



**METAPHORICAL EFFECTS OF ORGANOPESTICIDES CARBENDEZIM AND MALATHION ON THE GROWTH AND PHYSIOLOGICAL ACTIVITIES OF CYANOBACTERIA *NOSTOC COMMUNE*, VAUCHER INHABITING THE RICE FIELD OF BHANDARA DISTRICT (M.S.)**

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**ABSTRACT**

The effect of pesticides on Cyanobacteria (*Nostoc commune* vaucher ) has been analyzed in vitro in the laboratory, for which the pure culture of *Nostoc commune* (Voucher) was treated with various concentrations of two pesticides i.e. carbendazim and malathion and its metabolic effect was analyzed using parameters like concentration of proline and protein along with change in biomass production. The result obtained in the study indicates that amount of biomass and extracellular protein was increased with enhancing incubation time as well as with increase in concentrations of pesticides but decline after certain limit. The present data obtained cleared a way that the use of high concentrations and continuous use of organophosphorus pesticide causes detrimental effect on rice field cyanobacteria.

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**INTRODUCTION**

Several unique features of cyanobacteria such as cosmopolitan, pioneer, oxygenic photosynthesis, high biomass yield, growth on non-arable lands and on a wide variety of polluted water sources, generation of useful by-products and bio-fuels by them, enhancing the soil fertility, reducing green house gas emissions, have collectively offered these bio-agents as the precious bio-resource for sustainable development (Thajuddin and Subramanian, 2005). They are phototrophic, and naturally occur in several agro-ecosystems like paddy fields and from Antarctica to Arctic poles (Pandey et. al., 2002). Cyanobacterial biomass is the effective bio-fertilizer source to improve soil health and physico-chemical characteristics such as water-holding capacity and mineral nutrient status of the degraded lands (Nanjappan et. al, 2007.) As a beneficial microbe, cyanobacteria could play a potential role in the enhancement of agriculture productivity and mitigation of GHG emissions (Prasad et. al., 2005). It has been proposed that cyanobacteria could be the vital bio-agents in ecological restoration of degraded land (Singh et al. 1988). Cyanobacteria are the group of photosynthetic organisms which can easily survive on bare minimum requirement of light, carbon dioxide (CO<sub>2</sub>) and water (Brouwer et. al. 1999).

The term pesticide covers a wide range of compounds including insecticide, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulator (PGR) and others. There has been steady growth in the production of technical grade pesticide in India, from 5000 metric tons in 1958 to 102,240 metric tons in 1998. India ranks 10<sup>th</sup> in the world in pesticide consumption, as its total consumption amounts to about 500 million tons (Lari et. al. 2014). The Indian pesticides market is the 12th largest in the world with a value of US\$ 0.6 bn. which is 1.6% of the global market (Hundal B. S., 2006; Lari et. al. 2014, ) Despite increasing research efforts toward crop improvement by methods obviating the use of pesticides, agriculture remains heavily dependent on these chemicals (Gadkari, 1988). Cyanobacterial photosynthesis, growth and heterocysts differentiation is reduced or inhibited by herbicides and pesticides, such as, 2,4-D, atrazine, metsulfuron methyl (Berard and Benninghoff, 2001). The biochemical constituents of cyanobacteria depend on the nature of strains, physiological state of the culture & the environment (Mounika et. al. 2018). In correlation with these studies the present analysis was carried out to evaluate the effect of two pesticides i.e. carbendazim and malathion (diethyl (dimethoxy phosphinothioyl) thiobutanedioate) on the growth and physiological activities of cyanobacteria *Nostoc commune* Voucher.

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## MATERIAL AND METHOD

### Sample collection, isolation and identification

The culture of *Nostoc commune* was isolated from rice field of Lakhandur (Bhandara), then it was sub-cultured and pure culture of *Nostoc commune* Voucher in vitro was obtained. Microscopic observation and identification was done by spreading isolated culture on glass slide and observing it under high power microscope. Pure forms of cyanobacteria were identified on the basis of morphological characteristics mentioned in Bergey's Manual of Determinative Bacteriology and Bergey's Manual of Systematic Bacteriology, 2nd ed. Vol. 1 (Buchanan and Gibbons, 1994) and Desikachary (1959).

### Estimation of proline

Proline is a basic amino acid found in high percentage in basic protein. Free intracellular proline is said to play a role in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline, one of the hypotheses refers to breakdown of protein into amino acids and conversion to proline for storage. Many workers have reported a several-fold increase in the proline content under physiological and pathological stress conditions. Hence, the analysis of proline in plants has become routine in pathology and physiology division of agricultural sciences (Bates, 1973).

### Optimization of Biomass Production

Pure culture of *Nostoc commune* was inoculated in conical flask containing 100 ml BG-11 (Andersen, 2005) broth and incubated at 30°C for 15 days in continuously illuminated chamber at 4000 lux. The best growth medium was selected to carry out further experiments. At the stationary phase, *Nostoc commune* was harvested using centrifugation at 2000 rpm. Biomass was obtained by filtration using Whatman No. 1 filter paper and dried in hot air oven at 50°C for 2 hrs to remove extra moisture then total biomass was weighed. Same process was followed by control test and pesticide different gradient concentration.

### Protein estimation

The Lowry method (Lowry et al., 1951) was used to measure the protein content of the pretreated culture of *Nostoc commune*. The cells were pretreated with aluminum oxide for 5-min to release all the cellular protein followed by crushing. It resulted into total disruption of algal cells and longer periods of milling did not further increase the concentration of protein in the cell homogenates for liquid suspension. The obtained extract then analyzed for protein estimation.



Figure 1 Filtration and optimization of Biomass culture of *Nostoc commune*

## RESULT

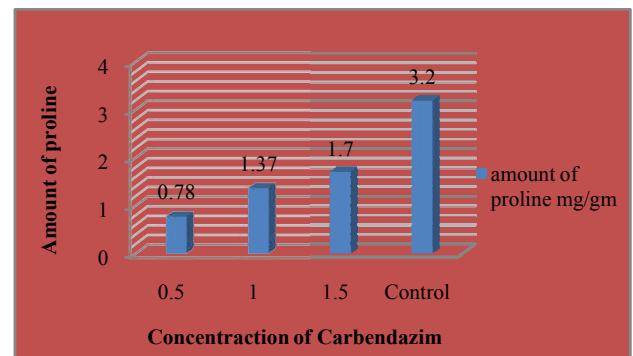


Fig 2 Amount of proline in culture extract of *Nostoc commune* in different concentrations of pesticide Carbendazim, after 15 days incubation

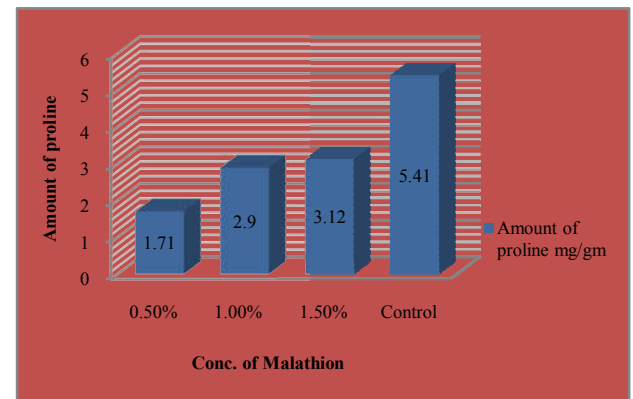
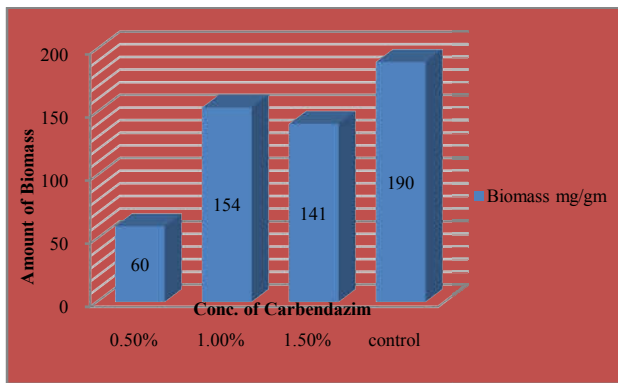
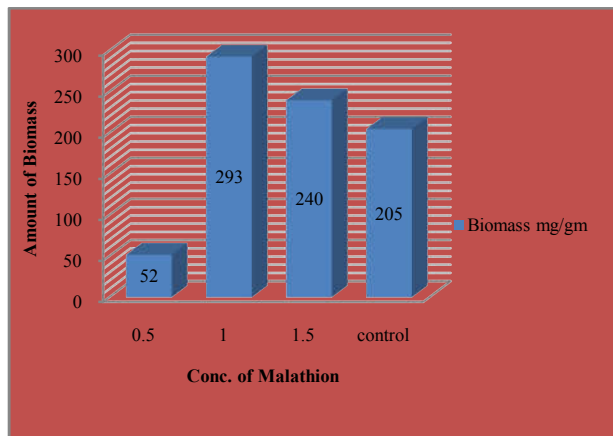


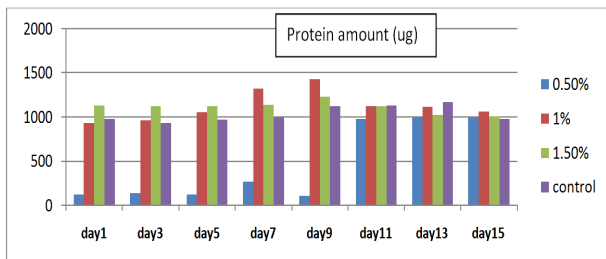
Fig 3 Amount of proline in extract of *Nostoc commune* in different concentrations of pesticide Malathion after 15 days incubation



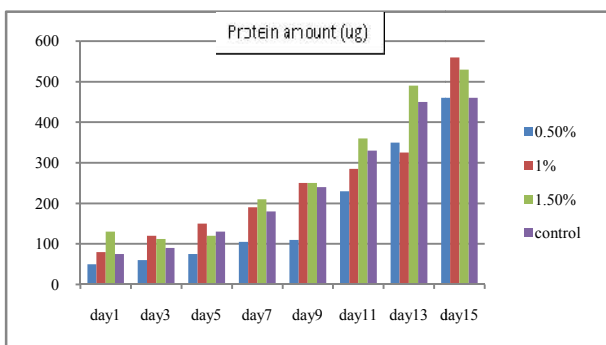
**Fig 4** Total biomass estimation of Nostoc commune in different concentrations of pesticide Carbendazim after 15 days incubation



**Fig 5** Biomass estimation of Nostoc commune in different concentration of pesticide Malathion after 15 days incubation



**Fig 5** Total proteins in culture of Nostoc commune in different concentration of pesticide Carbendazim during 15 days incubation



**Fig 6** Total proteins in culture of Nostoc commune in different concentration of pesticide Malathion during 15 days incubation

The data obtained in the present study reveals that proline content was increased as the concentrations of pesticide enhances but it remains lowered as compare to control of both the insecticides. Proline is a basic amino acid found in high percentage in basic protein. Free proline is said to play a role

in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline, one of the hypotheses refers to breakdown of protein into amino acids and conversion to proline for storage. Many workers have reported a several-fold increase in the proline content under physiological and pathological stress conditions.

The biomass and metabolite estimation of various incubation extract was evaluated using standard protocols and results obtained showed that biomass was increased as incubation period augmented as well as it depends on the concentrations of pesticide. It has been found that minimum biomass i.e. 52 mg was produced in 0.5% concentration of pesticide malathion after 15 days incubation in Nostoc commune. The biomass was maximum i.e. 293 mg at 1.0 concentration of pesticide during 15 day incubation. Results indicates that amount of proteins was increased up to 15 days incubation time but not significantly. Amount of protein was maximum i.e. 1430 ug at 9<sup>th</sup> day in carbendazim and 560 ug in malathion treated group respectively.

The biochemical constituents of cyanobacteria depend on the nature of strains, physiological state of the culture & the environment. Rosales et al. 2005, observed significant variation in protein content among the isolated of Nostoc sp. Due to this biochemical variation he could enabled to distinguished between the sub species in several cyanobacterial genera. There are certain factors including pesticide stress, which also influence the protein synthesis (Borbely et al. 1985).

cyanobacteria are nurtured by soil and in spite that most reverend providing health, fertility and microflora to the soil. Because of it exhibit novel properties such as bio indicator and bio-remediator in contaminated agriculture field due to pollutant stress at moderately. But Nostoc commune in general do not resist to a very high concentration of insecticides carbendazim and malathion as it is revealed in present analysis. However the effect of pesticide on the population of nitrogen fixing cyanobacteria in rice fields also depends on other insecticide concentration and flooding of water associated with paddy fields. More detailed field studies are needed, avoiding the use of high application rates more than recommended will likely increase the more tolerant cyanobacteria.

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