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Evaluation of the acute toxicity of the seeds of Anamirta cocculus (Linn.) and its piscicidal effect on three species of freshwater fish

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Abstract

Seeds of the Indian fish berry Anamirta cocculus (Linn.) are a potential piscicidal agent used for catching fish from the wild by native people. In the present study, laboratory evaluation of the acute toxicity of the seeds of A. cocculus was done at various time intervals on three species of freshwater predatory fishes viz., Clarias batrachus (Linn.), Channa striatus (Bloch.) and Mystus vitattus (Bloch.). The piscicidal agent was administered as a stomach poison in two different modes viz., after deep-frying and without heating. The LC50(s) and LC99(s) at different durations were calculated in both the modes of administration on all the three species tested. For C. batrachus, the LC50(s) at 1, 2, 24, 48, 72 and 96 hours (h) of piscicidal administration in the heated mode was 203.8463, 170.5797, 137.785, 100.5709, 85.4432 and 62.7660 mg/kg body weight and in the without heated mode 167.2705, 101.2334, 68.4538, 63.9773, 51.6782 and 50.2421 mg/kg body weight respectively. For C. striatus, the LC50(s) at 1, 2, 24, 48, 72 and 96 h in the heated mode was 109.2334, 77.4538, 57.9773, 44.2705, 34.6782 and 24.2421 mg/kg body weight and in the without heated mode 90.7660, 55.4433, 34.5709, 28.7685, 20.5798 and 15.3158 mg/kg body weight respectively. For M. vitattus the LC50(s) at the corresponding durations in the heated mode was 32.2706, 22.4433, 19.7686, 15.2883, 7.2517 and 3.4484 mg/kg body weight and in the without heated mode 26.8463, 17.2705, 10.0193, 7.9773, 4.9850 and 1.9082 mg/kg body weight respectively. LC99(s) in both the modes of administration in the respective exposure periods for all the three species tested were also calculated. The results reveals that of all the species tested, C. batrachus is the most resistant one towards the toxicity of A. cocculus followed by C. striatus and M. vitattus. The present study has also shown that these seeds may be used as a potent aquaculture management tool to eradicate unwanted wild fish from culture ponds before stocking.

INTRODUCTION

Since prehistoric times, cultures throughout the world have used piscicidal plants for fishing. According to ([1]), plants are virtually inexhaustible sources of structurally diverse and biologically active substances. Fossil records dates back the use of plants by human beings for various purposes including medical use at least to the middle Paleolithic age, some 60,000 years ago ([2]). Plants with insecticidial, piscicidal and molluscicidal properties have also been used widely by human beings ([3]). Fisher folks of various African countries extensively use many plants and plant products for capturing fish ([4,5]). Barbascos of ethanobotanical origin and their application in capturing fish have also been reported from other regions of the world such as South America ([6]), Nepal ([7]), India ([8,9]), etc. In addition to their use as traditional piscicidal agents for catching wild fish, plant derived fish toxicants are also used in aquaculture management for controlling the predatory and weed fishes. The eradication of these wild fishes form 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. In this aspect, the air-breathing predatory fish species are of particular importance as they are highly resistant to toxicants ([10]) and may survive in moist borrows and mud even when ponds are drained. The use of plant origin ichthyotoxicant as a fisheries management tool has been practiced in at least 30 countries ([11,12,13]). As the control and eradication of unwanted fishes in the pond require effective piscicides which are usually not easily accessible, farmers even use synthetic compounds including malachite green, sodium cyanide, antimycin, etc. and even pesticides ([14,15,16,17]). However, use of these compounds in culture ponds is seldom appreciated especially due to their long-term persistence in the ecosystem as well as in the cultured species. Therefore alternative piscicides such as botanicals, which are biologically degradable, and having piscicidal activities with shorter residual effects are being appreciated.

The seeds of the Indian fish berry Anamirta cocculus (Linn.) are one such piscicidal agent, which is used by the local people in different parts of the country for catching fish for human consumption. However, only few scientific reports regarding the piscicidal plants of India are available ([8,9]). Similarly studies regarding A. cocculus are also scanty ([18]). In this context, efforts have been made to evaluate the acute toxicity of the seeds of A. cocculus in the stomach poison mode in two ways of administration (viz., heated and without heating) using two freshwater catfishes viz., Clarias batrachus (Linn.) and Mystus vitattus (Bloch.) and the freshwater snakehead fish Channa striatus (Bloch.). While C. batrachus and C. striatus are voracious predatory air-breathing fishes causing considerable loss of yield in the culture pond, M. vitattus preys upon fry and fingerlings. It is also considered as a weed fish in culture ponds. Selection of the stomach poison mode for the administration of the toxicant is because of the fact that the rural folk utilize this route of administration of the piscicidal agent. Their routine method involves deep-frying the seeds and mixing it with minced fish feeds like earthworm, chicken intestine etc., before administration.

MATERIALS AND METHODS

FISHES AND THEIR MAINTENANCE

Healthy specimens of C. batrachus (130 ± 2 g body weight and 18 ± 1 cm length), C. striatus (78 ± 2 g body weight and 16 ± 1 cm length) and M. vitattus (15 ± 2 g body weight and 10 ± 1 cm length) were collected locally at Chidambaram, Tamilnadu and were reared separately in large plastic aquaria bearing well water. The fishes were acclimated to the laboratory condition for about 30 days (d).

FOOD AND FEEDING

The fishes were liberally fed with minced earthworm meatballs. While C. batrachus and C. striatus were fed with meatballs weighing 6 ± 0.42 g each, M. vitattus were given meatballs each weighing 3 ± 0.23 g everyday for a period of 3 hours (h), before the renewal of the medium. On average, while an individual C. batrachus consumed 8 ± 1 meatballs, an individual C. striatus had 6 ± 1 meatballs and M. vitattus consumed 4 ± 1 meatballs per feeding. Water was renewed after every 24 h with routine cleaning of the aquaria leaving no fecal mater, dead fish (if any) or unconsumed food.

PREPARATION OF THE TOXICANT

The hard shells of the dried fruits of A. cocculus were broken and the seeds were collected and were processed in two ways for administering it as stomach poison. One part of the seeds was ground well in a grinder without frying. The powdered seeds were administered in the proportion of mg/kg body weight of fish through earthworm meatballs. After preliminary experiments, A. cocculus laced meatballs for C. batrachus and C. striatus were prepared in such a way that the required concentration of the seed per fish was equally divided into 4 parts and were incorporated to the interior of 4 meatballs. For M. vitattus, the required concentration of the seed per fish was equally divided into two parts and was incorporated to the interior of two meatballs. The seeds were totally covered by the meatballs. Another part of the seeds was deep fried (heated) at 100 °C for 15 minutes and ground well. The required quantity of seed incorporated meatballs was prepared in the same way as that of the unheated seeds.

BIOASSAY

$LC_{50(s)}$ of A. cocculus as stomach poison to all the three species at various exposure periods viz., 1, 2, 24, 48, 72 and 96 h were estimated in two modes of administration (heated and without heating) following Finney's ([19]) method. In both the modes of administration, the experiments were done in triplicate for calculating the $LC_{50(s)}$ at each exposure period. For each exposure period, groups of 10 fish each were kept in plastic aquarium bearing 100 liters of well water having an ambient temperature 27 ± 1 °C, pH 7.2 ± 0.2, dissolved oxygen content 5.6 ± 0.2 mg/l, carbon dioxide 3 ± 0.2 mg/l, alkalinity 9.3 ± 0.1 ppm and total hardness 88 ± 2 ppm ([20]). Parallel controls were also kept without administering the seeds. The experimental groups were fed with the respective meatballs laced with the A. cocculus seeds 3 h prior to the renewal of the medium. Wherever needed, water in the respective sets was renewed after every 24 h. Dead fish were immediately removed and recorded. The fish was considered dead if it did not respond to prodding by a glass rode. No mortality was recorded in the control groups.

CALCULATION OF LETHAL CONCENTRATIONS AND STATISTICS

The mortality rates observed during the stipulated exposure periods were recorded and using this data various $LC_{50(s)}$ and $LC_{99(s)}$ were calculated by following Finney's ([19]) method along with the slope values. The heterogeneity of the data (if any) in each set was also checked using chi-square (χ^2) test.

RESULTS

BEHAVIOURAL CHANGES

In both the modes of administration of A. cocculus seeds, the acutely intoxicated fishes of all the three species exhibited violent swimming activities. They often came to the water surface and exhibited increased gulping activity. They also expressed highly increased opercular movements indicating respiratory distress and tried many times to jump out of the aquaria. Excessive amounts of mucus were also seen all over the body of the exposed fish. None of the control fish showed any of these behavioral changes. The dying ones in the experimental aquaria exhibited vertical positioning with head above the water surface. They also showed muscular twitching and tetany before death. Finally they lost balance, settled at the bottom of the aquaria and died. No mortality was observed in the control groups.

MORTALITY RATE (FIGS. 1 TO 6)

The average percentages of mortality of all the three species at various stages of toxicant administration in both the modes are given in Figures 1 to 6. Using these mortality rates, the $LC_{50(s)}$ and $LC_{99(s)}$ were calculated.

LETHAL CONCENTRATIONS (TABLES 1 TO 6)

The respective $LC_{50(s)}$ of A. cocculus at various stages of administration to all the three species are given in Tables 1, 3 and 5. Similarly, the respective $LC_{99(s)}$ are shown in the Tables 2, 4 and 6. The estimated $LC_{50(s)}$ lie within the 95% confidence limits and are given along with the slope and χ^2 values. $LC_{50(s)}$ are found to decrease with increasing exposure periods.

Figure 1

Figure 1: Percentage of mortality of and administered with seeds in different modes of administration at 1 h duration

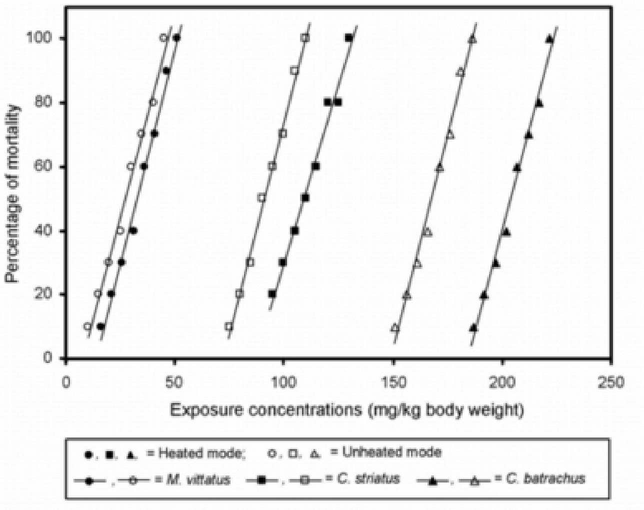

Figure 2

Figure 2: Percentage of mortality of and administered with seeds in different modes of administration at 2 h duration

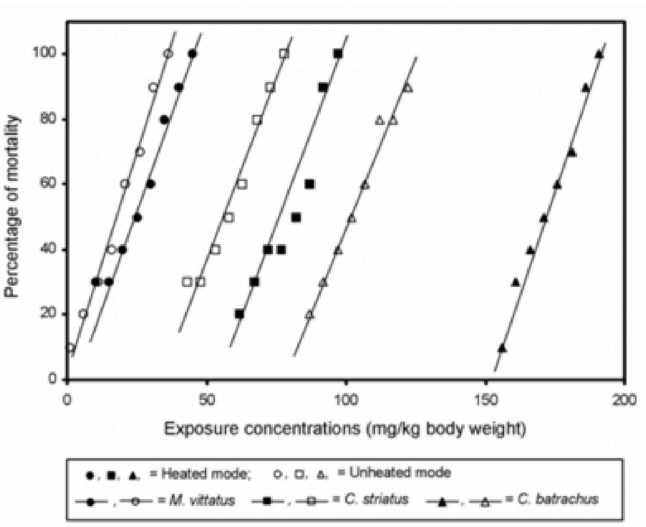

Figure 3

Figure 3: Percentage of mortality of and administered with seeds in different modes of administration at 24 h duration

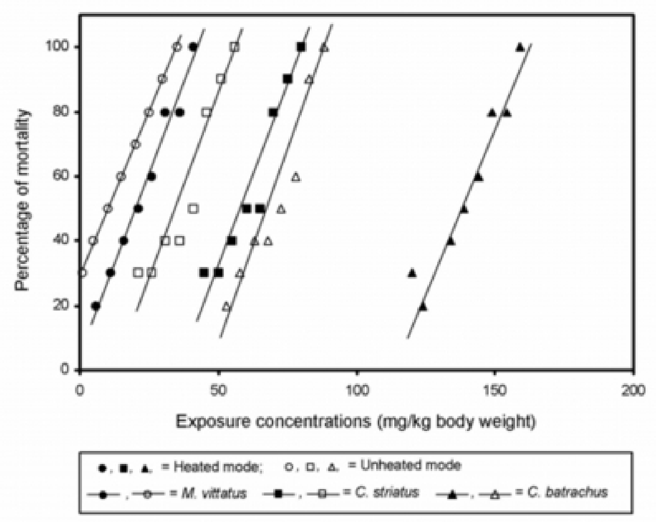

Figure 4

Figure 4: Percentage of mortality of and administered with seeds in different modes of administration at 48 h duration

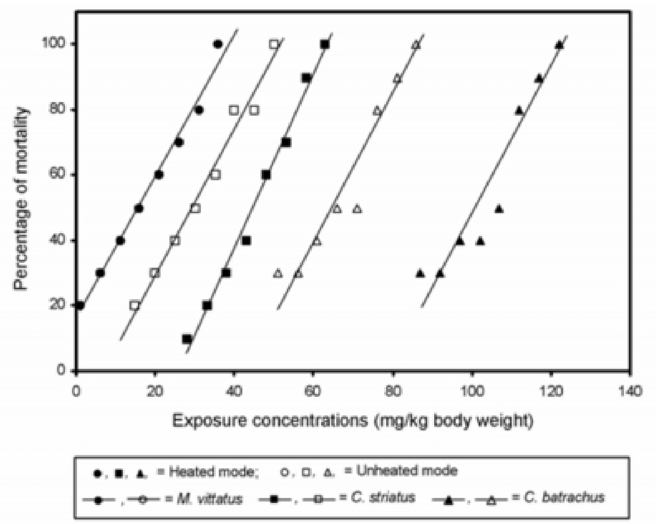

Figure 5

Figure 5: Percentage of mortality of and administered with seeds in different modes of administration at 72 h duration

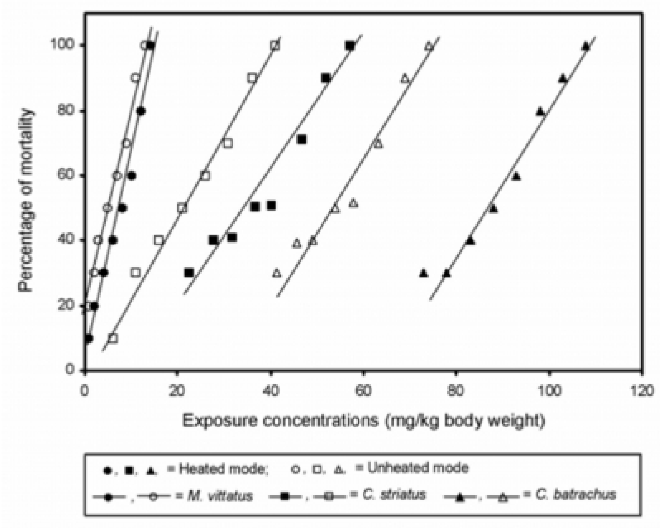

Figure 6

Figure 6: Percentage of mortality of and administered with seeds in different modes of administration at 96 h duration

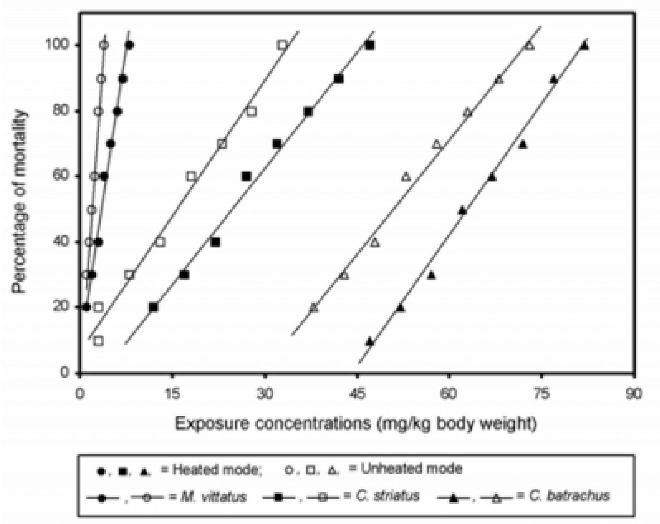

Figure 7

Table 1: LC of to in different modes of administration along with their 95% confidence limits, values and slopes at various time intervals

Duration (h)	$LC_{50(t)}$	Lower limit	Upper limit	χ^2	Slope
			Heated mode		
1	32.2706	28.1827	36.2607	1.209	1.4818
2	22.4433	16.4697	27.0587	2.015	2.4040
24	19.7686	14.1192	24.4129	1.500	2.4282
48	15.2883	9.4326	20.1736	1.480	2.6263
72	7.2517	5.5517	9.0479	1.986	2.7365
96	3.4484	2.3837	4.2876	0.669	3.2237
			Unheated mode		
1	26.8463	22.5109	31.1226	1.252	1.7116
2	17.2705	13.1826	21.2607	1.209	2.0345
24	10.0193	2.5680	14.7571	0.833	2.1549
48	7.9773	5.5878	9.8234	2.015	3.0154
72	4.9850	3.1298	6.5851	1.402	3.1723
96	1.9082	1.4408	2.3056	1.215	3.6729

Figure 8

Table 2: LC of to in different modes of administration along with their 95% confidence limits at various times intervals

Duration (h)	$LC_{99(t)}$	Lower limit	Upper limit
		Heated mode	
1	59.1996	51.1677	76.3458
2	52.6965	43.4398	75.3538
24	49.9844	40.6332	72.6054
48	44.4374	35.0965	67.6862
72	19.1769	15.4113	27.7456
96	9.5283	7.7538	13.6269
		Unheated mode	
1	56.0254	47.1430	75.6352
2	44.1996	36.1677	61.3459
24	38.6947	30.0507	61.0949
48	21.6849	17.5296	31.9842
72	16.5236	12.9714	25.0918
96	4.7635	3.9034	6.7756

Figure 9

Table 3: LC of to in different modes of administration along with their 95% confidence limits, values and slopes at various time intervals

Duration (h)	$LC_{50(t)}$	Lower limit	Upper limit	χ^2	Slope
			Heated mode		
1	109.2334	102.7855	114.4227	0.314	1.1733
2	77.4538	72.0662	82.3884	3.664	1.2291
24	57.9773	51.9909	62.7139	3.170	1.3206
48	44.2705	40.1826	48.2607	1.209	1.3214
72	34.6782	27.5806	39.8982	3.475	1.7734
96	24.2421	18.9185	28.4381	0.669	1.9123
			Unheated mode		
1	90.7660	86.5856	94.7384	1.129	1.1435
2	55.4433	49.4696	60.0586	2.015	1.3277
24	34.5709	28.8047	39.3330	3.890	1.6388
48	28.7685	23.1192	33.4128	1.500	1.8289
72	20.5798	15.8212	24.7901	1.736	2.2449
96	15.3158	11.3220	19.3548	1.590	3.3179

Figure 10

Table 4: LC of to in different modes of administration along with their 95% confidence limits at various times intervals

Duration (h)	$LC_{99(t)}$	Lower limit	Upper limit
		Heated mode	
1	148.0767	135.6771	181.0881
2	112.7723	101.5124	140.9403
24	93.1683	82.3266	120.4719
48	71.1996	63.1677	88.3459
72	61.1681	52.3813	84.5416
96	54.6415	45.7690	75.1345
		Unheated mode	
1	117.9028	109.8568	135.1754
2	89.7121	79.3238	115.4601
24	69.6592	58.7701	96.8861
48	62.9951	52.5207	88.6341
72	50.5164	41.5626	70.8329
96	42.5939	34.2321	61.0317

Figure 11

Table 5: LC of to in different modes of administration along with their 95% confidence limits, values and slopes at various time intervals

Duration (h)	$LC_{50(s)}$	Lower limit	Upper limit	χ^2	Slope
	Heated mode				
1	203.8463	199.5109	208.1226	1.252	1.0660
2	170.5797	165.8212	174.7901	1.736	1.0815
24	137.7685	132.1191	142.4128	1.500	1.1176
48	100.5709	94.8047	105.3330	3.890	1.1697
72	85.4432	79.4696	90.0586	2.015	1.1981
96	62.7660	58.5856	66.7384	1.129	1.2156
	Unheated mode				
1	167.2705	163.1826	171.2607	1.209	1.0745
2	101.2334	94.7855	106.4227	0.314	1.1886
24	68.4538	63.0662	73.3884	3.664	1.2642
48	63.9773	57.9909	68.7139	3.170	1.2849
72	51.6782	44.5806	56.8982	3.475	1.4304
96	50.2421	44.9185	54.4381	0.669	1.4593

Figure 12

Table 6: LC of to in different modes of administration along with their 95% confidence limits at various times intervals

Duration (h)	$LC_{99(s)}$	Lower limit	Upper limit
	Heated mode		
1	233.0254	224.1430	252.6352
2	200.5164	191.5626	220.8329
24	171.9951	161.5207	197.6342
48	135.6592	124.7701	162.8862
72	119.7122	109.3239	145.4604
96	89.9028	81.8568	107.1754
	Unheated mode		
1	194.1996	186.1677	211.3459
2	140.0767	127.6771	173.0880
24	103.7723	92.5124	131.9403
48	99.1684	88.3266	126.4720
72	91.3140	78.6030	126.7824
96	80.6415	71.7690	101.1345

DISCUSSION

From time immemorial, humans explored ways and means to divert poisons of biological origin to their own advantage. The pandemic need to find plants that work well as soap has been pursued by most native cultures and this experience of using various plants for their soap like properties might have lead to the universal discovery that chemicals released from some plants would also stun fish when used in a specific circumstance ([21]). A. cocculus (Linn.) is commonly known as fish berry or crow killer. It is a wild woody climber producing poisonous seeds that are being exploited by human beings for several purposes including hunting and fishing ([22]). The dried berries of A. cocculus have been used in India to stupefy fish ([23]) and are reported to contain picrotoxin ([24]), which being a product of biosynthesis are liable to potential degradation. As in many other cases, native fishermen or local people might have identified the piscicidal property of A. cocculus also. However, the traditional fishermen are not aware of the precise quantity of the seeds required to kill fish belonging to any particular species and therefore they usually apply much higher quantities than the optimum such liberal applications seems to be an option, which is ecologically unhealthy.

On the other hand, predatory and weed fishes pose serious threats to the cultured species and therefore their control is an essential part of the pond management. The problem becomes more aggravated in the case of the predatory fishes like C. batrachus and C. striatus as they possess accessory respiratory organs, which facilitate their survival even under extreme environmental conditions. According to Chiayvareesajja et al. ([25]) air breathing species are more tolerant to piscicidal materials and therefore the piscicidal activity should be tested in such species. Critical analysis of the $LC_{50(s)}$ and $LC_{99(s)}$ (Tables 1 to 6) reveals that C. batrachus is the most resistant of all the three species tested, followed by C. striatus and M. vitattus. The higher $LC_{50(s)}$ in case of C. batrachus and C. striatus could be attributed to the presence of accessory respiratory organs in them and this observation is in confirmation with the view of Chiayavareesajja et al. ([25]). Therefore, the advantage of selecting C. batrachus as one of the test organisms is that the concentration of the toxicant, which wood kill this species could wipe out almost all the unwanted species of fishes, provided the pond is treated before stocking.

Usually, for harvesting fish from the wild using plant origin piscicides, the plant part to be used as piscicide is thoroughly pounded and the macerated material is thrown into the water body from which the fishes are to be harvested. In such situations, the toxicants act as contact poisons and therefore are more active in standing water bodies. However, in case of A. cocculus, its traditional use as stomach poison facilitates its application in standing as well as running waters to catch fish. It may also be mentioned that the use of the piscicidal agents as contact poisons in natural aquatic ecosystems could probably cause the loss of non-target biodiversity in those ecosystems. In the present study, the highly cohesive mucous content of the earthworm balls holds the seeds well inside the meatballs and thereby prevents the spillage of the seeds directly into medium to a great extend. The mode of administration using the deep fried seeds in the present study is exactly in accordance with the method followed by the native people. However, by also administering the seeds without heating, a comparative assessment of the toxicities of the raw seeds and cooked seeds is made (Tables 1 to 6). The results indicate that the raw seeds are more poisonous than cooked ones as the former is having low $LC_{50(s)}$ at various time intervals. Even though the toxicity of the seeds is noticeably reduced after frying, the seeds become more acceptable to fish. This could be one of the main reasons why the local people fry the seeds before using it as a barbasco. Further, by heating, perhaps some of the active ingredients in the seeds might be getting degraded leading to increase in $LC_{50(s)}$. Such a heat-attenuated toxicity would be a desirable factor as the fish caught is traditionally used for human consumption.

According to Cagauan et al. ([3]), concentration causing 100% mortality forms the basis of calculating the piscicidal activity of the test plants. Banerjee ([26]) is of the view that determination of LC_{50} is essential for acute toxicity testing and also for routine bioassay experiments. In view of these reports, the present study provides a range of $LC_{50(s)}$ and $LC_{99(s)}$ of A. cocculus at various durations. As a fishing poison, the knowledge of $LC_{50(s)}$ and $LC_{99(s)}$ at various exposure periods would provide more provisions to the farmers in case they want to kill/eradicate the predatory and weed fishes within a convenient duration from culture ponds before stocking. Further, the mortality rates observed in the present study suggests a clear relationship between dose, mortality and exposure period. The concentration of the toxicant is directly proportional to the mortality rate. On the other hand, as the duration of exposure increases the lethal concentration decreases. As the calculated χ^2 values (representing heterogeneity) are less than the respective table values, the mortality counts are not significantly heterogeneous indicating variables such as individual resistance, etc. do not significantly affect the lethal concentrations as they lie within 95% confidence limits. The steepness of the slope is also an indication of large increase in mortality rate with relatively small increase in the concentration of the piscicidal agent.

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