J. BIOL. ENVIRON. SCI.,
2012, 6(16), 67-75

Differential Effects of Agricultural Pesticides Endosulfan and Tebuconazole on Photosynthetic pigments, Metabolism and Assimilating Enzymes of Three Heterotrophic, Filamentous Cyanobacteria

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ABSTRACT

Cyanobacteria are present abundantly in rice fields and are important in helping to maintain rice field fertility through nitrogen fixation. Application of agricultural pesticides for improving crop productivity has become necessary in the present day agricultural practices. This has resulted in either stimulatory or inhibitory effects on the soil microflora including nitrogen-fixing cyanobacteria. The response of three nitrogen fixing cyanobacterial species to two pesticides (Endosulfan, an insecticide and Tebuconazole, a fungicide) was hence selected. A tremendous reduction in the photosynthetic pigments, metabolic as well as enzymatic activities of all the three organisms in response to the pesticides was recorded after sixteen days. All the pigments, metabolites and enzymatic activities of *Anaabena fertilissima, Aulosira fertilissima* and *Westillopsis prolifica* were dropped by between 59 to 96% upon various doses of pesticide treatments. The results showed that *An.fertilissima* was more tolerant to both the pesticides, whereas between the two tested pesticides, Tebuconazole found higher toxicity to *Aul. fertilissima* and *W. prolifica.*

Keywords: ***Anabaena fertilissima, Aulosira fertilissima, e*ndosulfan, tebuconazole, *Westiellopsis prolifica.***

INTRODUCTION

Cyanobacteria belong to a group of ubiquitous photosynthetic prokaryotes possessing the ability of synthesize chlorophyll-*a* and carry out an important role in nutrient cycling and maintenance of organic matter in aquatic systems including lakes, rivers and wetlands. Nitrogen-fixing cyanobacteria are known to b a prominent component of the microbial population in wetland soils, especially rice fields, contributing significantly to the fertility as a natural biofertilizer (Kumar and Kumar 1998).

Pesticides appear to be very effective in controlling a wide range of pests that infect crop plants including rice, peppers etc. The effects of a few pesticides have been studied with respect to growth, photosynthesis, nitrogenase activity and carbon fixation etc. Suresh Babu *et al.* (2001) studied the effect of lindane on growth and metabolic activity of cyanobacteria. Moreover, several reports have been published on the comparative toxicity of herbicides and fungicides towards various test organisms such as blue-green alga (Abou-Waly *et al.,* 1991).

Besides, Nirmal Kumar and Rita (1996) carried out the impact of pesticides on nucleic acids of *Anabaena sp. 310.* Photosynthetic, biochemical and enzymatic investigation of *Anabaena fertilissima* in response to an insecticide-hexachloro-hexahydromethano-benzodioxathiepine-oxide was also studied by Kumar *et al.* (2009). However, there is little information available on the effect of these pesticides on algal communities (Abou-Waly *et al.,* 1991) and the susceptibility of cyanobacteria to toxicants such as herbicides, fungicides and heavy metals (Ferrando *et al.,* 1996). Present study is aimed to elucidate the differential effect of two classes of pesticides (insecticide and fungicide),three cyanobacterial species were treated with different concentrations of the pesticides and observed for changes in their pigment content, metabolic such as carbohydrates, proteins, amino acids, phenols, and enzymatic activities like nitrate reductase, glutamine synthetase and succinate dehydrogenase.

MATERIALS AND METHODS

For measurement of pigments, metabolites and enzyme activities, cultures were grown in nitrogen-deficient BG_{11} medium for heterocystous, nitrogen fixing forms. All the experiments were carried out in replicates. Samples were thoroughly homogenized and drawn during exponential phase of growth for further analysis. Endocel (35% Endosulfan) and Folicur (25.9% Tebucoazole) were procured from Excel Crop Care, Gujarat and Bayer Crop Science, Mumbai respectively (Table: 1).

LC_{50} values of the organisms for Endosulfan and Tebuconazole were determined in terms of quantitative estimation of chlorophyll-a and accordingly, various concentrations of the pesticides were used in all further

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experiments (Table: 2). Sterile cultures and conditions are maintained throughout the experimental period. Stock solution of both the pesticides were prepared in sterilized double-distilled water and added aseptically to the culture medium to the final concentrations indicated for each treatment.

Table 1. Properties of the pesticides used for the study.

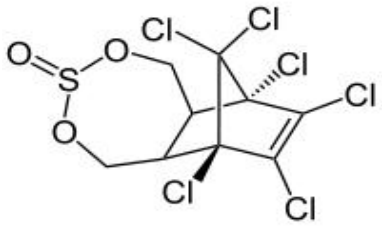
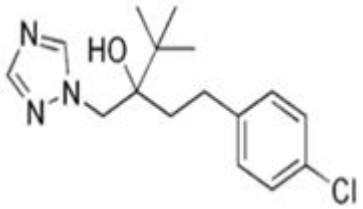
Properties	Endosulfan	Tebuconazole
Class	Organochlorine Insecticide	Triazole Fungicide
IUPAC name	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide	1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol
Chemical structure		
Trade names	Benzoepin, Endocel, Parrysulfan, Phaser, Thiodan, Thionex	Folicur

Table 2. LC_{50} values and pesticide treatments of the test organisms for Endosulfan and Tebuconazole.

Sr.no.	Xenobiotic compound	Organisms selected for study	LC_{50} values determined (ppm)	Treatments decided based upon LC_{50} (ppm)
1.	Endosulfan	*Anabaena fertilissima*	6	3 6 12
		Aulosira fertilissima	30	15 30 60
		Westiellopsis prolifica	20	10 20 40
2.	Tebuconazole	*Anabaena fertilissima*	15	7.5 15 30
		Aulosira fertilissima	30	15 30 60
		Westiellopsis prolifica	30	15 30 60

Incubation and maintenance of culture

Axenic cultures Anabaena fertilissima, C.B.Rao, *Aulosira fertilissima*, Ghose and *Westiellopsis prolifica*, Janet were obtained from National Facility for Blue-Green Algae, IARI, New Delhi, India and was cultured in BG-11 medium (Rippka 1979) and were kept to maintained room temperature (27±2°C), illuminated by 2500-3000 lux light for 12 hours.

Pigments measurement
Chl-a was measured spectrophotometrically in cell lysates after extraction in 80% acetone (Jeffrey and Humphrey 1975). Phycobiliprotein was measured as described by Bennett and Bogorad (1973).

Metabolite estimation
Carbohydrates were assayed quantitatively as per Roe (1955), total soluble proteins were determined as described by Lowry *et al.* (1951), amino acids were estimated by the method of Lee and Takahasi (1966), whereas phenols were measured according to Malick and Singh (1980).

Enzyme assays
The estimation of in vivo nitrate reductase activity was measured by the method of Sempruch et al. (2008), glutamine synthetase activity was done by γ-glutamyl transferase as described by Pamiljans et al.(1962) and succinate dehydrogenase activity, a major respiratory enzyme present in the thylakoid of the cyanobacteria was measured by the method of Kun and Abood (1949).

RESULTS AND DISCUSSION

Photosynthetic Pigments
Endosulfan and Tebuconazole treatments at various treated concentrations caused reduction in the chl-a content of the cells, which was found significant after 4 days of treatment. Highest treatments of Endosulfan reduced chl-a in *An.fertilissima* by 59% and 34%, while Tebuconazole reduced chl-a by 50% and 99% respectively after 4 and 16 days. Moreover, Endosulfan treatments reduced chl-a of *Aul.fertilissima* by 34% and 94%, whereas 50% and 95% of chl-a was observed upon Tebuconazole treatment. Chl-a of *W.prolifica* reduced by 95 and 93% respectively after 16 days upon treatments with both Endosulfan and Tebuconazole respectively (Fig 1a, 2a, 3a, 4a, 5a). The results were highly indicative of their inhibitory effects on photosynthetic activities of the cells. Corresponding to chl-a, carotenoids and phycobiliproteins decreased with increasing pesticide treatments. 39%, 90% and 85% reduction was recorded in carotenoid content of the respective organisms after Endosulfan treatment, while Tebuconazole declined carotenoids by 94%, 86% and 94% after 16 days (Fig 1a, 2a, 3a, 4a). Fall of carotenoids of the organisms explained that the pesticides not only accelerated the degradation but also blocked their synthesis. Phycobilin contents of the cells ceased significantly by 43%, 56% and 86% in presence of Endosulfan while Tebuconazole treatments led to reduction by 37%, 61% and 92% of *An.fertilissima, Aul.fertilissima* and *W.prolifica* after 16 days (Fig 1b, 2b, 3b, 4b, 5b). These water-soluble pigments were found to degrade at a faster rate than those of chl-a and carotenoids. The degradation of these photosynthetic pigments (phycocyanin, allophycocyanin and phycoerythrin) could be also attributed to the pesticide-thylakoid membrane-interaction. Mohapatra and Schiewer (2000) have demonstrated with organophosphorous insecticides that toxicant-membrane interaction is responsible for changes in fluorescence behavior and pigment content of *Synechocystis* PCC 6803. The findings are also in agreement with Mostafa and Helling (2002) who suggested that drop in chlorophyll-a, carotenoid and phycobiliprotein contents might be ascribed to the inhibition of pigment synthesis directly by the insecticide or accelerated degradation of pigments due to increased Active Oxygen Species (AOS) formation at various sites of the photosynthetic electron transport chain during stress. Moreover, the present results are also in consonance with the deleterious effects of other fungicides on chl-a, carotenoids and phycobiliproteins of marine microalgal communities investigated by Porsbring *et al.* (2009).

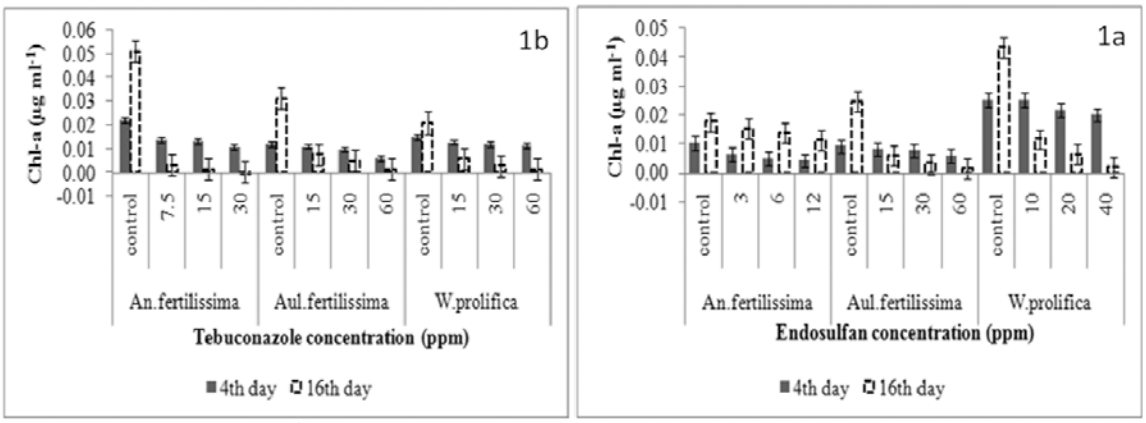


Figure 1. Concentration (µgml^{-1}) of Chl-a in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

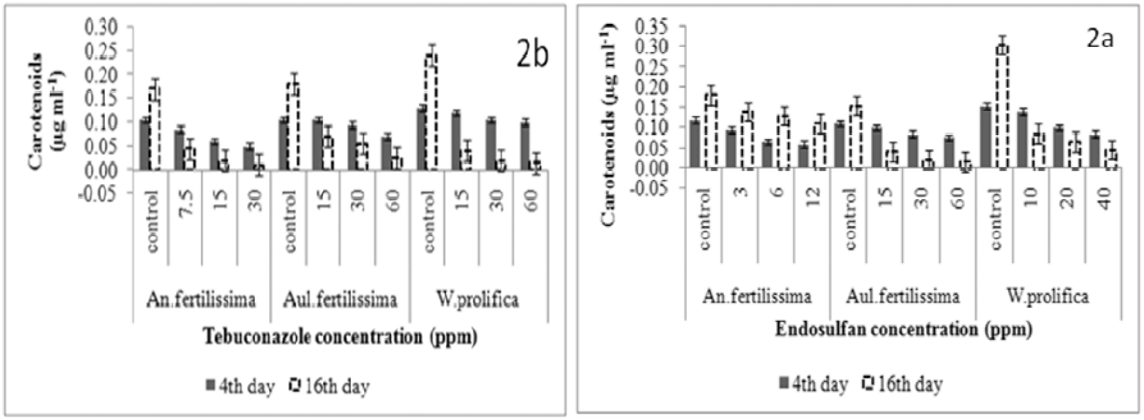


Figure 2. Concentration (µgml^{-1}) of carotenoids in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

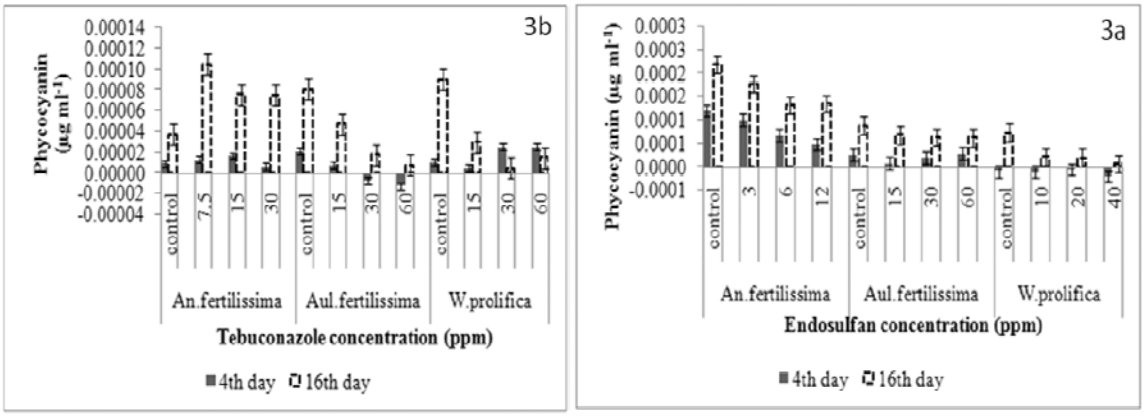


Figure 3. Concentration (µgml^{-1}) of phycocyanin in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

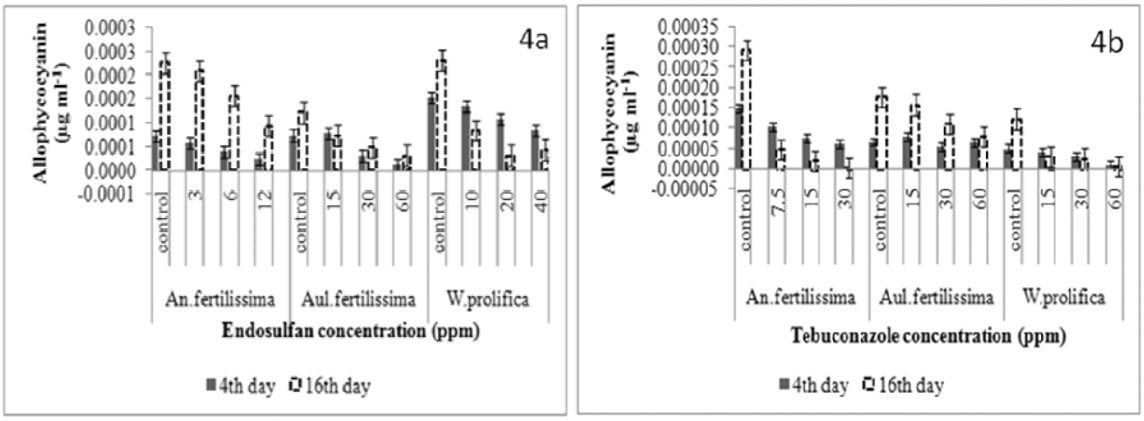


Figure 4. Concentration (µgml^{-1}) of allophycocyanin in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

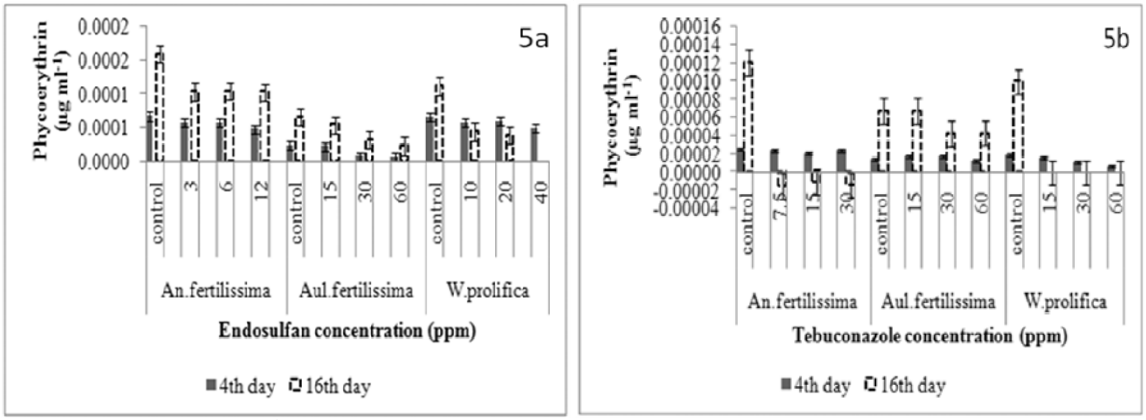


Figure 5. Concentration (µgml^{-1}) of phycoerythrin in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

Metabolites

The metabolic response of the three organisms to Endosulfan and Tebuconazole after 4 and 16 days respectively have been exhibited in Figures 6, 7, 8, and 9.

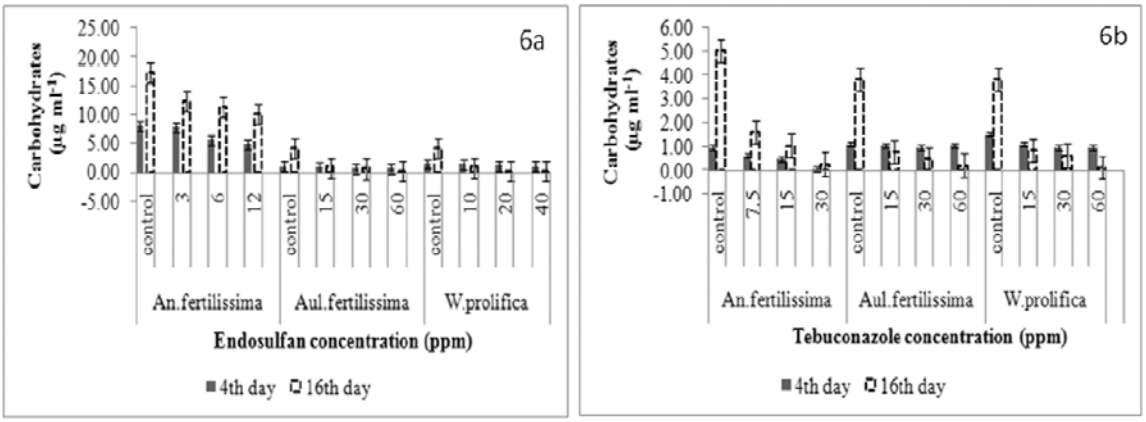


Figure 6. Concentration (µgml^{-1}) of carbohydrates in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

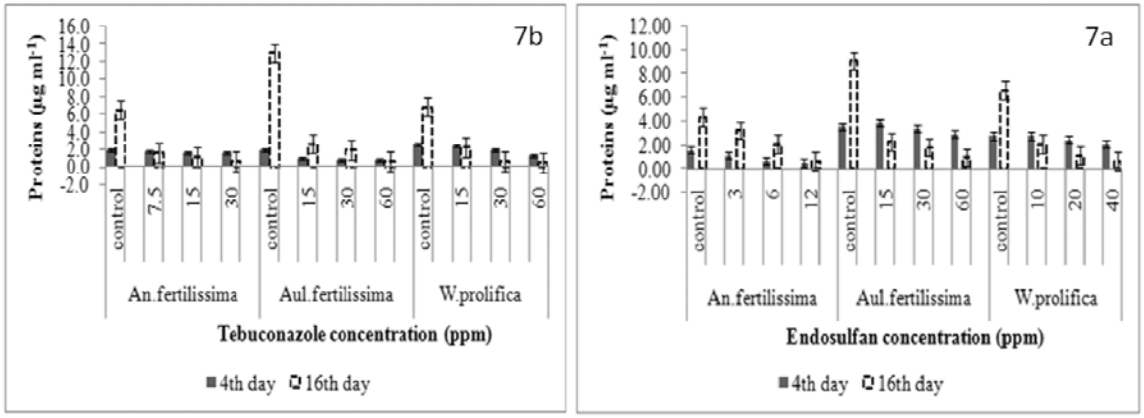


Figure 7. Concentration (µgml^{-1}) of proteins in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

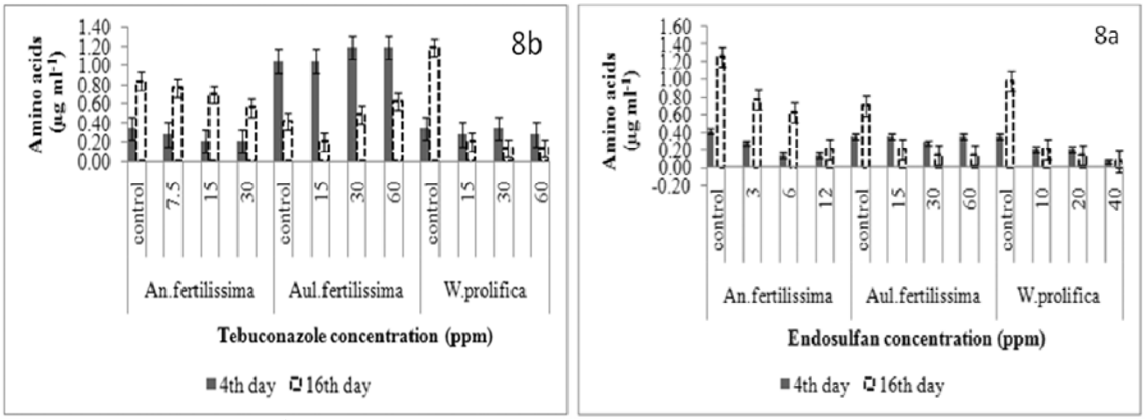


Figure 8. Concentration (µgml^{-1}) of amino acids in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

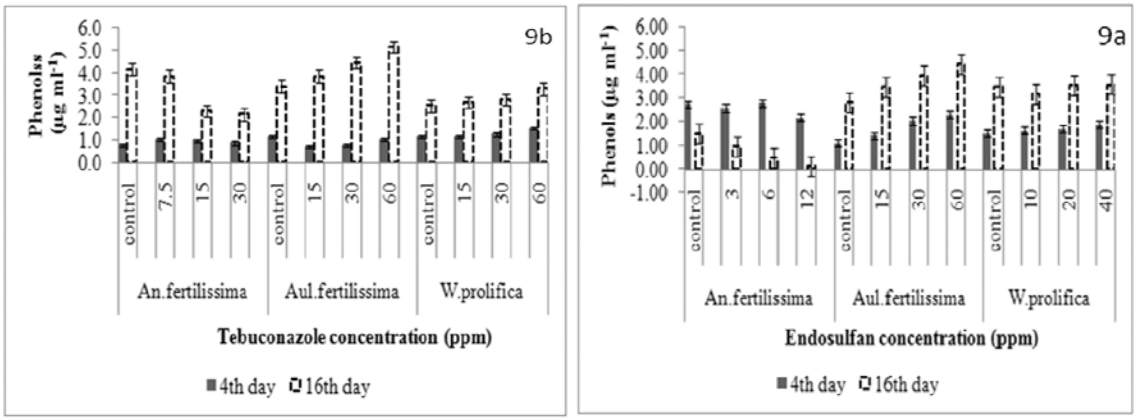


Figure 9. Concentration (µgml^{-1}) of phenols in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

A drastic reduction in carbohydrate content of *An.fertilissima, Aul.fertilissima* and *W.prolifica* was observed with rise in concentrations of the pesticides. Total carbohydrates of *An.fertilissima, Aul.fertilissima* and *W.prolifica* diminished by 42, 97 and 97% in Endosulfan treatments and 94, 95 and 96% in Tebuconazole treatments at the end of 16 days. Many studies have endorsed that pesticide adversely affects the carbohydrates in algae. Kumar et al. (2008) quoted similar observations while studying on Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria (*Aulosira fertilissima, Anabaena variabilis and Nostoc muscorum*).

Nirmal Kumar (1991) reported the inhibition of sugar contents of the algae by increasing doses of substituted urea herbicide N-(4-isopopylphenyl) N, N-dimethyl urea and stated that this retardation might be due to the interference of chemicals during photosynthetic process, which ultimately lapse the production of net gain of carbohydrates.

Pesticide stress had a pronounced effect on the production of proteins in cyanobacteria, a concentration and time dependent stress being observed. Protein levels of *An.fertilissima, Aul.fertilissima* and *W.prolifica* ceased by 85%, 89%, 90% and 90%, 95% and 93% respectively upon Endosulfan and Tebuconazole treatments after 16 days. Eladel *et al.* (1999) supported present findings that 3 mg l^{-1} of thiobencarb declined the protein content of *Protosiphon botryoides*. Kapoor *et al*. (1996) also stated that the interruption of protein synthesis could be due to the inhibition of enzymes and structural proteins essential for growth of the organism.

Time dependent inhibition of amino acids by Endosulfan and Tebuconazole was recorded. Endosulfan reduced amino acids content by 83, 80 and 93%, while Tebuconazole decreased amino acids by 33, -50 and 88% in *An.fertilissima, Aul.fertilissima* and *W.prolifica.* Measures (1975) elaborated that changes in amino acid concentration may be due to synthesis from endogenous precursors or to inhibition of normal catabolism. El-Ayouty and Ezzat (1991) also explained that higher concentrations of pesticides were inhibitory to total amino acids in *Nostoc muscorum*.

Phenols increased by 59% and 3% of *Aul.fertilissima* and *W.prolifica,* in presence of Endosulfan treatments. Similarly Tebuconazole shot up phenol content by 52% and 30% after 16 days of pesticide treatments. On the contrary, phenols of *An.fertilissima* declined by 93% and 49% respectively upon Endosulfan and Tebuconazole treatments. The findings also corroborated with those of Mallick and Rai (1994) who substantiated earlier that phenols could be used as protectants to the organisms during stress or drought conditions and further stated that this could be due to the possible conversion of primary metabolites into phenols as well as accumulation of detoxicants of fungicide during stress conditions.

Assimilating Enzymes

Nitrate reductase (NR), a key enzyme involved in nitrogen fixation was affected drastically in presence of both the pesticides. NR activity of *An.fertilissima, Aul.fertilissima* and *W.prolifica* declined by 77%, 90% and 95% in presence of Endosulfan while Tebuconazole reduced the activity by 90%, 93% and 93% respectively when assayed after 16 days. Adhikary et al. (1984) studied the effect of carbamate insecticide Sevin on the growth, survival and nitrogen fixation of *Anabaena spp*. and *Westiellopsis prolifica* and quoted similar results.

Glutamine synthetase (GS) leads to the conversion of ammonia to glutamine. GS activity also expressed a concentration dependent inhibition when treated with the pesticides. The activities of *An.fertilissima, Aul.fertilissima* and *W.prolifica* suppressed by 77%, 90%, 97% and 59%, 95%, 90% after endosulfan and Tebuconazole treatments respectively which has also been further supported by Rajendran et al. (2007) expressing a remarkable decrease in the GS activity to different pesticides.

Succinate dehydrogenase (SDH) enzyme is a major respiratory enzyme responsible for conversion of succinate to fumarate in the tricarboxylic acid cycle (TCA). But the treatment of these pesticides (Endosulfan and Tebuconazole) lowered the activities of *An.fertilissima, Aul.fertilissima* and *W.prolifica* by 95, 82, 95% and 89, 79, 98% respectively after 16 days. Similar inhibition of the enzyme succinate dehydrogenase activity was observed in the cultures of four Gram(+) bacteria, *Rhodococcus sp. AK 1*, *Bacillus cereus* Frankland & Frankland, *Bacillus subtilis* (Ehrenberg) Cohn, *Nocardia asteroides* and a Gram(-) bacterium, *Rhizobium leguminosarum* when treated with the fungicide tridenmorph by Kalam and Banerjee (1995). Moreover, Gary and Joan (1993) also reported the inhibition of succinate dehydrogenase in the fungi *R.solani* when treated with Thiazole carboxanilide fungicides. Enzymatic activities of the three selected organisms in response to Endosulfan and Tebuconazole have been represented in figures 10, 11 and 12 respectively.

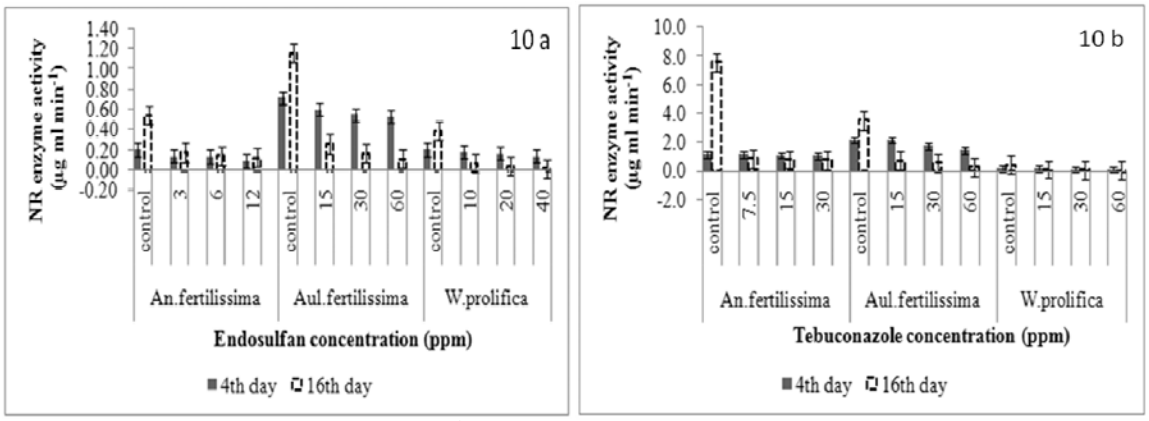


Figure 10. Nitrate reductase enzyme activity (µgml^{-1}/min) in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

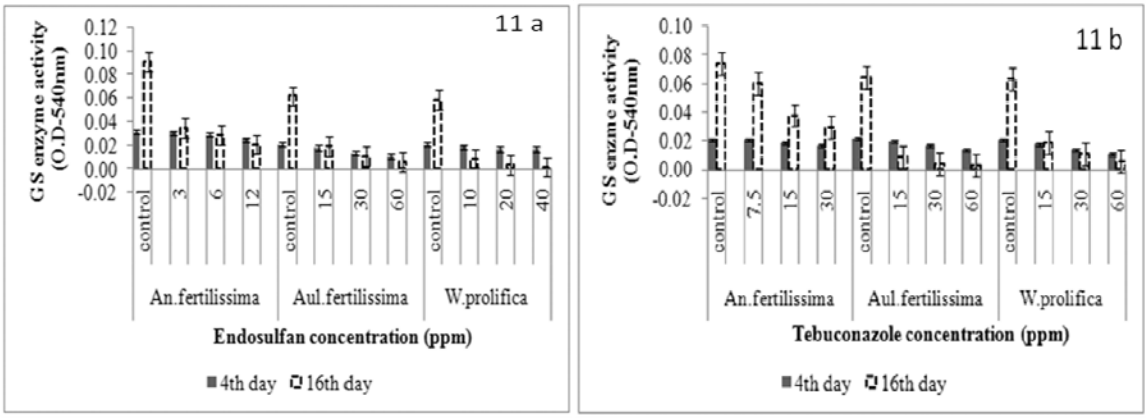


Figure 11. Nitrate reductase enzyme activity (µgml^{-1}/min) in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

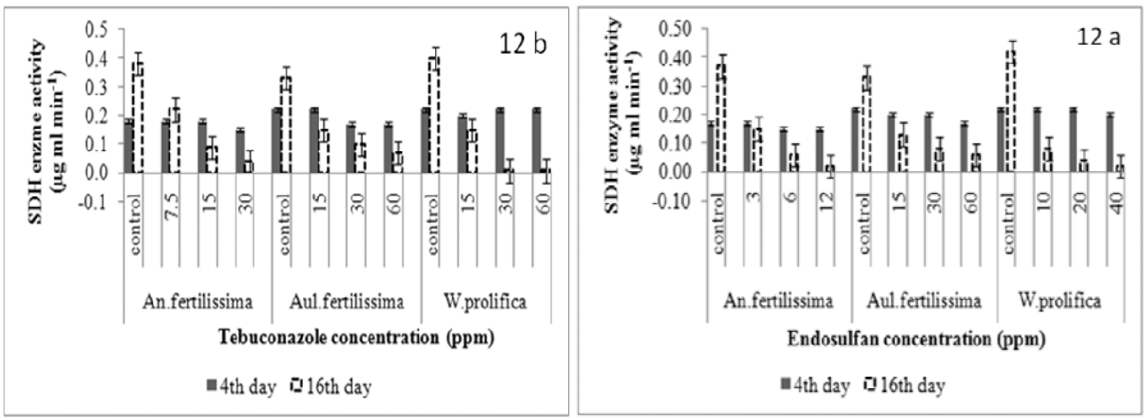


Figure 12. Glutamine synthetase enzyme activity (µgml^{-1}/min) in *An.feritlissma, Aul.fertilissima* and *W.prolifica* after 4 and 16 days at different doses of (a) Endosulfan and (b) Tebuconazole.

CONCLUSIONS

Experiments were conducted with a view to determining the deleterious and differential effects of pesticides on the photosynthetic pigments, metabolic and enzymatic activities of cyanobacteria. The results suggest that *Aulosira fertilissima* was the most susceptible organism to both the insecticide and the fungicide studied. The order of pesticide tolerance of each organism towards both the pesticides can be described as *Anabaena fertilissima* being more tolerant than *Westiellopsis prolifica* followed by *Aulosira fertilissima* being the least tolerant for endosulfan as well as Tebuconazole. However, application of the fungicide proved to be more inhibitory to the cultures than that of endosulfan. In conclusion, although Endosulfan and Tebuconazole are useful agents to control the growth of pests, their application to rice fields should be considered because of its toxicity to the heterocystous filamentous cyanobacteria.

ACKNOWLEDGEMENT

Authors thank the University Grants Commission (UGC), New Delhi for providing financial assistance.

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