

## Exploitation of piscicidal potential of *Anamirta cocculus* (Linn.) seed extracts for the eradication of predatory wild fish *Mystus vittatus* (Bloch.)



## Zoology

**KEYWORDS :** *Anamirta cocculus*, *Mystus vittatus*, biopiscicide, biopesticide

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### ABSTRACT

The present study aims to evaluate acute toxicity and piscicidal potential of methanol, chloroform and acetone extracts of *Anamirta cocculus* seeds by using the freshwater catfish *Mystus vittatus* as a model. Mortality caused by the three extracts against *M. vittatus* has been reported and that all the extracts of *A. cocculus* were active in killing the fishes. Eight different concentrations each of methanol, chloroform and acetone extracts were applied on different groups of *M. vittatus* and were observed that toxic effect of all the extracts was time as well as dose dependent. The 24h LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>99</sub> values of each extract was calculated by Finney's method. From the three extracts of *A. cocculus* methanol extract was the most potent one with lowest 24h LC<sub>50</sub> value of 9.951mg/L and the respective lowest 24h LC<sub>90</sub> and 24h LC<sub>99</sub> values of 11.749 and 13.452<sup>mg/L</sup>. The present investigation revealed that *A. cocculus* seed extracts can be used as the potential aquaculture management tool to eradicate unwanted fish fauna from culture ponds. Culture fingerlings can be released into the fish rearing ponds few days after applying *A. cocculus* seed extracts as a biopiscicide/biopesticide.

### INTRODUCTION

Natural products have played a major role in the development of certain essential piscicidal agents. The eradication of wild fishes in the culture ponds before the stocking of desired species is an important step in pond management as the former compete and/ or prey upon the latter. The use of plant origin ichthyotoxicant as a fisheries management tool has been practiced in at least thirty countries including India (Murphy and Willis, 1996). *Mystus vittatus* (Bloch, 1794) is a member of the Bagridae (*Siluriformes*) family that occurs widely throughout the Indian subcontinent (Froese and Pauly, 2006). It feeds on plants, shrimps, insects, molluscs and fishes (Bhatt, 1971; Pethiyagoda, 1991). This carnivorous nature of *M. vittatus* can damage the culture fish in various hatcheries. The fast multiplication of *M. vittatus* is also a serious concern in fresh water ecosystem, since they can eat away most important fish and other animal fauna in water bodies. For eliminating unwanted population of *M. vittatus* from cultured ponds, fish farmers have made several efforts, by making use of synthetic pesticides (piscicides) in their farms (Marking, 1992). Due to the long term persistence of synthetic pesticides in water bodies, the quality of water, fish and their status has been affected adversely (Cullen and Connell, 1992). A better alternative for these harmful synthetic pesticides is environmentally safe plant origin piscicides which are less expensive, biodegradable, readily available, easy to handle and safe to mankind and environment (Marston and Hostettmann, 1985; Singh *et al.*, 2010).

Traditionally the dried berries of *A. cocculus* have been used in India to stupefy fish and are reported to contain picrotoxin (Satya and Paridhavi, 2012). The preliminary work done by Jothivel and Paul (2008) suggested raw seeds of *A. cocculus* as a biopiscicide against weed fishes. The present work was carried out to investigate the piscicidal activity of methanol, chloroform and acetone extracts of seeds of *A. cocculus* against *M. vittatus*.

### MATERIALS AND METHODS

*M. vittatus* were collected from the Lalpet pond in Cuddalore District of Tamil Nadu. They were bought to the laboratory where they were washed with KMnO<sub>4</sub> solution (1 mg/L) for 5 minutes and then transferred to the acclimation tank having physicochemical parameters given in Table-1. The fishes having an average body length of 11.21± 0.23 cm, and body weight of 18± 0.23 g were selected for the present study. The seeds of *A. cocculus* were collected from Kerala forest, India and the dried seeds were broken and endosperms were collected. Five hundred grams of the powdered endosperms were packed in the thimble of the Soxhlet apparatus and were extracted separately with methanol, chloroform and acetone at 30-40°C for 24 hours.

Acute toxicity of each extract on test fish was measured in terms of LC<sub>50</sub> for 24hours. Static bioassay procedures, as outlined by the USEPA (2005) were followed. A minimum of 8 concentrations of each extract were applied on each group of fishes (30 fishes per group) (Table-3). Lethal concentrations (LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>99</sub>) at 95% fiducial level of upper and lower confidence were estimated according to Finney's (1971) method. Further the LC<sub>50</sub> value of each extract calculated from Finney's method was compared with the LC<sub>50</sub> calculations from graphical method. Chi-square ( $\chi^2$ ) test was used to check the heterogeneity of the data.

### RESULTS

By applying methanol, chloroform and acetone extracts of the seeds of *A. cocculus*, the acutely intoxicated fish generally exhibited violent swimming activities. They often wriggled to the water surface and exhibited increased gulping activity (Table-2). The present observations also indicated that the piscicidal activity of *A. cocculus* against *M. vittatus* was both time and dose dependent. There was significant negative correlation between exposure time and LC<sub>50</sub> of tested extract. The 24h LC<sub>50</sub>, LC<sub>90</sub> and LC<sub>99</sub> values of the three extracts were in the increasing order as, methanol extract < chloroform extract < acetone extract (Table-3). From the present study it was observed that methanol extract of *A. cocculus* seeds was the most potent with lowest LC<sub>50</sub> value of 9.951mg/L (Finney's method) and 9.937mg/L (Graphical method) as compared to chloroform and acetone extracts. Similarly LC<sub>90</sub> and LC<sub>99</sub> values of methanol extract were 11.749 and 13.452mg/L which was less than the other two extracts. There was no significant difference between the lethal concentration values, calculated from the two methods *i.e.*, Finney's method and graphical method. Further the order of toxicity at all the exposure periods was 3h < 6 h < 9h < 12h < 15h < 18h < 21h < 24h. The value of Lethal concentrations for the given 24h was LC<sub>50</sub> < LC<sub>90</sub> < LC<sub>99</sub> (Table-4). The estimated lethal concentrations for 24h lie within the 95% confidence limits and are given along with their  $\chi^2$  values (Table-3).

### DISCUSSION

As a piscicide, the knowledge of LC<sub>50</sub> of the three extracts would provide more information to the farmers in case they want to kill/eradicate the predatory and weed fishes within a convenient duration from culture ponds before stocking. The toxicity observation from the present study suggests that the concentration of toxicant is directly proportional to the rate of mortality and an increase in lethal concentration decreases the duration of mortality. Kinghorn and Evans (1975) demonstrated that the toxicological action of plant extracts may be due to the presence of alkaloids in them. The toxicity of methanol extract of *A. cocculus* seeds in the present study may also be

linked with the presence of alkaloids in them (Qadir and Paul, Annamalai University, India, Article submitted for publication). Gill *et al.*, (1991) described that the behavioral anomalies are due to inhibition of cholinergic impulse by the hydrolysis of neurotransmitter acetylcholine released during synaptic transmission. Present study is in agreement with Clotfelter *et al.*, (2006) who has also observed aggressive behavior in fighting fish, increased surface breathing and opercular movement in the stressed *M. vittatus* and has linked it to sustained respiratory discomfort by the toxicity of WPME (whole paper mill effluent). The methanol extract of *A. cocculus* in the present study has the highest potential to eradicate weed fish *M. vittatus* with 24h LC<sub>50</sub> values of 9.951 mg/L. Thus a potential biopiscicide can be prepared from the methanol, chloroform and acetone extracts of *A. cocculus* which can replace the chemical piscicides/ pesticides in the fish farms and can do less or no damage to the environment.

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**Table 1. Physico-chemical properties of water**

Temperature	26.5–30.0 °C
pH	7.5–7.6
Dissolved oxygen	7.6–8.4 mg/l
Carbon dioxide	4.7–5.3 mg/l
Alkalinity	111.3–113.3 mg/l
Hardness of water	141 ppm

**Table 2. Behavioral response of *M. vittatus* intoxicated with *A. cocculus* extracts at various exposure time intervals.**

Extract Exposure	Behaviors	Exposure time (hours)							
		3	6	9	12	15	18	21	24
Methanol	Loss of reflex	-	-	-	-	-	-	+	+
	Molting	-	-	+	+	-	-	-	+
	Discoloration	-	+	+	+	+	-	-	+
	Air gulping	+	+	+	+	+	-	-	+
	Erratic swimming	+	+	+	+	+	+	+	+
	Barbel deformation	-	-	-	-	-	-	+	+
	Excessive mucus secretion	-	-	-	+	+	+	+	+
		-	-	-	+	+	+	+	+
Chloroform	Loss of reflex	+	-	-	-	+	+	+	+
	Molting	-	-	+	+	-	-	+	+
	Discoloration	-	+	+	+	-	-	-	+
	Air gulping	-	+	+	+	-	-	-	+
	Erratic swimming	+	+	+	+	+	+	+	+
	Barbel deformation	-	-	-	+	+	+	+	+
	Excessive mucus secretion	-	+	+	+	+	+	+	+
		-	+	+	+	+	+	+	+
Acetone	Loss of reflex	+	-	-	-	+	-	+	+
	Molting	-	-	+	+	-	-	-	+
	Discoloration	-	+	+	+	-	-	-	+
	Air gulping	+	+	+	+	+	+	+	+
	Erratic swimming	+	+	-	+	+	+	+	-
	Barbel deformation	+	+	-	+	+	+	+	+
	Excessive mucus secretion	-	+	+	+	+	+	+	+
		-	+	+	+	+	+	+	+

**Table 3. 24 h LC50, LC90 and LC99 values (mg/L) of *A. cocculus* against *M. vittatus* with 95% confidence limits, and calculated c2 values.**

Method employed	Extracts	LC <sub>50</sub>	LC <sub>90</sub>	LC <sub>99</sub>	c <sup>2</sup>
		(LCL-UCL)	(LCL-UCL)	(LCL-UCL)	
Finney's method	Methanol	9.951 (9.659-10.196)	11.749 (11.364-12.352)	13.452 (12.712-14.742)	10.466*
	Chloroform	13.453 (13.153-13.725)	15.958 (12.136-21.846)	17.475 (16.783-18.734)	10.107*
	Acetone	18.768 (18.413-19.109)	21.834 (17.563-27.945)	24.348 (23.673-25.652)	9.759*
Graphical method	Methanol	9.937	11.738	13.438	10.393*
	Chloroform	13.420	15.924	14.457	10.102*
	Acetone	18.749	21.839	24.363	9.821*

\*Significant at P<0.05.

**REFERENCE**

Bhatt, V.S. 1971. Studies on the biology of some freshwater fishes, part VI. *Mystus cavasius* (Ham.). *Hydrobiologia*, 38: 289–302. | Clotfelter, D., Ethane, C. and Alison, R. 2006. Behavioral changes in fish exposed to phytoestrogens. *Environ. Pollut.*, 144: 833-839. | Cullen, M.C. and Connell, D.W. 1992. Bioaccumulation of chlorohydrocarbon pesticides in fish in the natural environment. *Chemosphere*, 25(11): 1579-1587. | Finney, D.J. 1971. *Probit analysis*. 3rd Edn. Cambridge University Press, Cambridge, UK. | Froese, R. and Pauly, D. 2006. *Fish Base 2006*. World Wide Web electronic publication. Available at: <http://www.fishbase.org>, pp. 47. | Gill, T.S., Pandey, J. and Tiwari, H. 1991. Individual and combined toxicity of common pesticides to teleost *Puntius conchionius* (Hamelton). *I. J. Exp. Biol.*, 29 (2): 145–148 | Jothivel, N. and Paul, V.I. 2008. Exploitation of acute toxicity of the seeds of *Anamirta cocculus* (Linn.) as a potential aquaculture management tool to eradicate unwanted fish fauna. *Asian Fish Sci.*, 21 (4): 457–467. | Kinghorn, A.D. and Evans, F.J. 1975. A biological screen of selected species of the genus *Euphorbia* for skin irritant effects. *Planta Medica*, 28: 325–335. | Marking, L.L. 1992. Evaluation of toxicants for the control of carp and other nuisance fishes. *Fish.*, 17: 6-13. | Marston, A. and Hostettmann, K. 1985. *Plant molluscicides*. *Phytochem.*, 24: 639-652. | Murphy, B.R. and Willis, D.R. 1996. Sampling with toxicants. In: Murphy B R, Willis D R (eds) *Fisheries techniques*. 2nd ed: American fisheries society: Bethesda, MD, pp: 303-333. | Pethiyagoda, R. 1991. *Freshwater fishes of Sri Lanka*. The Wildlife Heritage Trust of Sri Lanka, Colombo, pp. 362. | Satya, V. and Paridhavi, M. 2012. Ethano-botanical, phytochemical and pharmacological review of *Anamirta cocculus* (Linn.) *Wight and Arn. Int. J. Rev. Life Sci.*, 2 (1): 1–6. | Singh, S., Yadav, R. and Singh, A. 2010. Molluscicides from some common medicinal plants of eastern Uttar Pradesh. *I. J. Appl. Toxicol.*, 30(1): 1-7. | United States Environmental Protection Agency (USEPA). 2005. *Aquatic Toxicity Information Retrieve (AQUIRE)*, aquatic toxicology database. Available: [www.epa.gov/ecotox/](http://www.epa.gov/ecotox/) accessed: August 2003, pp. 138. |