Isolation of Cyanobacteria from water sample and study of its efficiency to degrade Organophosphorus Pesticide Malathion.

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Abstract:
This research focuses on the phycoremediation potential of cyanobacteria in degrading the organophosphorous pesticide malathion. Cyanobacterial strains isolated from water samples obtained from paddy field were cultivated using BG11 and Pringsheim's media. The study has three main objectives. Firstly, it investigates the impact of malathion on the growth of selected cyanobacterial strains, analyzing growth patterns over an 8-10 week period. Secondly, it examines the ability of cyanobacteria to utilize malathion as a phosphorous source, offering insights into their potential role in phosphorous pollution mitigation. Lastly, the research quantifies changes in phosphorous and pesticide residue levels within the culture media, providing a comprehensive understanding of nutrient dynamics during the incubation period. This research contributes to the field of phycoremediation by elucidating the interactions between cyanobacteria and malathion. The findings hold relevance for environmental scientist and ecologists involved in the sustainable management of pesticide-contaminated aquatic ecosystems.

Keywords: Phycoremediation, Cyanobacteria, Malathion, Organophosphorus pesticide, Residue analysis

Introduction:
In recent years, the pervasive use of pesticides has raised concerns about the environmental repercussions of their residues in aquatic ecosystems. Among these, organophosphorous pesticides, with malathion being a prominent representative, pose significant threats to water quality and ecosystem health. In response to the imperative of sustainable environmental management, the present study delves into the promising realm of phycoremediation, specifically exploring the potential of cyanobacteria in mitigating the impact of malathion contamination as an inexpensive and time efficient alternative to chemical and physical methods of decontamination. Independant research has shown that most organophosphorus xenobiotics can be utilized by microorganisms as a source of phosphorus or carbon or both (Karpouzas et al., 2006).
Cyanobacteria, often referred to as blue-green algae, are photosynthetic microorganisms that play pivotal roles in aquatic ecosystems. Harnessing the unique metabolic capabilities of cyanobacteria for environmental remediation, particularly in the context of organophosphorous pesticide contamination, represents a frontier in ecological research.
The selection of malathion as the target pesticide stems from its widespread application in agriculture, mosquito control, and public health programs (Low et al.,2013). Despite its efficacy in pest management, malathion's persistence in aquatic environments raises concerns about its adverse effects on non-target organisms and overall ecosystem integrity. This study seeks to shed light on the potential of cyanobacteria to serve as nature's custodians, addressing the challenges posed by malathion residues through their inherent abilities in biodegradation and nutrient assimilation.
Malathion, a widely used organophosphorus pesticide, exerts considerable ecological and health consequences. Its non-selective nature poses a threat to non-target species, disrupting ecosystems by affecting beneficial insects and aquatic organisms. Runoff from fields and wastewater treatment plants introduces malathion into water bodies, where its persistence raises concerns about cumulative effects on aquatic life (Bunzel et al., 2013). Soil microorganisms critical for nutrient cycling may suffer, impacting overall soil health. The pesticide's absorption by plants raises concerns about its transfer through the food chain, affecting herbivores and organisms feeding on contaminated plants. Prolonged use contributes to the development of pesticide resistance in target pests, necessitating the use of more toxic alternatives. On the health front, individuals exposed to malathion, such as farmers and pesticide applicators, face acute toxicity risks with symptoms ranging from nausea to respiratory failure. Long-term exposure, particularly for vulnerable populations, may lead to chronic health effects, including neurotoxicity and developmental issues. Monitoring pesticide residues in food is crucial, emphasizing the need for careful pesticide management strategies to mitigate these ecological and health impacts.
The objectives of this research are multifaceted, encompassing a nuanced exploration of cyanobacterial responses to malathion exposure. First and foremost, we aim to discern the impact of malathion on the growth dynamics of selected cyanobacterial strains. By subjecting these strains to controlled conditions over an extended timeframe, variations in growth patterns and morphological characteristics that will elucidate the resilience or vulnerability of cyanobacteria to malathion- induced stress.
In parallel, the study investigates the capacity of cyanobacterial strains to utilize malathion as a phosphorous source. This facet of the research taps into the potential of cyanobacteria not only as agents of pesticide degradation but also as

contributors to nutrient cycling in aquatic ecosystems. The examination of malathion assimilation by cyanobacteria may offer novel insights into their adaptive mechanisms and their role in nutrient dynamics within contaminated environments. A critical aspect of this investigation involves the quantification of residual phosphorous concentrations in the culture media post-exposure. By rigorously assessing the nutrient dynamics over the course of 8-10 weeks, we aim to gauge the effectiveness of cyanobacteria in mediating nutrient availability in the wake of malathion degradation. This information is pivotal for understanding the broader ecological implications of cyanobacterial responses to pesticide contamination.
In conclusion, this research attempts to contribute significantly to the evolving field of phycoremediation by utilizing the complex interaction between cyanobacteria and malathion. The outcomes hold not only scientific significance but also practical implications for the sustainable management of pesticide-contaminated aquatic ecosystems. As we embark on this exploration, the potential benefits of harnessing cyanobacterial prowess in addressing pesticide residues emerge as a ray of hope for the ecological well-being of our water resources.

Materials and Methods:

Collection of samples: Water sample was collected from the paddy field of ICAR-RCER complex, Patna.

Collection of pesticide: 50% malathion pesticide was purchased from Jamal Road, Patna, Bihar.

Isolation and Enumeration of cyanobacteria: Paddy field water sample was diluted up to 10^{-4} dilution and cultured in 250 mL Erlenmeyer flask. Into each flask 100 mL of liquid Pringsheim's medium was added with inoculum for enumeration. The culture flasks were kept under alternate 12 hour dark and light CFL light illumination for 20 days at room temperature. Samples were taken from flask and observed under microscope for algal population identification.
Cultured medium was streaked and re-streaked on BG11 agar medium to obtain isolated colonies of cyanobacteria which were identified by observing morphological characteristics under microscope.

Characterization of cyanobacterial isolates: The morphological characteristics of the selected cyanobacterial isolates were determined (Prescott, 1978). Morphological characteristics including colony colour, texture, elevation, margin, shape, opacity, Gram's stain was studied (Rippka et al., 1979).

Growth of cyanobacteria on phosphorus limited medium: Exponentially growing cells were inoculated into flasks containing medium with 1/10th of the original phosphorus concentration. The phosphorus-limited cells were cultured in a medium without and with different concentrations of malathion. The different concentrations of malathion added were 0.02, 0.2, 2, 20, 50 and 100 ppm. Samples were taken at 4 days interval for 20 days for cell count. After 20 days, samples were tested for malathion residue and phosphorus content of algal cells.

Cyanobacterial cell count: The number of cyanobacteria cells in the medium were counted using a Neubauer chamber and a light microscope at 4 days intervals for 20 days.

Estimation of phosphorus content of cyanobacterial cells: After 20 days incubation, the cyanobacterial cells were analysed for total phosphorus content of the cyanobacterial biomass in the control and malathion added culture flasks (Watanabe et al., 1965).

Analysis of malathion residue: After 20 days incubation, the medium was analysed to estimate the concentrations of malathion remaining (Norris et al., 1954; Vishweswriah et al., 1973; Babu et al., 2021).

Results:

Isolation and Enumeration of cyanobacteria from paddy field sample: The water sample collected from paddy field of ICAR-RCER, Patna when enriched in Pringsheim's medium showed prominent growth of green algae initially and then after microscopic cells which were spherical, green coloured, coiled filaments were observed in the culture medium and identified as Nostoc spp.

Fig: Microscopic view of paddy field sample.

Characterization of cyanobacterial isolates:

Table 1: Cultural and morphological characteristics of different isolate:

Isolate	Colour	Elevation	Texture	Shape	Gram's Stain
I	Green	Raised	Smooth	Circular	-ve
II	Green	Flat	Smooth	Circular	-ve

Fig 2: Cyanobacterial isolates obtained by streak plate method.

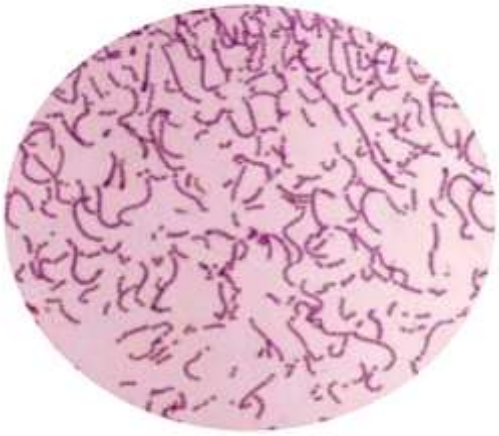

Fig 3: Gram Staining result of cyanobacteria isolate.

Growth of cyanobacteria on phosphorus limited medium: The selected isolates were cultured in Pringsheim's medium containing $1/10^{th}$ phosphorus content of the original composition. One culture flask was kept as control and six different flasks were added with 0.02, 0.2, 2, 20, 50 and 100 ppm concentrations of malathion respectively. The flasks were incubated under alternate 12 hours dark light illumination for 20 days.

Cyanobacterial cell count: Strain I and Strain II were counted at 4 days interval for 20 days. The data in **Fig 4** and **Fig 5** showed that the relationship between malathion concentration and the cyanobacterial growth is inversely related and the Nostoc strain I was observed to be slightly more tolerant to malathion.

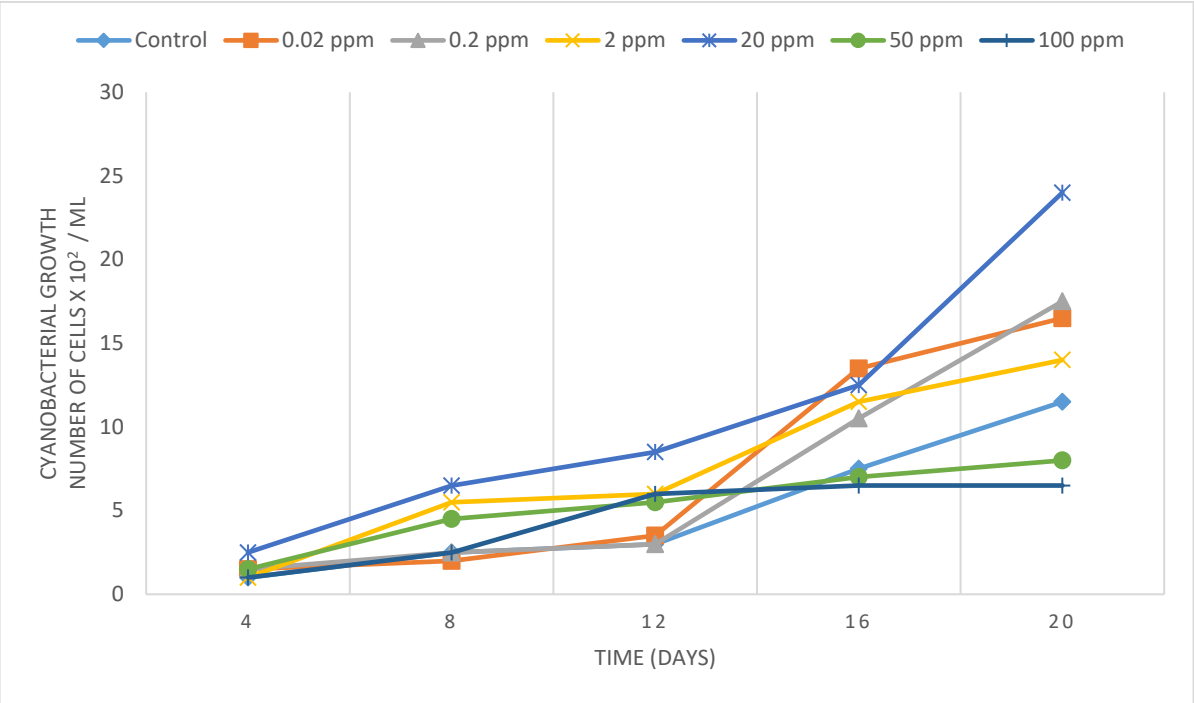

Fig 4: Effect of different malathion concentrations on the growth of Nostoc Strain I

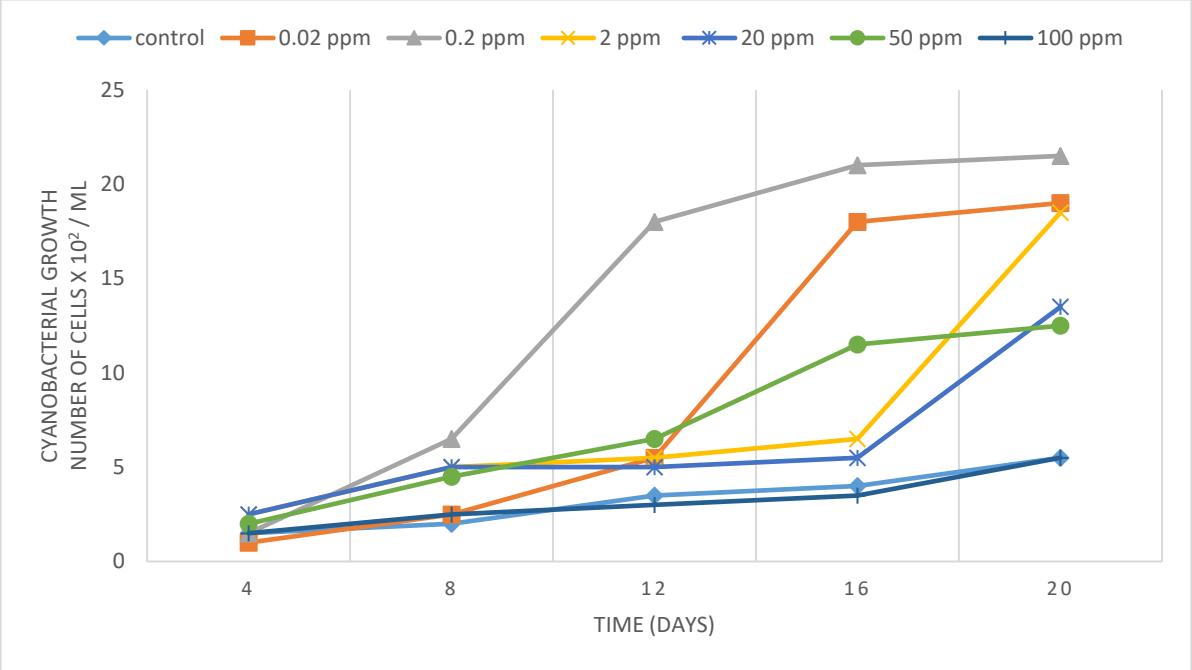

Fig 5: Effect of different malathion concentrations on growth of Nostoc Strain II

Effect of malathion on phosphorus content of cyanobacterial cells: The phosphorus content of cyanobacterial cells was estimated colorimetrically by ascorbic acid method (Watanabe et al., 1965). The ability of cyanobacteria to utilize malathion as phosphorus source was confirmed by estimating phosphorus content of algal biomass. The data can be interpreted as phosphorus amount present in P-limited cells was negligible whereas, those enriched with malathion had almost same amount of phosphorus as unlimited medium.

Table 2: Total phosphorus content (mg/g dry weight) of cyanobacterial biomass under different culture conditions

Cyanobacterial isolates:	Nostoc Strain I	Nostoc Strain II
Unlimitation	9.2±0.1	10.1±0.1
P- limitation	3.6±0.1[a]	2.2±0.1[a]
P- limitation with different concentrations of malathion		
0.02 ppm	11.2±0.0	6.9±0.0[b]
0.2 ppm	4.3±0.1[b]	8.8±0.1[b]
2 ppm	4.7±0.1[b]	11.6±0.1[b]
20 ppm	5.8±0.1[b]	13.7±0.1[b]
50 ppm	6.6±0.1[b]	14.0±0.1[b]
100 ppm	10.4±0.1[b]	15.9±0.1[b]

[a]Significant decrease compared with unlimitation condition.
[b]Significant increase compared with P-limitation condition.

Biodegradation of malathion by cyanobacteria: The estimation of malathion residue was done by colorimetric method (Babu et al., 2021). **Fig 6** showed that the two cyanobacterial strains were able to biodegrade malathion at different concentrations. It could be inferred from data that the strain I was more efficient than the strain II with the mean removal percentage of 65% and 54% respectively.

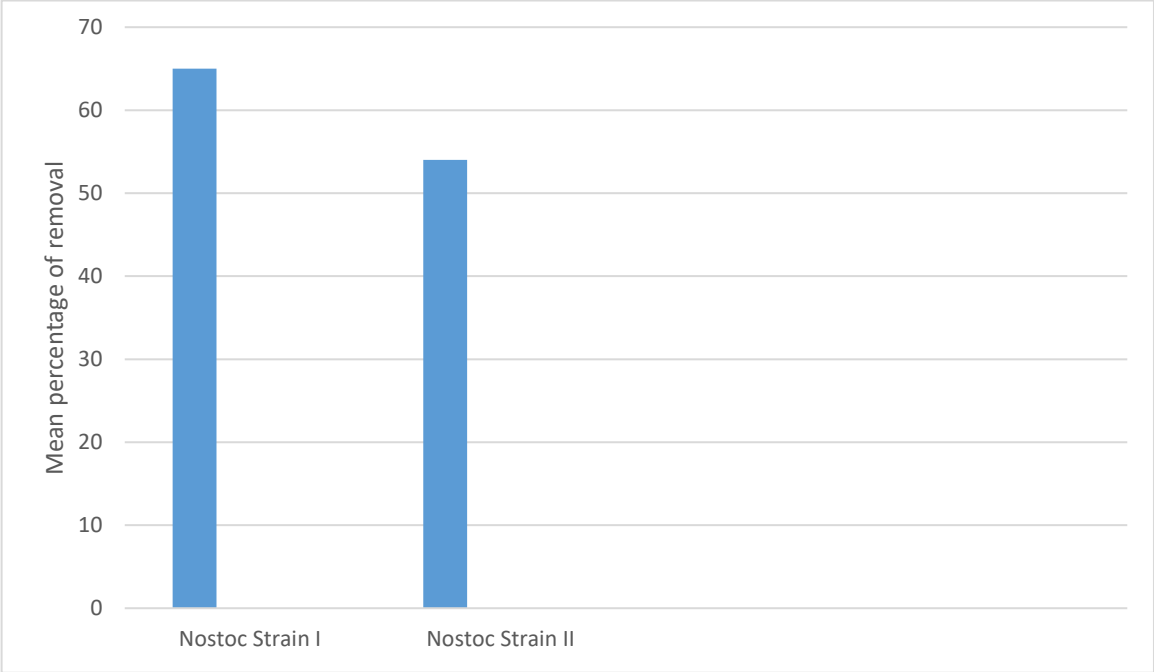

Fig 6: Efficiency of the two cyanobacterial strains to biodegrade malathion

Discussion:
It is clear from the results that the cyanobacterial strains growth had decreased as malathion concentration increased. This inverse correlation between malathion concentration and the algal growth agrees with Ibrahim et al.,2010 and Ghadai et al.,2010 who studied the effect of different concentrations (1–400 ppm) of organophosphorus pesticides on the growth of seven cyanobacterial strains. This inhibitory effect of malathion could be attributed to the adsorption of organophosphorus pesticide on the lipid rich cell membranes of the microalgal cells, thus, altering the membranes permeability (Rioboo et al., 2002) and diminishing photosynthetic activity (Manikar et al., 2013) as well as increasing reactive oxygen species (ROS) during stress (Mostafa et al., 2002). **Fig 6** revealed that Nostoc Strain I was more tolerant to different concentrations of malathion than the other. Highest tolerance of Nostoc Strain I could be as a result of its highest ability to biodegrade malathion (65%) at different concentrations. Results from **Table 2** revealed that the phosphorus content of cyanobacterial cells grown under P-limitation and malathion presence was much higher than that found in cells grown under same conditions but without addition of malathion revealing the ability of these cyanobacterial strains to degrade and utilize malathion as a sole phosphorus source. These results agree with Subramanian et al., 1994.

Conclusion:

In conclusion, this research delved into the pivotal realm of phycoremediation, focusing on the biodegradation of the organophosphorous pesticide malathion by cyanobacteria. Through rigorous experimentation with samples collected from diverse sources- we sought to elucidate the impact of malathion on the growth of selected cyanobacteria strains. Our findings unveiled a nuanced relationship, highlighting both resilience and susceptibility among the test strains in response to the pesticide.

Furthermore, our investigation explored the cyanobacteria strains' ability to utilize malathion as a phosphorous source, revealing a potential avenue for eco-friendly remediation strategies. The study not only expanded our understanding of the adaptive mechanisms within cyanobacteria but also underscored their role in environmental detoxification.

As we examined the residual levels of phosphorous and malathion in the media after 8-10 weeks, the research shed light on the efficacy of cyanobacteria in nutrient cycling. This holistic approach provides valuable insights for sustainable bioremediation practices. In summary, this research contributes essential knowledge to the intersection of cyanobacterial biology and environmental science, paving the way for informed strategies in addressing pesticide contamination and fostering ecological equilibrium.

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