

Effect of copper sulfate on eradication of snail's specie, *Oncomelania quadrasi*, in aquatic habitats having *Labeo rohita* as a selected fish

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Abstract

The snail's specie, *Oncomelania quadrasi*, is found abundantly in fresh water fish ponds of Punjab. It is an intermediate carrier / host of various digenetic trematode parasites which causes many serious fish diseases in aquaculture ponds. The purpose of this study was targeted for controlling these snails in aquatic environments through chemical control strategies using copper sulfate as an eradicator. Mainly three trials were conducted to determine the effectiveness of copper sulfate against this snail sp. in laboratory aquaria conditions. Trial I involved the copper sulfate treatment sprayed directly for reductions of snails present on the upper moist layer of soil. Trial II was demonstrated for reductions of the snail densities buried under the depth of 8-16 inch layer of soil. Trial III was managed to study the copper sulfate toxicity on the survival of snails and *Labeo rohita* present together in the same aquatic media. The study indicated that copper sulfate was effective for eradication of the snails in all conditions whether present on the wet edges / moist soil surfaces, buried in the soils or present in the aquatic environment with fish, however, with varying degrees and intensities.

Keywords: Snail, *Oncomelania quadrasi*, *Labeo rohita*, Copper sulfate, Molluscicide, Toxicity

Introduction

The *Oncomelania* snail species can commonly survive long periods of unfavorable conditions because of the presence of an operculum and are capable of producing high-density populations. They can spend their lives both in dry and aquatic conditions in a variety of different moist habitats including sluggish streams and irrigation canals (Hoffman, 1970). Phenomenally speaking, many freshwater snails' species act as the first intermediate hosts in the life cycle of the trematodes which are reasons for infections and diseases caused in human beings. The species of trematodes have also been known to persist within the fish host for up to four years, larger than the typical growing period (Hoffman, 1999). Infestation of fish with digenetic trematodes, commonly referred to as grubs, are a significant problem in aquaculture ponds. Fish stocks composed of small individuals may incur high mortality when infested with the trematodes, while larger fish are often rendered unmarketable due to the grubby appearance of the fish flesh (Lane and Morris, 2000). One method of preventing trematodes infestations among cultured fish is draining ponds for controlling the access to fish eating birds. It is practically not feasible, so most control efforts focus on eliminating the snail host (Venable *et al.*, 2000).

In order to prevent trematode infestations, it is necessary to disrupt the trematodes life cycle at one or more

stages. Therefore, the only viable approach to prevent trematode infestations in fish is reduction or elimination of snail populations in aquaculture ponds. This may be accomplished through chemical means (Venable *et al.*, 2000; Ledford 2003; Wang *et al.*, 2003). Molluscicide is an alternate chemical substance specifically used for destroying mollusks (Claudi and Mackie, 1994). Their mode of action includes the stressing of the water balancing system, toxic reactions to occur at gill membranes, lethal activity against mussels and disturbances in metabolism or physiological functions leading to their mortality (McCullough *et al.*, 1980; Sprecher and Getsinger, 2000). Molluscicides are typically classified as either oxidizing or non-oxidizing compounds. The first class of compounds include ozone, potassium permanganate, hydrogen peroxide, bromine, etc (Claudi and Mackie, 1994; Netherland and Getsinger, 1998; Sprecher and Getsinger, 2000) while the second class of compounds include quaternary and polyquaternary ammonium compounds, aromatic hydrocarbons; copper metal and their salts (Andrews *et al.*, 1982; Sprecher and Getsinger, 2000; Giovanelli *et al.*, 2002). Non-oxidizing molluscicides have a higher cost than oxidizing ones, but remain cost-effective due to lower amounts of application, shorter exposure periods and fast toxicity. A successful attempt was made to eradicate zebra mussels through lake-wide application of copper sulfate to

Lake Offutt, Offutt Air Force Base, Nebraska (URS Group, Inc. Final Summary Report, 2009).

Copper sulfate not only acts as a fungicide (Michaud and Grant, 2003) and algicide (Fitzgerald and Faust, 1963) but also is utilized as a molluscicide (Hoffman and Zakhary, 1953). Mortality is being caused through its high concentrations being toxic in nature, causing an ultimate disruption of structure of DNA and proteins, finally resulting in their nonfunctioning (de Oliveira-Filho *et al.*, 2004). The copper sulfate application rate of approximately 1.0 mg/L to Marsh rams-horn snails (*Planorbella trivolvis*) resulted in an average survival rate of 3% at water temperatures $\geq 21^{\circ}\text{C}$ (Mitchell and Hobbs, 2003). The mechanism of action for copper-based products is believed to target specific physiological processes such as electron transport in photosystem, cell division and nitrogen fixation (Cooke *et al.*, 1993; Senseman, 2007).

This research is based on laboratory trials involving chemical/molluscicide application, Copper sulfate against *Oncomelania* snails for their complete or partial eradication since these are

causing nuisance in the pond's aquatic environment of Central Fish Seed Hatchery as well as in cemented tanks of FR&TI, Lahore, Pakistan. This study will help to safeguard the fish mortality scenes especially of the fish fry and fingerlings resulting from trematode infections in fish ponds and tanks.

Material and methods

Three experimental trials (each of five days duration) were conducted in Chemistry Research Laboratory, Fisheries Research & Training Institute, Lahore for the present study. The sketch of the experimental design is shown in Table 1.

There were 10, 10 and 12 treatments of copper sulfate having one control with three replicates for each of the three trials, respectively. For this research, the required numbers of glass aquaria i.e., 30, 30 and 36 for Trial I, II and III, respectively each with a dimension of 1 x 2 x 1.5 cubic feet, were placed in the laboratory and each of these was filled with 4-16 inches of soil layer depending upon the nature of the trial.

Table 1: Experimental design.

Trials	Conditions applied
Trial -I	Moist conditions with snails at top soil surface
Trial – II	Moist conditions with snails buried under soil layers
Trial -III	Aquatic environment having both fish and snails

The snails (*Oncomelania quadrasi*) with an average length of 2.3 cm were collected from the fish ponds of Lahore Fish Seed Hatchery where they were found so abundant that they were causing a variety of problems/diseases to the fish fry and fingerlings. These were kept in a glass aquaria containing well fertile pond water and placed in the lab. till the start of the experiment. The fingerlings of Rohu (*L. rohita*) with an average weight 2.2 g and average total length of 4.1 cm were also procured from the Central Fish Seed Hatchery, Lahore. Prior to initiation of any experimental work, the fish were acclimatized to laboratory environment for at least 10 days. During this transient period fish were kept independently, separate from the snails in well aerated aquaria and were fed on artificial feed (Yvonnen *et al.*, 1987).

Trial- I

As per proposed pattern, the moist soil conditions were established for ten treatments (in triplicate) in thirty glass aquaria in the first trial (Table 1) along with one control with no chemical added. Nine different doses of copper sulfate for eradicating snails were established for Trial-I and II. Twenty snails were placed above the 4 inches moist soil layer and nine different doses of commercially available copper sulfate (60% pure, Brand: China) ranging from 0.15 g to 7.20 g were uniformly sprayed on the upper top layer of soil (Table 2).

The mortality rate of snails was continuously recorded after post application of copper sulfate for five days. This was done by putting each snail out of the aquaria using forceps, placing it on a watch glass and observing its body movements and conditions closely through eye contact.

Table 2: Copper sulfate doses applied in Trial -I, II and III.

Treatments	Trial - I Doses	Trial - II Doses	Trial - III Doses
T1	0.00 g	0.00 g	0.000 g
T2	0.15 g	0.15 g	0.025 g
T3	0.25 g	0.25 g	0.050 g
T4	0.45 g	0.45 g	0.100 g
T5	0.90 g	0.90 g	0.150 g
T6	1.80 g	1.80 g	0.200 g
T7	2.70 g	2.70 g	0.250 g
T8	3.60 g	3.60 g	0.300 g
T9	5.40 g	5.40 g	0.350 g
T10	7.20 g	7.20 g	0.400 g
T11	-	-	0.450 g
T12	-	-	0.500 g

The dead snails were not returned into the aquaria while the live ones were again subjected to the same conditions.

Trial-II

The trial-II had the same experimental conditions, with the same number of treatments, with the same doses of copper sulfate and the same number of snails with the exception that snails were buried inside the 16 inches soil layer for observing difference in results of mortality rates in comparison with trial-I (Table 2). Different copper sulfate doses in solution form were uniformly added to penetrate into the soil layer. The mortality rate of snails was continuously recorded in the same manner as the previous trial.

Trial-III

In Trial III, 4 inches moist soil layer was placed at the bottom of each tank. For establishing an aquatic environment, 50 liters of tap water was added to each aquarium of thirty-six treatments individually. Twenty snails were transferred into each experimental tank. At the end of the acclimatization period, ten fish (fingerlings of *L. rohita*) were also stocked into each glass aquarium. Regular aeration was done for the maintenance of optimum dissolved oxygen level in water. Twelve different doses of copper sulfate ranging from 0.025 g to 0.500 g were applied to the aquatic environment (Table 2) containing both fish and snails. A glass rod was used for stirring purposes for the uniform distribution of

doses in each aquarium. The mortality rate of snails and fish were continuously recorded. The daily records of the number of live and dead snails/fish were properly maintained and compared for each trial at the end of the experimental period for evaluating the conclusions.

Water quality parameters

Water quality from each glass aquarium of three trials was also monitored for the physicochemical parameters before, after the application of each copper sulfate dose and during the entire research period. Temperature, pH, carbon dioxide, dissolved oxygen, alkalinity, hardness and total dissolved solids (TDS) were mainly included, for taking into consideration following APHA. (2012).

Results

The experimental design of the present research could be better understood through Table 1 illustration notifying the three major conditions for snail/fish environment taken into consideration. The various doses of copper sulfate selected for the three trials are well explained through Table 2 which in turn is based on a series of experiments for evaluation of a right and safer copper sulfate dose for eliminating snails without harming the fish residing in that particular water body. All results obtained were based on snail and fish mortality during uniform intervals of time through observations made by the researchers.

The LC50 and LC100 results of snail mortality are depicted in Table 3 where it can simply be explained that in aquatic environment containing fish as an inhabitant, a least amount of copper sulfate was required for eradication of snails, however, it gradually increased where the snails were present at the naked top layer of soil and further increased with snails hiding under the soil layers. Moreover, fish were also

observed to move away from the copper sulfate concentrated application areas supporting the observations recorded by Spear and Pierce (1979).

Trial I results

In the first trial, ten different doses of copper sulfate were sprayed at the surface of soil in the moist condition. The results of % age mortality of snail in Trial I am shown in Fig. 1.

Table 3: 24 hours LC50 and LC100 Results.

Trials		LC50	LC100
I	Snails	3.00 g	7.20 g
II	Snails	10.00 g	13.40 g
III	Snails	0.75 g	1.45 g
III	Fish	1.50 g	2.50 g

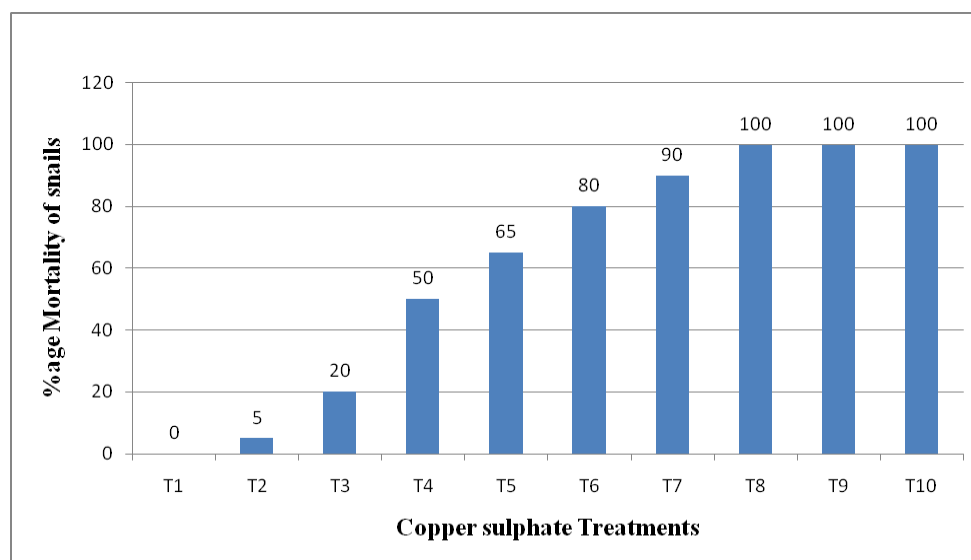


Figure 1: Results of Trial -I.

The copper sulfate was directly sprayed in the powdered form on the top most layer of the soil containing all the snails (20 in number) under experimentation.

The 24 hours LC50 and LC100 results were obtained as 3.00 g and 7.20 g, respectively. The snail mortality trends of Trial-I are depicted in Fig. 2.

This is quite obvious to observe from the trends that in T2 only 5% snail mortality was observed after the 3rd day which remained constant till the end of the trial and no further mortality was observed, however in T3, 5% snails were killed within 24 hours and these gradually increased to 20% till the 4th day and no more mortalities were further observed in this treatment. T4 and T5 showed very close trends starting from 10% each and gradually ending at 50% and 65%, respectively.

In T6 and T7, the initial mortalities were doubled and tripled, respectively from T5 and finally reached 80% and 90% at the end of the 5th day. The trends in T8 and T9 showed initial mortalities within 12 hours at 60% and 80% and reaching 100% i.e., total mortality within 60 and 40 hours, respectively.

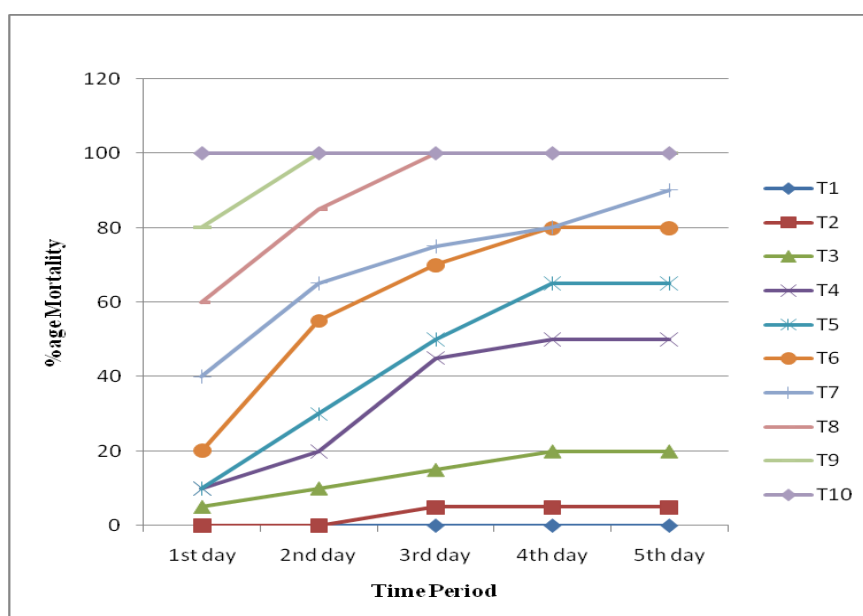


Figure 2: Snail's mortality trends in Trial-I.

The treatment T10 showed a very rapid and 100 % mortality of snails at once i.e., within half an hour of starting time. The snail mortality was in accordance with the findings of Mitchell (2002). There was no mortality of snail observed in T1 i.e. control during five days duration.

Trial II results

In the second trial, ten different doses of copper sulfate were applied to percolate inside the moist soil. Fig. 3 shows the results of snail mortality in Trial II.

The copper sulfate in the form of solution was allowed to sink into the layers of soil through the surface of soil with moist conditions containing twenty

snails buried inside the 16 inches layer of soil. The LC50 and LC100 results were evaluated to be at 10.00 g and 13.40 g, respectively. The snail mortality trends of Trial II are depicted in Fig. 4.

It was quite difficult to observe the trends of mortality in this trial since the snails were buried inside the soil layers which made this trial a real hectic one, however, the results were accomplished with tedious efforts (pulling up the snails one by one and then putting back

them in the same position). The trends depicted total survival of snails in T1, T2, and T3 while only 5% snails died in T4 and T5 on the 3rd day completing a figure of 10% and 15% till the end of the trial.

T6 started with 5% mortalities on the 6th day and finally ended at 35%. T7 and T8 showed quite similar trends starting with mortalities of 10% snails on the 2nd day and on the 5th day it remained at 50% and 75%, respectively.

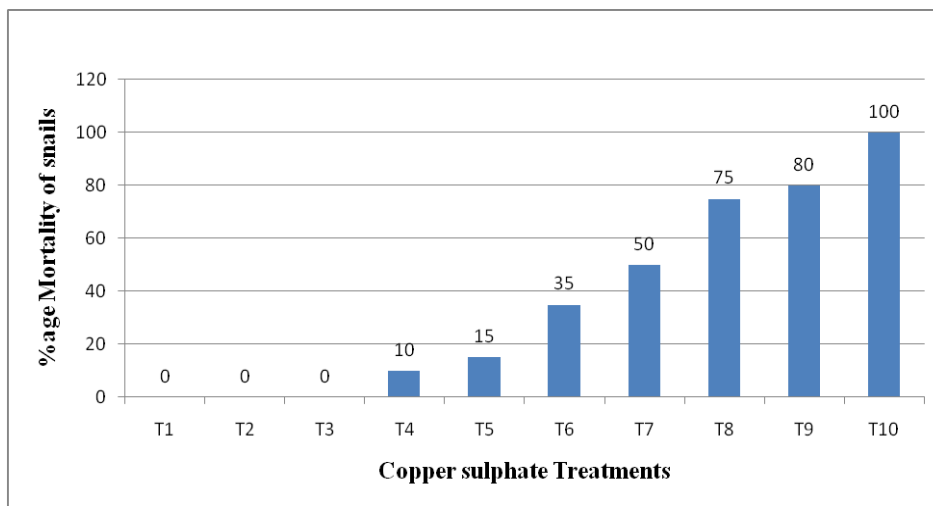


Figure 3: Results of Trial-II.

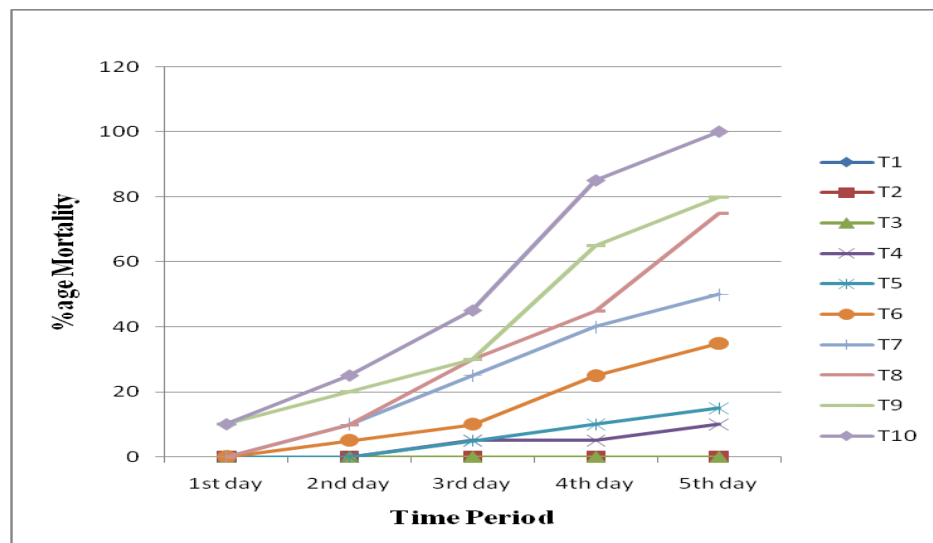


Figure 4: Snail's mortality trends in Trial -II.

The treatments T9 and T10 also started each with 10% mortalities but within 20 hours it finally reached 80% and 100% at the end of the 5th day.

Trial III results

Fig. 5 describes the results of snail mortality in Trial III. The LC50 and LC100 results were noted to be at 1.50 g and 2.50 g, respectively.

Fig. 6 represents a scenario of snail's mortality trends of Trial III after application of the prescribed doses of the chemical for this trial.

Since no chemicals were added to control treatment, T1, hence no mortality was observed there. This is quite evident from Fig. 6 that T1 and T2 showed full 100% survival of snails.

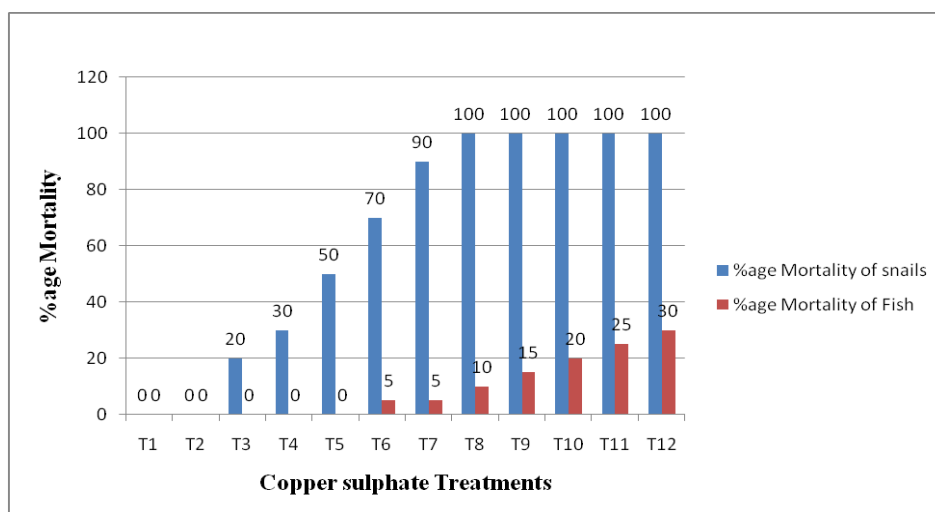


Figure 5: Results of Trial-III.

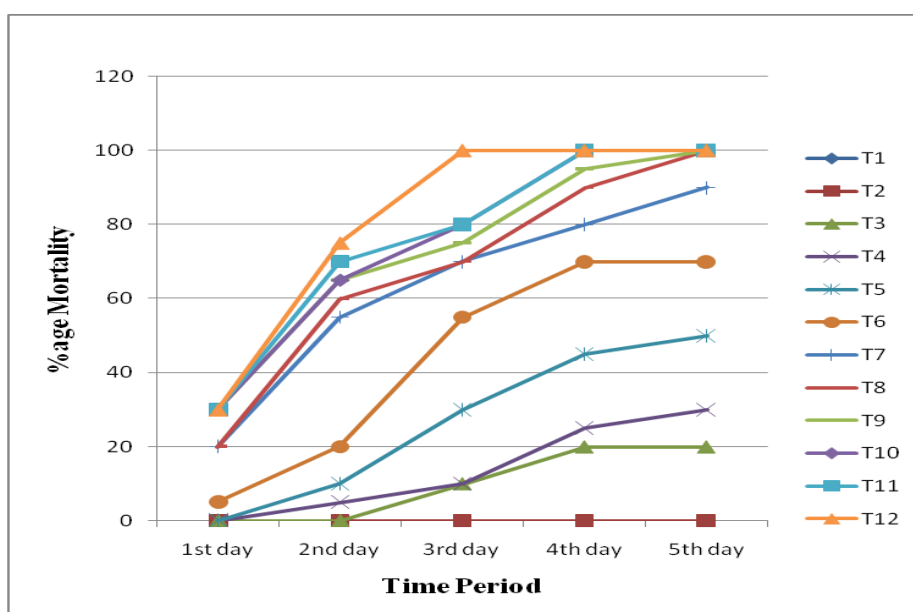


Figure 6: Snail's mortality trends in Trial-III.

The trends in T3, T4, T5 and T6 showed mortalities of 10%, 5%, 10% and 5% snails each on 3rd, 3rd, 2nd and 1st days and finally attained 20%, 30%, 50% and 70% mortalities until the 5th day. T7 started at 20% and ended at 90% while the trends in T8, T9, T10, T11 and T12 all started 30% snail mortality and ended at 100% on the 5th, 5th, 4th, 4th and 3rd days, respectively.

Fig. 7 represents the trends for fish mortality in the same trial. There were no fish mortalities in T1, T2, T3 and T4 while only 5% fish were finally found dead till the end of 5th day in T5, T6 and T7.

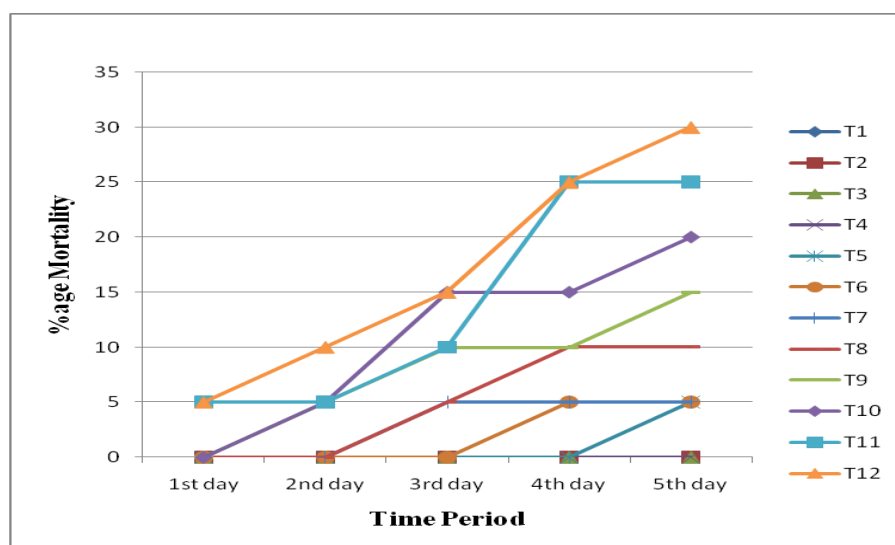


Figure 7: Fish mortality trends in Trial -III.

The rest of trials i.e., T8, T9, T10, T11 and T12 indicated a final mortality up to 10%, 15%, 20%, 25% and 30% fish, respectively.

It is pertinent to mention here that where 100% snail mortality was achieved i.e. in T8 with 0.3 g copper sulfate, only 10% fish were found dead till the end of 5th day. This experiment ended successfully giving an achievement regarding safest dosage selection of copper sulfate in terms of high percentages of healthy fish survival.

Physicochemical parameters

The variations among various physicochemical parameters including temperature, pH, carbon dioxide and dissolved oxygen in trials I, II and III which are self-explanatory are represented by Fig. 8.

These variations were not strong enough with each other and were found in the desirable ranges and nothing exceeded or dropped to a lethal limit after application of copper sulfate. However, it was observed that high temperature increased the toxicity of the chemical while the lower

temperature reduced the effect of the same which was in accordance with the observation recorded by Sorenson (1991).

The three physicochemical parameters i.e., Alkalinity, Hardness and TDS values during three Trials were also found in suitable water quality range requirements of an aquaculture operation and can be well explained through Fig. 9.

The total alkalinity remained between 240-270 ppm, the total hardness between 65-260 ppm and TDS between 513-840 ppm indicating a safer environment for application of the chemical without enhancing its toxicity to the fish corresponding to the studies made by Tucker and Robinson (1990).

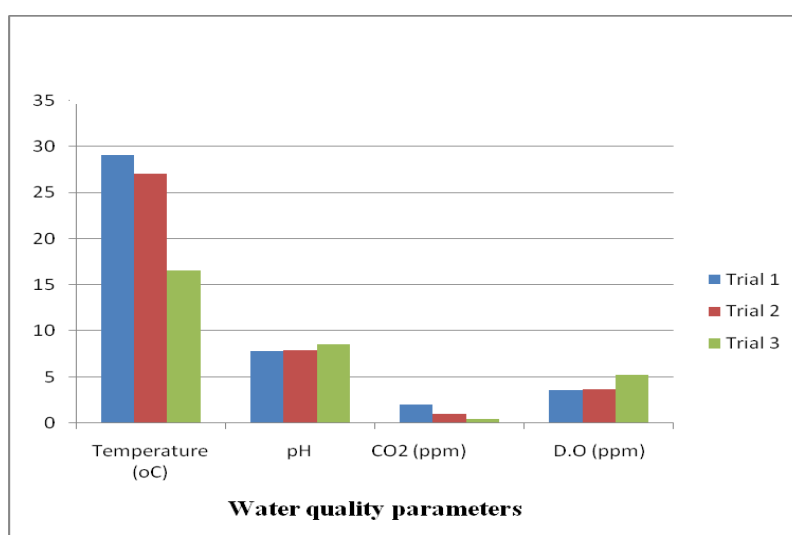


Figure 8: Temperature, pH, carbon dioxide and dissolved oxygen parameters.

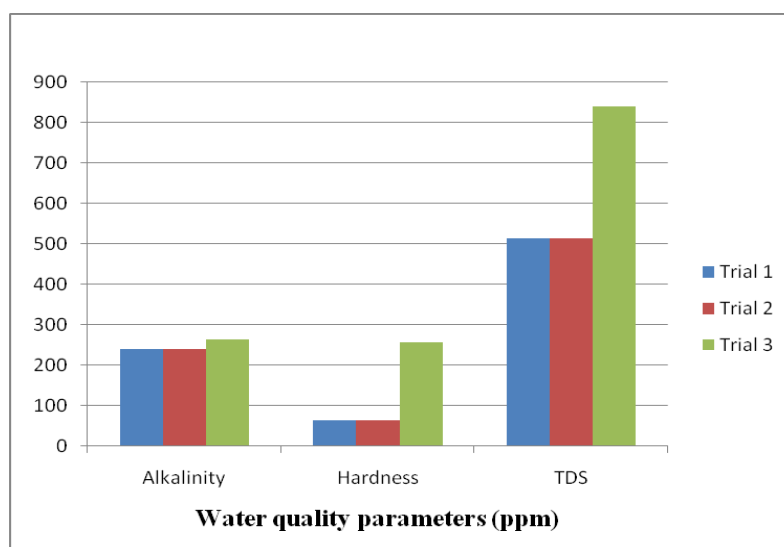


Figure 9: Alkalinity, hardness and TDS parameters.

Boyd and Tucker (1998) indicated that the pond waters with low pH and lower total alkalinity were more susceptible to fish loss from copper toxicity, however, metal toxicity decreases as pH increases with total alkalinity. The results of parameters indicated that the toxic effect of copper sulfate did not affect the water quality; however, it badly and directly affected snail survival causing their rapid mortality while leaving fish alive and healthy. Mostly fish (*L. rohita*) was found to be more resistant to the chemical as compared to the snail species (*Oncomelania quadrasi*) under consideration. Hence, the copper sulfate treatment was found significantly more pronounced in clearing the pond shorelines and aquatic environment by eradicating snails effectively.

Discussion

The results indicated that the copper sulfate treatments were very effective in eliminating snail species, *Oncomelania quadrasi* on moist soil surfaces as well as in water bodies. In all the three Trials, it was noted as a common observation that since copper sulfate was not added to the experimental tanks of the control treatment, no snail and fish mortality were taken into account. However, in all the others, there were snail and fish mortalities observed though with varying degrees. The results were in accordance with the findings of Mitchell (2002) who applied 589 g CuSO₄ on a 2-m swath per 10 linear meters of shoreline for effective elimination of rams-horn snail

Planorbella trivolvis. These findings and results were also supported by Cooke *et al.* (1993) who evaluated the efficacy of copper-containing algaecides being ineffective in waters with environmental conditions at temperatures lower than 15°C, while having pH and alkalinity on higher sides. He also suggested that the continuous use of these algaecides may result in an accumulation of copper in sediments consequently restricting their reuse and disposal.

The study indicated the usefulness and effectiveness of copper sulfate being employed as a molluscicide against snail species, *Oncomelania quadrasi*. This was completely in agreement with the results obtained by Mitchell (2002) who demonstrated that CuSO₄, when applied at 26.5–28°C, effectively killed majority of rams-horn snails, however, these results were contradictory and did not match those as shown by Haak *et al.* (2014) who concluded that copper sulfate could not effectively kill the adult Chinese mystery snails in his laboratory experiments. Avery *et al.* (2002) and Avery (2001) showed their concerns regarding copper toxicity and advised the fish farmers to avoid treatments in ponds with low alkalinity waters having total alkalinity approximately less than 150 ppm. They also recommended that farmers should not treat the ponds smaller than seven acres regardless of total alkalinity concentration. They also guided them that severe oxygen depletions can be resulted due to heavy

algal blooms caused through the use of copper sulfate in ponds. Masuda and Boyd (1993) reported that when copper chelated forms were applied, the total copper concentrations were higher and remained longer in solution than on application as copper sulfate only. On the contrary, David (2005) evaluated in his experimental results that copper sulfate did not appear to be toxic to the channel catfish and was safe between 2.5 and 5.0 ppm limits.

The overall research results led to a scenario depicting that the application of molluscicide, copper sulfate, in all three trials has shown promising and excellent results which are in accordance with the outcome of research conducted by Mitchell (2007). It was evaluated that the chemical was very effective for the eradication of snail species, *Oncomelania quadrasi*, in all conditions whether present on the wet edges of the soil, buried inside the bottom soil layers or present in the aquatic environment. Moreover, the fish were found more resistant to the moderate concentrations of copper sulfate toxicity as compared to snails which ended this research in great success.

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