effect, against freshwater air breathing predatory snakehead *C. punctatus* was investigated.

Materials and Methods

Collection and maintenance of test animals

The fish *C. punctatus* (Bloch.) (15.70 ± 1.30 cm in length, 14.00 ± 3.00 gm in weight) were collected locally from Ramgarh Lake in the Gorakhpur district of Utter Pradesh (India). The fishes were held in glass aquaria containing 100L of dechlorinated tap water for acclimatization to laboratory conditions for up to seven days. The water in the aquaria was changed every 24 hour, and maintained as follows: atmospheric temperature, 30.0 ± 1.0°C ; water temperature, 27.0 ± 1.5°C ; pH, 7.0-7.2; dissolved oxygen, 7.5-8.0 mgL^{-1}; free carbon dioxide, 4.7-5.8 mgL^{-1}; bicarbonate alkalinity, 105.0-106.0 mgL^{-1} (APHA et al. 1998).

Collection and preparation of latex extracts

Nerium indicum (Family Apocynaceae) was collected locally from the botanical garden of D.D.U. Gorakhpur University, Gorakhpur and identified by Prof. S.K. Singh, Plant Taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, U. P. (India).

The yellowish milky latex of *N. indicum* was drained into glass tubes by cutting the stem apices. The latex was centrifuged at 1000 g for 20 minutes to remove the resin, this resin free latex lyophilized at –40°C, and the lyophilized powder stored for further use. The wet weight of one ml latex of *N. indicum* was 1.06 gm and dry weight (lyophilized at –40°C) was 0.160 gm. One gm. freeze-dried latex powder was mixed with 100 ml of methanol (MeOH), acetone (AcO), chloroform ($CHCl_3$) or

diethyl ether (Et_2O), respectively. Each solution were left for one hour at room temperature, and then centrifuged at 5,000 g for 25 minutes. Supernatant liquid poured in petridish and the solvent evaporated at room temperature. Then dried powder was thoroughly washed with their mother solvent and then filtered. The filtrate was evaporated to dryness with the help of vacuum pump. Dried powder *Nerium indicum* latex extracts of 0.460 gm (MeOH), 0.400 gm (AcO), 0.395 gm ($CHCl_3$) and 0.350 gm (Et_2O) were obtained, which were used in the remaining experiments.

Dose-response relationship

Toxicity experiment was performed according to the method of Singh and Agarwal (1988). The fishes were exposed for 24h, 48h, 72h or 96 hour at four different concentrations of *N. indicum* latex extract. Concentrations (w/v) of *N. indicum* latex extracts used for toxicity experiments were 8, 12, 16 and 20 mgL^{-1} for methanol (LE_{MeOH}); 11, 15 19 and 23 $mgL^{-1}$for both chloroform (LE_{CHCl3}) and acetone (LE_{AcO}); 15 19, 23 and 27 mgL^{-1} for diethyl ether (LE_{Et2O}). Six aquaria were set up for each dose and each aquarium contains ten fishes in six litre of de-chlorinated tap water. Control animals were kept in similar condition without any treatment.

Mortality was recorded every 24h throughout the 96h exposure period. Fishes were considered dead if they failed to respond to vigorous poking with glass rod. Dead fishes were removed from test aquaria as soon as possible in order to prevent their body decomposition. Lethal concentration (LC values), upper and lower confidence limits, slope value, 't' ratio, 'g' factor and heterogeneity were calculated through probit log analysis method by using POLO computer programme of Russel et al. (1977).

Treatment of animal for biochemical studies

The acclimatized fishes were treated with sub lethal doses (40% and 80% of LC_{50}) of LE_{MeOH} for 24h or 96h exposure periods (The LE_{MeOH} had maximum piscicidal activity). Six aquariums were set up for each dose and each aquarium contained 10 fishes in 6-liter dechlorinated tap water. After 24h or 96h the test animals were removed from aquaria and washed with water and killed by severe blow on head. Control animals were held in similar condition without any treatment. The dead animals were dissected and liver and muscle collected to measure total protein, total free amino acid, nucleic acid, glycogen, pyruvate, lactate levels and activity of protease, alanine aminotransferase, aspartate aminotransferase, acid phosphatase, alkaline phosphatase, acetylcholinesterase enzymes.

Each experiment was replicated six times and the values have been expressed as mean of six replicates. Student 't' test was applied to locate significant changes with controls (Sokal and Rohlf 1973).

Withdrawal experiment

In order to see effect of withdrawal of treatment of LE_{MeOH}, the fishes were exposed for 96h to 80% of the LC_{50} (96h) and the one half of the animal was sacrificed and total protein, total free amino acid, nucleic acid, glycogen, pyruvate, lactate levels and activity of protease, alanine aminotransferase, aspartate aminotransferase, phosphatase, acetylcholinesterase enzymes were measured. The other half was transferred to treatment free water, which was changed every 24h for the next seven days. Following to this, total protein, total free amino acid, nucleic acid, glycogen, pyruvate, lactate levels and activity of protease, alanine aminotransferase, aspartate aminotransferase, phosphatase, acetylcholinesterase enzymes were also measured in liver and muscle tissues of fish. Control animals were kept in same condition without any treatment.

Protein

Protein levels were estimated according to the method of Lowry et al. (1951), using bovine serum albumin as standard. Homogenates (5 $mgml^{-1}$, wv^{-1}) were prepared in 10% trichloroacetic acid (TCA).

Total free amino acids

Total free amino acids level was estimated according to the method of Spices (1957). Homogenates (10 $mgml^{-1}$, wv^{-1}) were prepared in 95% ethanol, centrifuged at 6,000 g. Standard curves using the same procedure were drawn with know amounts of glycine.

Nucleic acids

Estimation of DNA and RNA was performed, by methods of Schneider (1957), using diphenylamine and orcinol reagents, respectively. Homogenates (10 $mgml^{-1}$, wv^{-1}) were prepared in 5% TCA, centrifuged at 5,000 g for 20 min.

Protease

Protease activity was estimated by the method of Moore and Stein (1954). Homogenate (50 $mgml^{-1}$, wv^{-1}) was prepared in cold distilled water.

Glycogen

Glycogen was estimated by the method of Van der Vies (1954). Homogenate (10 $mgml^{-1}$, wv^{-1}) was prepared in cold 5% TCA. The optical density was compared with a set of glucose standard of varying concentrations.

Pyruvate

Pyruvate was measured according to the method of Friedemann and Haugen (1943). Homogenate (50 $mgml^{-1}$, wv^{-1}) was prepared in 10% TCA. Sodium pyruvate was used as standard.

Lactate

Lactate was estimated according to Huckabee (1961) method. Homogenate (50 $mgml^{-1}$, wv^{-1}) was prepared in 10% cold TCA. Sodium lactate was used as standard.

Aminotransferase activity

Alanine aminotransferase and Aspartate aminotransferase activities were determined by the method of Reitman and Frankel (1957). Homogenate (50 $mgml^{-1}$, wv^{-1}) was prepared in 0.25 M cold sucrose solution.

Phosphatase activity

Acid and alkaline phosphatase activities were determined according to the method of Andersch and Szcypinski (1947). Tissue homogenates (2%, wv^{-1}) were prepared in ice-cold 0.9% sodium chloride. ñ- Nitro phenol was used as standard.

Acetylcholinesterase activity

Acetylcholinesterase activity was measured by the method of Ellman et al. (1961). Homogenate (50 $mgml^{-1}$, wv^{-1}) was prepared in 0.1 M-phosphate buffer, $_{p}$H 8.0 for 5 min in an ice bath. The change in optical density at 412 nm, caused by the enzymatic reaction, was monitored for 3 min at 25°C. Protein estimation was done by the method of Lowry et al. (1951).

Results

Exposures to the various extracts of *N. indicum* latex caused significant behavioural changes in the snakehead *C. punctatus*. After introduc-

tion of extracts, fish immediately settled down at the bottom of the aquaria. Within 5-10 minutes, the fishes appeared to suffer the effects of suffocation and they came to the water surface gasping the air. The initial 30 minutes was a period of hyper activity, during which fishes become restless. They came at the surface of water for gasping the air and then settle down at the bottom. After 30-60 minutes, their swimming activity slowed, they settled at the bottom of the aquaria and formed clusters. Their rate of operculum movement also increased. Mucous secretion from skin and respiration through gill also increased. After some time of treatment the operculum movement of fish progressively decreases although they tried to stay at the water surface. This was obviously hypoxia, where sufficient quantity of oxygen is not available for cellular respiration. In case of LE_{MeOH}, treatment, hypoxia and mucous secretion was the most pronounced. At higher doses, after 10-12 hours, all the activities of fishes slowed down and they permanently settled at the base of aquaria and finally died.

Toxicological experiments

All the *N. indicum* latex extracts (LE) have potent piscicidal activity against freshwater air breathing predatory snakehead *C. punctatus*. Their rank order of toxicity at all exposure periods ranging from 24h to 96h was as follows, MeOH > $CHCl_3$ > AcO > and Et_2O extract of *N. indicum* latex.

Table 1. Toxicity (LC_{50}) of different organic solvent extracts of *N. indicum* latex against common freshwater predatory snakehead *C. punctatus* at different time intervals

Solvents	Exposure periods	Effective dose(LC_{50}, mgL^{-1})
Methanol	24h	17.02±2.10
	48h	14.22±1.20
	72h	11.60±0.79
	96h	10.42±0.87
Acetone	24h	18.49±1.39
	48h	15.82±0.88
	72h	14.56±0.78
	96h	13.81±0.78
Chloroform	24h	18.91±1.70
	48h	15.19±0.77
	72h	14.31±0.75
	96h	13.53±0.79
Diethyl ether	24h	23.06±1.66
	48h	20.51±1.23
	72h	18.40±0.98
	96h	17.31±0.86

There was no mortality in control groups; batches of ten fishes were exposed to four different concentrations of each solvent extracts of *N. indicum* latex; concentrations given are the final concentrations (wv^{-1}) in aquarium water; regression coefficient showed significant ($P<0.05$) negative correlation between exposure time and different LC values; effective doses were expressed as LC_{50}±confidence limits

Table 1 shows, the toxicity of all the LE was time as well as dose dependent, and there was a significant ($P<0.05$) negative correlation between LC values and exposure periods i.e. LC_{50} values of LE_{MeOH} decreased from 17.02 mgL^{-1} (24 h) > 10.42 mgL^{-1} (96 h). Similarl trends were also observed in the case of diethyl ether, acetone and chloroform extracts.

Biochemical experiments

Exposure of fish to sub lethal doses of LE_{MeOH} caused significant biochemical changes in liver and muscle tissue of snakehead *C. punctatus* (Tables 2 and 3). Student't' test and analysis of variance showed that these alteration were significantly ($P < 0.05$) time and dose-dependent.

Total protein level was reduced to 32% and 36%; DNA level was reduced to 59% and 40%; RNA level was reduced to 30% and 42%; total free amino acid levels was induced to 194% and 192%; glycogen level was reduced to 55% and 37%; pyruvate level was reduced to 58% and 59%; lactate level were increased to 170% and 160% of controls in liver and muscle tissue of fish *C. punctatus* respectively, after treatment with 80% of 96 hour LC_{50} of LE_{MeOH} (Table 2). While protease enzyme activ-

Table 2. Changes in different biochemical concentrations in liver (L) and muscle (M) of snakehead *C. punctatus* after 24h and 96h exposure to 40% and 80% of 24h and 96h LC_{50} of methanolic extract of *N. indicum* latex and 7th days after withdrawal

Parameters		Concentrations					
		Control	24h, 40% LC_{50} (6.81 mgL^{-1})	24h, 80% LC_{50} (13.62 mgL^{-1})	96h, 40% LC_{50} (4.17 mgL^{-1})	96h, 80% LC_{50} (8.34 mgL^{-1})	Withdrawal
Total proteinµg mg^{-1}	L	131.20	74.50*	54.50*	65.60*	42.60*	126.80+
	M	150.90	90.50*	75.40*	82.90*	54.30*	143.40+
Total free amino acidµg mg^{-1}	L	9.80	10.30*	13.10*	11.20*	14.80*	9.10+
	M	16.90	18.30*	23.70*	20.60*	28.30*	19.00+
DNAµg mg^{-1}	L	35.90	31.40*	27.90*	28.70*	20.60*	32.30+
	M	34.90	25.50*	24.30*	23.70*	14.20*	31.70+
RNAµg mg^{-1}	L	36.00	21.20*	19.80*	16.90*	11.10*	34.50+
	M	37.10	22.70*	20.00*	18.30*	15.60*	35.00+
Glycogenmg gm^{-1}	L	2.04	1.54*	1.21*	1.51*	1.12*	1.94+
	M	1.70	1.36*	1.11*	1.03*	0.64*	1.43+
Pyruvateµg mg^{-1}	L	2.34	1.76*	1.50*	1.59*	1.36*	2.26+
	M	1.99	1.45*	1.25*	1.45*	1.17*	1.81+
Lactateµg mg^{-1}	L	1.50	1.66*	2.16*	1.95*	2.55*	1.58+
	M	1.28	1.41*	1.82*	1.54*	2.05*	1.37+

Values are mean of six replicates; data were analysed through student's test; *, significant ($P<0.05$), when treated groups were compared with controls; +, significant ($P<0.05$), when withdrawal groups were compared with treated groups

ity was increased to 142% and 154%; ALAT activity was increased to 183% and 171% and AAT activity was increased to 159% and 151%; activity of acid phosphatase was inhibited to 66% and 74%; activity of alkaline phosphatase was reduced to 75% and 80%; AChE enzyme activity was inhibited up to 43% and 34% of controls in liver and muscle tissue of fish *C. punctatus* respectively, after treatment with 80% of 96 hour LC_{50} of LE_{MeOH} (Table 3).

After seven days in a clean enclosure following exposure to 80% of 96hour LC_{50} of LE_{MeOH} (Tables 2 and 3), there was significant ($P < 0.05$) recovery observed in the total protein, total free amino acid, nucleic acid, glycogen, pyruvate and lactate levels and in the activity of protease, ALAT, AAT, acid phosphatase, alkaline phosphatase and AChE enzyme were observed in both liver and muscle tissue of fish *C. punctatus.*

Discussion

By changing a large number of behavioural responses, fishes try to resist changes in their environment, and in this case, reduce the harmful

Table 3. Changes in different enzyme activities in liver (L) and muscle (M) of snakehead *C. punctatus* after 24h and 96h exposure to 40% and 80% of 24h and 96h LC_{50} of methanolic extract of *N. indicum* latex and 7^{th} days after withdrawal

Parameters		Concentrations					
		Control	24h, 40% LC_{50} (6.81 mgL^{-1})	24h, 80% LC_{50} (13.62 mgL^{-1})	96h, 40% LC_{50} (4.17 mgL^{-1})	96h, 80% LC_{50} (8.34 mgL^{-1})	Withdrawal
Proteasem moles tyrosine mg $protein^{-1}.h^{-1}$	L	0.50	0.56^{*}	0.62^{*}	0.60^{*}	0.77^{*}	0.52^{+}
	M	0.34	0.38^{*}	0.47^{*}	0.40^{*}	0.51^{*}	0.37^{+}
Acid phosphataseñ-Nitrophenol.30min^{-1}· mg $protein^{-1}$	L	0.14	0.14^{*}	0.11^{*}	0.12^{*}	0.09^{*}	0.14^{+}
	M	0.13	0.12^{*}	0.11^{*}	0.12^{*}	0.10^{*}	0.13^{+}
Alkaline phosphataseñ-Nitrophenol.30min^{-1}· mg $protein^{-1}$	L	0.49	0.48^{*}	0.42^{*}	0.43^{*}	0.37^{*}	0.46^{+}
	M	0.48	0.45^{*}	0.40^{*}	0.43^{*}	0.39^{*}	0.44^{+}
Alanine aminotransferasem moles pyruvate· mg $protein^{-1}$. h^{-1}	L	3.83	4.55^{*}	5.34^{*}	4.99^{*}	7.01^{*}	4.33^{+}
	M	3.01	3.45^{*}	4.77^{*}	3.68^{*}	5.16^{*}	3.31^{+}
Aspartate aminotransferasem moles pyruvate· mg $protein^{-1}$. h^{-1}	L	1.50	1.64^{*}	2.08^{*}	1.81^{*}	2.39^{*}	1.73^{+}
	M	1.32	1.42^{*}	1.89^{*}	1.50^{*}	1.99^{*}	1.59^{+}
Acetylcholinesterase µ mol 'SH'.min^{-1}· mg $protein^{-1}$	L	0.19	0.11^{*}	0.09^{*}	0.12^{*}	0.08^{*}	0.18^{+}
	M	0.25	0.17^{*}	0.12^{*}	0.14^{*}	0.08^{*}	0.24^{+}

Values are mean of six replicates; data were analysed through student's test; *, significant ($P<0.05$), when treated groups were compared with controls; $^{+}$, significant ($P<0.05$), when withdrawal groups were compared with treated groups

effects of the *N. indicum* latex extract. The initial increase in opercular movement seems to be an indication of the stress felt by the fish exposed to latex extracts. The increased mucous secretion in fish seen after treatment is a defense response by which fish try to reduce entrance of latex extract through body surface. This copious mucus secretion formed a thin film on delicate and sensitive gill tissue thus minimizing exchange of gases, particularly intake of oxygen (Sambasiva Rao 1999).

Animal behaviour is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances (Bullock et al. 1977). From our results, it is evident that active compounds in LE inhibits acetylcholinesterase activity. This enzyme is present in synaptic regions and mediates transmission of impulses by breaking acetylcholine into acetic acid and choline (O'Brien 1976). The acetylcholine at neural and neuromotar regions upon accumulation causes 'hyper-excitability (Siva Prasada Rao 1980), which in turn might also influence behaviour pattern. Similar behavioural responses were also observed in organophosphate and carbamate pesticides exposed fishes (Gill et al. 1991).

However, AChE inhibition is not the only factor contributing to abnormal behaviour patterns. In fact, there exists a close relationship between animal behaviour, coordination of brain, loss of body equilibrium and nervous function and the metabolic and physiological state of an organism (Sambasiva Rao 1999). Our results suggest that the LE_{MeOH} caused significant alteration in fish metabolism at the tissue level. No such behavioral symptoms and death occurred in control groups, indicating that no factor other than m:LE_{MeOH} were responsible for altered fish behavior and mortality.

Toxicity data showed a significant positive correlation between fish mortality and exposure periods. This could be the result of several factors, which may be acting separately or conjointly (Singh and Agarwal 1988). This possibility cannot be over ruled in present study. The differences in toxicity of different solvent extracts may be due to the variation in rate of absorption, their translocation to target site, the extent of activation and their excretion.

After exposure to sublethal concentration of LE_{MeOH}, fishes were stressed. During stress, fish need more energy to detoxify the toxicants and try to minimize their toxic effect. Carbohydrate represents the principle and immediate energy source while protein is the energy source to spare during chronic periods of stress (Umminger 1977). The depletion of the protein fractions may be due to their degradation and possible utilization of degraded products for metabolic purposes. The increase in free amino acids level was the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Sambasiva Rao 1999). The decreases in total protein level and increases in total free

amino acid level in both the tissues suggest the high protein hydrolytic activity due to elevation of protease activity. Depletion of glycogen may be due to direct utilization for energy generation, a demand caused by latex extract induced hypoxia. Liver glycogen levels are depleted during acute hypoxia or physical disturbances in the fish (Heath and Fritechard 1965).

The level of tissue lactate content is act as an index of anaerobiosis, which might be beneficial for animal to tolerate hypoxic condition during stress (Thoye 1971), where as the pyruvate level in tissue can be taken as a measure of aerobic condition. In liver and muscle, both aerobic and anaerobic conditions are likely to operate depending on availability of molecular oxygen and other physiological needs imposed by other factor. Pesticides are also inhibited energy production by suppressing aerobic oxidation of carbohydrate leading to energy crisis (Kohli et al. 1975).

The fish were stressed, as evidenced by the increase in lactate content while decrease in pyruvate content in all the tissue, which suggests a shift towards anaerobiosis as a consequence of hypoxia, created under pesticides toxic impact leading to respiratory distress (Siva Prasad Rao 1980). The aspartate and alanine amino transferase function as a link between carbohydrate and protein metabolism by catalyzing the interconversion of strategic compounds respectively (Martin et al. 1983). After exposure, the activity of both the aminotransferases were highly elevated in both the tissues (table 3), confirming the augmentation of stress as a consequence of LE_{MeOH}. Glycogen, which is ultimate energy source, decreases (table 2) resulting in higher demand for carbohydrate and their precursors to keep the glycolytic and TCA cycles at sustained levels to cope the energy demands during stress condition. Since the amino acid level also increased (table 2) it is evident that both ALAT and AAT activities are being stepped up to be in line with the increasing energy demands. In liver and muscle tissue both, ALAT predominates over AAT where the feeding of amino acids into energy cycle is more through alanine-pyruvate pathway representing anaerobic tendency of the tissues.

Vorbrodt (1959) has reported that phosphatase is an important enzyme of animal metabolism, which plays an important role in the transport of metabolites across the membranes. Since, LE_{MeOH} has anti-phosphatase activity. So the reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis (Pilo et al. 1972) and also involved in the synthesis of certain enzymes (Sumner 1965).

Thus, it is believed that *N. indicum* latex extracts may be a useful substitute of synthetic piscicides in killing snakehead fishes from culture ponds. This is environmentally safe because their toxic effect is reversible within seven days after exposure.

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