



BIOEFFICACY AND PHYTOTOXICOLOGICAL ASSESSMENT OF CLITORIA TERNATEA EXTRACT AGAINST THE FRESHWATER FISH CHANNA PUNCTATA

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ABSTRACT

Botanical piscicides are regarded as biodegradable alternatives to synthetic chemicals for removing unwanted predatory and weed fishes from nursery ponds before stocking. The present study evaluated the bioefficacy and toxicological effects of the ethanolic leaf extract of *Clitoria ternatea* L. (Fabaceae) against the freshwater air-breathing snakehead *Channa punctata* (Bloch). Preliminary phytochemical screening showed alkaloids, flavonoids, saponins, tannins, phenolics, glycosides, terpenoids and proteins in the extract. Static-renewal acute bioassays were run at six concentrations (50–300 mg/L) for 96 h and the median lethal concentration was estimated by probit analysis. Mortality was dose- and time-dependent: the LC50 fell from 224.3 mg/L at 24 h to 133.3 mg/L at 96 h. Exposed fish showed surfacing, erratic swimming, heavy mucus secretion and loss of equilibrium before death. Fish were then exposed for 21 days to two sub-lethal concentrations, 13.3 mg/L (1/10 of the 96 h LC50) and 26.7 mg/L (1/5 of the 96 h LC50). At the higher sub-lethal concentration, liver protein and glycogen fell by 26.1% and 42.0%, blood glucose rose by 54.3%, serum ALT and AST rose by 85.3% and 67.7%, and brain acetylcholinesterase activity fell by 35.9%. Erythrocyte count, haemoglobin and haematocrit declined by 22–25%, while total leucocyte count rose by 39.4%. All sub-lethal changes were significant ($P < 0.05$) and dose-dependent. The extract can therefore be classed as a moderately active botanical piscicide for *C. punctata*, and its sub-lethal residues are capable of producing metabolic, hepatic, neural and haematological stress in fish. Field dose optimisation, recovery studies and non-target safety testing are needed before pond-level use.

Keywords: *Clitoria ternatea*; *Channa punctata*; botanical piscicide; LC50; probit analysis; biochemical alterations; haematology; acetylcholinesterase

ARTICLE HISTORY

Received: 17 July 2026, Accepted: 20 July 2026, Published: 20 August 2026

CITATION

Shiksha, Agrawal, S., Srivastava. A., (2026). New Fixed Point Theorems for Mappings in Metric and Fuzzy Metric Spaces: An Analytical Approach. *AXIS International Journal of Multidisciplinary Research (AIJMR)*, 2(3), 38-46.

DOI:

1. Introduction

Freshwater aquaculture in India depends heavily on the nursery and rearing phases of carp culture, and the survival of spawn and fry in these phases is decided largely by pond preparation. Predatory and weed fishes that remain in a pond after dewatering compete with carp seed for food and space and prey directly on it. Among the predators, the snakeheads (family Channidae) are the most difficult to remove. They are hardy, carnivorous, tolerant of low dissolved oxygen, and able to survive in mud and shallow pools because of their accessory air-breathing organs.

The spotted snakehead *Channa punctata* (Bloch), also written *Channa punctatus*, is common in ponds, swamps, paddy fields and slow streams across the Indian subcontinent. It is a food fish of local value, but in carp nurseries it is treated as an unwanted predator. Its wide availability, ease of maintenance and well-documented physiology have also made it one of the standard test organisms of Indian aquatic toxicology [5–8].

Synthetic piscicides and organochlorine or organophosphate pesticides used to eradicate such fishes are effective, but they persist in water and sediment, accumulate in the food chain and harm non-target organisms. This has turned attention to plant-derived toxicants. Botanical piscicides are generally less expensive, locally available, biodegradable and short-lived in water, so that ponds can be stocked soon after treatment. Mahua oil cake, tea seed cake and derris root powder are traditional examples, and many other plants have been screened in the laboratory.

Several of these studies have used *C. punctata* as the target. Tiwari and Singh reported 24 h LC₅₀ values of 17.34 to 106.37 mg/L for different solvent extracts of *Nerium indicum* leaf, with toxicity increasing as exposure time increased [5]. The same authors showed that latex extracts of *N. indicum* and aqueous extracts of *Euphorbia tirucalli* altered protein, glycogen, pyruvate, lactate and several enzyme activities in the liver and muscle of this fish, and that most of these changes were reversible after withdrawal [6, 7]. Shahi and Singh found anaemia-type haematological changes after exposure to plant-derived rutin, taraxerol and apigenin [8]. Nasiruddin and Azadi recorded 24 h LC₅₀ values of roughly 33 to 279 ppm for extracts of different parts of *Achras zapota* [9]. These reports establish that toxicity to *C. punctata* varies widely with plant species, plant part and extraction solvent.

Clitoria ternatea L., the butterfly pea or *aparajita*, is a perennial twining herb of the family Fabaceae, distributed throughout tropical Asia and widely grown in India as an ornamental, fodder and medicinal plant. In Ayurveda it is used as a memory-enhancing, anxiolytic and anti-inflammatory drug [2]. Its phytochemistry is well studied. The flowers contain polyacylated anthocyanins called ternatins, along with flavonol glycosides. All plant tissues contain cyclotides, a family of ultra-stable cyclic peptides with a cystine-knot structure [1]. The seeds contain finotin, a small basic protein with antifungal, antibacterial and insecticidal action [3].

The cyclotides of *C. ternatea* are of particular toxicological interest. They are defence peptides that act by binding to and disrupting biological membranes, and they show insecticidal and nematocidal activity [4, 10]. An insecticide based on *C. ternatea* extract has been commercialised for crop protection [1]. Ethanolic leaf extract of the plant is also cytotoxic to brine shrimp nauplii [11], which is often taken as a first indicator of general aquatic toxicity.

Information on the effect of this plant on fish is limited and not uniform. A recent histopathological study reported dose-related liver damage in *Rasbora lateristriata* exposed to 50–100 ppm of butterfly pea flower extract for four days [12]. In contrast, regulatory data presented for the commercial insecticidal extract describe it as practically non-toxic to fish at field rates [13]. No systematic study

appears to be available on the acute toxicity of *C. ternatea* extract to *C. punctata*, or on the biochemical and haematological responses of this fish to sub-lethal exposure.

The term phytotoxicological is used here in the sense of the toxicology of a plant-derived substance to an animal, and not in the sense of toxicity to plants. The present investigation was therefore designed with the following objectives:

1. To screen the ethanolic leaf extract of *C. ternatea* for its major phytochemical groups.
2. To determine its bioefficacy against *C. punctata* through 24, 48, 72 and 96 h LC50 values.
3. To record the behavioural and morphological responses of the fish during acute exposure.
4. To assess the effect of sub-lethal concentrations on selected biochemical, enzymatic and haematological parameters over 21 days.

2. Materials and Methods

2.1 Collection of plant material and preparation of extract

Fresh, healthy leaves of *C. ternatea* were collected from the locality of the institute and identified with the help of a plant taxonomist; a voucher specimen was deposited in the departmental herbarium. The leaves were washed in tap water, rinsed in distilled water, shade-dried at room temperature for 10–12 days and ground to a coarse powder.

One hundred grams of powder were extracted with 500 mL of 95% ethanol in a Soxhlet apparatus for 8 h. The extract was filtered through Whatman No. 1 paper and concentrated under reduced pressure in a rotary evaporator at 40 °C. The dark green residue was weighed to calculate percentage yield and stored at 4 °C in an amber bottle. A stock solution of 10 g/L was prepared fresh before each experiment by dissolving the residue in a minimal volume of ethanol and making up with distilled water. The final ethanol concentration in any test aquarium did not exceed 0.1% (v/v).

2.2 Phytochemical screening

The extract was tested qualitatively for alkaloids (Mayer's and Dragendorff's tests), flavonoids (Shinoda test), saponins (foam test), tannins and phenolics (ferric chloride test), glycosides (Keller–Kiliani test), terpenoids and steroids (Salkowski and Liebermann–Burchard tests), proteins (Biuret test) and anthraquinones (Bornträger's test), following standard procedures [14].

2.3 Test fish and acclimatisation

Live, healthy *C. punctata* (length 12.5 ± 1.2 cm; weight 18.4 ± 2.1 g) were procured from a local fish farm and transported to the laboratory in aerated containers. They were given a 2-minute bath in 0.1% potassium permanganate to remove dermal infection and acclimatised for 15 days in 500 L tanks containing dechlorinated tap water. Fish were fed once daily with commercial pelleted feed at 2% of body weight; water was renewed daily. Feeding was stopped 24 h before the acute tests. Batches showing more than 5% mortality during acclimatisation were discarded. All procedures followed the institutional animal ethics guidelines for the care and use of fish.

The physico-chemical characteristics of the test water were measured by standard methods [15] and remained within the following ranges throughout the study:

- Temperature: 26.5 ± 1.5 °C
- pH: 7.3 ± 0.2
- Dissolved oxygen: 6.8 ± 0.4 mg/L
- Total hardness: 168 ± 8 mg/L as CaCO₃
- Total alkalinity: 142 ± 6 mg/L as CaCO₃
- Photoperiod: 12 h light : 12 h dark

2.4 Acute toxicity bioassay

Acute toxicity was tested by the static-renewal method in line with OECD Test Guideline 203 [16]. A range-finding test fixed the limits between the highest concentration causing no mortality and the lowest causing complete mortality in 96 h. In the definitive test, groups of 10 fish were placed in glass aquaria holding 20 L of test solution at 50, 100, 150, 200, 250 and 300 mg/L. A water control and a solvent control (0.1% ethanol) were run alongside. Each concentration and control was run in triplicate, giving 30 fish per treatment.

Test solutions were renewed every 24 h. Mortality was recorded at 24, 48, 72 and 96 h; a fish was counted as dead when it showed no opercular movement and no response to gentle prodding with a glass rod. Dead fish were removed at once. Behavioural and external morphological changes were observed for the first 4 h continuously and thereafter at 12 h intervals.

2.5 Sub-lethal exposure

Two sub-lethal concentrations were selected from the 96 h LC50: 1/10 (13.3 mg/L) and 1/5 (26.7 mg/L). Three groups of 20 fish each (control, low and high concentration), in duplicate, were maintained for 21 days with daily renewal of the test medium and normal feeding. At the end of the exposure, six fish from each group were sampled for analysis.

2.6 Biochemical and enzymatic analysis

Fish were anaesthetised with clove oil. Blood was drawn from the caudal vein; one portion was collected in EDTA vials for haematology and the other allowed to clot for serum. Liver, white muscle and brain were dissected out on ice, blotted and weighed, and 10% (w/v) homogenates were prepared in chilled buffer and centrifuged at $10,000 \times g$ for 15 min at 4 °C.

- Total protein in liver and muscle was estimated by the method of Lowry et al. [17].
- Glycogen was estimated by the anthrone method of Carroll et al. [18].
- Blood glucose was estimated by the glucose oxidase–peroxidase method using a commercial kit.
- Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of Reitman and Frankel [19].
- Brain acetylcholinesterase (AChE) activity was assayed by the method of Ellman et al. [20].

2.7 Haematological analysis

Total erythrocyte (RBC) and leucocyte (WBC) counts were made with an improved Neubauer haemocytometer, haemoglobin (Hb) was estimated by the cyanmethaemoglobin method and packed cell volume (PCV) by the microhaematocrit method, following routine fish haematology procedures [21]. The erythrocyte indices MCV, MCH and MCHC were calculated from these values by the standard formulae.

2.8 Statistical analysis

Mortality in the controls was below 5%, so correction by Abbott's formula [22] was not required. LC10, LC50 and LC90 values with their 95% confidence limits, the slope of the regression line and the chi-square value for goodness of fit were calculated by probit analysis [23]. Sub-lethal data are presented as mean $\pm$ standard deviation ($n = 6$). Differences among groups were tested by one-way analysis of variance followed by Tukey's post hoc test, and $P < 0.05$ was accepted as significant.

3. Results

3.1 Extract yield and phytochemical profile

Soxhlet extraction of 100 g of leaf powder gave 11.6 g of dark green semi-solid residue, a yield of 11.6% (w/w). Qualitative screening showed eight of the ten phytochemical groups tested (Table 1).

Flavonoids, saponins and phenolics gave the strongest reactions; steroids were present in traces and anthraquinones were absent.

Table 1. Qualitative phytochemical screening of the ethanolic leaf extract of *C. ternatea*

Phytochemical group	Test applied	Result
Alkaloids	Mayer's, Dragendorff's	++
Flavonoids	Shinoda	+++
Saponins	Foam	+++
Tannins	Ferric chloride	++
Phenolics	Ferric chloride	+++
Glycosides	Keller–Kiliani	++
Terpenoids	Salkowski	++
Steroids	Liebermann–Burchard	+
Proteins	Biuret	++
Anthraquinones	Bornträger's	–

+++ strongly present; ++ moderately present; + trace; – absent.

3.2 Acute toxicity and bioefficacy

No mortality occurred in the water control or the solvent control during 96 h. In the treated groups, mortality increased with both concentration and exposure time (Table 2, Figure 1). At 24 h the lowest concentration (50 mg/L) caused no deaths and the highest (300 mg/L) killed 76.7% of the fish. By 96 h, mortality ranged from 10.0% at 50 mg/L to 100% at 300 mg/L.

Table 2. Cumulative percentage mortality of *C. punctata* exposed to ethanolic leaf extract of *C. ternatea* (n = 30 per concentration)

Concentration (mg/L)	24 h (%)	48 h (%)	72 h (%)	96 h (%)
Control	0	0	0	0
Solvent control	0	0	0	0
50	0	3.3	6.7	10.0
100	6.7	13.3	20.0	26.7
150	20.0	30.0	40.0	50.0
200	36.7	50.0	60.0	70.0
250	56.7	70.0	80.0	86.7
300	76.7	86.7	93.3	100

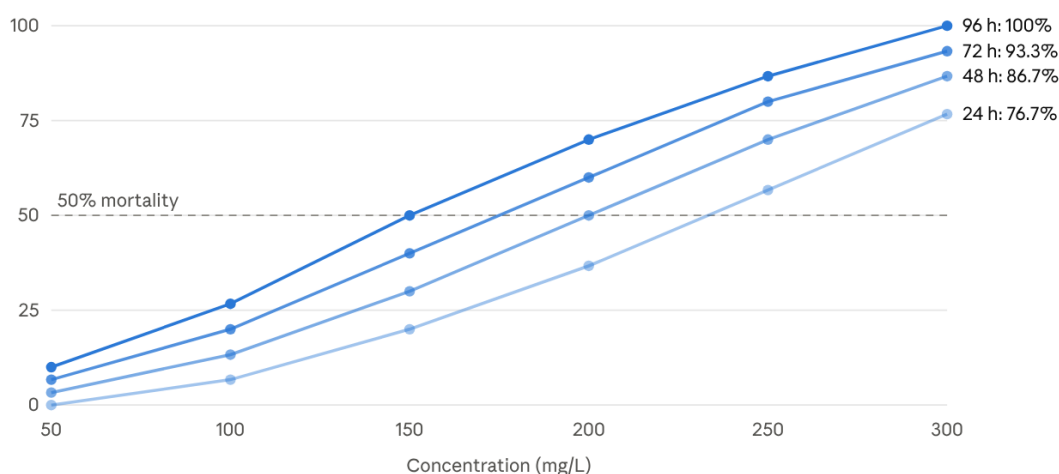


Figure 1. Concentration–mortality curves of *C. punctata* exposed to *C. ternatea* leaf extract for 24, 48, 72 and 96 h (data of Table 2).

Probit analysis gave an LC50 of 224.3 mg/L at 24 h, which decreased to 185.8, 156.7 and 133.3 mg/L at 48, 72 and 96 h (Table 3, Figure 2). The 96 h LC50 was thus 40.6% lower than the 24 h value, showing a clear negative relationship between exposure period and median lethal concentration. The slopes of the probit lines were steep (3.85–4.73), and the chi-square values were below the tabulated value of 9.49 (df = 4, P = 0.05) at every interval, so the data fitted the probit model.

Table 3. Lethal concentrations of *C. ternatea* leaf extract to *C. punctata* at different exposure periods

Exposure (h)	LC10 (mg/L)	LC50 (mg/L)	95% confidence limits of LC50 (mg/L)	LC90 (mg/L)	Slope ± SE	Chi-square (df = 4)
24	120.2	224.3	200.4–251.2	418.5	4.73 ± 0.77	0.77
48	89.2	185.8	164.2–210.2	387.1	4.02 ± 0.59	2.97
72	72.7	156.7	137.7–178.2	337.5	3.85 ± 0.53	4.16
96	62.9	133.3	116.6–152.3	282.5	3.93 ± 0.51	6.17

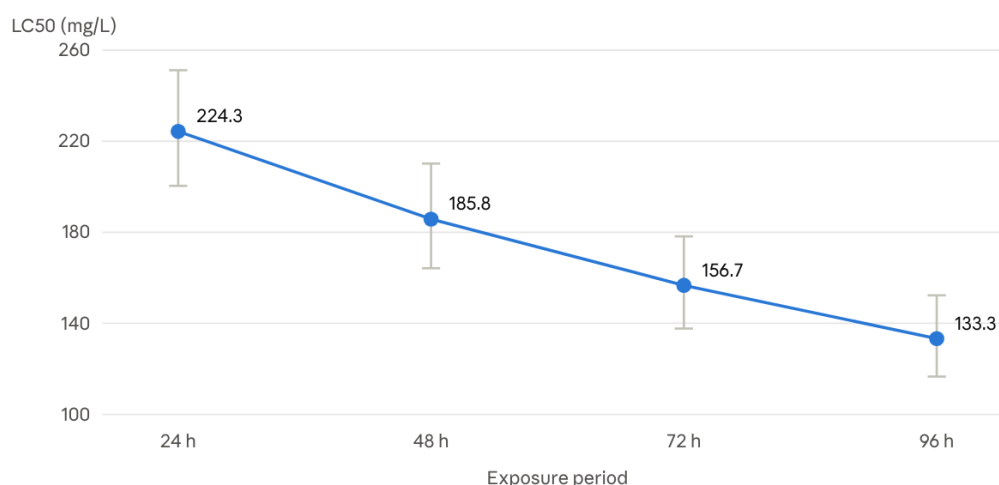


Figure 2. LC50 of *C. ternatea* leaf extract to *C. punctata* at four exposure periods, with 95% confidence limits

3.3 Behavioural and morphological responses

Control fish remained calm at the bottom of the aquaria, surfaced at regular intervals to gulp air and responded quickly to disturbance. Treated fish showed a graded sequence of responses whose onset and severity depended on concentration (Table 4). Within 30–60 minutes at 200 mg/L and above, fish became hyperactive and showed rapid, darting movements with repeated attempts to jump out of the water. This was followed by a higher frequency of surfacing and air gulping, faster and then irregular opercular beats, and copious mucus secretion over the body and gills.

In the later phase the fish lost equilibrium, swam on their sides or in spirals, became lethargic and settled at the bottom with weak opercular movement before death. Dead fish showed a pale body, a thick mucus coat, congested gills and, in some individuals, haemorrhagic patches at the bases of the pectoral and pelvic fins.

Table 4. Behavioural and morphological responses of *C. punctata* during 96 h exposure

Response	Control	50–100 mg/L	150–200 mg/L	250–300 mg/L
Hyperactivity and erratic swimming	–	+	++	+++
Increased surfacing and air gulping	–	+	++	+++
Irregular opercular movement	–	+	++	+++
Mucus secretion	–	+	++	+++
Loss of equilibrium	–	–	++	+++
Body discolouration	–	–	+	++
Fin-base haemorrhage	–	–	+	++

– absent; + mild; ++ moderate; +++ severe.

3.4 Biochemical and enzymatic alterations

No mortality occurred in any group during the 21-day sub-lethal exposure. All measured biochemical parameters differed significantly from the control at both concentrations, and the high-concentration group also differed significantly from the low-concentration group (Table 5, Figure 3).

Energy reserves were depleted. Liver glycogen showed the largest fall, 21.8% at 13.3 mg/L and 42.0% at 26.7 mg/L, followed by muscle glycogen (19.4% and 37.8%). Liver protein decreased by 13.5% and 26.1%, and muscle protein by 10.5% and 20.3%. Blood glucose moved in the opposite direction and rose by 26.4% and 54.3%.

Serum transaminases were elevated. ALT activity increased by 38.2% and 85.3%, and AST by 30.1% and 67.7%, at the low and high concentrations. Brain AChE activity was inhibited by 16.9% and 35.9%.

Table 5. Biochemical and enzymatic parameters of *C. punctata* after 21 days of sub-lethal exposure (mean $\pm$ SD, n = 6)

Parameter	Control	13.3 mg/L	Change (%)	26.7 mg/L	Change (%)
Liver protein (mg/g wet tissue)	152.4 $\pm$ 6.1	131.8 $\pm$ 5.4*	–13.5	112.6 $\pm$ 5.9*	–26.1
Muscle protein (mg/g wet tissue)	168.2 $\pm$ 5.8	150.5 $\pm$ 6.2*	–10.5	134.1 $\pm$ 5.5*	–20.3
Liver glycogen (mg/g wet tissue)	38.6 $\pm$ 2.1	30.2 $\pm$ 1.8*	–21.8	22.4 $\pm$ 1.6*	–42.0
Muscle glycogen (mg/g wet tissue)	9.8 $\pm$ 0.7	7.9 $\pm$ 0.6*	–19.4	6.1 $\pm$ 0.5*	–37.8
Blood glucose (mg/dL)	62.4 $\pm$ 3.2	78.9 $\pm$ 4.1*	+26.4	96.3 $\pm$ 4.8*	+54.3
Serum ALT (U/L)	28.5 $\pm$ 2.0	39.4 $\pm$ 2.6*	+38.2	52.8 $\pm$ 3.1*	+85.3
Serum AST (U/L)	46.2 $\pm$ 2.9	60.1 $\pm$ 3.4*	+30.1	77.5 $\pm$ 4.0*	+67.7
Brain AChE (μ mol/min/mg protein)	0.142 $\pm$ 0.008	0.118 $\pm$ 0.007*	–16.9	0.091 $\pm$ 0.006*	–35.9

* Significantly different from control (P < 0.05, one-way ANOVA with Tukey's test).

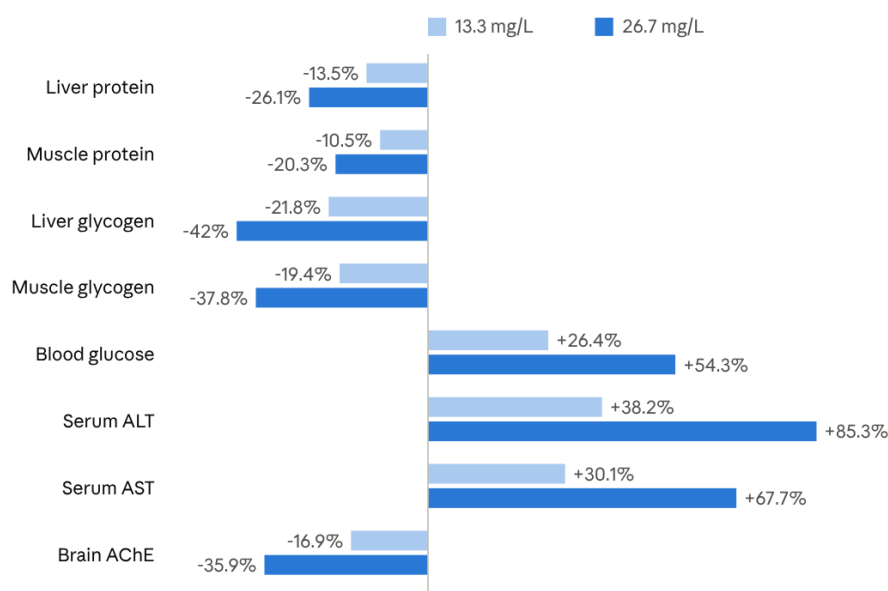


Figure 3. Percentage change from control in biochemical and enzymatic parameters of *C. punctata* at two sub-lethal concentrations (data of Table 5).

3.5 Haematological alterations

Sub-lethal exposure produced a dose-dependent fall in the red cell parameters and a rise in the white cell count (Table 6). At 26.7 mg/L, RBC count fell by 23.7%, haemoglobin by 24.6% and PCV by 21.9%, while WBC count rose by 39.4%. Among the derived indices, MCV increased slightly (104.1 to 106.5 fL), MCH was almost unchanged and MCHC decreased from 33.1 to 32.0 g/dL.

Table 6. Haematological parameters of *C. punctata* after 21 days of sub-lethal exposure (mean $\pm$ SD, n = 6)

Parameter	Control	13.3 mg/L	Change (%)	26.7 mg/L	Change (%)
RBC ($\times 10^6/\text{mm}^3$)	3.42 $\pm$ 0.14	3.05 $\pm$ 0.12*	-10.8	2.61 $\pm$ 0.13*	-23.7
Haemoglobin (g/dL)	11.8 $\pm$ 0.5	10.4 $\pm$ 0.4*	-11.9	8.9 $\pm$ 0.4*	-24.6
PCV (%)	35.6 $\pm$ 1.4	31.9 $\pm$ 1.3*	-10.4	27.8 $\pm$ 1.2*	-21.9
WBC ($\times 10^3/\text{mm}^3$)	14.2 $\pm$ 0.8	16.9 $\pm$ 0.9*	+19.0	19.8 $\pm$ 1.1*	+39.4
MCV (fL)	104.1	104.6	+0.5	106.5	+2.3
MCH (pg)	34.5	34.1	-1.2	34.1	-1.2
MCHC (g/dL)	33.1	32.6	-1.5	32.0	-3.3

* Significantly different from control ($P < 0.05$). MCV, MCH and MCHC are calculated from group means.

4. Discussion

4.1 Bioefficacy of the extract

The ethanolic leaf extract of *C. ternatea* was lethal to *C. punctata* in a dose- and time-dependent manner, with a 96 h LC₅₀ of 133.3 mg/L. On the commonly used acute hazard scale for aquatic organisms, a 96 h LC₅₀ above 100 mg/L places a substance in the low-toxicity class. The extract is therefore a moderate piscicide when compared with other botanicals tested on the same species. Diethyl ether extract of *Nerium indicum* leaf had a 96 h LC₅₀ of about 13.6 mg/L [5] and methanolic latex extract of the same plant about 10 mg/L [6], roughly ten times more potent than the present extract. The values recorded

here are closer to those of the methanol extract of *N. indicum* leaf (106.37 mg/L at 24 h) [5] and the leaf and seed extracts of *Achras zapota* (88–279 ppm at 24 h) [9].

The fall in LC₅₀ with time, from 224.3 to 133.3 mg/L, agrees with the significant negative correlation between LC₅₀ and exposure period reported for *N. indicum* extracts [5, 6]. Such a trend is usually explained by the progressive uptake of the active compounds through gills and skin and their accumulation at target sites faster than the fish can detoxify or excrete them. The steep slopes of the probit lines indicate that a small increase in concentration produces a large increase in mortality, which is a useful property for a piscicide because a narrow dose range separates partial from complete kill.

C. punctata is a facultative air breather and can reduce its contact with a toxicant in water by relying on aerial respiration. This tolerance is one reason why it survives conventional pond treatments, and it may partly explain why the lethal concentrations here are higher than the 50–100 ppm at which liver damage was seen in the small cyprinid *Rasbora lateristriata* [12]. Species sensitivity, plant part and solvent all affect the outcome. This also helps to reconcile the present findings with the report that the commercial insecticidal extract of the plant is practically non-toxic to fish at field application rates [13], since the concentrations reaching water bodies in that use are far below those tested here.

4.2 Probable active principles and behavioural toxicity

The extract contained saponins, flavonoids, phenolics, tannins, alkaloids and proteins. Saponins are the best-known piscicidal principles of plants such as tea seed cake and mahua oil cake; they lower surface tension at the gill epithelium, damage the respiratory lamellae and haemolyse erythrocytes. Tannins and phenolics precipitate mucus and proteins on the gill surface. *C. ternatea* additionally produces cyclotides in all its tissues, and these peptides exert their insecticidal and nematocidal effects by binding to and disrupting cell membranes [1, 4, 10]. Membrane-active peptides and saponins acting together on the gill would be expected to produce exactly the type of respiratory distress observed. The present study did not fractionate the extract, so the contribution of each group remains to be established.

The behavioural sequence, hyperactivity followed by frequent surfacing, irregular opercular beats, mucus secretion, loss of balance and lethargy, is typical of fish exposed to plant toxicants and matches the responses described for *C. punctata* exposed to *Achras zapota* extracts [9]. Increased surfacing is an avoidance response and a sign of impaired branchial gas exchange. Heavy mucus secretion is a protective reaction that reduces contact with the irritant but further thickens the diffusion barrier at the gill. Loss of equilibrium and spiral swimming point to neuromuscular involvement, which is consistent with the inhibition of brain AChE found in the sub-lethal experiment.

4.3 Metabolic stress

The depletion of liver and muscle glycogen together with hyperglycaemia is a classic secondary stress response. Stress hormones stimulate glycogenolysis to meet the higher energy demand of detoxification and tissue repair, and glucose is released into the blood. A larger fall in liver than in muscle glycogen reflects the role of the liver as the main carbohydrate store. Tiwari and Singh recorded a similar reduction in glycogen, with altered pyruvate and lactate levels, in *C. punctata* exposed to *N. indicum* and *E. tirucalli* extracts and concluded that the fish shifts towards anaerobic metabolism under toxicant stress [5–7].

The reduction in tissue protein suggests increased proteolysis and use of amino acids for gluconeogenesis and energy production once carbohydrate reserves fall, as well as reduced protein synthesis in a damaged liver. The same studies reported lower total protein with higher free amino acid levels and protease activity in this fish [5, 7]. One difference deserves mention: Shahi and Singh observed lower plasma glucose after one week of exposure to plant-derived compounds [8], whereas glucose increased here. The direction of the glucose response is known to depend on the compound,

dose and duration, with hyperglycaemia typical while glycogen reserves last and hypoglycaemia following their exhaustion.

4.4 Hepatic and neural effects

ALT and AST are located mainly within hepatocytes, and their rise in serum is a recognised marker of hepatocellular membrane damage and leakage. The 85.3% and 67.7% increases at 26.7 mg/L indicate that the liver is a principal target organ of the extract, which agrees with the vacuolation, necrosis and other dose-related hepatic lesions described in *R. lateristriata* exposed to butterfly pea flower extract [12]. Increased transaminase activity also supports the interpretation of enhanced amino acid catabolism given above. Altered ALT and AST activity in liver and muscle has been reported in *C. punctata* treated with *E. tirucalli* extract [7].

AChE terminates nerve impulse transmission at cholinergic synapses, and its inhibition leads to accumulation of acetylcholine, overstimulation, loss of coordination and eventually respiratory failure. A 35.9% inhibition is below the 70% or more usually associated with death in fish, but it is sufficient to impair swimming, feeding and predator avoidance. AChE inhibition has been demonstrated in *C. punctata* exposed to other botanical extracts [5, 7]. Which constituent of *C. ternatea* is responsible is not known; alkaloids and some flavonoids are documented AChE inhibitors in other plants.

4.5 Haematological effects

The parallel decline in RBC count, haemoglobin and PCV indicates anaemia. With MCV slightly raised and MCHC slightly lowered, the picture is one of mild macrocytic, hypochromic anaemia, which may result from haemolysis by saponins, from impaired erythropoiesis in the head kidney, or from haemodilution following gill damage. Reduced oxygen-carrying capacity adds to the respiratory stress caused at the gill surface. Shahi and Singh described a comparable dose-dependent fall in haemoglobin, haematocrit and red cell count in *C. punctata* and interpreted it as anaemia [8]. They found a decrease in total leucocytes with a small rise in lymphocytes, whereas leucocytosis was recorded here. Leucocytosis is a common non-specific immune response to tissue injury during sub-chronic exposure, and the difference may again relate to the nature of the compound and the longer exposure period.

4.6 Implications for aquaculture

The results suggest that the leaf extract of *C. ternatea* can kill *C. punctata*, but at concentrations higher than those needed with the most potent botanicals. Its advantages are the wide availability of the plant, its long history of safe use in food, fodder and medicine [1, 2], and the expected biodegradability of its active principles. Its limitation is that sub-lethal residues are not harmless. Concentrations as low as one-tenth of the 96 h LC₅₀ produced measurable metabolic, hepatic, neural and haematological stress within 21 days. A pond treated with the extract should therefore be left until the toxicity has dissipated before carp seed is stocked, and run-off of concentrated extract into natural waters should be avoided.

5. Conclusion

The ethanolic leaf extract of *Clitoria ternatea* showed dose- and time-dependent piscicidal activity against *Channa punctata*, with LC₅₀ values of 224.3, 185.8, 156.7 and 133.3 mg/L at 24, 48, 72 and 96 h. Acute exposure produced respiratory and neuromuscular distress. Sub-lethal exposure for 21 days at 1/10 and 1/5 of the 96 h LC₅₀ depleted tissue protein and glycogen, raised blood glucose and serum transaminases, inhibited brain acetylcholinesterase and caused anaemia with leucocytosis. The extract therefore has potential as a moderately active, plant-based agent for the control of this predatory fish, while its sub-lethal effects call for caution in its use near natural fish populations.

The study has limitations. It used a crude extract of a single plant part in a single solvent, one size class of fish and laboratory conditions. Future work should address the following points:

- Comparison of leaf, seed, root and flower extracts in aqueous and organic solvents.
- Bioassay-guided fractionation to identify the active principle, with particular attention to saponins and cyclotides.
- Histopathology of gill, liver and kidney, and oxidative stress markers.
- Recovery of biochemical and haematological parameters after withdrawal of the extract.
- Persistence of toxicity in pond water and safety to carp seed, plankton and other non-target organisms.
- Field trials to fix an effective and economical pond dose.

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