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Piscicidal and Anti AChE Activity of Medicinal Plant *Jatropha gossypifolia* (Family-Euphorbiaceae)

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Abstract: Use of plant based pesticides is less disastrous, more eco-friendly, contain easily biodegradable molecules which are more target specific than the highly persistent broad-spectrum synthetic chemical and left no residues in the environment. The aim of this study was to find out the plant origin piscicides from common endemic plants. The latex powder and Apigenin extracted from *Jatropha gossypifolia* plant belonging to the family Euphorbiaceae were found to have strong piscicidal and anticholinesterase (anti-AChE) activity on the fish *Channa punctatus*. The anti-AChE activity as well as the mortality caused by the latex powder and Apigenin were time as well as dose dependent. It has been asserted that the inhibition of the enzyme might be through activation of protein kinase-C.

Key words: Apigenin · *Jatropha gossypifolia* · Piscicide · Trash Fishes · Predatory Fishes

INTRODUCTION

Indian freshwater aquaculture has evolved itself from the stage of a domestic work in the eastern states of West Bengal, Orissa and Arunanchal Pradesh to that of industry and has become an important component of Indian fishery contributing about one third of total fish production of the country and share about 95% of total aquaculture production. So 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 [1]. Some fishes are uneconomic, small in size that naturally occur and accidently introduced in fish culture pond along with carp spawn and predate the fry and fingerling stage of carp fishes, called weed fishes. The majority of common weed fishes are active competitors with the major carps for the food available in the pond.

Due to their faster growth rate, predatory fishes share and better utilize cultured carp habitats and their food [2]. 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 [3] and by this, predatory fishes adversely affect the aquaculture production, so removal of unwanted fish population from the culture carp ponds is necessary before the seed of cultured carps was added.

For controlling weed fishes, fish farmers often use synthetic piscicides e.g. chlorinated hydrocarbons such as dialdrin, aldrin, endrin and organophosphates such as phosphamidon, dimethyl dichlorovinyl phosphate [4] PCP-Na [5], malachite green [6] and sodium cyanide [7].

Unlimited use of synthetic pesticides for the control of predatory and trash fishes, results in the excess inflow of toxic chemicals, mainly in to the aquatic ecosystem [8, 9] and adversely affect aquatic flora and fauna [10-12].

Plants are virtually inexhaustible natural resource of structurally diverse and biologically active substances [13,14]. Recent worked on the use of botanical pesticides because they were easily biodegradable and leave no residue in the environment [15]. Many plants belonging to different families, which posses a number of compounds such as saponins, tannins alkaloids alkenyl phenols, di- and triterpinoids etc. have effective molluscicidal, insecticidal and piscicidal properties [16-20].

The plant *Jatropha gossypifolia* (Euphorbiaceae) is known as belly ache bush. The plant originated from Brazil and it is now cultivated in Tropical countries throughout the world. The roots, stems, leaves, seeds and fruits of the plant have been widely used in traditional

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folk medicine in many parts of the World [21]. In the other side aqueous extract of latex, stem bark and leaf of this plant have potent molluscicidal and larvicidal activity [16, 22-25].

The aim of this study was to explore the piscicidal activity and mode of action of freeze dried latex powder and active compound Apigenin extracted from leaf of *Jatropha gossypifolia* against freshwater air breathing predatory fish *Channa punctatus* that cause special problem because they may survive in moist burrows, even when the ponds are drained.

MATERIALS AND METHODS

Collection of Plant Materials: The leaf of plant *Jatropha gossypifolia* were collected from Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur and plant was identified by Prof. S.K. Singh, Taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, (U.P.) India, where the voucher specimen is deposited. The latex was obtained from *Jatropha gossypifolia* by cut marking of old trunk of the plant. At the site of cutting trunk, the creamy latex was secreted and collected in test tubes.

Collection and Maintenance of Test Animals: Freshwater fish *Channa punctatus* (63.86±1.50 g body weight; 18.37±1.34 cm. in total length) were collected from Ramgarh Lake of Gorakhpur (U.P) India, the collected fish were maintain in plastic tank containing 100 L of de-chlorinated tap water for acclimatization to laboratory condition for 21 days. The aquarium water was aerated continuously by aerator. The dead fishes were removed from the aquarium to avoid any type of contamination.

Preparation of Freeze Dried Latex Powder of *Jatropha gossypifolia*: The 60 ml of latex was collected in six test tubes, all test tubes was set in Lyophilizer (Freeze dryer) for freeze drying. After 40-45 hour running of freeze dryer the latex was converted into powder form and then stored into UV protector desiccators for further study.

Extraction of Apigenin from Leaf: The Apigenin was isolated from the leaf of *Jatropha gossypifolia* by the method of Subramanian *et al*. [26]. The leaves of *Jatropha gossypifolia* were washed properly by tap water and cut the leaf by scissors and then dried in shady place and further dried in an incubator at about 35°C temperature, the dried leaves were powdered by electric Grinder. About 50 g powder of leaves was subjected in Soxhlet extraction unit with about 250-300 ml ethyl alcohol for about 72h at 30-40°C, when extraction was completed then filtered and a little amount of crude yellow powder was obtained. After addition of NaOH and HCl Apigenin was obtained which crystallized by methanol. Apigenin extracted from *Jatropha gossypifolia* leaves were confirmed by UV spectra data of Dordevice *et al.* [27]. The chemical structure of Apigenin is illustrated in (Fig. 1).

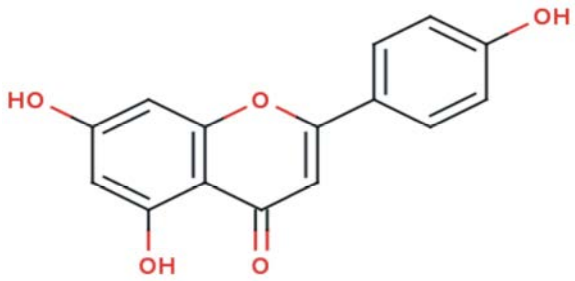

Fig. 1: Chemical structure of Apigenin

Treatment Condition for Toxicity Testing: Toxicity experiments were done according to Agarwal *et al.* [28]. Ten experimental fish *Channa punctatus* were kept in a glass aquarium containing 12 L de-chlorinated tap water, the physicochemical characteristics of experimental water was Temperature 28-30°C, pH 7.1-7.3 dissolved oxygen 6.8-7.0 mg/l, carbon dioxide 3.8-6.0 mg/l, alkalinity 105-109 ppm. Water was changed every 24 hour. Fishes were exposed for 24h, 48h, 72h and 96h at four different concentrations (w/v) of Apigenin and freeze dried latex of *Jatropha gossypifolia*. Six aquaria were set up for each dose, control animals were kept in similar condition without any treatment. Mortality was recorded after every 24h up to 96h exposure periods. Dead animals were removed to prevent decomposition of dead and decaying animal body in experimental aquaria.

Toxicity data obtained from this study was computed through POLO computer program of Robertson *et al.* [29].

Estimation of AChE Activity: The acclimatized fishes were treated within 40% and 80% of LC_{50} 24h, 48h, 72h or 96h of freeze dried latex powder of *Jatropha gossypifolia* and Apigenin, Six aquaria were set for each dose and each aquarium contained ten fishes in 12 L de-chlorinated tap water.

Acetylcholinesterase (AChE) activity was estimated by the method of Elman *et al*. [30]. Nervous tissue homogenate (50 mg/l) was prepared in 0.1M-Phosphate buffer, pH 8.0 and homogenized for 5 min. in an ice both.

Glutathionin was used as standard. The Enzyme activity has been expressed in (µm SH hydrolyzed/min/mg protein). Each experiment was replicated at least six times and data has been expressed as mean ±SE. student t-test were applied for locating significant differences [31].

Withdrawal Experiment: In order to see the effect of withdrawal of treatment of freeze dried latex powder of *Jatropha gossypifolia* and compound Apigenin to freshwater fish *Channa punctatus* exposed for 96h of 40% and 80% of LC_{50} and one half of the animal was scarified and the activity of enzyme acetylcholinesterase (AchE) were measured. The other half was transferred to freeze dried latex and Apigenin free water which was changed every 24h for the next 6 days. Then the activity of enzyme acetylcholinesterase (AChE) was measured in nerve tissues of fish *Channa punctatus*.

RESULTS

Effect on Behavioral Changes and Poisoning Symptoms: Behaviorally, fishes start scratching their nostril at the bottom of aquarium, firstly came at the water surface for engulfing air. Within 20-30 minutes, fishes try to escape from test aquaria. Within 35 min., their movement was slowed down, but they continue to swim near the water surface. Thereafter, fishes show irregular, irritating and sometime jerky movement that was increases as exposure period increased. At higher doses after 10-20 hours, loss of body equilibrium and hemorrhage and salivation, which manifested itself as reddish color in head, opercular region and finally fishes were died. Control fishes were free from such type of behavioral changes.

Dose-Mortality Response: The LC_{50} values of the aqueous extracts of freeze dried latex powder of *Jatropha gossypifolia* and Apigenin for periods ranging from 24h or 96h of fish *Channa punctatus* are shown in (Table 1&2). The toxicity was time as well as dose dependent, as there was a significant negative correlation between LC_{50} values and exposure periods. Thus, the LC_{50} of freeze dried latex powder of *Jatropha gossypifolia* was decreased from 62.31 mg/l (24h) > 57.25mg/l (48h) > 52.47 mg/l (72h) > 50.95 mg/l (96h) (Table 1) and LC_{50} of Apigenin is decreased from 100.76 mg/l (24h) to 97.86 mg/l (48h) to 95.19 mg/l (72h > 90.94 mg/l (96h) to fish *Channa punctatus*, respectively (Table 2).

The slope values were steep and heterogeneity factor was less than 1.0 indicates that the result found to be within the 95% confidence limits to LC_{50} values. The regression test ('t' ratio) was greater than 1.96 and the potency estimation test ('g' value) was less than 0.5 at all probability levels (Table 1&2).

Table 1: Toxicity (LC values) of freeze dried latex powder of *Jatropha gossypifolia* against freshwater fish *Channa punctatus* at different time intervals

		Limits			
Exposure period (in hours)	LC values (mg/L)	Lower	Upper	Slope value	Heterogeneity
24	LC_{10}=53.51	45.86	56.49	9.37±6.66	0.32
	LC_{50}=62.31	59.52	68.98		
	LC_{90}=86.56	66.92	94.91		
48	LC_{10}=46.77	34.45	51.05	4.58±5.24	0.01
	LC_{50}=57.25	53.46	61.28		
	LC_{90}=70.09	64.23	94.98		
72	LC_{10}=44.63	33.71	48.65	8.24±6.19	0.46
	LC_{50} =52.47	47.66	55.07		
	LC_{90}=61.68	58.28	72.06		
96	LC_{10}=40.70	20.79	46.67	3.14±4.54	0.06
	LC_{50}=50.95	40.89	54.39		
	LC_{90}=60.79	57.21	76.08		

•Batches of 10 fishes were exposed to four different concentrations of *Jatropha gossypifolia*

•Concentrations given are the final concentrations (w/v) in laboratory conditions.

•Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values

•LCL= lower confidence limit; UCL=Upper confidence limit

Table 2: Toxicity (LC values) of extracted compound Apigenin against freshwater fish *Channa punctatus* at different time intervals

Exposure period (in hours)	LC values (mg/L)	Limits		Slope value	Heterogeneity
		Lower	Upper		
24	LC_{10}=88.74	74.54	93.05	3.24±7.38	0.01
	LC_{50}=100.76	97.36	107.80		
	LC_{90}=114.41	107.23	149.11		
48	LC_{10}=85.55	67.71	90.66	5.95±6.98	0.03
	LC_{50}=97.86	93.73	102.67		
	LC_{90}=111.94	105.37	143.18		
72	LC_{10}=81.74	53.78	88.31	9.37±6.98	0.03
	LC_{50}=95.19	87.23	99.39		
	LC_{90}=110.85	104.04	154.29		
96	LC_{10}=79.76	53.81	86.39	6.48±7.78	0.05
	LC_{50}=90.94	79.96	94.47		
	LC_{90}=103.69	99.28	123.65		

•Details are given in Table 1

Table 3: Inhibition of Acetylcholinesterase (AChE) in the nervous tissue of fish *Channa punctatus* exposed to sublethal doses (40% and 80% LC_{50} 24h) of freeze dried Latex powder and Apigenin at different exposure periods

Exposure period (in hours)	Active compounds	AChE activity (µm SH hydrolyzed/min/mg protein)			
		Control	40 % of LC_{50}	80 % of LC_{50}	Recovery (144h after withdrawal)
4	Latex powder	0.092±0.0035	0.077±0.0025*	0.058±0.0001*	0.089±0.0028
		(100)	(84)	(63.0)	(96)
	Apigenin	0.092±0.0032	0.079±0.0013*	0.060±0.0007*	0.090±0.0025
		(100)	(86)	(65)	(97)
48	Latex powder	0.090±0.0025	0.067±0.0024*	0.047±0.0006*	0.088±0.0028
		(100)	(74)	(52)	(97)
	Apigenin	0.091±0.0023	0.069±0.0028*	0.050±0.0015*	0.089±0.0025
		(100)	(75)	(55)	(97)
72	Latex powder	0.093±0.0025	0.056±0.0018*	0.033±0.0014*	0.090±0.0026
		(100)	(60)	(35)	(96)
	Apigenin	0.092±0.0035	0.058±0.0016*	0.038±0.0016*	0.090±0.0032
		(100)	(63)	(43)	(97)
96	Latex powder	0.090±0.0022	0.048±0.0012*	0.028±0.0015*	0.087±0.0025
		(100)	(53)	(31)	(96)
	Apigenin	0.091±0.0025	0.050±0.0015*	0.021±0.0002*	0.088±0.0026
		(100)	(55)	(23)	(96)

* Significant at, ($P<0.05$), when student 't' test was applied between control and treated groups.

Table 3 show that treatment of fish with sub-lethal dilution of freeze dried latex powder and Apigenin for 24h, 48h, 72h, or 96h, caused significant ($p<0.05$) inhibition of AChE activity in the nervous tissue of C*hnna punctatus*. In vivo treatment with 40% of LC_{50} of freeze dried latex powder and Apigenin reduced the AChE activity to 84% and 86% of control, respectively while 80% of 24h LC_{50} of freeze dried latex powder and Apigenin, reduced activity to 63% and 65% of control, respectively (Table 3). After 96h, 40% of LC_{50} and 80% of LC_{50} of freeze dried latex powder treatment period reduced the AChE activity to 53% and 31% of control, respectively. While 40% and 80% of 96h LC_{50} of Apigenin reduced the activity to 55% and 23% of control, respectively (Table 3).

Table 3 also shows that 144h after termination of treatment with toxic material there was near complete recovery in the AChE activity of fish. Student 't' test showed that even through 144h after treatment termination, the activity of enzyme AChE was different also for control, it is statistically insignificant.

DISCUSSION

The data demonstrate that the latex powder and Apigenin extracted from *Jatropha gossypifolia*, have strong piscicidal and anticholinesterase properties. The toxicity and anticholinesterase activity of the latex powder was more than Apigenin. This indicates that principal cause of the death of fish could be anticholinesterase inhibition.

Members of the family Euphorbiaceae contain a group of compound known as Phorbol esters, Apigenin, Jatrophane etc. which are reported to promote activity of the enzyme protein kinase-C [32, 33], which specifically phosphorylates Serine and threonine residues in proteins [33] since the active site of Acetylcholinesterase (AChE) contain a serine residue [34] it is possible that the inhibition of AChE observed by us could be due to the phosphorylation of the active site of AChE. We therefore believe that latex powder and active compound Apigenin may eventually be of great value for the control of predatory and weed fishes from culture pond.

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REFERENCES

1. Katiha, P.K., 2000. Freshwater aquaculture in India: status, potential and constraints. Workshop Proceeding. National Center for Agriculture Economics and Policy Research, 7: 98-108.
2. Jhingran, V.G., 1983. Fish and Fisheries in India. 2ed edition, Hindustan Publishing Corporation, New Delhi, India.
3. Chakraborty, D.B., A.C. Nandy and M.T. Philipose, 1972. Barringtonia acutangula L. Gaertn. As a fish poison. Indian J. Exp. Biology, 10: 78-80.
4. Hickling, C.F., 1962. In: Fish Culture. Faber and Faber, London.
5. Terazaki, M., P. Tharnabuppa and Y. Nakayama, 1980. Eradication of predatory fishes in shrimp farms by utilization of Thai tea seed. Aquaculture, 19: 235-242.
6. Gribgratok, S., 1981. The role of cyanide on the fisheries. Thai Fisheries Gazette, 34:499-506 (In Thai).
7. Marking, L.L., 1992. Evaluation of toxicants for the control of carp and other nuisance fishes. Fisheries, 17: 6-12.
8. Bhaskaran, P., S. Palanichamy, S. Vijaylakshmi and M.P. Balasubramanian, 1989. Effect of mineral fertilizers survival of the fish *Oreochromis mossambicus*. Environ. Ecol., 7: 463-465.
9. Kalavathy, K., A.A. Sivakumar and R. Chandran, 2001. Toxic effect of the Pesticide dimethoate on the fish *Sarotheradon mossambicus*. J. Ecol. Res. Bioconserv., 2(1-2): 27-32.
10. Pant, J.C. and I. Singh, 1983. Inducement of metabolic disfunction by carbamate and organophosphorus compound in a fish *Pantius conchonius*. Peste. Biochem. Physiol., 20: 294-298.
11. Hodson, P.V., 1988. The effect of metal metabolism on uptake, deposition and toxicity in fish. Aquat. Toxicol., 11: 3-18.
12. Johl, M.S. and A. Dua, 1995. Hemental lepidological and toxicological studies in *Channa punctatus* (Bloch) upon exposure to an organochlorine pesticide, endosulphon. Bull Environ. Contam. Toxicol., 55(6): 916-921.
13. Istvan, U., 2000. Semi-natural products and related substances and alleged botanical pesticides. Pest Management Sci., 56(8): 703-705.
14. Shahi, Jaya and Ajay Singh, 2010. A comparative study on piscicidal activity of synthetic pesticides and plant origin piscicides, to fish *Channa punctatus*. World journal of zoology 5(1): 20-24.
15. Singh, A., D.K. Singh, T.N. Mishra and R.A. Agrawal, 1996. Molluscicides of plant origin. Biological Agricultural and Horticulture, 13: 205-252.
16. Singh, S.K., R.P. Yadav and A. Singh, 2010. Molluscicides from some common medicinal plants of Eastern Uttar Pradesh, India. J Appl. Toxicol., 30: 1-7.
17. Okunji, C.O. and M.M. Iwu, 1988. Control of schistosomiasis using Nigerian medicinal plants as molluscicides. International Journal of Crude Drug Research, 26: 245-252.
18. Chauhan, S., Jaya Shahi and Ajay Singh, 2011. Ecofriendly management of *Lymnea acuminata*, Snail vector of Fascolesis in liver stock in Eastern Uttar Pradesh. Global veterinaria, 7(1): 10-18.

19. Singh, S.K. and A. Singh, 2010. Toxicity effect of *Alstonia scholaris* plants to fingerlings of Labeo rohita (Hamilton) in different conditions. World journal of zoology, 5(1): 41-46.
20. Singh, P. and A. Singh, 2012. Evaluation of latex extract of *Euphorbia roylena* for its piscicidal and muricidal activities. World journal of Agricultural sciences, 8(5): 520-524.
21. Pravathi, V.S., B.S. Jyothi, T. Lakshmi, B.P. Srivastav and R. Karthikayan, 2012. Scholars research library. Der Pharmacia Letter, 4(l): 256-262.
22. Yadav, R., V.K. srivastava, R. Chandra and A. Singh., 2002. Larvicidal activity of latex and stem bark of *Euphorbia tyrucally* plant on mosquito *Culex quinquefasciatus*. J. of Communicable Dis., 34(4): 264-269.
23. Yadav, R., V.K. Srivastava and A. Singh., 2003. Toxicology of Apigenin (Flavonoid) Extracted from the latex of *Jatropha gossypifolia* against the larvae of mosquito *Culex quinquefasciatus* Biological control of insect pest. Ignacim and Jayraj, Published by Phoenix publishing house PVT.LTD, pp: 227-331.
24. Yadav, R.P., S. Tiwari and A. Singh, 2005. Toxic effects of taraxrol extracted from *Codiaum variegatum* on metabolism of fresh water snail *Lymnea acuminata* and non-target fish. Iberus, 23(1): 1-13.
25. Yadav, R.P. and A. Singh, 2006. Toxic effects of Jatropha gossypifolia and its binary and tertiary combinations with other plants molluscicides in natural ponds. Iberus, 24(2): 47-54.
26. Subramanian, S.S., S. Nagarjuna and N. Sulochana, 1971. Flavonoids of Jatropha gossypifoli. Phytochemistry, 10: 1690.
27. Dordevice, S., Mcakic and S. Amra, 2000. The extraction Apigenin and Lutiolin from the sage Salvia officinalis L. from Jordon. The Scientific J. Facta Universitatis, 1(5): 87-93.
28. Agarwal, R.A. and D.K. Singh, 1988. Harmful gastropods and their control. Acta Hydrochim. Hydrobiol., 16: 113-138.
29. Robertson, R.M. Russel, H.K. Preisler and M.E. Saven, 2007. Bioassay with Arthropods: polo, a new computer program. C.R.C. Francis and Taylor, pp: 1-224.
30. Elman, G.L., K.D. Courtney, V. Jr. Andress and F.R.M. Stone 1961. A new and rapid colorimetric determination of AChE activity. Biological Pharmacology, 7: 88-98.
31. Sokal, R.R. and F.J. Rohlf, 1973. Introduction to biostatistics. San Francisco, W.H. Freeman and Co., pp: 368.
32. Evans, F.J. and M.C. Edwards, 1987. Activity correlation in the phorbol ester series. Botanical J. Linnaean Soci., 94: 231-246.
33. Aitken, A., 1987. The activation of protein kinase C by dephnane, ingenane and tigliane diterpenoid esters. Botanical J. Linnaean Soci., 94: 247-263.
34. Koelle, G.B., 1975. Anticholinesterase agents. In: The Pharamacological basis of therapeutics (L.S. Goodman and A. Gillman Eds.) Macmillan Publishing Co., New York, pp: 404-466.