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Research Article

Evaluating growth and biochemistry of *Westiellopsis prolifica* in response to Malathion

Jyotishmita Dutta* & P P Baruah

Department of Botany, Gauhati University, Guwahati 781014, Assam, India

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Correspondence
Jyotishmita Dutta
✉ jyotishmitadutta90@gmail.com

Abstract
The present endeavour was aimed to investigate the chronic response of *Westiellopsis prolifica* to an organophosphate insecticide malathion at different concentrations 30, 60 and 90 ppm. The influence of malathion on growth(biomass), pigments(chlorophyll-a, carotenoid), release of metabolites such as protein and carbohydrate was analysed for a period of 16 days under aseptic laboratory conditions. Results revealed enhancement in chlorophyll-a production at 30 ppm on 4th day ($p<0.05$) and 8th day ($p>0.05$), from the day of inoculation. On the other hand, there was a significant decrease in the carotenoid, protein and carbohydrate content with increase in malathion concentration in a time and dose dependent manner. However, a little but insignificant increase in biomass was recorded on the 4th day at 30 ppm concentration over the control. The study revealed that the reduction in biomass, protein and carbohydrate content with the increase of malathion concentrations was an indication of its toxicity to the test cyanobacterium which is one of the natural biofertilizers in the rice field ecosystem.

Keywords: Cyanobacterium; Organophosphate; Malathion; Westiellopsis prolifica..

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Introduction

Pesticides are synthetic organic compounds used in almost all the crop fields to control unwanted but selective pests (1). There are various types of pesticides widely used throughout the tropic which includes organophosphate, organochlorine, carbamate and pyrethroid groups. These are chemical compounds and are stimulatory, inhibitory or neutral depending on the nature of the chemicals, its concentration and the time of treatment (2). The use of pesticides has become an integral part of modern agricultural practices. Though they are being used for killing some targeted organisms, it ends up affecting the non targeted and beneficial organisms too. Thus, continuous addition of these chemicals to the rice fields affects the dynamic equilibrium of the soil environment (3) hampering the growth of beneficial soil microbes including nitrogen fixing cyanobacteria. It has been reported that 10-15 % yield loss in rice production is accounted for insect/ pest related causes (4). To overcome this loss,

different insecticides are used throughout the globe and malathion is one of them.

According to Patil and David (5), Malathion is a non-systemic, wide spectrum organophosphate insecticide used in both agricultural and non-agricultural practices. There are reports which inform that malathion has adverse effects on a number of algal groups and cyanobacterial species (6, 7).

Cyanobacteria are photosynthetic prokaryotes which has the ability to grow in both terrestrial and aquatic environment. They are important in agricultural fields as they can fix nitrogen, produce growth hormones, enhance soil fertility etc. The wide distribution of cyanobacteria reflects a broad spectrum of physiological properties and their tolerance capacity to environmental stresses including pesticidal toxicity (8).

W. prolifica, is abundantly found in all agricultural fields in general and rice fields in particular (9). It is one of the dominant cyanobacterial genera in the rice field soils of India (10, 11). They are of great economic and ecological significance due to their ability of fixation of elementary nitrogen (12, 13). The genera was reported to increases the soil fertility with humus and nitrogen content. It is also used as a 'diazotroph biofertilizer' (14). Reports suggest that regular exposure of cyanobacteria to pesticides have some deleterious effects on them (15).

It is essential to understand factors which hampers nitrogen fixation in rice fields. Weedicides, fungicides and pesticides which are used for protection of the crops in the rice fields are reported to have adverse effect on the cyanobacterial population. Thus in the present study, the effect of a commonly used pesticide, malathion on the growth and biochemistry on a common cyanobacterial biofertilizer (*W. prolifica).*

Material and Methods

Isolation and maintenance

The test organism, *W. prolifica* was isolated from the rice field soils of Assam. The pure cultures of the species was grown and maintained in BG-11 media without nitrogen at a temperature of 25±2 °C, under a light intensity of 2000-3000 lux intensity and alternating with light/dark cycles of 14/10 hours.

Pesticide used

The pesticide used for the study was malathion (50%EC), an organophosphate insecticide which is commonly used in rice fields is obtained from a chemical shop in Guwahati (Assam). The molecular formula and chemical name of malathion is $C_{10}H_{19}O_6PS_2$ and O, O-dimethyl phosphorodithioate of diethyl mercaptosuccinate. The stock solution of the pesticide was prepared by adding appropriate dilution of 50%EC malathion to 1000ml of sterilized double distilled water. From the stock solution, a desired amount of pesticide was added aseptically to the culture tubes to get the final concentrations of 30 ppm, 60 ppm and 90 ppm based on the LC_{50} value.

LC_{50} value

The test species was subjected to various concentrations from 10 ppm-120 ppm, from which the LC_{50} value was calculated in terms of chlorophyll-a following the method of (16) and recorded to be 60 ppm. Based on the LC_{50} value, various concentrations of 30 ppm, 60 ppm and 90 ppm were taken along with the control (without pesticide) to do the analysis.

Biomass estimation

Biomass was estimated following (17). 20 ml of the culture was centrifuged and filtrated. The filtered was properly washed with double distilled water for removing the adhering pesticides in the samples. The harvested wet pellet weighted around 4.5mg. The washed material was oven dried at 80 °C for 72 hr. The test was done in triplicate for both treated and control sets and at an interval of 4 days

Chlorophyll-a estimation

The homogenate cyanobacterial suspensions were washed with double distilled water to remove the traces of pesticides. The suspension was then homogenised with 10 ml of 95% methanol using mortar and pestle and boiled in a water bath for 30 minutes at 65^0C temperature. The samples were than cooled and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was read against 95% methanol as blank at 663nm in a uv-visible spectrophotometer 119 (SYSTRONICS). The value obtained was calculated according to the formula of (18).

Carotenoid estimation

Total carotenoid was calculated following (19). For calculating the total carotenoid, the samples were first washed with double distilled water and then suspended in 80% acetone and incubated overnight at 4^0 C. Next day, the samples were centrifuged and the supernatant was read in the spectrophotometer at 480 nm against 80% acetone as blank.

Protein estimation

1 mg of algal biomass was taken in a test tube and to it 1 ml of 1N NaOH was added and then the tubes were kept in a boiling water bath for 10 minutes. After that, the tubes were cooled and 5 ml of reagent A was added (prepared by adding 1 ml freshly prepared 1% Na-K tartarate solution in 50 ml of 2% Na_2CO_3 solution containing 0.5% CuSO4)

and incubated at room temperature for 10 min. This was followed by the addition of 0.5 ml of reagent B (Folin reagent), mixed properly and incubated at room temperature for another 30 min. The absorbance of the supernatant was read at 650 nm against folin reagent as blank. The amount of protein content was determined by comparing it with the standard curve of protein prepared with brovin serum albumin as a standard following (20)

Carbohydrate estimation

For carbohydrate estimation, 0.5ml of homogenised algal suspension was taken in test tube and volume was made to 1ml by addition of distilled water. It was followed by addition of 4ml anthrone reagent to the tubes (prepared by addition of 100mg of anthrone with 1gm thiourea in 100ml of 75% sulphuric acid). The tubes were then kept at boiling water bath for 10 minutes. Finally, the tubes were brought to room temperature and absorbance was read at 625 nm. The final carbohydrate content was calculated from the standard graph prepared with glucose as standard following (21).

Statistical analysis

The experiment was carried out in triplicates and was expressed as mean ± standard deviation (N=3). The significant test were carried out with one way

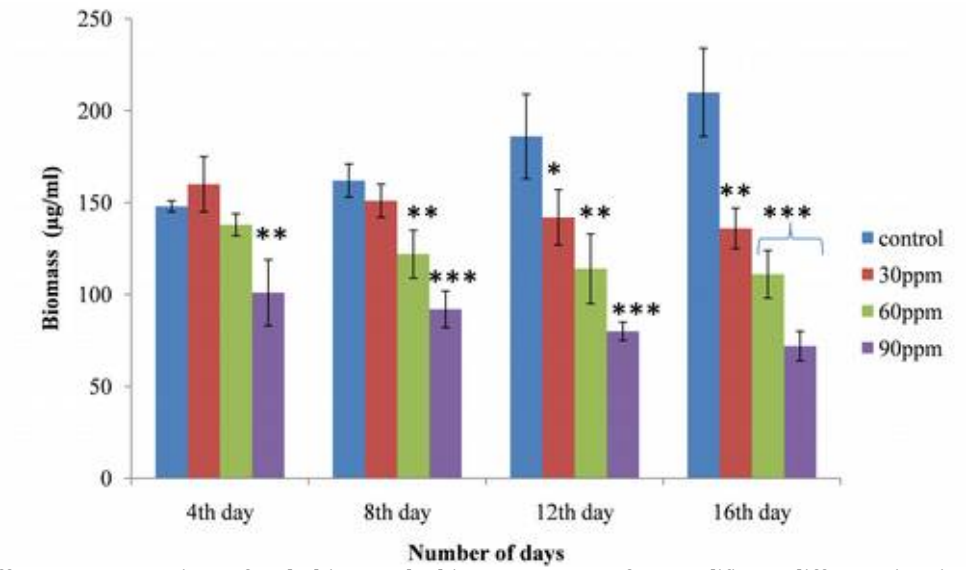

Fig. 1. Effect of different concentrations of malathion on the biomass content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p<0.05, **p<0.01, ***p<0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

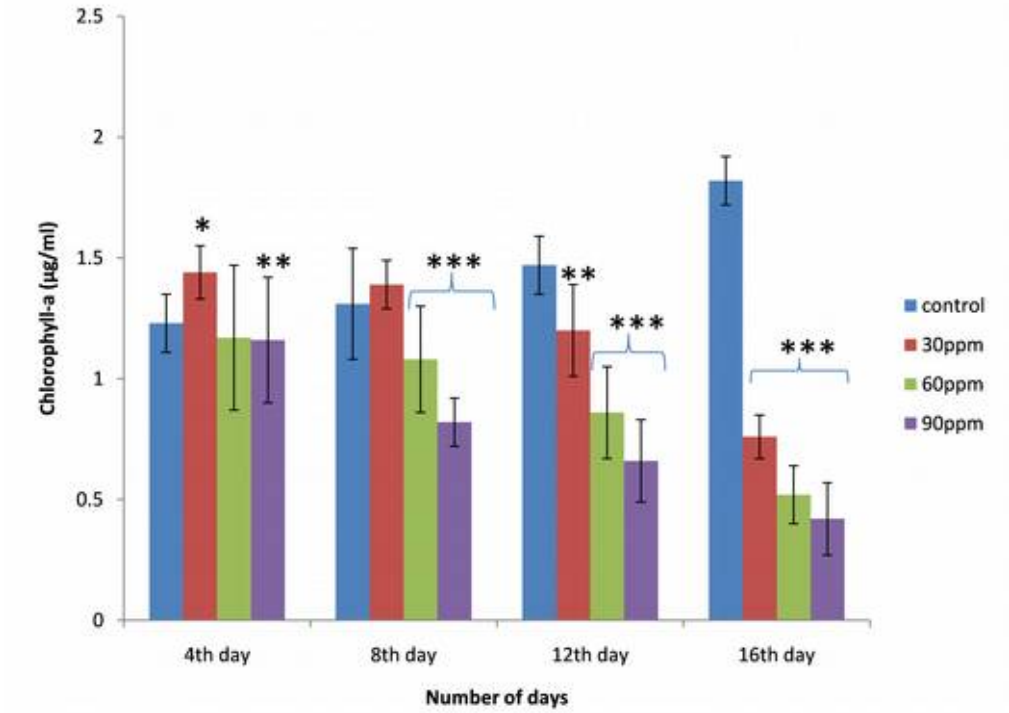

Fig. 2. Effect of different concentrations of malathion on the chlorophyll-a content of *W. prolifica* at different time intervals . The values are presented as mean ± SD of three replicates. Asterisks (*p<0.05, **p<0.01, ***p<0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

analysis of variance (ANOVA) followed by post hoc (Dunnet) test (P<.05 for significant) using software Graph Pad prism 5.01.

Results

Biomass

The biomass content of *W. prolifica* with malathion treatment is shown in the Fig. 1. From the figure, it was observed that the biomass content of the test species decreases from lower to higher concentrations up to 16th day from the day of inoculation, over the control, but on the 4th day, at 30 ppm concentration there was a little but insignificant increase in the biomass content compared to the control. The growth was found to be highest in the control set on the 16th day (210±12) µg/ml and the lowest growth was observed at 90ppm on the 16th day of treatment (72±16) µg/ml (P<0.001).

Chlorophyll-a

The growth of the test organism in terms of Chlorophyll-a is depicted in Fig. 2. From the figure, chlorophyll-a production was found to be slightly higher than the control at 30 ppm on the 4th day (p<0.05) and on 8th day (p>0.05) from the day of inoculation. There was a gradual reduction in chlorophyll-content from lower dose to higher dose of pesticide on the 12th and 16th day. The highest chlorophyll-a content was found in the

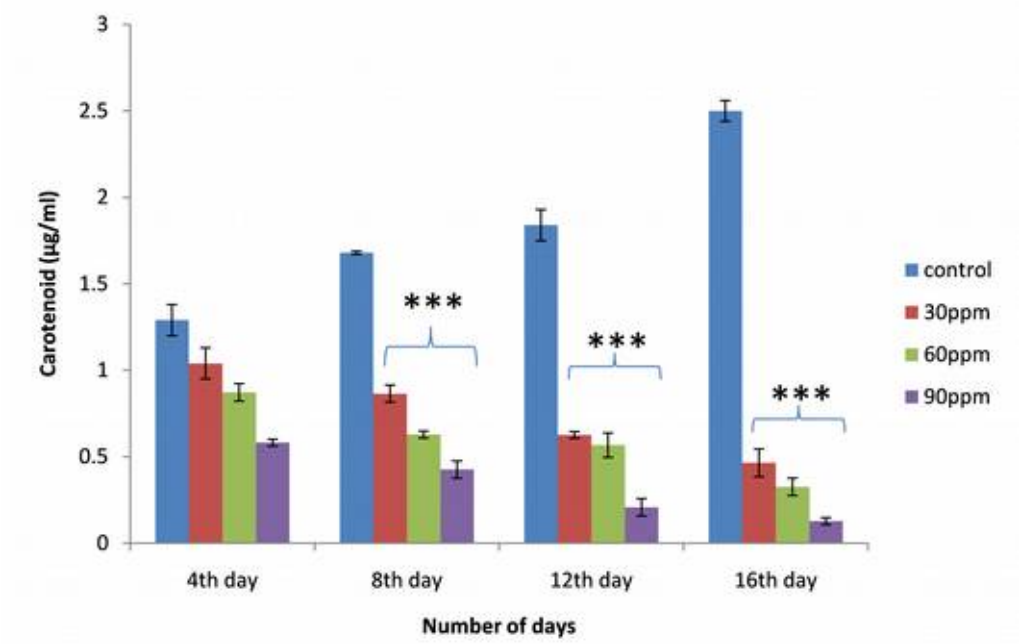

Fig. 3. Effect of different concentrations of malathion on the carotenoid content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p<0.05, **p<0.01, ***p<0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

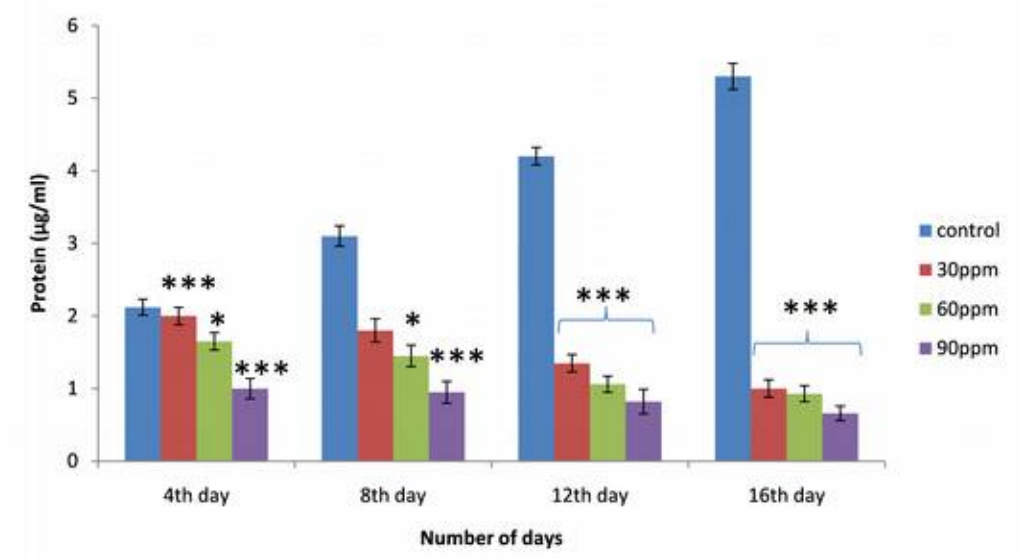

Fig. 4. Effect of different concentrations of malathion on the protein content of *W. prolifica* at different time intervals. The values are presented as mean ± SD of three replicates. Asterisks (*p<0.05, **p<0.01, ***p<0.001) above the histogram bars depicts significant variation in the pesticides treated samples over the control

untreated set that is in the control, on the 16th day (1.82 ± 0.1 µg/ml) and the highest inhibitory concentration was found to be 90 ppm with lowest reduction of chlorophyll-a on the 16th day (0.42 ± 0.15 µg/ml) ($p<0.001$).

Carotenoid

Results obtained after exposing the test organism to different concentrations of malathion is presented in Fig. 3. A remarkable degree of Carotenoid inhibition was observed in *W. prolifica* after exposure to malathion treatment for a period of 16 days. Carotenoid content of the test species was observed to be affected by the pesticide in a time dose dependent manner. In the control sets, an exponential growth was observed up to the 16th day from the day of inoculation throughout the experiment period. The highest carotenoid content was observed in the control during the 16th day (2.5 ± 0.06 µg/ml) and lowest reduction was observed on the 16th day (0.12i88z ± 0.02 µg/ml) at 90 ppm ($p<0.001$).

Protein

The effect on the protein content of the test species with malathion treatment is shown in Fig. 4. There was a gradual reduction in the protein content with increase in pesticide concentration in a time and dose dependent manner. The highest protein content was found in the control sets (5.3±0.18) µg/ml and lowest at 90 ppm (0.66±0.1) µg/ml on the 16th day ($p<0.001$).

Carbohydrate

Results of carbohydrate content obtained after exposing the test organism to varying malathion concentrations is depicted in the Fig. 5. The carbohydrate content was found to be decreasing gradually from lower to higher pesticide concentration with increase in time period. There was a significant reduction in carbohydrate content at 90 ppm on 12th day ($p<0.001$) concentration and on 16th day ($p<0.001$) from the day of inoculation.

Discussion

The effect of Malathion on the biomass of *W. prolifica* as observed in Fig. 1 shows that it has a significant effect on the growth of the species. The pesticide showed deleterious effect on the biomass content of the test species. The biomass decreases considerably with the increase in concentration of the pesticide with time. On the other hand, in the control sets, a steady growth was observed up to the 16th day from the day of inoculation. The decrease in biomass might be due to the inhibition of photosynthesis by the application of the pesticide (22). This observation supports the findings of (23) while working on *Anabaena variabilis* exposed to malathion pesticide. However, an insignificant increase in biomass at 30 ppm on 4th day might be an adaptive measure adopted by the test organism at low pesticide concentration (24).

Malathion treatment in *W. prolifica* affects its chlorophyll-a content in different blue green algae by taking part in light absorption and photochemistry (25). The chlorophyll-a content of the species after treatment with the pesticide was seen to be enhanced at low concentration of

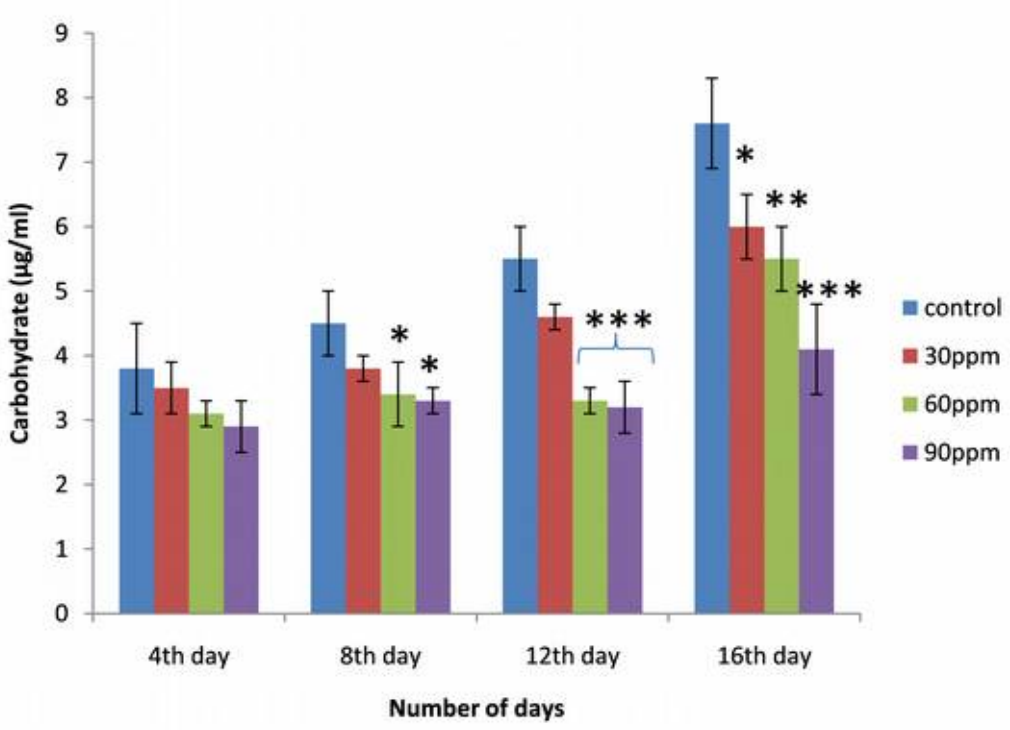

Fig. 5. Effect of different concentrations of malathion on the carbohydrate content of *W. prolifica* at different time intervals . The values are presented as mean ± SD of three replicates. Asterisks (*$p<0.05$, **$p<0.01$, ***$p<0.001$) above the histogram bars depicts significant variation in the pesticides treated samples over the control.

pesticide *i.e.* at 30 ppm and there was an increase in the pigment up to the 8th day from the day of inoculation compared to the control (Fig. 2). With gradual increase in the concentration of the pesticide with time, the content of chlorophyll-a decreased. This is an indication that photosynthesis is inhibited at higher pesticide concentration (4). The inhibition of photosynthesis accelerates degradation rate of pigments to meet the energy demands of the cells by producing carbon skeleton in the cell (26). Similar to our findings, (27,28) also reported that the effect of malathion on cyanobacterial and algal populations has stimulatory effect at lower concentration (30ppm) and inhibitory at higher concentration (90ppm) of the pesticide.

Carotenoid which is an accessory pigment of cyanobacteria plays an important protective role in photo-oxidative damage and also serves as light harvesting pigment during photosynthesis (29). The carotenoid pigment which is associated with the photosynthetic process was seen to be decreasing with increase in pesticide concentrations and time throughout the study (Fig. 3). The decrease in carotenoid content specifies that the pesticide not only accelerated the degradation process but also blocked their synthesis (30). This result is in consonance with the findings of (17), where he observed similar effect of carotenoid content in *Anabaena cylindrical* with molinate.

Protein is an important metabolite in cyanobacteria. The data obtained, highlights gradual decrease in protein content with increase in pesticide dose with time (Fig. 4.) This decrease in protein content with increase in time could be due to the inhibition in the synthesis of enzymes and structural proteins which are necessary for the growth of cyanobacteria (30). This result is in agreement with the findings of (4, 31).

It was recorded that increase in concentration of pesticide drastically retarded the carbohydrate content of *W. prolifica* (Fig. 5), and it could be possibly due to the conversion of sugars into other metabolites (32). This observation supports the findings of (33), who observed reduction in carbohydrate content of *Anabaena fertilissima, Aulosira fertilissima and W. prolifica* with increase in Endosulfan and Tebuconazole pesticides. On the 16th day, though carbohydrate content decreased as compared to the control but the value was observed to be higher than that of the values recorded for 4th, 8th and 12th days of incubation periods at 30, 60 and 90 ppm of the concentrations of the pesticides. It could be attributed to the decrease in protein synthesis leading to accumulation of carbohydrate and lipids inside the cells (34) and the results were in concomitant with that of the findings of (35) on *Anabaena variabilis* treated with malathion.

Conclusion

From the results obtained after evaluating the effects of malathion on the growth parameters of *W. prolifica*, it was observed that malathion has adverse effect on all the tested parameters of the test organism. A lower concentration of malathion for a short time of exposure though caused enhancement of the biomass and chlorophyll-a in *W. prolifica* but the increase was not significant over the control. With gradual increase in pesticide concentration with time there was an overall reduction in the tested parameters. Considering the adverse affect on the natural biofertilizer like *W. prolifica*, it is suggested to restrict the use of malathion in the rice field ecosystems.

Author's contributions

Both the authors participated in the design, planning, and implementation of the study and were also instrumental in the writing of the manuscript.

Conflict of interest: The authors declare that they have no conflict of interests.

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