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Toxicity effect of copper oxychloride on an Aquatic fern, *Azolla pinnata* R. Br.

R. Santhi

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Research Article

Abstract : In this study, the toxic effects of a heavy metal containing fungicide, copper oxychloride, on the water fern, *Azolla pinnata* was assessed in the laboratory condition. *Azolla*, an aquatic macrophyte is an ideal biofertilizers for agriculture and also used as feed for livestock, bioremediation of waste water and reclamation of saline soils. Toxicity test was employed using six different concentrations (0, 0.03, 0.06, 0.09, 0.12 and 0.15 mg/l) along with the control, for an observation period of 7 days. To verify water fern tolerance to copper oxychloride, the effects on morphological changes, number of plants produced and growth appearance were recorded daily. During 7 days of exposure, *A. pinnata* can survive with fungicide concentration of 0.03 mg/l, but all the macrophytes died in 0.15 mg/l concentration at the end of 7 days of exposure. The results clearly showed that the copper oxychloride at higher concentrations and longer exposure cause phyto toxicity in the water fern, *A. pinnata*.

Keywords: *Azolla pinnata*, Heavy metals, toxicity, copper oxychloride, tolerance, metal pollution

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Present Address
Dr. R. Santhi
Head, Department of Zoology,
Pioneer Kumaraswamy College,
Nagercoil, Tamil Nadu.
Email: rsanthib@gmail.com

Submission of manuscripts info:
biotekeditor@yahoo.com

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1. Introduction

The fungicides have wide application in agro ecosystems and they are always components of agricultural run of entering freshwater bodies. The non-target species affected by copper based fungicides includes mites (Michaud and Grant, 2003) entomopathogenic fungus (Mc Coy and Lye, 1996) and nematodes (Jaworsha and Gorczyca, 2002). The fate of toxic substances in soil and aquatic environment depends on processes like non-specific absorption, specific absorption, precipitation, dissolution, oxidation, reduction, phyto chemical reaction, biotic process etc. (Huang, 1993).

A. pinnata is a freshwater macrophytes, species of fern known by several common names, including feather mosquitofern and water velvet. Rajendran and Reuben, (1998) showed that *A. pinnata* greatly reduced both oviposition and adult emergence of *Culex quinquefasciatus* and *Anopheles culicifacies* gills but not larval survival. Laboratory culture of preliminary experiment was conducted to assess the ability of the *A. pinnata* to survive when exposure to different concentrations of diesel was shown by Al-Baldawi *et al*., (2012). Arora (2006) found that tolerance and phytoaccumulated of chromium by three *Azolla* species and also the same result was found by Cohen-shoel, (2002). Studies have shown that the most popular fresh water macrophytes used in toxicity test is the duckweed (family Lemnaceae) and the small free floating aquatic fern *Azolla* (family Azollaceae). Their small size, simple morphology, very fast growth rates, cosmopolitan in distribution and ability to grow under varying ecological conditions are attributes that has made them promising test species (Lewis, 1993).

The main aim of the present study was to determine the toxicity of a heavy metal containing fungicide copper oxychloride on *A. pinnata* in laboratory condition. It also assesses the tolerance of the aquatic fern to heavy metal pollution.

2. Materials and Methods

Aquatic ferns of *A. pinnata* was bought from Vivekananda Kendra, Kanyakumari District in Tamilnadu. *A. pinnata* were maintained in laboratory under controlled conditions (Proper aeration, pH, temperature and light). Different concentration of copper oxychloride solutions were prepared for this experiment. Macrophytes were exposed to different concentration of copper oxychloride ranging from 0.03, 0.06, 0.09, 0.12 and 0.15 mg/l. For toxicity test triplicates were maintained for each concentration along with control. 20 healthy fronds were introduced into each concentration. Physical observation for 7 days was done to determine the ability of the macrophytes to survive and resist the copper oxychloride contaminant. The physico-chemical parameters of water were observed before starting this experiment Table –1.

Table –1: Physico- chemical parameters of water

Sl. No	Parameters	Concentration
1.	Temperature (^{o}C)	26
2.	Hydrogen Ion concentration (pH)	6.8
3.	Dissolved oxygen content (ml/l)	4.24
4.	Total Alkalinity (ppm)	10
5.	Salinity (ppm)	1.436
6.	Chlorinity (ppm)	0.0617

The percentages of dead plants were calculated based on the number of *A. pinnata* died over total number of *A. pinnata* (Table - 2).

$$\% \text{ of dead plants} = \frac{\text{No. of Dead plants}}{\text{Total Number of Plants}} \times 100$$

3. Results

First a range of 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l copper oxychloride were used. After first day of exposure it was observed that *A. pinnata* was died for all the concentrations of copper oxychloride. This is due to the higher concentrations of copper oxychloride to the macrophytes. Hence these macrophytes were exposed another sets of copper oxychloride ranges from 0.03, 0.06, 0.09, 0.12, and 0.15 mg/l. During the 7th days of exposure it was observed that *A. pinnata* can survive 0.03 mg/l concentration. 10 percent *A. pinnata* died 0.06 mg/l concentration on 5th days of exposure and 50 percent mortality was observed 0.12mg/l concentration

on 5th days of exposure. During the 7th days of exposure 100 percent mortalilty was recorded at only 0.15 mg/l concentration and 50 percent mortality is seen 0.09 mg/l. In *A. pinnata* exposed to copper oxychloride for 5th day, 50 percent mortality was recorded in the concentration of 0.12 mg/l and the same result was also recorded at 0.15 mg/l concentration on the 3rd day. In control multiplication and growth of macrophytes was observed throughout the experiment. Visible damages were observed in the macrophytes during the treatement in different concentration of copper oxychloride. Chlorosis (a colour change of green to yellow) was observed on 0.06 mg/l concentration on 4th days of exposure. The growth was inhibited from the third day on 0.09 mg/l concentration and 80 percent mortality was recorded at the concentration of 0.12 mg/l after seventh day exposure. *Azolla* changed its colour from yellow to brown indicates the death of the macrophytes.

Table-2: Mortality response of *A. pinnata* to Copper oxychloride

Concentration (mg/l)	Mortality (%)						
	Day1	Days 2	Days 3	Days 4	Days 5	Days 6	Days 7
0.03	0	0	0	0	0	0	0
0.06	0	0	0	0	10	10	20
0.09	0	10	20	20	30	40	50
0.12	0	20	30	40	50	70	80
0.15	20	40	50	60	80	90	100

4. Discussion

Aquatic macrophytes are more suitable for waste water treatment due to their fast growth, ability to pollutant uptake and better purification effects due to direct contact with contaminated water. The results of the toxicity studies indicate clear-cut non-target effects of a heavy metal containing fungicide, copper oxychloride on an aquatic macrophytes such as *A. pinnata*. *Typha, Eichhornia, Azolla, Lemna* and other aquatic macrophytes are some of the wetland plants that provide an enriched culture zone for the microbes involved in the degradation of heavy metals (Rai, 2008). Earlier results of water fern, *Azolla* is a small, free floating, can fix nitrogen by its symbiotic partnership with Anabaena (Kannaiyan *et al;* 1997). For this reason *Azolla* has been used as a green manure to improve soil fertility and rice production (Wagner, 1997).

Sela *et al.*, (1989), Rai and Tripathi (2009) have been reported that *Azolla* has high capacity to accumulate toxic elements such as mercury, chromium, cadmium, copper, nickel and zinc. Zhang *et al.*, (2008) have been studied that the three species of water fern *A. caroliniana, A. filiculoides* and *A. pinnata* has the ability to uptake arsenic from water and tolerate arsenic concentration in various solution. In the present study the aquatic macrophyte *A. pinnata* tolerate in the copper oxychloride upto 0.03 mg/l concentration on 7 days of exposure. The exposure of heavy metals suppressed the vegetative growth and sporulation in different species of *Azolla* depends on the tolerance species as well as the concentration of heavy metals was showed by Arora *et al.*, 2006. Rai 2008 observed 27 to 39.9 percentage suppression of *A. pinnata* growth in the presence of various treatments of cadmium and mercury (1.0 and 3.0 mg/l) and also growth reduction in *A. caroliniana* is due to the presence of mercury was reported by Bennicelli et al., 2004. In the present study copper oxychloride solution at low concentration (0.03 mg/l) was essential to develop the fronds of *A. pinnata* but in higher concentration (0.12 mg/l) rapid development of chlorosis the fronds colour changed into yellow and reduction in the growth of *A. pinnata* were observed.

Copper is considered to be one of the most toxic trace metal to plants, although it is required as an essential elements for metabolic and physiological processes (Xia and Tian, 2009). The tolerance and concentration capacity of *Azolla* to different metal ions has been reported in earlier studies also. In 1985, Yong – Huang and Weizhen from China studied tolerance of four *Azolla* Species to Cu, Mn, Fe, Zn, Mo,Co, Cd etc. under laboratory conditions it was found that concentration capacity of *Azolla* for metals affected its growth only slightly without any detrimental effect or not at all. This study also shows that low concentration (0.03 ml/l) of this fungicide can the capacity to tolerate the metal and its production only slightly affected during the 7^{th} days exposure.

Madhaiyan *et al*.,(2006) suggested that the application of various insecticides and fungicides show low toxicity effects on the doubling time of *Azolla*, compared with herbicides. Kannaiyan (1989) reported that carbofuran have little effect on the relative growth rate in *A. pinnata*, such kind of result was also obtained in metal stressed *A. fillculoides* by Khosravi *et al*., (2005). Growth behavior of *Azolla pinnata* at various salinity levels and induction of high salt, tolerance studies showed adaptation involved the development of a capability in the plants to regulation concentration has been reported by Rai and Rai (1999).

Copper is used today as a chemotherapeutic agent in aquaculture in the world (GESAMP, 1997). However, the high level of copper in aquatic environments is due to industrial or agricultural wastes. Fungicides used in agro ecosystem affect aquatic organisms directly once they are drained into water bodies. The complexity of copper flux in the aquatic ecosystems is not fully understood but its impact has been clearly established. A common freshwater macrophyte *A. pinnata* is significantly affected by copper oxychloride at higher concentration and this species is cosmopolitan in distribution may be considered as a bioindicator of copper pollution by its greater degree of tolerance, compared to other aquatic macrophytes.

Heavy metal containing fungicides like copper oxychloride are toxic to aquatic organisms as they leach into the aquatic environment, persisting there for very long periods of time. The heavy usage of this fungicide gets dispersed into the aquatic environment and is transported into different matrices at low tropic levels. The chronic exposure may cause reduced growth, shorter life span, reproductive problems, reduced fertility and behavioural changes were observed in *A. pinnata*. In the present work, *Azolla pinnata* "a green gold mine" (Wagner, 1997) was chosen to study the toxicity effect of copper oxychloride. This study obviously showed that this aquatic macrophytes have tolerate different concentration of copper oxychloride. So, the macrophyte *A. pinnata* is considered to be a best tolerate of fungicide in the aquatic system.

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