Asian Fisheries Science **21**(2008):457-467
https://doi.org/10.33997/j.afs.2008.21.4.009

Asian Fisheries Society, Manila, Philippines

Exploitation of Acute Toxicity of the Seeds of *Anamirta cocculus* (Linn.) as a Potential Aquaculture Management Tool to Eradicate Unwanted Fish Fauna

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Abstract

Acute toxicity studies on the seeds of the Indian berry *Anamirta cocculus* (Linn.) were carried out using the air-breathing catfish *Clarias batrachus* (Linn.). The acute toxicity was tested for 1, 2, 24, 48, 72 and 96 hours of exposure and the respective LC_{50} and LC_{99} values were estimated. The results indicated the powerful piscicidal property of the seeds of *A. cocculus.* The 1, 2, 24, 48, 72 and 96 hours LC_{50} values of the piscicide were 501, 256, 220, 194, 186 and 181 ppm, respectively. The toxicity was found to depend on the concentration of the toxicant as well as on the duration of exposure. On the other hand, the LC_{50} values were found to be inversely proportional to exposure duration. Being a potent piscicidal agent, seeds of *A. cocculus* may be used for eradicating un-wanted wild fishes, especially the sturdy air breathing species like *C. batrachus*, from aquaculture ponds.

Introduction

Poisons are widespread in plants and humans have often tried to turn them into their own advantage by utilizing these poisonous properties.

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Poisons derived from plants are used for hunting, fishing, against depredation by wild animals and even in tribal warfare in Africa (Neuwinger 1998). Most of the indigenous cultures across the globe have used poisonous plants (ichthyotoxicants or piscicides) to catch fish by making use of the various chemicals present in plants to stun or stupefy them. Reports describing the utilization of plants as piscicides by Polynesian cultures have appeared as early as 1862 (Burton et al. 2003). Fisher folks of Nigeria and other African countries extensively use many plants and plant products for capturing fish (Seigler 2002; Fafioye et al. 2004). Similarly, in South America numerous piper species are used ethnobotanically as barbascos (Schultes & Rafauf 1990). As far as South East Asia is concerned, Kulakkattolickal (1987) has conducted a survey of piscicidal plants found in Nepal. Studies pertaining to piscicidial plants in India are mainly centered on members of Euphorbiaceae and Apocynaceae (Singh & Singh 2002; Tiwari & Singh 2005).

In addition to their use as traditional piscicidal agents for catching fish, plant derived fish toxicants are widely used in aquaculture management also for eradicating unwanted/wild species of fishes including the predatory ones. Demand for good piscicides that are cheap, efficient and safe for the users and end consumers has increased tremendously with the expansion of aquaculture. Therefore, attention has to be placed on alternative plants with intrinsic piscicidal properties and preference has to be given to such plants, which are cost effective, locally available, easy to prepare, apply and biodegradable.

Indian berry, *Anamirta cocculus* (Linn.) (Menispermaceae) is such a wild plant and its seeds (berry) are potential piscicidal agents. Even though the rural folks use *A. cocculus* for catching wild fish from natural water bodies for human consumption in various states of India, scientific studies regarding its toxicity towards various species of fishes are extremely scanty (Dumen et al. 2001). In this context, the present study is an attempt to evaluate the acute toxicity of the seeds of *A. cocculus* with a view to use it as a potential tool in aquaculture management to eradicate weed fishes and predatory ones. The selection of *Clarias batrachus* (Linn.) (Claridae) as the test organism was based on three reasons viz. (i) it is distributed throughout the Indian subcontinent; (ii) even though it is a table fish, it is considered as an aggressive predatory wild fish causing severe economic loss to the fish farmer especially in carp culture system; and (iii) it is a very sturdy intruder in culture ponds especially due to the bimodal respiration facilitated by accessory respiratory organ as well as gills (Munshi 1980; Chandra & Banerjee 2003). Farmers are very keen to eradicate this sturdy fish from culture ponds and a concentration of the piscicidal agent that kills this fish would wipe out most (if not all) of the unwanted wild fish species from the culture pond.

Materials and Methods

Collection and maintenance of fish

Irrespective of the sex, healthy specimens of *C. batrachus* having 150 to 160 g body weight and 20 to 22 cm length were collected locally at Chidambaram, Tamilnadu, India and were reared in large plastic aquaria bearing well water. The fish were acclimated to laboratory condition for 30 days. During this period, they were fed with minced goat liver every day for a period of three hours (h), before the renewal of the medium. Water was renewed every 24 h with routine cleaning of the aquaria leaving no fecal matter, dead fish (if any) or unconsumed food.

Piscicidal agent

The piscicide used in the present study were the ripe, dried seeds of *A. cocculus*. This plant is usually known as Indian berry, which is a woody climber with heart shaped leaves.

Preparation of the toxicant

The hard shells of the dried fruits of *A. cocculus* were broken using a hammer and the seeds were taken out with the help of a needle. The collected seeds were ground into fine particles using a grinder. The ground powder was thoroughly mixed with well water in required quantity (ppm) for use as contact poison.

Estimation of LC_{50}

Median lethal concentrations (LC_{50}) of *A. cocculus* to *C. batrachus* at various exposure periods viz. 1, 2, 24, 48, 72 and 96 h were estimated according to Finney's (1971) method by exposing the fish to the medium containing the piscicide in the required proportion. A fish was considered dead if it did not respond to prodding using a glass rode. The mortality rates during the stipulated exposure period were recorded and using this data various LC_{50} and LC_{99} values were calculated along with the slope values. The heterogeneity of the data (if any) in each exposure group was checked using chi-square (χ^2) test.

For the estimation of LC_{50} at various exposure periods, experiments were conducted in triplicate for every exposure period. Each set in each exposure period (3 × 6 = 18 sets) composed of 10 fish each was exposed to 100 liters of the experimental medium, prepared by mixing the ground seeds in the required ppm proportion in well water. The various physico-chemical parameters of the well water, during the tenure of the experiment, were estimated following APHA et al. (1989). Parallel control groups were also kept without the addition of *A. cocculus* seeds. Feeding was withheld 24 h prior to the commencement of the experiment and the fasting continued throughout the tenure of the experiment. The experimental as well as the control media were renewed after every 24 h leaving no faecal matter or dead fish (if any).

Results

Upon exposure to the lethal concentrations of *A. cocculus* seeds, toxic stress on the fish was immediately manifested in the form of restlessness and increased release of mucus into the medium. Respiratory distress was evident from the highly increased opercular movements and gulping activity. As the exposure period increased, the surfacing activity also increased till the exposed fish started losing their balance and equilibrium posture. After the initial restlessness, the fish started jerky, erratic and sometime violent swimming movements. They tried to escape from the aquaria by jumping. Later they exhibited a characteristic vertical position with the head above the water surface along with extended barbels and moving in the same position accompanied by muscular twitching and tetany. After a short while they again went inside the water column to reappear and repeat the same type of movements. Before death, the fish lost balance and moved very violently. Finally all the body activities decreased and they settled down at the bottom and died. No such behavioral changes or mortality were observed in the control groups.

The physico-chemical parameters of the well water used for the bioassay are shown in table 1. The average mortality rates observed at various stages of exposure in the various sets of experiments conducted in triplicate are given in table 2. The observed mortality rate in the respective exposure periods were utilized to estimate the respective LC_{50} values (Table 3) within the 95% confidence limits along with the slope and χ^2 (for heterogeneity) values. The calculated χ^2 values were found to be less than the respective table values. The mortality rates were found to be dependent on both concentrations and exposure periods. While mortality was found to increase with an increase in exposure period as well as concentration, LC_{50} values were inversely proportional to exposure periods. The calculated LC_{99} values (Table 4) of *A. cocculus* were found to cause almost 100% mortality of the test organism. Therefore these concentrations were taken as the absolute lethal concentrations causing 100% mortality in the respective exposure periods.

Table 1. Physico-chemical parameters of the well water used for the experiment

Parameter	Average ± SD
Temperature	26 ± 1 °C
pH	7.4 ± 0.2
Dissolved oxygen	6.1 ± 0.3 mg•L^{-1}
Carbon dioxide	3 ± 0.5 mg•L^{-1}
Alkalinity	9.5 ± 0.1 ppm
Hardness	234 ± 2 ppm

Table 2. Mortality rates of *C. batrachus* exposed to various concentrations of the seeds of *A. cocculus* (averages of experiments done in triplicate)

D (h)	EC (ppm)	N	PM	D (h)	EC (ppm)	N	PM
1	470	10	10	48	177	10	10
	480	10	20		182	10	30
	490	10	30		187	10	40
	500	10	40		192	10	40
	510	10	60		197	10	50
	520	10	80		202	10	60
	530	10	90		207	10	80
	540	10	100		212	10	100
2	225	10	20	72	169	10	20
	235	10	20		174	10	20
	245	10	30		179	10	30
	255	10	50		184	10	40
	265	10	60		189	10	50
	275	10	70		194	10	60
	285	10	80		199	10	90
	295	10	100		204	10	100
24	205	10	20	96	165	10	10
	210	10	30		170	10	20
	215	10	40		175	10	30
	220	10	50		180	10	40
	225	10	50		185	10	60
	230	10	60		190	10	70
	235	10	80		195	10	90
	240	10	100		200	10	100

Note: D = duration of exposure; EC = exposure concentration; h = hour(s); N= number of fish exposed; PM = percentage of mortality; ppm = parts per million.

Table 3. LC_{50} values of *A. cocculus* to *C. batrachus* at different periods of exposure with their 95% confidence limits, calculated χ^2 values and respective slopes

Hours of exposure	LC_{50}	Lower limit	Upper limit	χ^2	Slope
1	501.5023	493.5649	509.1492	0.914	1.0466
2	256.2216	246.2699	265.4149	1.753	1.1209
24	220.4266	214.4177	225.7605	2.677	1.0814
48	193.7715	188.7087	198.7284	3.037	1.0826
72	185.6805	181.0188	190.3293	3.410	1.0797
96	181.2705	177.1826	185.2607	1.209	1.0684

Table 4. LC_{50} and LC_{99} values of *A. cocculus* to *C. batrachus* at various exposure periods along with 95% confidence limits

Exposure concentration	Hours of exposure					
	1	2	24	48	72	96
LC_{50}	501.5023	256.2216	220.4266	193.7715	185.6805	181.2705
Upper limit	509.1492	265.4149	225.7605	198.7284	190.3293	185.2607
Lower limit	493.5649	246.2699	214.4177	188.7087	181.0188	177.1826
LC_{99}	552.7666	321.5764	259.0916	228.2599	217.5992	208.1996
Upper limit	584.3445	368.9419	293.2199	255.5365	240.7716	225.3459
Lower limit	537.6877	301.4016	246.3947	217.1722	207.6002	200.1677

Discussion

Fishing poisons are also known as piscicides or barbascos. The use of piscicidal plants for harvesting fishes has been practiced all over the world by various indigenous ethnic groups in different geographical regions such as Amazon basin (Heizer 1953; Acevedo-Rodriguez 1990; Schultes & Raffauf 1990; Calixto et al. 1998), Bolivia (Mućoz et al. 2000), Ecuador (Bachmann et al. 1993), Guyana (Van Andel 2000), India (Jhingran 1991), Nepal (Kulakkattolickal 1987), Thailand (Chiayvareesajja et al. 1997) and Venezuela (Pittier 1926). The basis in using piscicidal plants in general is that human consumption of fish poisoned by these piscicides agents has no major reported negative effects even though direct consumption of these plant origin piscicides are reported to have adverse effects (Cannon et al. 2004). As in many other cases, native fishermen or local people might have also identified the toxicity of *A. cocculus*. However, the traditional ethnic groups and rural folks are not aware of the precise quantity of the material required to kill fish in a particular body (quantity) of water. Therefore the native fishermen usually apply a much higher quantity than the optimum to catch fish for human consumption. This may also cause the loss of biodiversity in natural aquatic ecosystems. In this state of affairs, the present acute toxicity study is a step towards efficient and optimal utilization of locally available, natural and cheap botanicals. Further, the absence of behavioral alterations and mortality in the control groups vividly indicate that seeds of *A. cocculus* are responsible for the behavioral alterations and mortality in the experimental groups.

Being a sensitive aquatic organism, laboratory bioassays with fish as sentinel species could provide valuable information about the toxicity of a single chemical or a mixture of them. Even though in most cases the actual situation of the stressed environment corresponds to sub lethal or chronic conditions (de la Torre et al. 2002), in the present study importance has been given to acute toxicity because the purpose is to use *A. cocculus* as a potential piscicidal agent. Banerjee (1993) has also opined that knowledge of LC_{50} testing is an essential pre-requisite for the study of acute toxicity of toxicants. Moreover, in addition to its common use as a piscicide, in the present study emphasis is given to the use of the seeds of *A. cocculus* as a potential tool in aquaculture management to eradicate unwanted wild fishes. Due to the same reason, concentrations of the piscicide causing 100% mortality have also been tested for the various exposure periods and were found to be more or less the same as that of LC_{99}. Such absolute mortality data at various exposure periods would provide more convenience to the farmers as they can eradicate the wild fish within a convenient duration. Cagauan et al. (2004) is also of the view that the concentration causing 100% mortality is the basis of the piscicidal activity of the test plant. Further, by providing LC_{50} and LC_{99} values at different exposure periods, the farmers can select the least optimum concentration according to their requirements as the caught fish are traditionally used for human consumption without any reported major ill effects. We also suggest that the use of the seeds of *A. cocculus* as a piscicide be limited only to aquaculture ponds as it may cause the loss of biodiversity if used in natural water bodies.

Lethality caused by *A. cocculus* seeds indicates a clear and significant relationship between mortality rate and dose/exposure period. The increase in mortality with an increase in exposure period as well as concentration may be due to different factors such as rate of penetration of active moieties, nature of slope and concentration of the active moieties, etc. which may be acting separately or conjointly (Tiwari et al. 2003). The increased concentration of the piscicide in the test medium might have resulted in increased uptake of the active moieties into the body of the fish. This could be one of the reasons for an increase in mortality due to increased toxicant concentration. On the other hand LC_{50} values and exposure periods are inversely proportional i.e., as exposure period increases, LC_{50} decreases. Chi-square tests for goodness of fit show that the calculated χ^2 values are less than the respective table values. This signifies that the mortality counts are not significantly heterogeneous and variables such as individual resistance do not significantly affect the lethal concentration values as they lie within 95% confidence limits. The steepness of the slope (Tiwari et al. 2003) indicates that there is a large increase in mortality rate with a relatively small increase in the concentration of the toxicant.

The presence of predatory and weed fishes in a culture pond is detrimental in the case of carp culture and is a serious problem for aquaculture managers. These unwanted fishes adversely affect the cultured fish in the pond due to their predatory nature, faster growth, better utilization of feed and habitats. The problem becomes more aggravated in the case of the predatory fishes with accessory respiratory organs (e.g. *C. batrachus*), as they can escape netting operations by burying themselves into the bottom mud or cracks and crevices on the walls of the pond. Therefore, it is absolutely essential to eradicate this otherwise valued fish along with the other wild varieties during preparation of pond for aquaculture and the only effective way to get rid of these air-breathing predatory species is through the application of piscicidal agents especially in non-drainable ponds.

In an effort to eliminate the unwanted species, farmers even resort to the use of many synthetic pesticides (Marking 1992; Tiwari & Singh 2005) such as agro-pesticides, sodium cyanide (Cagauan et al. 2004) and other synthetic compounds including chlorinated hydrocarbons and organophosphates (Terazaki et al. 1980; Gribgratok 1981; Mukherjee et al. 2000; Das & Mukherjee 2003). Most of the synthetic piscicides have long-term persistence in the ecosystem leading to their bioaccumulation in the cultured fish (Arasta et al. 1996). This would in turn adversely affect the health of the fish as well as the consumer (Cullen & Connell 1992; Waliszewski et al. 1999; Cagauan et al. 2004; Tiwari & Singh 2005). The chemical makeup of the seeds of *A. cocculus* reportedly includes picrotoxin (Fabricant & Farnsworth 2001). Due to all these reasons, there is a need to explore environmental friendly alternative fish toxicants and a better alternative to the harmful synthetic piscicides is the environmentally safe plant origin piscicides such as the seeds of *A. cocculus* that are less expensive, easy to handle and safe for the user and environment. Being a product of biosynthesis, it is potentially biodegradable too.

Conclusion

Clarias batrachus is an air-breathing sturdy catfish widely distributed in the Indian subcontinent and thrives well even in the oxygen deficient muddy swamps, ponds, paddy fields, etc. due to its possession of an accessory respiratory organ. Even though it is a cherished table fish, it is considered as an undesirable wild fish especially in carp culture ponds due to its predatory habit. Farmers are keen to eradicate this otherwise valuable fish along with other wild species before introducing the preferred species. Evaluation of the acute toxicity of the seeds of *A. cocculus* using *C. batrachus* at various exposure periods has revealed its potential piscicidal activity. Being a product of biosynthesis, *A. cocculus* seeds may be used as an efficient ichthyotoxicant in aquaculture management with minimum environmental impacts.

Acknowledgement

The authors are grateful to the Annamalai University authorities for providing the laboratory facilities and to Dr. K.K.N. Nair, Scientist, Division of Botany, KFRI, Kerala, India, for identification of the plant.

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Received: 28 July 2007; Accepted: 16 April 2008