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Effects of Insecticide-Difenoconazole and Fungicide-Chloropyrifos on *Azolla cristata* and *Salivinia natans*

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ABSTRACT

A study was carried out to investigate the effect of an insecticide difenoconazole (cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether) and fungicide chloropyrifos (Diethoxy-sulpanylidene-(3, 5, 6-trichloropyridin-2-yl) oxy-5-phosphane) on the chlorosis, leaf split and survival rate of Azolla cristata and Salvinia natans at concentrations of 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, and 0.5ml per 4 litre of water. The inhibition was found to be dose dependent. The fungicide chloropyrifos was found to be detrimental to the developmental of Azolla cristata and Salvinia natans. However, the deleterious effects of difenoconazole on Azolla cristata and Salvinia natans were low. The experimental trials showed the significant reduction in growth and survival rate of Azolla cristata due to chloropyrifos treatment. Further, chloropyrifos exposure was considerably affecting the photosynthetic pigments of plant. The strong inhibitory effect on the growth and photosynthetic pigments could be related with chloropyrifos induced inhibition. In contrast to this, Salvinia natans, showed resistence and can withstand toxic effects of different pesticides. The results of our study showed that Azolla cristata have low tolerance to all concentrations of chloropyrifos and high tolerance to difenoconazole at the different concentrations, as compared to the Salvinia natans which showed more tolerance to the lower concentrations of chloropyrifos and different concentrations of difenoconazole.

Key Words: Azolla cristata, Growth rate, Photosynthetic pigments, Salvinia natans, Suvival rate

1. INTRODUCTION

FAO has defined the term of pesticide, “As any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transportation or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies” [1]. The subclasses of pesticides include herbicides, insecticides, fungicides, rodenticides, pediculocides and biocides[1].

Fungicides are defined as the biological agents or the chemical compounds whose presence is having negative influence on the growth and development of fungi or their spores [2]. Population is increasing at an alarming rate. In order to sustain the increasing demands of growing population more and more pressure is put on the agricultural land and more pesticides are introduced into the immediadiate living environment. Initial legislative authority for managing the use of pesticides was enacted in 1910 [3]. Pesticide use has increased 50-folds since 1950 and 2.3 million tons (2.5 million short tons) of industrial pesticides are now used every year, of which 45% is consumed in Europe, 24% in USA and 25% in rest of the countries [4]. In India the pesticidal consumption is low 0.5kg ha^{-} in Korea and Japan the pesticidal consumption is 6.6 and 12.0kg ha^{-}[5]. Indiscriminate use of pesticide can develop resistance among pests, necessitating a new pesticide. Indiscriminate use of pesticide can develop resistance among pests, necessitating a new pesticide. Alternatively, an increased dose of the pesticide can be used to counteract the resistance, which will further worsen environment. The studies framed out by Shahate (2011) stated the effects of different concentrations of pesticides with respect to growth rate, pigment composition and photosynthetic activity of *Azolla cristata* and *Salvinia natans* under laboratory conditions and it was revealed that pesticide uptake generally increased with the increase of the incubation period of the applied chloropyrifos and difenoconazole at all concentrations [6]. Chloropyrifos and difenoconazole induced adverse effects on the survival, morphological and metabolic behaviour of *Azolla cristata* and *Salvinia natans*. The growth rate was decreased with increasing time and concentrations, compared to the control. It has been reported that pesticide inhibited the relative growth rate, pigment content and promoted the splitting of the plant body [7]. Insecticide carbofuran (furadan) has been found to significantly increase the chlorosis, which resulted in decreased photosynthetic activity of *Azolla cristata* [8]. It has been reported that addition of pesticides to the growth media substantially reduced the survival rate of *Salvinia natans* by affecting the photosynthetic activity due to the reduction of chlorophyll in the plant leaves [9]. Kole (1999) revealed that most of pesticides undergo photochemical transformation to produce metabolites which are relatively toxic to the free-floating macrophytes and the toxicity increased with time [10].

This study detailed the impacts and fate of pesticides-Chloropyrifos (fungicide) and Difenoconazole (insecticide) on *Azolla cristata* and *Salvinia natans* collected from world famous Dal Lake.

2.MATERIAL AND METHODS

2.1 Material

Two pesticides namely chloropyrifos and difenoconazole were used in different concentrations, to evaluate the changes in the morphology and survival rates of the two free-floating aquatic plants, *Azolla cristata* and *Salvinia natans*.

2.1.1 Chloropyrifos

Chloropyrifos is a Fungicide commonly known as Dursban having molecular formula $CH_{11}Cl_3NO_3PS$ and IUPAC name Diethoxy-sulpanylidene-(3, 5, 6-trichloropyridin-2-yl) oxy-5-phosphane.

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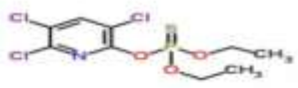
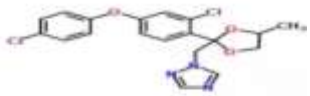


Structure of chloropyrifos

2.1.2 Difenoconazole:

It is a crystalline organophosphate insecticide commonly known as Difenoconazole having molecular formula is $C_{19}H_{17}Cl_2N_3O_3$ and IUPAC name cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether.



Structure of difenoconazole

2.1.3 *Azolla cristata*

Azolla are weeds in many parts of the world, entirely covering some water bodies. *Azolla* is a macrophyte, floating on the surface of water by means of numerous small closely overlapping scale-like leaves with their roots hanging in the water. They are in symbiotic relationship with the cyanobacterium *Anabaena azollae*, which fixes atmospheric nitrogen, giving the plant access to the essential nutrients.

2.1.4 *Salvinia natans*

Salvinia, a genus in the family Salviniaceae, is a floating fern. Recent sources include both *Azolla cristata* and *Salvinia* in Salviniaceae, although each genus was formerly given its own family. *Salvinia*, like the other ferns in order Salviniales are heterosporous, producing spores of differing size. *Salvinia* are small, free floating aquatics with creeping stems, branched, and bearing hairs on the leaf surface papillae but no true roots.

2.1.5 Methods

Dose-response study on aquatic plants was a trial based and entirely depends out on developing the protocol for the present study. Work of Shahate *et al.*, (2011) and Raja *et al*., (2012) were reviewed for the development of protocol. Efforts were made to detect the nominal dosage rate which was also done by using an array of concentrations spread over wide range. Trials were focused on the concentration above the nominal dosage. After the development of the protocol, the dose-response study was carried out .Fresh plant samples of *Azolla cristata* and *Salvinia natans* were collected from different areas of Dal Lake. Different concentrations of the pesticides (0.01, 0.05, 0.1, 0.5, 0.25, 0.025, 0.005 ml per 4 liters of water) were taken in 5 liter buckets and were allowed to stand for an exposure period of 3 weeks (21 days). In each bucket, 20 fronds of these free-floating plants were subject to this dose-response study. Observations related to chlorosis, leaf damage and survival were recorded on daily basis and noted down for further evaluation and discussion.

3.RESULTS AND DISCUSSION

The different pesticides used in the present work showed varying effects on *Azolla cristata* and *Salvinia natans*. A range of concentrations (0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 ml per 4 litre of water) of chloropyrifos and difenoconazole were used to verify the optimal level which can be used without affecting the viability of *Azolla cristata* and *Salvinia natans.* The effects on the survival, leaf split and chlorosis of *Azolla cristata* and *Salvinia natans* were analysed after the treatment. These observations were derived from the fronds grown in the control and the results were expressed in percentage (%).

3.1 Effect of chloropyrifos

It is evident from the Fig. (1.1) chlorosis of *Azolla cristata* increased significantly with the increase in the time period of treatment. Maximum chlorosis (100%) was observed on the day 19, treated with 0.25ml chloropyrifos followed by 0.5ml which resulted in 98% chlorosis in *Azolla cristata*. The least chlorosis was observed at 0.01ml of chloropyrifos during the entire study period. 0.025ml and 0.1ml resulted in 90% chlorosis followed by 0.005ml and 0.05ml which accounted to 80% of chlorosis in *Azolla cristata*.

The chlorosis of *Salvinia natans* increased significantly with the increase in the time period of treatment. The maximum chlorosis (100%) was observed on day 21 treated with 0.25ml and 0.5ml of chloropyrifos, followed by 0.025ml and 0.05ml which resulted in 90% chlorosis. The minimum chlorosis (30%) was observed at 0.01ml of chloropyrifos during the entire study period Fig. (1.2).

The leaf split for *Azolla cristata* was observed for all concentrations at different stages of time. At 0.25ml, 0.1ml and 0.5ml of chloropyrifos, leaf split was observed 100% within 21 days of treatment. The plant exhibited a minimum leafsplit (30%) at 0.01ml of chloropyrifos. 0.05ml resulted in 70% leaf split followed by 0.005ml and 0.05ml which accounted to 60% and 50% leaf split, respectively Fig. (1.30).

In *Salvinia natans* the leaf split increased significantly with the increase in the time period. The maximum effect (100%) was observed on day 20, treated with 0.5ml of chloropyrifos followed by 0.1ml which resulted in 90% leaf split in *Salvinia natans*. Minimum leaf split (20%) was observed at 0.01ml and 0.005ml of chloropyrifos during the entire period of treatment. 0.05ml and 0.25ml resulted in 50% chlorosis followed by 0.025ml which accounted to 40% leaf split in the plant Fig. (1.4).

The survival rate of *Azolla cristata* decreased significantly with the increase in the time period. The maximum survival rate was 95% observed at 0.01ml of chloropyrifos during the entire study period. Minimum survival rate (0%) was observed on the day 19, treated with 0.25ml of chloropyrifos. 0.05ml and 0.005ml resulted in 20% survival rate followed by 0.025ml and 0.1ml of chloropyrifos which accounted to 10% survival rate in *Azolla cristata* Fig.(1.5).

Salvinia natans showed a gradual decrease in the survival rate at all the concentrations of chloropyrifos at different stages of time (Figure 1.6). The maximum survival rate (70%) was observed at 0.01ml on the day 21 of treatment. Minimum survival rate (0%) was observed on the day 20, treated with 0.25ml and 0.5ml of chloropyrifos. 0.05ml, 0.1ml and 0.025ml accounted to 30%, 15% and 10% survival rate, respectively within the entire study period.

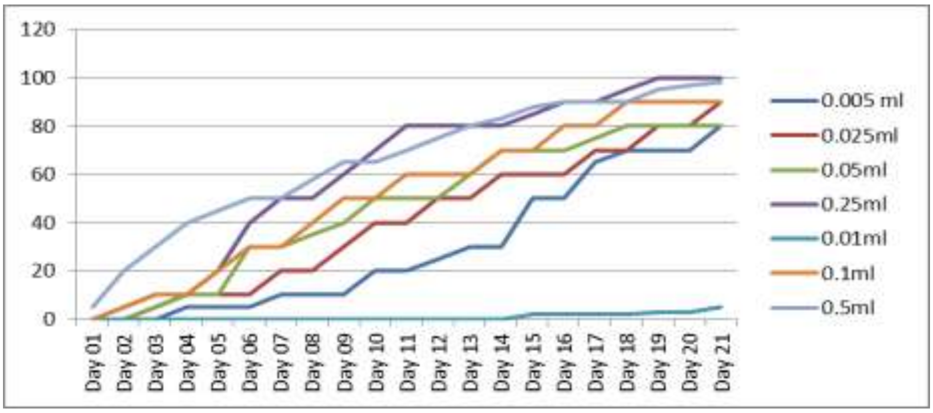


Figure 1.1 showing effect of chloropyrifos on the chlorosis of *Azolla cristata* in different concentrations.

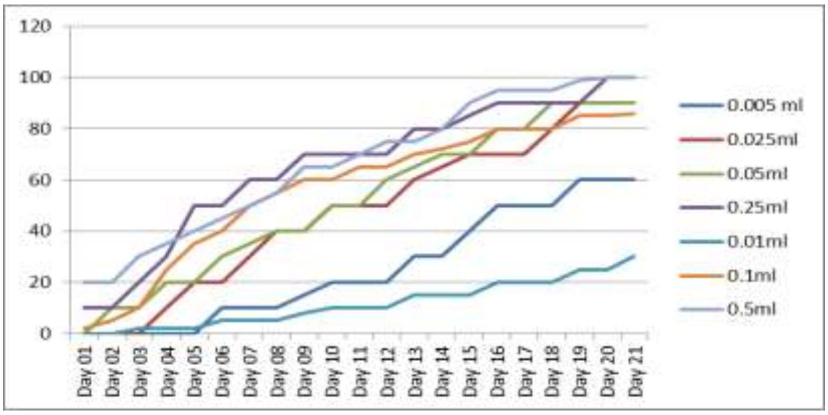


Figure 1.2 showing effect of chloropyrifos on the chlorosis of *Salvinia natans* in different concentrations.

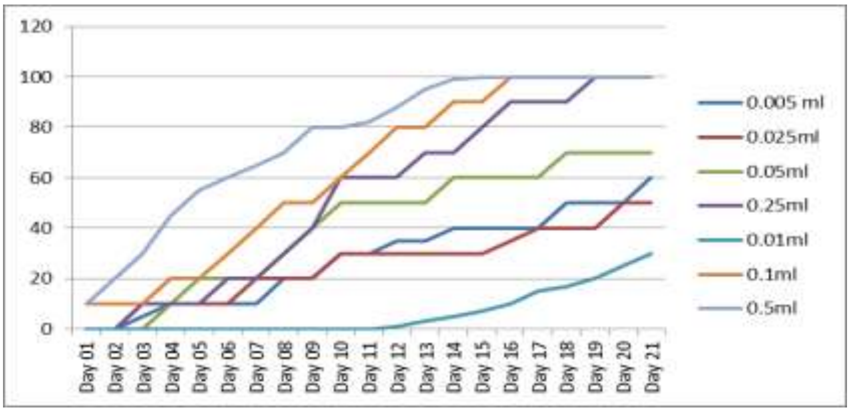


Figure 1.3 showing effect of chloropyrifos on the leaf split of *Azolla cristata* in different concentrations.

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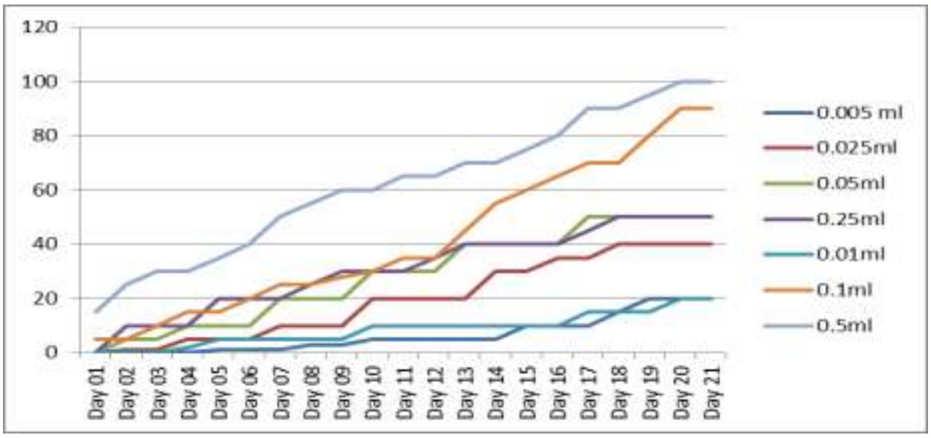

Figure 1.4 showing effect of chloropyrifos on the leaf split of *Salvinia natans* in different concentrations.

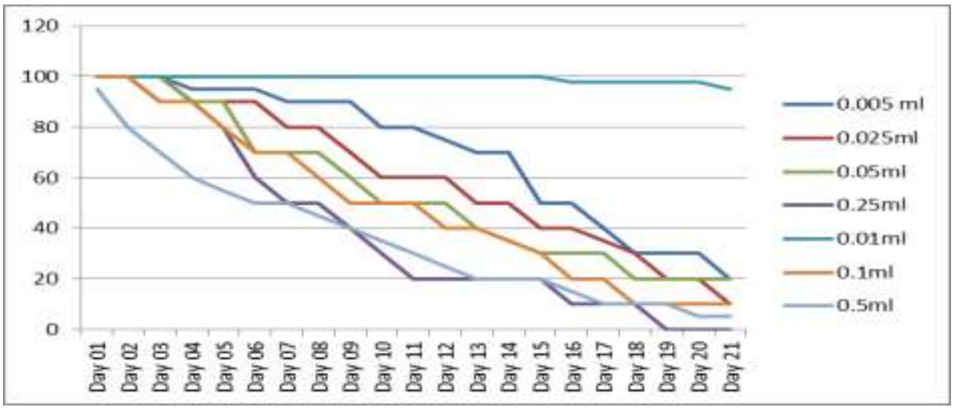

Figure 1.5 showing effect of chloropyrifos on the survival rate of *Azolla cristata* in different concentrations.

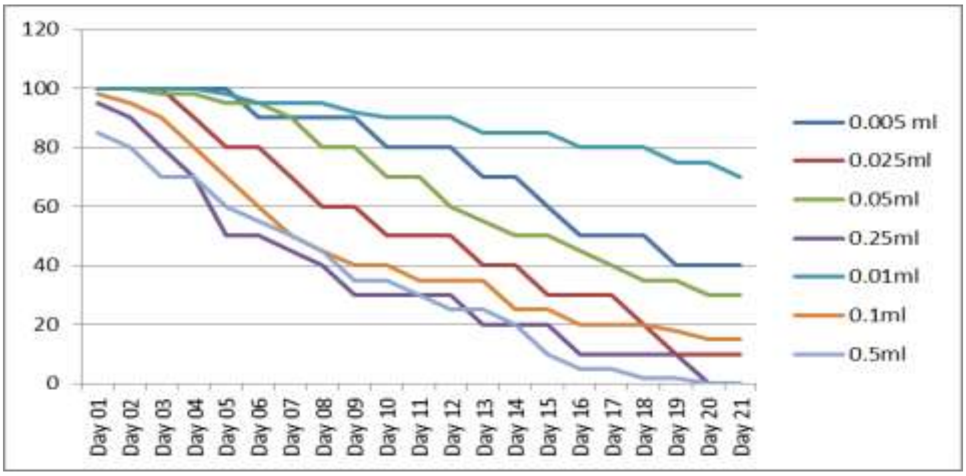

Figure 1.6 showing effect of chloropyrifos on the survival rate of *Salvinia natans* in different concentrations

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3.2 Effect of Difenoconazole

Azolla cristata appeared sensitive to 0.25ml and 0.5ml of difenoconazole. The chlorosis was exhibited as 2% at 0.25ml and 5% at 0.5ml, during the entire study period. However, no effect was observed for the other concentrations Figure (2.1). *Azolla cristata* appeared sensitive to 0.5ml of difenoconazole. 2% chlorosis was observed on the day 21 of the treatment. However, no effect was observed for the other concentrations Figure (2.3).The data reflects that for 0.5ml and 0.1ml of difenoconazole, the chlorosis of *Salvinia natans* reached only 3% and 2%, respectively observed on the day 19 of the treatment. However, no effect was observed for the other concentrations Figure (2.2). *Salvinia natans* did not show any sensitivity to any of the concentrations of difenoconazole during the entire treatment period Figure (2.4).

Survival rate of *Azolla cristata* was observed to decrease by 5% at 0.5ml concentration of difenoconazole during the entire study period Figure (2.5). However, *Salvinia natans* did not exhibit any sensitivity to any of the concentrations of difenoconazole within 21 days of treatment Figure (2.6).

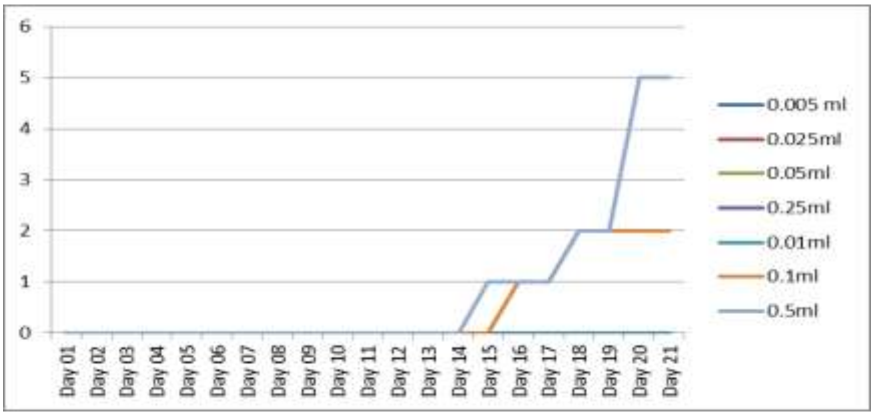

Figure 2.1 showing effect of difenoconazole on the chlorosis of *Azolla cristata* in different concentrations.

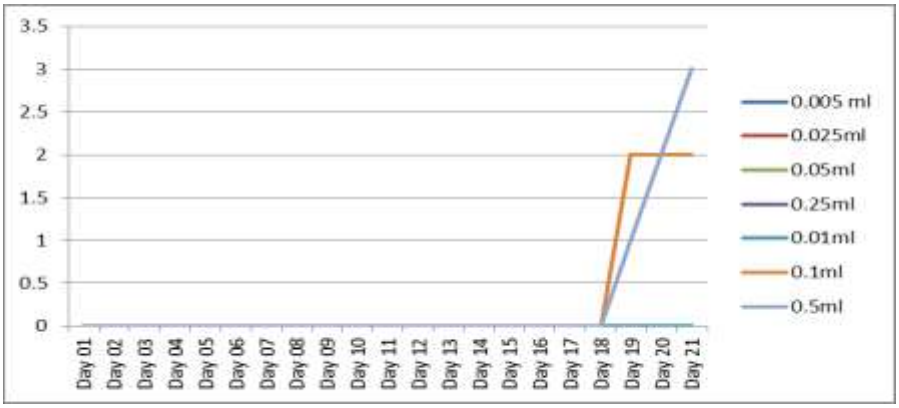

Figure 2.2 showing effect of difenoconazole on the chlorosis of *Salvinia natans* in different concentrations.

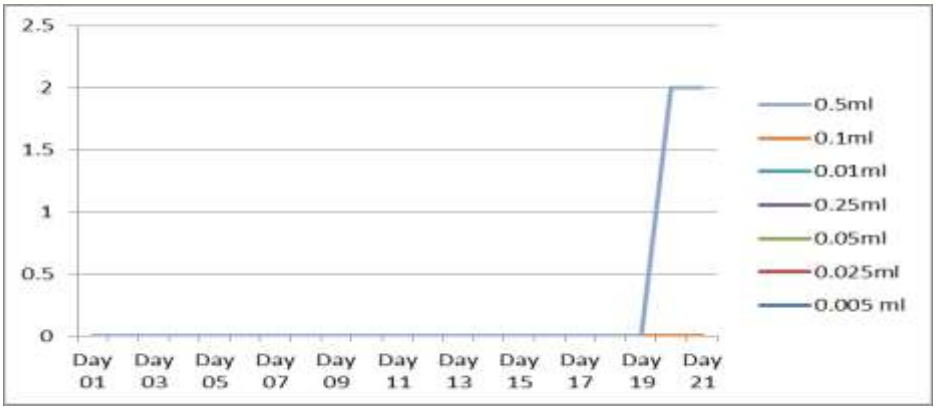

Figure 2.3 showing effect of Difenoconazole on the leaf split of *Azolla cristata* in different concentrations.

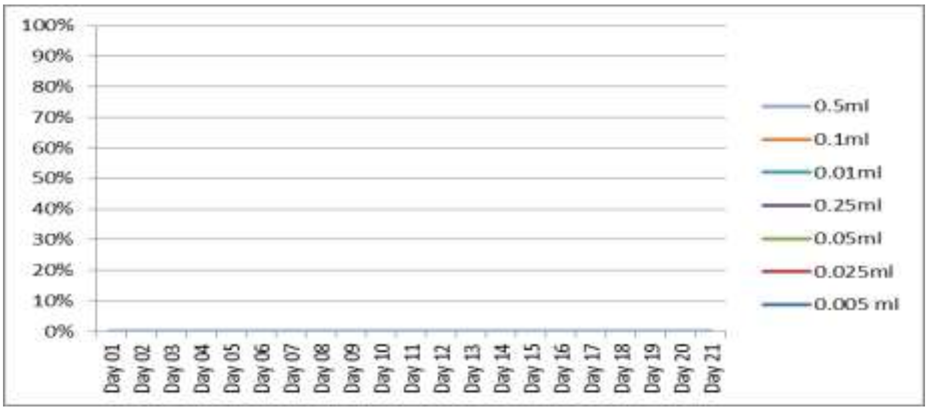

Figure 2.4 showing effect of Difenoconazole on the leaf split of *Salvinia natans* in different concentrations.

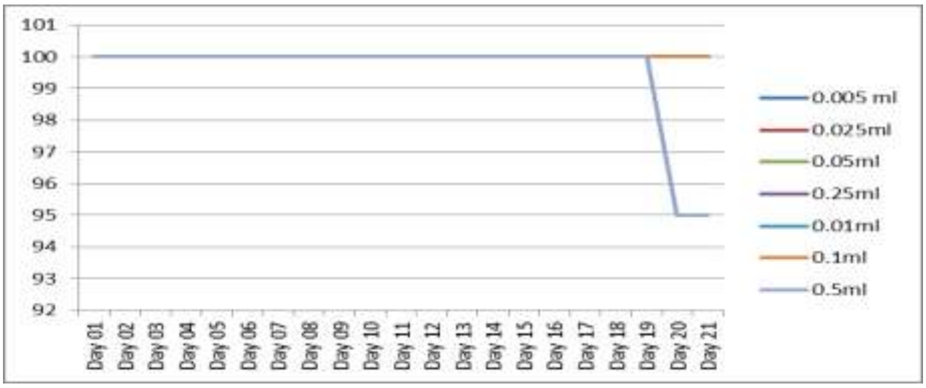

Figure 2.5 showing effect of Difenoconazole on the survival rate of *Azolla cristata* in different concentrations.

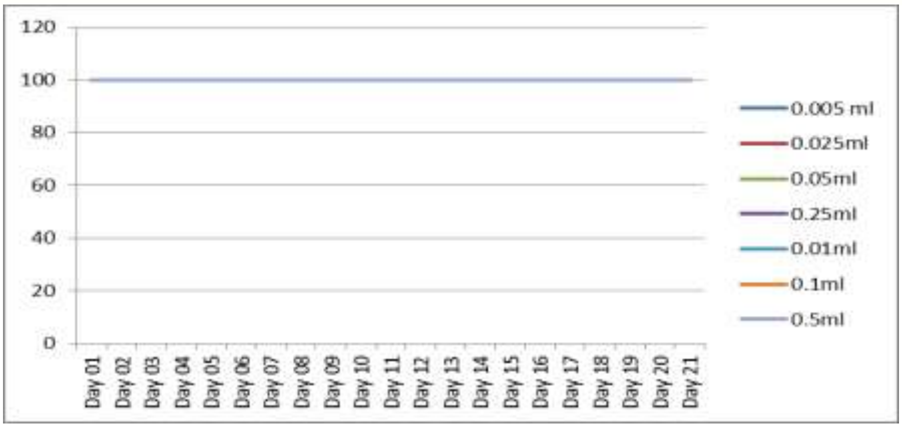

Figure 2.6 showing effect of Difenoconazole on the survival rate of *Salvinia natans* in different concentrations.

The range of concentrations (0.005, 0.025, 0.05, 0.25, 0.01, 0.1, 0.5 ml) of chloropyrifos and difenoconazole were applied without affecting the viability of *Azolla cristata* and *Salvinia natans*. Chloropyrifos exposure to *Azolla cristata* and *Salvinia natans* resulted in the reduction in relative growth rate and survival rate at all the concentrations compared to the control. Along with inhibition in the growth of *Azolla cristata* and *Salvinia natans*, pesticide application also resulted in increasing the chlorosis and leaf split in the plant within the treatment period of 21 days. It was observed that increasing the dose (concentration) of chloropyrifos, resulted in increasing the percentage of chlorosis and leaf split and decrease in the growth and survival rate in both the plants. The impact of fungicide (chloropyrifos) in the low doses (concentrations) was observed to be low while the impact of higher concentrations was adverse and the damage was immediately observed within 2 to 5 days of exposure. Higher concentrations were totally detrimental to the plant bodies and survival rate decreased drastically and the damaging effect of chloropyrifos on photosynthetic pigments (chlorosis) and leaf split of the plants was noticed within 3 days of treatment. The reduction in survival rate might be due to pesticide which affects the tissue binding process in *Azolla cristata* and *Salvinia natans* at higher concentrations and by the accumulation of these pesticides in the plant tissues [11]

The impact of insecticide (difenoconazole) was less as compared to the fungicide (chloropyrifos). The plants did not show any response to the small dosage of the insecticide in terms of chlorosis, leaf split and survival rate. The effect of difenoconazole on the survival rate of *Azolla cristata* and *Salvinia natans* was evident at higher doses (0.25ml and 0.5ml) which were observed in the plant body in the form of chlorosis, leaf split and survival rate.

During the present study, it was observed that an increase in the concentration of pesticides resulted in increasing the inhibitory effects on the growth and survival rate of both the macrophytes, because of the accumulation of the pesticides in the tissues and the heavy use of pesticides (higher dose) reduced the growth and survival rate of the plants, which is in agreement with the studies of a similar trend. Rajendra (2000) also observed that many pesticides and their residues got accumulated in aquatic plants thereby decreasing their growth and survival by inhibiting the photosynthetic activities of the plants.

During the present study, it was observed that the treatment with chloropyrifos and difenoconazole at varied concentrations inhibited the relative growth rate, reduced the pigment content and promoted the splitting of the leaves, which in turn interfered with their photosynthetic performances and thus, reduced the survival rate of the plants. This observation is in agreement with the findings of Raja *et al*., (2012). Moreover, Singh also reflected in his studies that the anti-enzymatic activity and the mortality caused by the pesticide application were time and dose dependent [12], which was also observed during the present investigation.

4.CONCLUSION

The study on *Azolla cristata* and *Salvinia natans* was devoted to investigate the impacts of chloropyrifos and difenoconazole *on the chlorosis, leaf split and survival rate of Azolla cristata and Salvinia natans* at different concentrations. The results demonstrated differential response in terms of growth, survival, leaf split and chlorosis of *Azolla cristata* and *Salvinia natans* in response to chloropyrifos and difenoconazole. The fungicide (chloropyrifos) showed to be deleteriously affecting the activities in the *Azolla cristata* and *Salvinia natans*. It adversely depleted the chlorophyll content, leading to a marked increase at lower concentration and the effect gradually increases at higher concentrations. Decrease in the survival rate also increased with increase in the concentrations of chloropyrifos. The inhibition was found to be dose dependent. On the contrary, the deleterious effects of difenoconazole on *Azolla cristata* and *Salvinia natans* were low.

The different treatment trials showed that chloropyrifos affect *Azolla cristata* and caused significant reduction in growth rate, contents of photosynthetic pigments and survival of the plant. The strong inhibitory effect on the growth and photosynthetic pigments could be related with chloropyrifos induced inhibition. In contrast to this *Salvinia natans, showed resistence and can withstand toxic effects of different pesticide.*It was obvious from the results obtained throughout this study that *Azolla cristata* showed low tolerance to all concentrations of chloropyrifos and high tolerance to difenoconazole at the different concentrations, as compared to the *Salvinia natans* which showed more tolerance to the lower concentrations of chloropyrifos and different concentrations of difenoconazole.

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