Indian Journal of Natural Products and Resources
Vol. 4 (1), March 2013, pp. 48-53

Comparative study on the haematological effect of synthetic and plant origin pesticides on fish *Channa punctatus*

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Received 30 July 2011; Accepted 18 December 2012

Application of synthetic pesticides is one of the methods used to increase agriculture production, animal husbandry and welfare of mankind. Due to their long-term persistence, slow degradability toxicity to other organisms and accumulation inside the fish body, synthetic pesticides adversely affect the environment. To solve this problem, studies have been carried out on the possibility of using plant origin pesticides. In the present investigation, a study has been carried out to compare the hematological effect of synthetic pesticides, Furadan and plant origin pesticides, viz. rutin, taraxerol and apigenin. Fishes were exposed to different sub-lethal concentration of these compounds for one week. Thereafter, blood was assayed for selected haematological parameters (haematocrit, haemoglobin, red blood cell counts, white blood cell counts, total plasma protein and plasma glucose concentration). Sub-lethal concentrations of these compounds caused a dose dependent decrease in haemoglobin values coupled with a decrease in haematocrit values and red blood cell counts. The total white blood cell counts and the differential white blood cell counts were decreased except for the lymphocytes. Plasma protein and glucose were also lower in exposed fish when compared with control. The haematological indices, MCH, MCHC and MCV were also lowered. Results indicate that Furadan are more toxic than rutin, taraxerol and apigenin.

Keywords: Natural pesticides, Furadan, Rutin, Taraxerol, Apigenin, Toxicity, Fish, *Channa punctatus.*

IPC code; Int. cl. (2011.01)–A61K 36/00, A01N 25/00.

Introduction

The use of synthetic pesticides has increased with the growing awareness about their utility in agriculture production, animal husbandry, postharvest technology and in the public health and welfare of mankind. The intensive use of synthetic pesticides in agricultural fields and public health operation systems has resulted in serious environmental hazards[1,2]. Due to their long term persistence, slow degradability in the water, toxicity to other organisms[3] and accumulation inside the fish body, synthetic pesticide adversely affect the aquatic environment[4,5]. To minimize environmental pollution by pesticides, efforts are being made to find pesticides from plant origin because plants are virtually inexhaustible source of structurally diverse and biologically active substances[6,7]. A large number of biologically active compounds of various classes have been tested for insecticidal, piscicidal and molluscicidal activities[8-17]. The family Euphorbiaceae embraces about 7,000 species distributed all over the temperate and tropical world[18] and produces milky irritating juice which contains some bioactive ingredients like diterpene together with aleuritolic acid, oleanolic acid and betulin diacetate sesquiterpene-coumarin and a quinoid-type diterpenoid which negate physiological activities in aquatic organisms[19-20]. Taraxerol, apigenin and rutin extracted from *Codium variegatum* Blume and *Jatropha gossypifolia* Linn. of family Euphorbiaceae were tested on fish *Channa. punctatus*, which is an important fish of India capture fishery.

Most of the pesticides both plant origin and chemical, applied in agricultural field reach water bodies through runoff affecting aquatic flora and fauna (specially fishes). The lipophilicity of synthetic pesticide indicates that these chemicals will be absorbed by fish even from very low concentrations in water[21]. Persistent chemical molecules with long half-life periods found in chemicals pose a threat to fish and also to the human population consuming the affected fish[22-28].

Despite toxicity of these compounds, we are interested in knowing the mode of action and long term effect of pesticides on non-target organism. Haematological study is important for toxicological research because haematological alterations have rapid evaluation of the chronic toxicities of a compound[29]. Haematological values are widely used

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to determine systematic relationships and physiological adaptations including the assessment of the general health condition of animals and are more quickly reflected in the poor condition of fish than in other commonly measured variables. Most studies on the effects of pyrethroids are confined to reporting biochemical and physiological changes and very little attention has been paid to the haematological modulations induced by these pesticides[30].

Furadan (Carbofuran, 2, 3-dihydro-2, 2-dimethyl-7-benjofuranylmethylcarbamate) is one of the most effective carbamate pesticides, effective against corn and cotton insects and pests of potato. The aim of this study is to compare haematological effect of taraxerol, apigenin and rutin with furadan, a powerful chemical pesticide because it cannot be put to commercial use without a study of these aspects as well. Based on the results, the use of the less toxic pesticide could be promoted among the agriculturists. It is possible to substitute chemical pesticides with pesticides of plant origin.

Materials and Methods

Collection of experimental animal

Fish *Channa punctatus* (wt.29.21±1.83 g; length 14.5±1.20 cm) were collected from the Ramgarh Lake of Gorakhpur district. The collected fishes were maintained in glass aquaria containing 100 L de-chlorinated tap water for acclimatization to laboratory conditions for one week. The water in aquaria was aerated continuously then dead animals were removed to avoid any contamination.

Extraction of taraxerol from *C. variegatum* Blume

Pure taraxerol was isolated from the stem bark of *C. variegatum* by the method of Chatterjee and Banerjee[31]. During process the stem bark was dried in an incubator at about 37°C then powdered with the help of mechanical device. The dried powdered stem bark (2 kg) was extracted in Soxhlet apparatus with petrol, for about 70 h and a little amount of concentrated solution was obtained. After evaporation of the solvent by vaccum pump, the isolated compound in dried form was obtained. Taraxerol is soluble in organic solvents such as $CHCl_3$ and $CHCl_3$ – MeOH. Identification of the Taraxerol ($C_{32}H_{48}O_9$) was confirmed from NMR data of Lee *et al*[32].

Extraction of apigenin from *Jatropha gossypifolia* Linn.

Pure apigenin was isolated from the leaves of *J. gossypifolia* by the method of Subramanian *et al*[33]. Leaves were washed properly with the water and dried in incubator at 37°C. The dried leaves were then powdered and about 50 g powder was subjected to extraction through Soxhlet apparatus with about 250-300 mL ethyl alcohol for about 72 h at 20-40°C when extraction was completed, a little amount of crude yellow powder was obtained. After adding NaOH and HCl apigenin was obtained which is crystallized by methanol. Apigenin was confermed by the UV spectral data of Dordevic *et al*[34].

Rutin ($C_{27}H_{30}O_{16}$) plant origin pesticide was purchased from Sigma Chemical Company, USA.

Furadan (Carbofuran, 2, 3-dihydro-2,2-dimethyl-7-benjofuranyl methylcarbamate), a synthetic pesticide was purchased from Sigma Chemical Company, USA.

Toxicity experiment

Toxicity experiments were performed by the method of Singh & Agarwal[35]. Ten experimental animals *Channa punctatus* were kept in glass aquaria containing 6 L dechlorinated tap water. Fishes were exposed for 24, 48, 72 and 96 h at 4 different concentrations of single and binary combinations of rutin, taraxerol and apigenin and chemical pesticide furadan. Six aquaria were set up for each dose, control animals were kept in similar condition without any treatment. Mortality was recorded after every 24 h to 96 h exposure periods.

Toxicity data obtained from this study was computed through POLO computer program of Robertson *et al*[36].

Haematological experiments

A total of 50 healthy *Channa punctatus* were used for this study. They were divided into 5 groups, the first group was kept in pesticide free water and served as control and the second one was exposed to 40% of LC_{50} (0.076 mg/L) of furadan. The third, fourth and fifth group was exposed to 40% of LC_{50} (0.64 mg/L, 1.02 mg/L, 1.44 mg/L) of rutin, taraxerol and apigenin, respectively. The water quality parameters of the water used in the test determined by standard methods of APHA[37] (Table 1).

The exposure period lasted 14 days, after which blood samples were taken from the control and experimental fish. Approximately 2 mL of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5 mg ethylate diamine tetr acetic acid (EDTA) as anticoagulant; it was properly mixed and used for haematological analysis. All haemtological parameters such as haematocrit value, haemoglobin content, total leukocyte count, erythrocyte count and blood biochemistry such as serum glucose, serum protein, serum calcium and serum chloride was

estimated by an auto analyzer Sysmex fully computerized automatic blood cell counter (KX-21).

Results were expressed as mean ± SE of three replicates and differences between means were considered to be significant when $P<0.05$.

Results

Toxicity experiments

Toxicity data of rutin ($LC_{50:}$ 1.60 mg/L in 24h), taraxerol ($LC_{50:}$ 2.56mg/L in 24h), apigenin ($LC_{50:}$ 3.61mg/L in 24h) and furadan ($LC_{50:}$ 0.19 mg/L in 24h) (Table 1) was reported by Shahi and Singh[38].

Haematological observation

The effect of exposure to sub-lethal concentrations of furadan, rutin, taraxerol and apigenin for haematological parameters of *Channa punctatus* was studied. The haematological parameters for the treated fish and those of the fish from the control groups after 14 days showed significant difference. The synthetic pesticide furadan is highly toxic than plant origin pesticides. It was observed that there is a significant increase in white blood cell count, lymphocyte, monocyte and neutrophills ($P<0.05$) as compared to the control group (Table 2). Results concerning red blood cell counts exhibited a decrease in their numbers, mean corpuscular haemoglobin content (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were significantly ($P<0.05$) decreased than control groups. Results of the haemoglobin and haematocrit values decreased significantly (Table 3). A significant depression was

Table1— Comparative LC_{50} values (mg/L) of rutin, apigenin, taraxerol and furadan with their fiducial limits against freshwater fish *Channa punctatus* at different time intervals

	Plant origin pesticides						Chemical pesticide	
	Rutin		Taraxerol		Apigenin		Furadan	
Exposure Periods (Hours)	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL
24	1.60	1.45-1.91	2.56	2.41-2.86	3.61	3.17-5.18	0.19	0.160-0.239
48	1.39	1.20-1.69	2.35	2.18-2.54	3.18	2.83-4.06	0.11	0.100-0.141
72	1.30	1.15-1.45	2.19	1.95-2.34	2.79	2.41-3.38	0.07	0.062-0.981
96	1.12	0.93-1.24	2.02	1.73-2.15	2.40	1.86-2.73	0.05	0.048-0.064

LCL - Lower confidence limit; UCL - Upper confidence limit; LC_{50} - Lethal concentration for 50 % population of the exposed fish

Table 2— Comparative leukocyte and its differential counts of freshwater fish *Channa punctatus* after one week exposure of sub-lethal doses of rutin, taraxerol, apigenin and furadon

	Synthetic pesticide		Plant origin pesticides		
Parameters	Control	Furadon	Rutin	Taraxerol	Apigenin
	Total Leukocyte Count				
WBC (10^3 mm^3)	138.0	205.5*	184.0*	167.0*	152.0*
	Differential Leukocyte Count				
Lymphocyte (%)	104.0	132.7*	123.3*	115.0*	109.0*
Monocyte (%)	22.0	44.1*	36.8*	31.5*	26.0*
Neutrophill (%)	12..0	28.0*	23.5*	21.2*	17.0*

WBC-White blood corpuscles; *, Significance at $P<0.05$ level, when student's 't' test were applied between treated and control group

Table 3—Comparative erythrocyte counts, haemoglobin content and haematocrit value of freshwater fish *Channa punctatus* after one week exposure of sub-lethal doses of rutin, taraxerol, apigenin and furadon

	Synthetic pesticide		Plant origin pesticide		
Parameters	Control	Furadon	Rutin	Taraxerol	Apigenin
Haematocrit (%)	28.1	11.2*	17.2*	21.4*	25.1*
Haemoglobin (g/dL)	13.6	5.2*	8.34*	10.1*	12.1
	Erythrocyte Count				
RBC (10^6 mm^3)	4.17	1.92*	3.24*	3.12*	3.87*
MCHC (g/dL)	34.9	28.2*	30.9*	32.8*	33.1
MCH (Pg)	57.4	31.7*	38.7*	39.7*	42.2*
MCV (µg)	135.9	112.0*	117.8*	122.1*	128.4*

RBC-Red blood corpuscles; MCHC-Mean corpuscular haemoglobin concentration; MCH-Mean corpuscular haemoglobin (Pg); MCV- Mean cell volume. *, Significance at $P<0.05$ level when student's 't' test were applied between treated and control group

Table 4—Comparative data of levels of non-cellular blood constituents of freshwater fish *C. punctatus* after one week exposure of sub-lethal doses of rutin, taraxerol, apigenin and furadan

		Synthetic pesticide	Plant origin pesticide		
Parameter	Control	Furadon	Rutin	Taraxerol	Apigenin
Serum Glucose(mg%)	67.4	43.5*	54.2*	60.9*	64.4
Serum Protein (gm%)	4.97	2.35*	3.2*	3.8*	4.12
Serum Calcium(mg%)	22.7	10.8*	12.5*	14.3*	17.3*
Serum Chloride(m.eq/l)	137.4	105.2*	113.9*	118.6*	122.6*

*, Significance at $P<0.05$ level when student's 't' test were applied between treated and control group

observed in serum total protein, glucose, calcium and chloride in experimental groups as compared to control group (Table 4).

Discussion

According to Barton[39] stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic state. If severe or long lasting, the response then becomes maladaptive and threatens the fish health and wellbeing. Therefore, in the presence of stressors (contaminants/pollutants), blood parameters and blood chemistry can be employed as standard laboratory test to determine diseased conditions and metabolic disturbances in fish[40]. In the present investigation, it has been observed that low level sublethal exposure of these compounds affects haematology. Omoregie *et al.* reported that toxicants and pollutants have significant effects which can result in several physiological dysfunctions in fish[41]. Dysfunction in the fish induces changes in blood parameters.

In fishes, a change of the blood cell distribution is also correlated with the changes in environmental conditions[42-43]. The exposure of *C. punctatus* to sublethal concentrations of rutin, taraxerol and apigenin caused a significant decrease in erythrocyte count, haemoglobin and haematocrit of the fish. The decrease in haemoglobin concentration is similar to those reported in *C. gariepinus* exposed to cassava effluents and tobacco (*Nicotiana tobaccum* Linn.) leaf extracts[44-45]. This pattern of response may be attributed to haemolysis which results in haemodilution, a means of diluting the haemoconcentration of the extracts thus reducing the effect of the toxicants/pollutant in its system[46-47].

This effect on freshwater fish *C. punctatus* might have been achieved through failure or suppression of normal mechanisms promoting erythropoiesis and/or deficiency of some factors required for the maturation of the red cell. The causes of leucopenia observed in the present study are supposed to be according to the degeneration, depression, depletion and destruction of the blood forming materials by these compounds. The observed depiction in the haemoglobin and haematocrit values in the fish could also be attributed to the lysing of erythrocytes[29]. Thus, the significant reduction in these parameters is an indication of severe anaemia. In the values obtained in the haematological indices, slight fluctuations were recorded in the MCV and MCHC but there was significant change in the MCH due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis[48].

The white blood cells in fish respond to various stressors including infections and chemical irritants[49]. Thus increasing or decreasing numbers of white blood cells are a normal reaction on the exposure of toxicants[50]. In the present investigation the increase in WBC (leukocytosis) may have resulted from the excitation of defense mechanism of the fish to counter the effect of the toxicant[51]. A sharp decrease was observed in percentage neutrophills, this was attributed to tissue damage. Finally a slight but significant increase of lymphocyte was recorded in this investigation. This is in agreement with the findings of Sampath *et al.*, when they exposed the Nile tilapia *O. niloticus* to a toxic environment[47].

Plasma protein were found to decrease, this could be attributed to renal excretion or impaired protein synthesis or due to liver disorder[52]. On the other hand, the observed decrease of plasma protein could also result from the breakdown of protein into amino acids first and possibly into nitrogen and other elementary molecule. Similar reduction in protein has also been reported by Ibrahim *et al.* when brown *Hisex chickx* fed *Azadirachta indica* A. Juss. ripe fruit[53]. A change in plasma glucose was attributed to the changes in carbohydrate metabolism. Blood glucose has been employed as an indicator to environmental stress[54].

It has been observed that plasma osmolarity and monovalent ion concentrations remain relatively constant for a variety of teleosts. It is generally recognized that sodium, chloride, potassium and calcium are primarily for osmoregulation in fishes

either by exerting osmotic effect (sodium and chloride) or by effective uptake and excretion (calcium and potassium). These elements also serve electrochemical, enzymatic and structural functions.

Calcium serves a number of functions in fish. In bony fishes, it combines with phosphorus for the deposition of bone. It is possible that bone serves as a reservoir of calcium for plasma and tissues. Additionally, calcium appears to be important in reproductionand in mitochondrial function[55,57].

Conclusion

The toxicity and physiological data clearly state that the synthetic pesticide furadan is more toxic than plant origin biologically active compound rutin, taraxerol and apigenin against freshwater fish *Channa punctatus.*

Acknowledgements

One of the authors Jaya Shahi is thankful to University Grants Commission, New Delhi for financial assistance.

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