Lethal toxicity assessment of castor bean (*Ricinus communis*) seed as fish toxicant and its residual effect on water quality in aquaculture

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Abstract

The present study was conducted to evaluate the minimum concentration and lethal toxicity of castor bean seed (CBS) effect on tilapia (*Oreochromis mossambicus*: 3.81±0.01 cm, 1.76±0.2 g) for 24 hours in glass aquaria with four treatments with three doses i.e. T1 (225 mgL⁻¹), T2 (250 mgL⁻¹), T3 (275 mgL⁻¹) followed by residual effect of CBS on Amur carp (*Cyprinus carpio haematopterus*: 4.32±0.03 cm, 1.82±0.4 g) for 14 days in same glass aquaria with same treatments and one control (without CBS). The absolute mortality rate was recorded highest in T3 (95%) and lowest in T1 (60%). The 50% mortality was recorded within 3-4 hours of CBS powder application (ca.235 mgL⁻¹ - 240 mgL⁻¹) which can be considered as LD₅₀ of CBS powder. The mortality rate of Amur carp decreased below 10% after the 7th day of CBS application in all the treatments. Water qualities were within the optimum range except for pH which decreased to acidic levels (5.7 - 6.8) in all the treatments. Nitrogen and phosphorus ratio in water increased with the increase of CBS doses which could be due to more nitrogen input through CBS. It is concluded that CBS can be used as a potential piscicide in aquaculture with minimal residual effects with optimum doses at 225-250 mgL⁻¹ which subsequently will act as organic manure upon decomposition as culture progress.

Keywords: Amur carp, Castor bean, LD₅₀, N:P ratio, Tilapia, Toxicity

INTRODUCTION

Aquaculture is the fastest-growing food producing sector throughout the world with an annual growth rate of 5.8% (FAO, 2020). The aquaculture industry is facing limitations to utilize freshwater resources due to the ever-increasing population, water scarcity, land acquisition cost, and indiscriminate use of drugs and chemicals. Some of the unutilized village and roadside ponds can be brought under aquaculture through cleaning of weeds and removal of unwanted fish species by application of piscicidal agents, preferably of plant origin. This can be one of the judicious approaches for the enhancement of fish production through effective utilization of available nutrients and the ecological niches in a pond. Bioaccumulation and biomagnifications of drugs and chemicals used in aquaculture have led to various health issues in fish as well as in humans. To avoid such ill effects, plant-derived toxic components had

been introduced in India (Chatterjee and Ganguli, 1993; Das *et al.*, 2017). Plants belonging to different families are also having different kinds of toxic substances like alkenyl phenols, alkaloids, saponins, tannins, di and triterpenoids, which can be used to control the unwanted organisms and predatory fish species in aquaculture (Tiwari and Singh, 2003; Katewa *et al.*, 2005). Toxicity of such substances usually reduces within 10-15 days and the restoration of ecological status can be done upon decomposition of such substances which will enhance nitrogen and phosphorous ratio (N:P) in the pond (Mahapatra and Thosar, 1999; Das *et al.*, 2017).

Besides the use of different plant derivatives like mohua oil cake, tea seed cake, derris root powder, tobacco waste, castor bean seed powder, etc. can be a good source of piscicide for application in aquaculture (Das *et al.*, 2018). The extensive cultivation of castor in arid and semi-arid states leads to India as the

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top castor producing country in the world (Anjani, 2014). Generally, castor bean contains a high amount of oil (40%), ricin (1%-5%) and ricinine (0.3%-0.8%) (Johnson *et al.*, 2005) and also contains toxic glycoproteins, ricinoleic acid (12-hydroxyoleic acid) and the alkaloid ricinin (EFSA, 2008). Total four types of toxic ricins (A, B, C, and D) appear in small and large grains of castor bean plant (Lin and Li, 1980; Woo *et al.*, 1998). Ricin acts as a cytotoxic protein which usually inhibits the protein system that causes cell death (Audi *et al.*, 2005). Therefore, powder of castor bean seed can be used as a potent piscicidal agent in aquaculture (Mandal and Das, 2019).

The improvement and regeneration of the productive status of the pond system are usually dependent on the available nutrient status of the system. The increase in N:P ratio and acceleration of primary productivity; both depend on the availability of carbon, nitrogen and phosphorus level. The application of organic manure or inorganic fertilizer in pond culture systems is basically intended to enhance the plankton population, productivity level as well as promotion of fish growth (Chakrabarti and Jana, 1998). Therefore, besides toxicity, castor bean seed powder can be also used as good plant-derived manure in aquaculture due to the presence of 4.3% total nitrogen, 1.8% phosphorus and 1.3% potassium (Ghimire, 2002). The application of manure can boost the available nutrient sources in water and also enhance productivity and plankton populations (Garg *et al.*, 1998). So, plant-based manure can be a good managerial approach in terms of increase of culture efficiency and reducing of cost of fish production.

Usually, *Oreochromis mossambicus* is considered as low-cost fish and in least demand for aquaculture as the species hampers the culture systems due to its prolific breeding behavior and competition with other species with respect to utilization of food and ecological niches (NFDB, 2015). The uncontrolled overpopulation of tilapia is considered as one of the major challenging issues in the

aquaculture sector in India. Indian aquaculture practice is dominated by carp species. Amur common carp has a potential contribution to total aquaculture production. It is considered a good candidate species due to its high growth performance, slender body, hardy nature and better feed utilization efficiency (Basavaraju and Reddy, 2013). Various plant-derived, low cost and locally available materials became popular as piscicides particularly in the moderately intensive inland aquaculture in India and throughout the globe. The herbal piscicide like mohua oil cake is used widely in intensive aquaculture. Currently, the increased market price of good quality mohua oil cake is directed to choose alternative herbal piscicide. Therefore, the present study aimed to investigate the acute toxicity of castor bean powder on tilapia and its residual toxicity on Amur carp so as to act as an alternative, low-cost phyto-piscicide to costly mohua oil cake in carp aquaculture.

MATERIALS AND METHODS

The present study was conducted for 15 days duration (May, 2019) in the College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab (30°54'21.5" N, 75°48'04.7" E, MSL:247m). Comparative evaluation of different dosages of castor bean seed powder was carried out to evaluate the minimum concentration of the CBS for absolute mortality (acute toxicity assessment) on advanced fry of *Oreochromis mossambicus* followed by the residual toxicity assessment of CBS on advanced fry of Amur carp (*Cyprinus carpio haematopterus*). After the acute toxicity assessment, Amur carp fry was stocked in the same treatments in which CBS was applied earlier for the residual toxicity assessment. The powder form of castor bean seed was used for toxicity study. Raw castor bean seed (CBS) was procured from Gujarat, India. Castor bean seed was properly sun-dried to remove the moisture and ground to get fine powder form. CBS powder was packaged in air sealant plastic bag for further application.

Minimum concentration of the CBS for absolute mortality

The toxicity study was carried out in triplicates for 24 hour with three treatments in an experimental glass aquarium (56.63 L each). All the aquaria were cleaned and washed properly as well as disinfected with potassium permanganate (KMnO_4) solution (2 mgL^{-1}). Three different doses (225 mgL^{-1} , 250 mgL^{-1} and 275 mgL^{-1}) of powdered castor bean seed (CBS) were applied in three treatments T1 (225 mgL^{-1}), T2 (250 mgL^{-1}) and T3 (275 mgL^{-1}), respectively. Healthy pre-acclimatized tilapia fry ($3.81 \pm 0.01 \text{ cm}$, $1.76 \pm 0.2 \text{ g}$) was stocked @15 numbers in each aquarium.

Gradual decline of CBS effect

After the acute toxicity assessment on tilapia, the gradual decline of CBS effect (residual toxicity assessment) was carried out on Amur carp for 15 days with three same treatments and one control treatment in triplicates in the same experimental glass aquarium (56.63 L each). Healthy pre-acclimatized Amur carp fry ($4.32 \pm 0.03 \text{ cm}$, $1.82 \pm 0.4 \text{ g}$) was stocked @ 30 numbers in each aquarium. Amur carp juveniles were procured in well-oxygenated plastic bags from the farm of Central Institute of Freshwater Aquaculture, Kaushalyaganga, Bhubaneswar, Odisha, India. The fishes were conditioned for 2 hours in earthen pond condition. After which, fishes were treated with a mild common salt dip treatment ($15\text{--}20 \text{ gL}^{-1}$) for 2 min and transferred to a big cemented circular pool. Finally, fishes were transferred to experimental aquariums under aerated condition. The dead fishes were cleaned up and healthy fishes were stocked to maintain the appropriate stocking density.

Mortality, external body signs and behavioral response

Mortality of tilapia was recorded at every one hour interval for acute toxicity test upto 10 hours of CBS application, whereas, mortality of Amur carp was recorded at every alternate day during the study period. Dead fish

was removed from the aquaria by hand net and counted subsequently. The external body signs like colour, mucus, red patches, scale loss, rid gills and hemorrhages were observed in dead fishes. Responses like gasping on the surface of water and changes in swimming patterns were also monitored in both the test fishes.

Collection and analysis of water samples

Water samples were collected at three days interval. The water samples were collected from each aquarium at a fixed time (09.00-10.00 AM) of a day following all the precautions. Water quality parameters were analyzed following the standard methods (APHA, 1995). Temperature ($^{\circ}\text{C}$) and pH of water were measured using a digital pH meter (Cole-Parmer pH Tester). Biological oxygen demand (BOD_5), dissolved oxygen (DO), total alkalinity (TA), and total hardness (TH) were determined following the standard formula (APHA, 1995). Ammonia-nitrogen ($\text{NH}_3\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$) and ortho-phosphate ($\text{PO}_4\text{-P}$) were estimated in UV-vis-Spectrophotometer (HACH DR 6000 model).

Water bacteriological assay

Microbial densities in water were enumerated at every five days intervals. Serial dilution of water samples from each treatment was prepared and $100 \mu\text{L}$ of proper dilution was spread on a plate. Total aerobic heterotrophic bacteria (AHB), ammonifying bacteria (AB), ammonia oxidizing bacteria (AOB) and phosphate solubilizing bacteria (PSB) were enumerated through the spread plate method (Sanders, 2012). The population of aerobic heterotrophic bacteria was grown in nutrient agar medium supplemented with 1.0% w/v NaCl (Eaton *et al.*, 1998). The composition of the medium for ammonifying bacteria and ammonia oxidizing bacteria was used as per Alexander (1977). PSB are capable of utilizing tri-calcium phosphate. The production of a clear zone around the colonies of the organisms was an indication of the presence of PSB. The composition of modified Pikovskaya's agar medium was used as per the medium used by Rao (1978).

Statistical Analysis

Statistical analysis of the data was carried out using SPSS v. 20. One-way ANOVA and post-hoc Duncan's Multiple Range Test (DMRT) with statistical significance set at $\alpha=0.05$, were used throughout. All data were checked for normality and homogeneity of variance before analysis.

RESULTS

Mortality of tilapia and Amur carp

The hourly mortality rate showed that the absolute mortality of tilapia occurred on 6th hours of CBS application in T1, whereas, it was recorded on 7th hours and 9th hours in T3 and T2, respectively (Fig. 1a). A significant difference ($P<0.05$) was observed among the treatments. The mortality rate was higher in all the treatments upto first 3 hours of CBS application, whereas, the marginal rate of mortality reduced upto the 10th hour of CBS application in the respective treatment groups (Fig. 1b, 1c, 1d). The cumulative mortality rate of tilapia was showed a significant difference ($P<0.05$) among the treatments and the highest mortality rate was observed in T3 (72%) and lowest in T1 (42%) (Fig. 1b, 1c, 1d). The cumulative mortality rate increased over time and 50% of tilapia mortality was observed within 3-4 hours of CBS application in T2 and T3, whereas, it was 5-6 hours in T1. The residual effect of CBS toxicity on Amur carp showed

that $\geq 50\%$ mortality was observed on the 3rd day of CBS application (mortality T1-50.00%, T2-53.00% and T3- 46.67%). The mean difference among the treatment was not significant ($P>0.05$). The mortality rate reduced to less than 10% in T1 and T2 on the 12th day of the study period (Fig. 1e). The observations on the external behavior of both tilapia and Amur carp on the application of CBS indicated that tilapia became inactive gradually, lost their balance and gasping on the surface of the water (Table 1). Also, Amur carp showed erratic movement and released more mucus in water in all the CBS treatments. Amur carp did not show any abnormal behavior and responses in the control treatment.

Water quality parameters and microbial populations

All the water quality parameters are presented in Table 2. The surface water temperature remained within 25-27°C during the period of the experiment. Water temperature increased up to the 1st half, after which it decreased during the rest of the study period. The pH of water increased gradually upto the first 5 days after which it declined in all the treatments except in the control group and remained in alkaline conditions (7.13-8.2) with a significant ($P<0.05$) treatment difference (Fig. 2a). pH was highest in control and lowest in T2. A similar trend was observed for alkalinity,

Table 1. External behavior and body sign of tilapia and Amur carp

	Tilapia			Amur carp		
	T1	T2	T3	T1	T2	T3
Loss of balance	Low	Medium	High	Medium	Medium	High
Erratic movement	Medium	High	High	Low	Medium	High
Gasping at water surface	High	High	High	Low	High	High
Loss of scales and fins	Less	More	More	Less	More	More
Gill colour	Light brown	Reddish brown	Dark brown	Faint reddish	Dark brown	Dark brown
Mucus secretion	High	High	High	Low	Medium	High
Red patches	Less	Less	More	Less	Less	More
Scale loss	More	More	More	Less	Less	More
Hemorrhages	Less	More	More	Less	Less	More

Table 2. Water quality variables in different treatments (Mean±S.E.)

Variables	Treatment (Mean±S.E.)			
	T1	T2	T3	C
Temp. (°C)	27.30	27.32	27.35	27.31
pH	7.23-8.20	7.13-8.10	7.16-8.20	7.86-8.13
DO (mgL ⁻¹)	5.04±0.65 ^{ab}	4.59±0.63 ^a	4.42±0.63 ^a	7.39±0.08 ^c
BOD ₁ (mgL ⁻¹)	2.48±0.38 ^c	2.08±0.28 ^{bc}	1.91±0.23 ^b	1.64±0.05 ^a
TA (mgL ⁻¹)	236.38±24.80 ^a	239.83±24.56 ^a	231.00±27.07 ^a	334.78±2.02 ^b
TH (mgL ⁻¹)	222.67±6.25 ^a	211.22±8.82 ^a	210.72±9.20 ^a	237.61±1.54 ^b
NH ₃ -N (mgL ⁻¹)	0.058±0.01 ^b	0.069±0.015 ^c	0.077±0.018 ^d	0.024±0.000 ^a
NO ₃ -N(mgL ⁻¹)	0.386±0.062	0.421±0.07	0.403±0.067	0.116±0.007
O-PO ₄ (mgL ⁻¹)	0.055±0.006 ^b	0.064±0.007 ^b	0.064±0.008 ^b	0.031±0.001 ^a
N:P ratio	7.76±0.45 ^c	7.42±0.47 ^b	7.35±0.48 ^b	4.66±0.45 ^a

Mean with different superscripts within row differs significantly (P<0.05)

Abbreviation: Temp.- Temperature, DO- Dissolved oxygen, BOD- Biological oxygen demand, TA- Total alkalinity, TH- Total hardness, NH₃-N- Ammonia –nitrogen, NO₃-N- Nitrate-nitrogen, O-PO₄- Ortho-phosphate, N:P- Nitrogen and phosphorus ratio

hardness and BOD₁ level in the water (Fig. 2b, 2c, 2d). The dissolved oxygen level decreased upto 5 days after which it increased in all the treatments except in the control group in which the trend remained almost stationary (Fig. 2e).

Total inorganic nitrogen concentration increased but remained more or less stationary with a significant difference (P<0.05) among the treatments. The ammonia-nitrogen concentration increased during the first 5 days and then it continued to decline (Fig. 3a). The nitrate-nitrogen concentrations increased over time in all the treatments except in control with significant difference (P<0.05) among the

treatments (Fig. 3b). The ortho-phosphate concentration increased in all the treatments except in the control and showed a significant difference (P<0.5) among the treatments (Fig.3c). A similar trend was noticed with a significant difference (P<0.05) for the N:P ratio in all the treatment groups (Fig. 3d).

Populations of AHB, AB, AOB and PSB gradually increased in all the treatments except control after the 5th day of the study period with a significant difference (P<0.05) among the treatments. The mean population of AHB, AB, AOB and PSB were highest in T3 followed by T2, T1 and C (Table 3).

Table 3. Microbial populations in water in different treatments (Mean±S.E.)

Variables	CFU (log No. mL ⁻¹)			
	T1	T2	T3	C
AHB	3.04±0.01 ^b	3.14±0.01 ^c	3.23±0.01 ^d	2.71±0.01 ^a
AB	2.99±0.02 ^b	3.13±0.01 ^c	3.26±0.01 ^d	2.74±0.02 ^a
AOB	2.88±0.02 ^b	3.12±0.01 ^c	3.26±0.01 ^d	2.77±0.02 ^a
PSB	2.94±0.02 ^b	3.16±0.01 ^c	3.28±0.02 ^d	2.73±0.01 ^a

Mean with different superscripts within row differs significantly (P<0.05)

Abbreviation: AHB- Aerobic heterotrophic bacteria, AB- Ammonifying bacteria, AOB- Ammonia oxidizing bacteria, PSB- Phosphate solubilizing bacteria

Fig. 1. Temporal changes of hourly mortality rate of tilapia in different treatments (a), hourly mortality rate vs. cumulative mortality rate in T1 (b), T2 (c), T3 (d) and residual effect of CBS on mortality rate of Amur carp (e)

Fig. 2. Temporal changes of pH (a), alkalinity (b), hardness (c), BOD₁(d) and DO (e) in different treatments

Fig. 3. Temporal changes of $\text{NH}_3\text{-N}$ (a), $\text{NO}_3\text{-N}$ (b), Ortho-phosphate(c), and N:P ratio (d) in different treatments

DISCUSSION

The hourly mortality rate of tilapia showed that the absolute mortality rate was faster in T1 (225 mgL^{-1}) compared to the rest of the treatments. The cumulative mortality rate of tilapia was highest in T3 (275 mgL^{-1}) followed by T2 (250 mgL^{-1}) and T1 (225 mgL^{-1}). A more than 50% mortality of tilapia observed on 3-4 hours of CBS application, this implied that the positive toxic effect of CBS which can be used as a potent piscicidal agent besides other piscicide like mohua oil cake, tea seed

cake etc. (Mondal and Das, 2019). Therefore, the lethal dose of CBS may be considered within $225\text{-}250 \text{ mgL}^{-1}$ to act as a potent piscicidal agent in aquaculture. The higher cumulative mortality rate in 275 mgL^{-1} compared to other treatments suggested that the absolute mortality (100%) of tilapia was achieved with the increase in time (9th to 10th hours). This might be due to the presence of high level of toxic antimetabolites glycoprotein and ricin with increasing level of CBS dose as the polynomial relationship

(Fig. 4) was established between cumulative mortality rate of tilapia and the linear relationship with CBS dose (Fig. 5) as well as also with the duration of exposure. Aslania *et al.* (2007) reported that castor bean intoxication has a relatively long latency period. The 50% mortality of tilapia was achieved within 3- 4 hours of CBS powder application within the dose 235 mgL⁻¹ to 240 mgL⁻¹ which can be considered as LD₅₀ (Lethal Dose, 50%) of CBS powder.

Audi *et al.* (2005) reported that the presence of A-chain of glycoprotein lectin in ricin have the negative effect on fish cell as those inhibit the protein synthesis which causes the blocking of ribosomal activity leads cell death. As a result, the hourly mortality rate was faster and the cumulative mortality rate was slower due to the delayed response of CBS toxicity. Whereas, the mortality rate of Amur carp reduced from the 3rd day of the study, this may be related to the gradual reduction of the toxic effect of CBS. The findings are in agreement with earlier reports in *Labeo bata* (Mondal and Das, 2019) and higher animal like rat (Cook *et al.*, 2006). The absolute mortality of tilapia, reduced mortality of Amur carp and reclamation of water quality were more identical on the 6th day of the study as the mortality rate of Amur carp reduced $\leq 10\%$ which suggested that the toxic effect of CBS continued upto 10 days in aquaculture practices (Fig. 6). The duration of the residual toxicity, as well as reclamation of water quality in later, was in conformity with the

recommendation of the Aquaculture Authority (1999) which suggested the fish can be restocked in aquaculture system after 10 days of piscicide application.

In the present study, the decrease of pH in CBS treatments might be due to the gradual decomposition of CBS which led to mineralization of organic matter (Krachler *et al.*, 2009; Mondal and Das, 2019). The decomposition of organic matter reduced the dissolved oxygen level in water. The reduction of alkalinity level in water was parallel to pH level in water. Also, the BOD level increased due to the presence of high microbial load of the heterotrophic bacterial population as negative polynomial relationships were established between dissolved oxygen, BOD₁ and heterotrophic bacteria (Fig. 7). The increased level of total inorganic nitrogen and ortho-phosphate concentrations might be because of the high level of nitrogen and phosphorus in CBS (Ghimire, 2002; Mondal and Das, 2019).

The increased N:P ratio in all the CBS treatments increased gradually and remained identical between 7.35-7.75 which was within the optimal level (4:1 to 8:1) in the aquaculture production system (Ghosh and Chattopadhyay, 2005). This was established with the polynomial relation between N:P ratio and mineralizing bacterial population (Table 4). Mandal and Das (2019) reported that CBS treatment caused phosphorus limitation to maintain congenial N:P ratio in the aquaculture system.

Table 4. Polynomial relationship between N:P ratio and mineralizing bacterial populations

	T1	T2	T3
N:P vs. AHB	$y = -4.883x^2 + 11.97x + 2.147$; $R^2 = 0.885$	$y = -0.947x^2 + 3.872x + 4.348$; $R^2 = 0.552$	—
N:P vs. AB	$y = -8.982x^2 + 19.81x - 2.567$; $R^2 = 0.947$	$y = -6.599x^2 + 18.26x - 1.987$; $R^2 = 0.873$	$y = -2.535x^2 + 8.838x + 1.694$; $R^2 = 0.972$
N:P vs. AOB	$y = 6.013x^2 - 7.899x + 9.044$; $R^2 = 0.467$	$y = -5.619x^2 + 15.18x - 0.261$; $R^2 = 0.984$	$y = -2.322x^2 + 7.880x + 2.479$; $R^2 = 0.992$
N:P vs. PSB	$y = 7.493x^2 - 11.74x + 10.97$; $R^2 = 0.401$	$y = -3.952x^2 + 11.56x + 0.713$; $R^2 = 0.976$	$y = -2.061x^2 + 7.312x + 2.484$; $R^2 = 0.973$

Fig. 4. Polynomial relationship between cumulative mortality rate of tilapia with hours of observation

Fig. 5. Linear relationship between cumulative mortality rate of tilapia with CBS powder dose

Fig. 6. Linear relationship between mortality rate of Amur carp and duration of experiment

Fig. 7. Polynomial relationship between dissolved oxygen, biological oxygen demand with aerobic heterotrophic bacterial population in water

The lethal dose (LD_{50}) of castor bean seed powder might be considered within 235 mgL^{-1} to 240 mgL^{-1} . The optimum level of castor bean seed powder application might be within 225 mgL^{-1} to 250 mgL^{-1} as higher dose 275 mgL^{-1} showed no significant difference with 225 mgL^{-1} and 250 mgL^{-1} in terms of absolute mortality of tilapia. The toxicity level of castor bean seed powder reduced over time as evident from the reduced mortality rate of Amur carp and the N:P ratio in water enhanced through the activity of the mineralizing bacterial population. Though the mortality rate of tilapia and residual effect on Amur carp were higher in the higher dose of CBS (275 mgL^{-1}), nutrient mineralization and N:P ratio did not exert any

significant difference with lower doses (225 mgL^{-1} and 250 mgL^{-1}). Therefore, the application rate of CBS powder as a potential piscicidal agent may be considered within 225 mgL^{-1} to 250 mgL^{-1} in the aquaculture production system for maintaining the suitable ecological status.

Conflict of interest: Authors have no conflict of interest in this study.

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