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The effect of iterated use of Chlorpyrifos on three species of cyanobacteria isolated from ricefields

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ABSTRACT
The present paper describes the effect of a single and an iterated use of organophosphorous insecticide chlorpyrifos on three species nitrogen fixing cyanobacteria. Treatment levels of 0.5 µg/ml-4.0 µg/ml were applied once or twice with two weeks interval. Graded concentrations higher than 2.5 µg/ml showed affect on the growth of cyanobacteria. Maximum yellowing was seen to be at 4 µg/ml in all three species. The reduction in bili protein started at 2.5 µg/ml concentration of the insecticide. Three algal species showed varying degrees of sensitivity to the insecticide. Calculated no observed effect concentration (NOEC) values after treatments were 0.021, 0.024 and 0.075mg/ml for first application and 0.17 and 0.19 and 0.064mg/ml after second application for *Anabaena sp*, *Nostoc sp and Oscillatoria sp* respectively. Normal agricultural use of chlorpyrifos (250gm/1000lit/hectare) in rice fields will likely to be toxic to these ubiquitous nitrogen fixing cyanobacteria. Low dose application 2.5 µg/ml enables growth of more tolerant cyanobacteria as biofertilizer.

Introduction

Cyanobacteria are prokaryotes comprising unicellular to multicellular microorganisms that carryout oxygenic photosynthesis [1, 2]. The favorable conditions provided by the rice fields for nitrogen fixation by these organisms leads to enhanced plant available N in soil and yield improvement [3, 4]. The other beneficiary affects of cyanobacteria are their influence on physical and chemical and biological properties of soil has got huge significance [5, 7]. Extracellular substances liberated by cyanobacteria modulate pH, temperature and also playing a role in evaporation of methane generation therefore are directly or indirectly entail in the productivity of rice ecosystem [8]. The Green Revolution led to an intensification of agricultural practices and the use of pesticides [9] and fertilizers increased considerably throughout the years repeated application of the same pesticide has been reported to enhance the growth of the related specific decomposing microorganisms and cause the rapid inactivation of the pesticide [10]. However there are reports indicating that repetitive application of pesticides did not lead to the buildup of the degrading microflora. This was observed in laboratory experiments with carbofuran [11]. Chlorpyrifos is most popular amongst the organophosphate insecticides. It is most widely applied in crops due to its broad spectrum of activity and low cost. Depending on the rate of application its use generally varies but in some cases both high concentration and low concentration doses have been used. Extensive and regular use of pesticides in modern rice cultivation is reported to adversely affect the diversity, biology or even sustainability of cyanobacteria often leading to their complete elimination from the field ([12-13] Padhy 1985, Singh *et al*., 2003). Thus strategy is required to improve the ecological viability of biofertilizer strains of cyanobacteria under pesticide stress. As every pesticide used in agricultural practices affects the growth of non target soil microorganisms therefore the aim of this work was to establish the differential toxicity affects of the selected rice field pesticide on growth and survivability potentials of three species of rice field cyanobacteria and also to explore the effect of iterated use of this pesticide on nitrogen fixing cyanobacteria.

Cyanobacterial Cultures

The axenic cultures of nitrogen-fixing cyanobacteria, viz., *Anabaena sp*, *Aulosira sp* and *Westiellopsis sp* were isolated from the rice fields . The cyanobacteria were grown under controlled illumination of 40µEm-2s-1 at 27±1°C in a nitrogen-free BG11 liquid medium at pH 7.0±0.2 under aerobic and static conditions. All inoculations were carried out under aseptic conditions and the cultures were periodically checked for any contamination. Only axenic cultures were used for experimental studies.

Pesticide

The pesticides chosen for the study were Chlopyrifos (20% EC) (Table 1) obtained from Northern minerals limited, Haryana and Bayer CropScience limited, Mumbai respectively. Three concentrations of selected pesticide were used for the present investigation to analyze the response in *Anabaena sp, Aulosira sp, and Westiellopsis sp* (Table 2) on determining LC50. Stock solution of pesticide was prepared in sterilized double-distilled water and added aseptically to the culture medium to the final concentrations indicated for each treatment.

Biochemical Characeristics

Samples were taken after every four days up to sixteen days for the determination of pigments, metabolites, and enzyme activity. Analytical grade (Merck Ltd, and Himedia Ltd, India) chemicals were used throughout the study. Each experiment was conducted in replicates of three and their ±SD values were calculated. The pigments included were chlorophyll-*a* [14], carotenoids [15] and phycobilin pigments [16]. The changes in metabolites content like total carbohydrates [17], proteins [18], phenols [19] and amino acids [20] were measured. Nitrate Reductase (NR) activity in vivo was estimated by the method of Sempruch *et al.*

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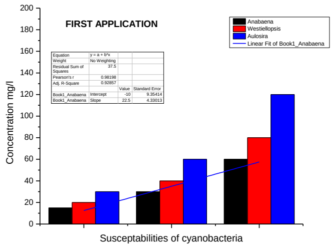
[21] while Glutamine Synthetase (GS) enzyme was extracted in Tris HCl buffer (pH 7.5) and estimated by slight modification of the method described by Pamiljans *et al.* [22]. The estimation of in vivo succinate dehydrogenase (SDH) activity was measured by the method of Kun and Abood [23].

Results

Fig 1 Graphical representation for Differential susceptibilities of three heterocystous (LC 50) filamentous cyanobacteria, *Anabaena sp*, *Aulosira sp*, and *Westiellopsis sp* to chlorpyrifos (First application and second application)



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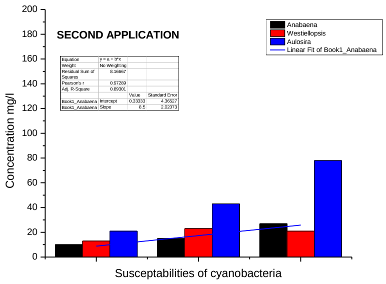

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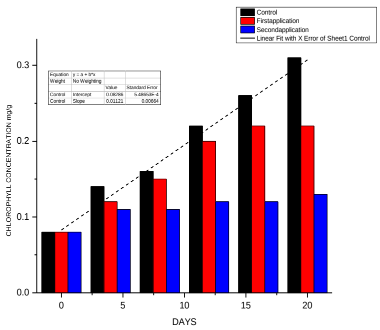

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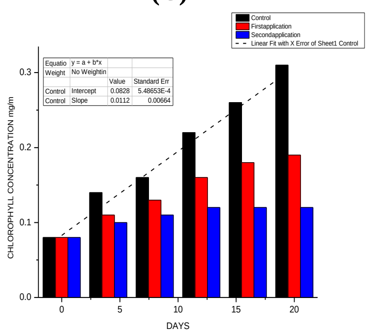

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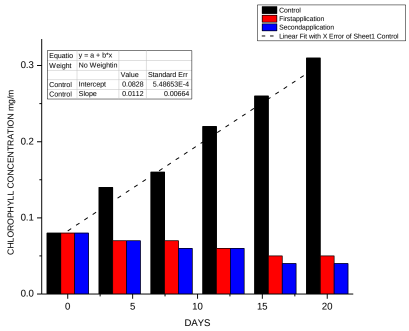

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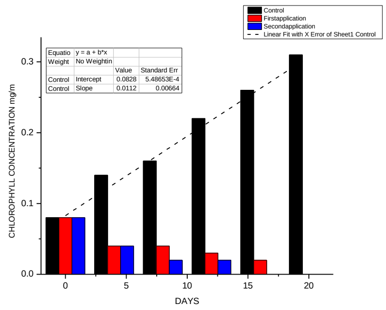

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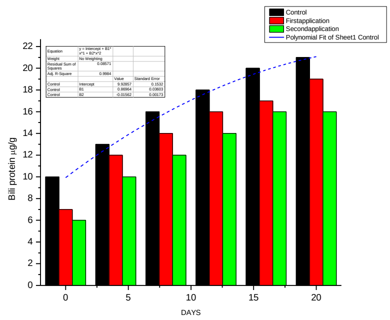

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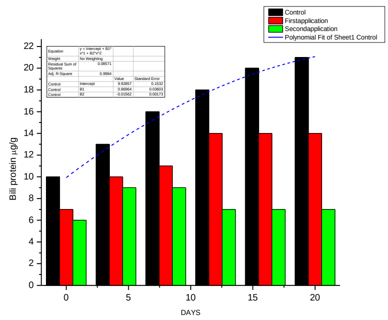

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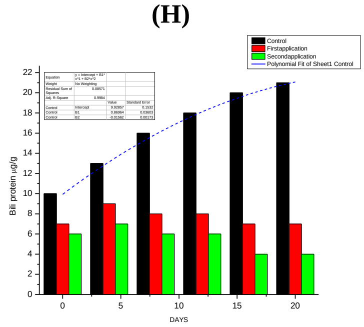

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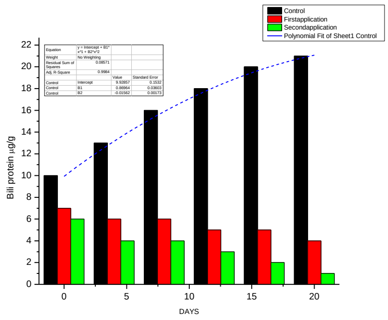

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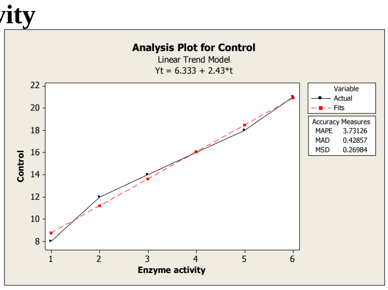
Enzyme Activity



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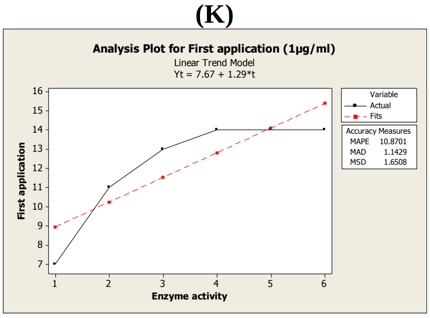

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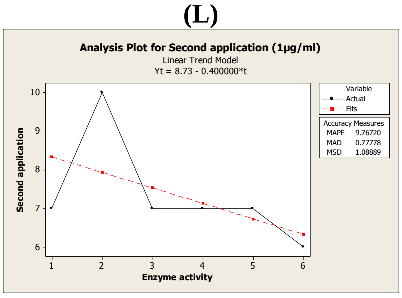

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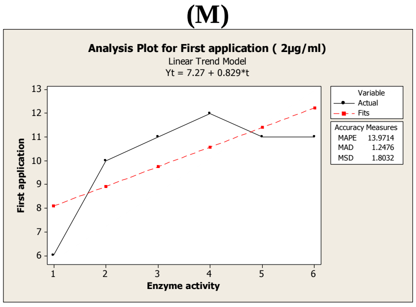

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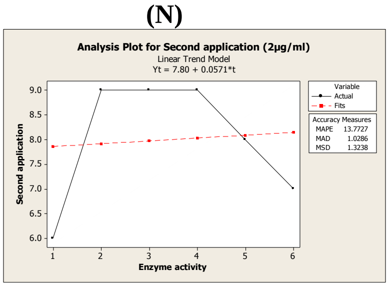

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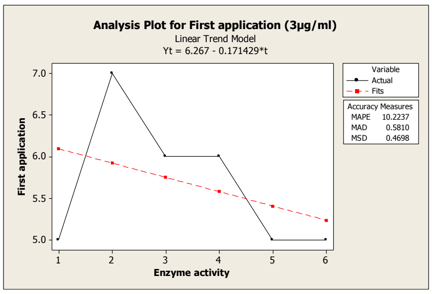

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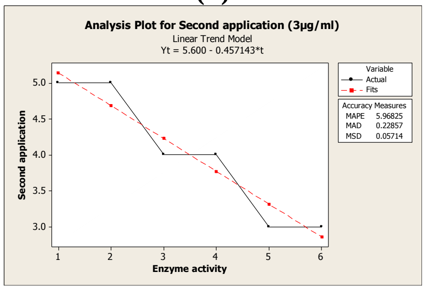

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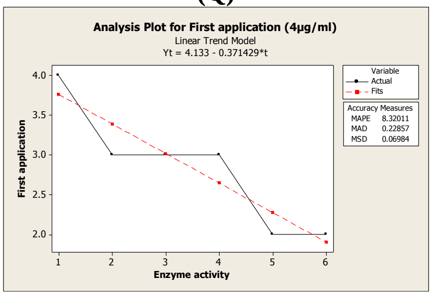

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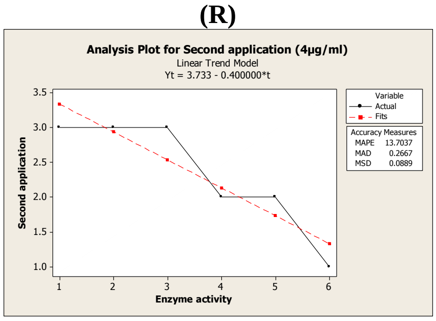

(S)

Result

The highest chlorophyll content was recorded in the untreated cells and the maximum reduction in the chlorophyll content was registered with Anabaena sp 66% at 4µg/ml followed by Aulosira sp 57% at 4µg/ml and Westiellopsis sp 48% at 4µg/ml. Carotenoid content in all the three strains was affected carotenoid content was depleted more with the second application of pesticide by 80% at 4µg/ml. When compared with Anabaena sp less reduction of carotenoids was observed in westiellopsis sp 62% at 4µg/ml followed by Aulosira sp 71% at 4µg/ml concentration. The phycobiliprotein content was adversely affected than chlorophyll content. Affect on carbohydrates was found to be higher in all tested periodic intervals of three species. In Anabaena sp there is a decrease of 81%, 76% and 60% for Aulosira sp and Westiellopsis sp at 4µg/ml in the second application.

Discussion

The iterated use of cyanobacterial population has been considered to be inhibitory at high doses [24]. The present data obtained cleared a way that the use of high and continuous use of pesticide causes detrimental effect on BGA. The growth in terms of chlorophyll-a was greatest in untreated cells, which might be due to inhibition on the photosynthetic activity of cyanobacteria by the continuous use of pesticide. Growth rate was less than 50% in maximum concentrations of pesticide. Primary and secondary application of the tested pesticide affected total carotenoids of Anabaena sp, Aulosira sp and Westiellopsis sp. The content of these pigments was decreased after 16days at highest concentration of pesticide. Sufficient literature is not available on the effect of continuous spraying of pesticides on cyanobacteria. Pesticides generally may increase or decrease the carbohydrate content of the BGA. The data obtained in the present paper reveals that carbohydrate content was decreased to a maximum at higher concentrations of the pesticide.

In conclusion it appears that all three strains of cyanobacteria in general and Anabaena sp in particular do not resist to a very high concentration of pesticide chlorpyrifos. However the effect of pesticide on the population of nitrogen fixing cyanobacteria in rice fields also depends on other pesticide concentration and flooding of water associated with paddy fields. More detailed fields studies are needed, avoiding the use of high application rates more than recommended will likely increase the more tolerant cyanobacteria.

Acknowledgements
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Table 1 Physico-chemical properties of Chlorpyrifos

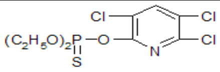
Pesticides	Empirical formula	Chemical name and number (Chemical abstract Service)	Chemical structure	Molecular weight (g.mol $^{-1}$)	Melting point (°C)
Chlorpyriphos (Insecticide)	$C_9H_{11}Cl_3NO_3PS$ CAS No: 2921-88-2	O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate		350.62	42-43.5°C

Table 2 Differential susceptibilities of three heterocystous filamentous cyanobacteria, *Anabaena sp*, *Aulosira sp*, and *Westiellopsis sp* to chlorpyrifos

Pesticide	Concentration mg/l		
	Anabaena sp	*Aulosira sp*	*Westiellopsis sp*
Chlorpyrifos	15 30 60	20 40 80	30 60 120