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A Comparative Study on the Piscicidal Activity of Synthetic Pesticides and Plant Origin Pesticides, to Fish *Channa punctatus*

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Abstract: Heavy use of pesticides, changes the qualities of water. Pesticides in agricultural runoff affect all the aquatic organisms. Fishes are common indicators of water qualities status. This study was done to check whether plant origin pesticides are less toxic to fish and other organisms, compared to synthetic pesticides. Bioassay of a plant origin compounds (i.e. Rutin, Taraxerol and Apigenin) and a carbamate, Furadan were separately done on freshwater fish *Channa punctatus*. The 96h LC_{50} of Furadan is 0.05 mg/L and Rutin, Taraxerol and Apigenin is 1.128, 2.407 and 2.021 mg/L, respectively. Even thought 96h LC_{50} of binary combinations (1:1 ratio) of these compounds are also higher than Furadan. The plant based compounds are less toxic to fish than, a carbamate pesticides. In conclusion, natural products have gained greater importance, since it is believed that the natural compounds are ecologically sound and culturally more acceptable than synthetic pesticides.

Key words: *Channa punctatus* · Rutin · Taraxerol · Apigenin · Furadan · Piscicidal activity

INTRODUCTION

The intensive use of synthetic pesticides in agricultural fields and public health operation systems has resulted in serious environmental hazards [1, 2]. The freshwater bodies adjoining to agriculture field are continuously being contaminated by the toxic wastes of chemical pesticides [3-5] and pose a potential direct threat to freshwater organism, particularly to sensitive animals, such as fishes and prawns [6-8].

Recent emphasis is on the use of natural pesticides because they are easily biodegraded and leave no residues in the environment [9-12]. Plants are virtually inexhaustible source of structurally diverse and biologically active substances [13]. Several plants belonging to different families, which possess a number of compounds, as saponins, tannins, alkaloids, alkenyl phenols, di- and triperpenoids etc. have effective molluscicidal, insecticidal and piscicidal properties [10 - 15].

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). 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 [16].

The family Euphorbiaceae embraces about 7,000 species distributed all over the temperate and tropical world [17] 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 [18,19]. Taraxerol, Apigenin and Rutin were extracted from *Codium variegatum* and *Jatropha gossypifolia* plants. These compounds are reported as potent molluscicides [20, 21]. These compounds tested on fish *Channa punctatus*, which is an important fish of India capture fishery and share the habitat with freshwater snails.

The aim of this study was to establish whether Taraxerol, Apigenin and Rutin have any non-target effect on fish and to compare its toxic impact with furadan, a powerful chemical pesticide. 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.

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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 1 week. The water in aquaria was aerated continuously. The dead animals were removed from the aquaria to avoid any contamination.

Collection of Plant Materials: The bark of plant of *Codium variegatum* and leaf of *Jatropha gossypifolia* were collected from the botanical garden of DDU, Gorakhpur University, Gorakhpur, Uttar Pradesh, India.

Extraction of Taraxerol from *Codium Variegatum:* Pure taraxerol was isolated from the stem bark the stem bark of *C. variegatum* by the method of Chatterjee and Banerjee [22]. The stem bark of *C. variegatum* dried in an incubator at about 37°C and dried bark was powdered with the help of mechanical device. The dried powdered stem bark (2 kg) of *C. variegatum* was extracted in Soxhlet apparatus with petrol, for about 70 hours and a little amount of concentrated solution were 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.* [23].

Extraction of Apigenin from *Jatropha Gossypifolia:* Pure apigenin was isolated from the leaf of *Jatropha gossypifolia* by the method of Subramanian *et al.* [24]. Leaf of *Jatropha gossypifolia* were washed properly with the water and dried in incubator at 37°C. The dried leaves were then powdered. About 50 gram powder of leaf were subjected to extraction through Soxhlet apparatus with about 250-300 ml Ethyl alcohol for about 72 hours 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 extracted from *Jatropha gossypifolia* leaf was confermed by the UV spectral data of Dordevic *et al.* [25].

Rutin was ($C_{27}H_{30}O_{16}$) (EC NO-205-814-1) purchased from Sigma Chemical Company, USA.

Table 1: Concentrations used for toxicity experiments

Compound	Concentrations used (mg/l)
Plant Origin Pesticide	
Individual	
Rutin	1.0, 1.25, 1.50, 1.75
Taraxerol	2.0, 2.25, 2.50, 2.75
Apigenin	2.0, 2.50, 3.0, 3.50
Binary Combinations (1:1 ratio)	
Rutin + Taraxerol	0.4, 0.6, 0.8, 1.0
Rutin + Apigenin	0.6, 0.8, 1.0, 1.2
Apigenin + Taraxerol	1.2, 1.4, 1.6, 1.8
Chemical Pesticide	
Furadan	0.05, 0.09, 0.10, 0.30

Furadan: (Carbofuran. 2,3-dihydro-2,2-dimethyl-7-benjofuranyl methylcarbamate): It has low mammalian toxicity but is one of the most effective carbamate insecticides. Furadan is effective against corn, cotton insects and pests of potatoes.

Treatment Condition for Toxicity Testing: Water parameters were within the following ranges: temperature 27-30°C, pH 7.2-7.4, dissolved oxygen 6.5-7.0 mg/l, carbon dioxide 4.0-6.0 mg/l, alkalinity 105-109 ppm. Water was changed at every 24h.

Toxicity experiments were performed by the method of Singh and Agarwal [26]. Ten experimental animals *Channa punctatus* were kept in glass aquaria containing 6 Ltrs. dechlorinated tap water. Fishes were exposed for 24 hrs, 48 hrs, 72 hrs and 96 hrs at 4 different concentrations of single and binary combinations of Rutin, Taraxerol and Apigenin and chemical pesticide furadan (Table 1). Six aquaria were set up for each dose, control animals were kept in similar condition without any treatment. Mortality was recorded after every 24 hrs up to 96 hrs exposure periods. Fishes shows irregular, erratic and some times jerky movement that was increase as exposure period increases. Dead animals were removed to prevent the decomposition of body in experimental aquarium, which may cause rapid death in the remaining population of snail.

Toxicity data obtained from this study was computation through POLO computer program of Robertson *et al.* [27].

RESULT

Effect on Behavioural Changes and Poisoning Symptoms: Behavioral and physical change was seen after few minutes of exposure to the compounds. The skin colour of the fishes becomes light grey, the black spots on fins were also found to loose their intensity under the

Table 2: Comparative LC_{50} values (mg/L) of different concentrations of Rutin, Apigenin, Taraxerol and Furadan with their fiducial limits against freshwater fish *Channa punctatus* at different time intervals

	Plant Origin Pesticide				Chemical Pesticide			
	Rutin		Taraxerol		Apigenin		Furadan	
Exposure Periods	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL
24h	1.60	1.45-1.91	2.56	2.41-2.86	3.61	3.17-5.18	0.19	0.160-0.239
48h	1.39	1.20-1.69	2.35	2.18-2.54	3.18	2.83-4.06	0.11	0.100-0.141
72h	1.30	1.15-1.45	2.19	1.95-2.34	2.79	2.41-3.38	0.07	0.062-0.981
96h	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 percent of the exposed fish

Table 3: Comparative LC_{50} values (mg/L) of different concentrations of Rutin + Taraxerol, Taraxerol + Apigenin and Rutin + Apigenin in 1:1 ratio with their fiducial limits against freshwater fish *Channa punctatus* at different time intervals

	Plant Origin Pesticide				Chemical Pesticide			
	Rutin+Taraxerol		Taraxerol+Apigenin		Apigenin+Rutin		Furadan	
Exposure Periods	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL	LC_{50} values	Limits LCL-UCL
24h	0.80	0.69-1.00	1.78	1.65-2.14	1.09	0.95-1.49	0.19	0.160-0.239
48h	0.64	0.48-0.84	1.65	1.52-1.94	0.91	0.76-1.15	0.11	0.100-0.141
72h	0.53	0.37-0.64	1.51	1.39-1.67	0.77	0.57-0.90	0.07	0.062-0.981
96h	0.44	0.31-0.52	1.37	37.87-45.71	0.65	0.48-0.74	0.05	0.048-0.064

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

exposure. Behaviorally, fishes start scratching their nostril at the bottom of aquarium and frequently came at the water surface for gasping air. Within 15-30 minutes, fishes try to escape from test aquaria. After 30 minutes, their movement was slowed down, but they continue to swim near the water surface. Their after, fish shows irregular, erratic and some times jerky movement that was increase as exposure period increased. At higher doses after 10-12 hours, loss of body equilibrium and haemorrhage occurred, which manifested itself as reddish colour in head region and finally fishes were died. Control fishes were free from such behavioural changes.

Toxicity Experiments: The toxicity of these compounds was time and dose-dependent. There was a significant negative correlation between LC values and exposure periods. Thus with increase in exposure periods the LC_{50} values decreased from 1.60 mg/l (24h) to 1.128 mg/l (96h), 2.562 mg/l (24h) to 2.021 mg/l (96h) and from 3.612 mg/l (24h) to 2.407 mg/l (96h) in case of Rutin, Taraxerol and Apigenin, respectively (Table 2). In binary combination the LC values decreased from 1.787 mg/l (24h) to 1.378 mg/l (96h), 1.096 mg/l (24h) to 0.650 mg/l (96h) and 0.806 mg/l (24h) to 0.441 mg/l (96h) in case of Taraxerol + Apigenin, Rutin + Apigenin and Rutin + Taraxerol in (1:1) ratio, respectively (Table 3). Similarly the LC values decreased from 0.19 mg/L (24h) to 0.05 mg/L (96h) in case of furadan, but the LC values are much smaller than Rutin, Taraxerol and Apigenin.

DISCUSSION

Various forms of abnormal behaviours were observed in *C. punctatus* when exposed to different concentrations of Rutin, Taraxerol and Apigenin. By changing a large number of behavioural responses, fishes try to resist the change in aquatic environment and reduce the harmful effect of stem bark extracts. These include change in skin

colour and start scratching their nostril at the bottom of aquarium and frequently came at the water surface for gasping air. Some of these behavioural responses have been reported by Ologe and Sogbesan [28], Wade *et al.* [29] and Oti [30] for *Barbus Occidentalis* exposed to *Euphorbia heterophylla, O. niloticus* exposed different concentration of cassava effluent under laboratory conditions and *Heteroclarias* exposed to water extract of bark of *Thevetia peruviana*, respectively. The nature and rapidity of onset of fish behavioural responses indicated that these compounds become active at the neuromuscular system of fish *C. punctatus*. Animal behaviour is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances [31]. The stressful breathing behaviour exhibited by fish may be as a result of respiratory impairment due to effect of toxicant on the gills.

Mortality caused by all the compounds showed a significant positive correlation between dose and mortality. It may be due to increase of extract concentration in water resulted in more intakes of their active moieties in fish body. Data also showed the significant negative correlation between LC values and exposure periods. It could be due to several factors [32] which may be acting separately or conjointly. Stability (life span) of active moieties in environment and their detoxification rate in animal body also alters the mortality and exposure periods, relationship [33, 34].

Newer biological pesticides are developed to replace deleterious chemical pesticides. Even though chemical pesticides are target specific and effective, but have bad impact on the environment. Plant based pesticides contain active principles with low half-life period and their effects on the environment are not too detrimental [35]. In the present study, the plant origin pesticide Rutin, Taraxerol and Apigenin is less toxic to fish compared to furadan. The 96h LC_{50} of plant origin pesticides is much higher than furadan indicating the less toxic nature of the plant based pesticide.

To reduce the chemical load on the environment, it is suggested that use of plant based pesticides should be encouraged [36]. However, care should be taken to use even the plant based pesticide at moderate levels. Furthermore, plant based pesticides disintegrate easily into constituent elements without leaving any indelible impression in different regions of the environment [37].

It was concluded that the natural products have gained greater importance, since it is believed that the natural compounds are ecologically sound and culturally more acceptable than synthetic pesticides.

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