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Isolation and Characterization of Cyanobacteria from Paddy Field Soil

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Original Research Article

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Abstract: Six cyanobacterial strains were isolated from pesticide exposed paddy field soil by enrichment method. The isolate exhibited unicellular and filamentous character and are designated and identified as JMCTTKC1-*Phormidium pachydermaticum*, JMCTTKC3-*Oscillatoria chalybea*, JMCTTKC4-*Oscillatoria tenuis*, JMCTTKC5-*Oscillatoria ornata*, JMCTTKC6-*Chroococcus dispersus* and JMCTTKC7-*Phormidium tenue*. The effect of 0.05% of lambdacyhalothrin was analysed on chlorophyll-a and protein content of the cyanobacterial isolates in mineral medium at different time intervals. The maximum level of chlorophyll-a content JMCTTKC4-*Oscillatoria tenuis*-39.3500µg/mL and protein content JMCTTKC6-*Chroococcus dispersus*- 99.9µg/mL was recorded on the 9th day sample. At the same time decreased level of chlorophyll-a content JMCTTKC1-*Phormidium pachydermaticum*-3.8597µg/mL and protein content JMCTTKC3-*Oscillatoria chalybea* 24.73µg/mL was observed on 13th day sample. From this above observation it was clearly noted that the selected isolates are capable of degrading Lamdacyhalothrin pesticide and used its metabolites as a sole carbon and nitrogen source for their growth.
Keywords: Paddy, Lamdacyalothrin, Chlorophyll-a and Protein.

INTRODUCTION

Green evolution changes the agriculture practices have resulted in severe increase in pesticides usage worldwide. Synthetic pyrethroids (SPs) are the chemical analogs of pyrethrins, which are compounds that are present in the flowers of *Chrysanthemum cinerariaefolium*.

This chemical class of pesticide is used worldwide in more than 320 million hectares, and in addition, it is the third most employed class of insecticide [1]. Over the decades, the usage of Lamdacyhalothrin has been gradually increased globally, especially with the phaseout of organophosphates use in residential home and some agricultural applications [2]. The extensive use of Lamdacyhalothrin has resulted in serious environmental contamination problems [3]. Numerous reports revealed that, the Lamdacyhalothrin is ubiquitous in water sources from either residential or agricultural runoff [4-7]. As a result, humans have an increased risk of exposure to Lamdacyhalothrin. The pesticide enters humans via ingestion of food or drinking of water or inhalation, or dermal contact [8-10]. Although Lamdacyhalothrin has relatively low mammalian toxicity, there is still caution with regard to human exposure [8, 11]. A number of studies have demonstrated that large dose exposures in mammals may cause significant toxicity and health effects, including neurotoxicity [12], genotoxicity [13, 14] cytotoxicity [14-15], and endocrine disruption which can damage mammalian reproduction [16-18]. Furthermore, chronic exposure to cyhalothrin even low level exposures may be associated with an elevated risk of mutagenicity [19] carcinogenicity [20] as well as childhood leukemia [21]. Additionally, Lamdacyhalothrin is also highly toxic to aquatic invertebrates and fish [22]. Its half-life varies from 17 to 110 days in water [23]. The large amounts of evidence suggest Lamdacyhalothrin has posed a great threat to human health and also for ecosystems [24].

Rice is an important cereal crop of the Asian countries. In India, rice is cultivated in about 44.3 million hectares producing 141 million metric tons of grains annually [25]. Lamdacyhalothrin is applied on a large scale in rice fields of Tamil Nadu state of India as a broad spectrum pyrethroid insecticide for the control of foliar insects. Once used, it eventually reaches the soil surface and accumulates nearly up to 15 cm of top soil layer [26]. This layer is the vital site of the highest microbial activities for maintaining soil fertility [27]. But the indiscriminate use of pesticides causes great damage to the beneficial microorganisms in the paddy field [28]. The non-or slow degradable characteristics of pesticides justifies their long persistence in the environment, which may not only lead to ecological damage to crops [29] but also tremendously harm some

beneficial organisms particularly the natural nitrogen-fixing cyanobacterial population growing in soil. Cyanobacteria, a group of ubiquitous photosynthetic prokaryotes perform two key biological functions: oxygenic photosynthesis and nitrogen fixation, and enrich the soil fertility, particularly with nitrogen and humus contents [30]. Non heterocystous cyanobacteria, which are predominantly found in paddy fields [31], may also fix atmospheric nitrogen under aerobic conditions [32]. To overcome the synthetic pesticide hazards, the biodegradation plays an alternative approach to control pesticide residues because of its cost-effective and ecofriendly properties. A few reports are available the degradation of Lamdacyhalothrin by cyanobacterial isolates. Therefore, there is an urgent need for effective strategies to remove cyhalothrin from environment. So, the present work mainly focused on the Lamdacyhalothrin degradation with the following objectives. (1) Isolation of cyanobacterial strains from pesticide exposed paddy field soil samples by enrichment method (2) Morphological Identification of isolates (3) Study the effect of Lamdacyhalothrin pesticides on growth parameters such as chlorophyll a and protein on the isolated cyanobacterial strains.

MATERIALS AND METHODS

Soil sample collection

The pesticide exposed paddy field soil samples were collected in a aseptic manner at a depth of 5-10cm according to the 'V' shaped method at different sites of paddy field at Paithur, Attur, Salem, Tamil Nadu, India. This particular field of choice is exposed to continuous application of Lamdacyhalothrin for more than 10 years. The samples were brought to the laboratory within six hours for further processing. The collected samples were spread in an aluminium trays and dried at room temperature to the point of soil moisture suitable for sieving. After sieving to a maximum particle size of <2mm mesh and these soil samples were stored at 4°C until further use.

Chemicals

The synthetic pyrethroid pesticide used in the present study was commercial grade pesticide, Lambda cyhalothrin 5% EC w/v named KARATE® (Syngenta Agrochemicals, India Private Limited) procured from the local market Attur, Salem, Tamil Nadu, India. All other chemicals and reagents used in this present study were of analytical grade and purchased from Hi-Media Pvt Ltd Mumbai, India.

Isolation, purification and identification of cyanobacteria from pesticide exposed paddy field soil sample:

10 g of pesticide exposed paddy field soil sample was added to 90ml of mineral medium (MM, pH 6.8–7.0) containing (g/L) Na_2HPO_4, 5.8; KH_2PO4, 3.0; NaCl, 0.5; NH_4Cl, 1; and MgSO4, 0.25, spiked with 0.05% pesticide (Lamdacyhalothrin) in 250ml Erlenmeyer flask for the isolation of cyanobacteria. The flasks were placed on a rotary shaker and incubated at 30 ± 2°C, 121 rpm for 7 days. After seven days, 10-fold dilutions of cultures were prepared and 100µL of each dilution was spread on agar plates (1.2 % agar) containing nitrogen-free BG-11 medium was used as growth medium [33]. The agar plates were kept at 25± 2°C in 2000 Lux light intensity with 16/8 h photo periods. After 7–10 days of inoculation visible blue green colonies were observed and characterized by a bright field microscope. 15–20 days of old colonies were subsequently used to establish liquid cultures.

The isolated pure cyanobacterial strains were maintained in BG11 medium. All cultures were shaken twice daily to prevent cells from clumping to accelerate the growth process. All inoculations were carried out under aseptic conditions, and the cultures were periodically monitored for any biological contamination. The axenic cultures were maintained in an exponential growth phase by regularly sub-culturing into fresh medium under same culture conditions. A bright field microscope (Olympus, model no: STC-313BPD) made in Japan with an attached camera was used to study the morphology of the isolates. A measurement of the cell dimensions was performed using Dewinter Biowizard 4.1 software. The isolated cyanobacteria were identified according to standard monograph cyanophyta [34] (Fig-1).

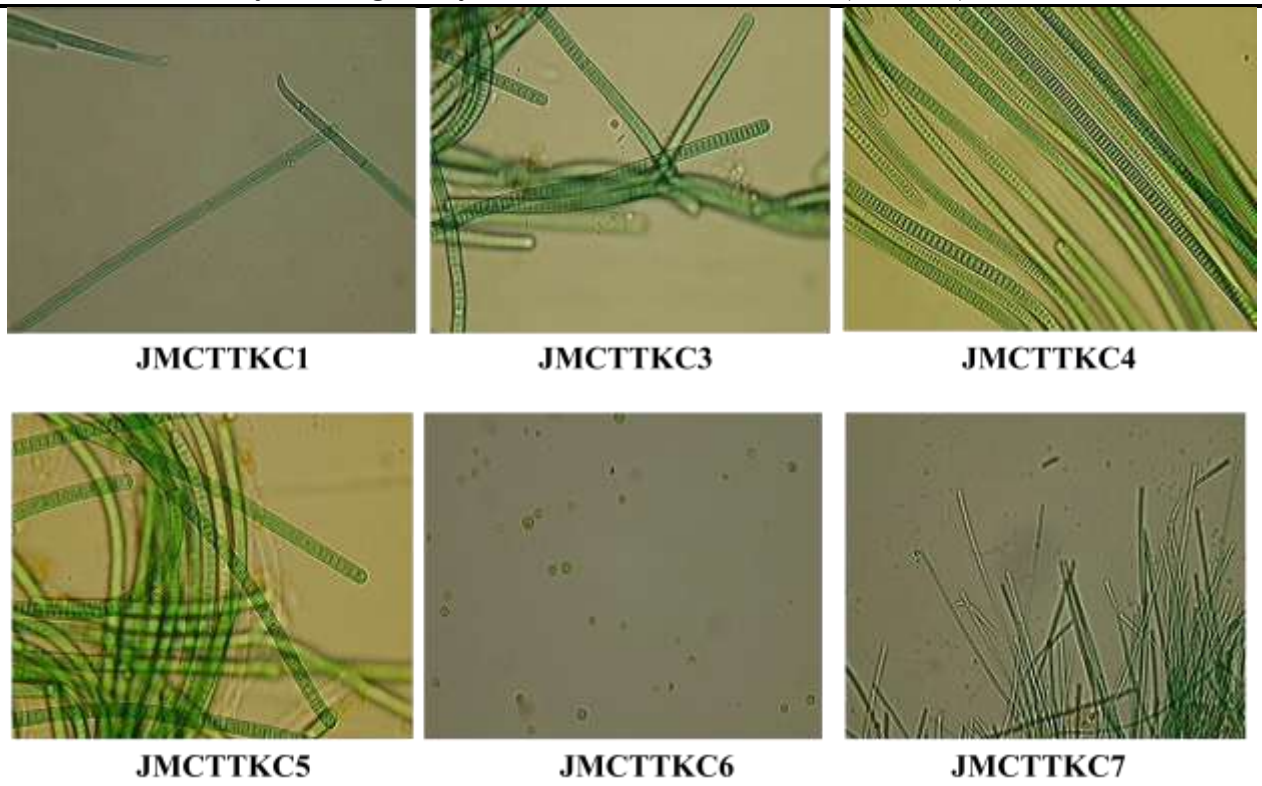

Fig1: Lamdacyhalothrin pesticide degrading cyanobacterial isolates JMCTTKC1-*Phormidium pachydermaticum*, JMCTTKC3-*Oscillatoria chalybea*, JMCTTKC4-*Oscillatoria tenuis*, JMCTTKC5-*Oscillatoria ornata*, JMCTTKC6-*Chroococcus dispersus* and JMCTTKC7-*Phormidium tenue*

Experimental design

The 0.3% inoculums of efficient pesticide degrading cyanobacterial isolates such as JMCTTKC1, JMCTTKC3, JMCTTKC4, JMCTTKC5, JMCTTKC6 and JMCTTKC7 were inoculated separately on Erlenmeyer flask containing 30ml of mineral medium supplemented with 0.05% of Lamdacyhalothrin as a sole carbon and nitrogen source. The cultures were incubated at 25±2 °C for 13 days, 2000 Lux light intensity with 16/8 h-photo period and were gently shaken by the hand on alternate days. The inoculated sample were recovered from the culture media at different intervals such as 1st, 3rd, 5th, 7th, 9th, 11th and 13th days aseptically, filtered and washed thrice by sterilized deionized water to remove the remaining insecticide residues and to estimate the chlorophyll-a and protein content. The mineral medium containing 0.05% Lamdacyhalothrin alone served as control. All experiments were performed in duplicate, and the average values were considered for further analysis

Growth measurement

Chlorophyll-a content

Extraction was made using 5mg dry weight of the selected cyanobacterial strains separately in 10ml 90% acetone in the test tube that was placed in a water bath at 65°C for 30 minutes. The pellet was discarded and the λ max of the supernatant was observed at 650nm and 665nm against 90% acetone as blank [35].

Protein content

The selected cyanobacteria mass (1mg) was taken individually in a test tube and 1ml of 1N NaOH was added to it. The test tube was placed in a boiling water bath for 10 minutes. The blank / sample solution were taken and added 5ml of Reagent A (prepared by adding 1ml freshly prepared l% Na-K tartarate solution containing 0.5% CuSO4 into 50ml 2% Na2CO3 solution and incubated at room temperature for 10 minutes. 0.5ml of reagent B (Folin reagent) was added and once again incubated at room temperature for 15 minutes. The absorbance of the supernatant was observed at λ650nm. Protein content was evaluated from the concentration of BSA solution known from standard curve [36].

RESULTS AND DISCUSSIONS

Totally six cyanobacterial strains were isolated from pesticide exposed paddy field soil in mineral medium with 0.05% of Lamdacyhalothrin, Paithur, Attur, Salem, Tamil Nadu, India. These isolated strains were capable of degrading and utilizing pyrethroid pesticide as a sole carbon nitrogen source. The isolates exhibits as a unicellular and filamentous characters and their pure form were maintained in liquid BG11 medium at 25±2°C, 2000 Lux light intensity with 16/8 h-photo period and were gently shaken by the hand on alternate days to maintain homogeneity. The vital cyanobacterial strains were identified as JMCTTKC1-*Phormidium pachydermaticum*, JMCTTKC3-

Oscillatoria chalybea, JMCTTKC4-*Oscillatoria tenuis*, JMCTTKC5-*Oscillatoria ornata*, JMCTTKC6-*Chroococcus dispersus* and JMCTTKC7-*Phormidium tenue*.

Morphotaxonomy of Isolates

JMCTTKC1-*Phormidium pachydermaticum*, this organism exhibit as thallus character outer surface dull blue green, inside brown, filament 6-10µ broad, straight or undulating, sheath at first thin, later thick irregularly lamellated, lamella short, irregularly disposed. Outside more or less rough not coloured blue by chlor–zinc-iodide; trichome blue green 5-6µ broad not constricted at the cross walls, end straight, not attenuated, not capitates cells nearly quadrate or up to ½ as long a broad , septa not granulated; end cell slightly convex or obtuse conical, with slightly thickened outer membrane. JMCTTKC3-*Oscillatoria chalybea*, is a dark blue-green thallus, trichome straight or lightly or irregularly spirally coiled, slightly constricted at the cross-walls attenuated at the spex and somewhat bent, 8-13µ broad, blue–green cells ½-1/3 times as long as broad , rarely as long as broad, 3.6-8µ long, septa not granulated, end cell obtuse, not capitates, without calyptra. JMCTTKC4-*Oscillatoria tenuis*, is a blue-green or olive-green thallus, slimy, trichome straight, fragile slightly constricted at the cross-walls, 40-10µ broad, blue green, sometimes bent at the ends, not attenuated at the apices, not capitate; cells up to 1/3 as long as broad, 2.6-5µ long, at the septa mostly granulated; end cells more or less hemispherical with thickened outer membrane. JMCTTKC5-*Oscillatoria ornata*, is a dark blue-green thallus; trichome spirally coiled at the ends, constricted at the cross-walls, 9-11µ broad, dull blue-green cells ½-1/6 as long as broad, 2–5µ long, cross walls granulated; apices slightly attenuated; end cells rounded, not capitate without thickened membrane. JMCTTKC6-*Chroococcus dispersus*, is a unicellular nature, the cells are 4-8, 16 or more in a tabular mucilaginous free swimming colony, with round margins, either solitary and then widely separated from each other or in groups isolated from each other, light or brilliant blue-green without sheath 5-6µ dia, colonies or groups 15-20µ distant, individual envelopes often totally gelatinized, not lamellated colorless. JMCTTKC7-*Phormidium tenue*, is pale blue–green thallus, trichome straight or slightly bent, densely entangled, slightly constricted at the cross walls, attenuated at the ends, 1-2µ broad, pale blue green, sheath thin, diffluent coloured violet by chlor–zinc-iodide, cells up to 3 times longer than broad, 2.5-5µ long, septa not granulated, cross–walls not commonly visible, end–cell acute-conical, calyptras absent.

Effect of Lamdacyhalothrin on the cyanobacterial growth

Chlorophyll-a content

In order to study the effect of Lamdacyhalothrin on the chlorophyll-a content of the selected cyanobacterial strains such as JMCTTKC1, JMCTTKC3, JMCTTKC4, JMCTTKC5, JMCTTKC6 and JMCTTKC7 has inoculated separately on mineral medium supplemented with 0.05% Lamdacyhalothrin for 1st, 3rd, 5th, 7th, 9th, 11th and 13th days under aseptic condition (Fig-2)**.** The chlorophyll-a content of the inoculated strains exhibited the gradual increasing from the 3rd, 5th, 7th and 9th day intervals. The maximum amount of chlorophyll-a content was observed on the ninth day sample, however the chlorophyll-a content was quit vary with one another of the selected strains were as follows the JMCTTKC4-39.3500µg/mL, followed by JMCTTKC6-33.45434µg/mL, JMCTTKC5-28.31141µg/mL, JMCTTKC7-25.7753µg/mL, JMCTTKC3-15.4793µg/mL and JMCTTKC1-14.236535µg/mL.

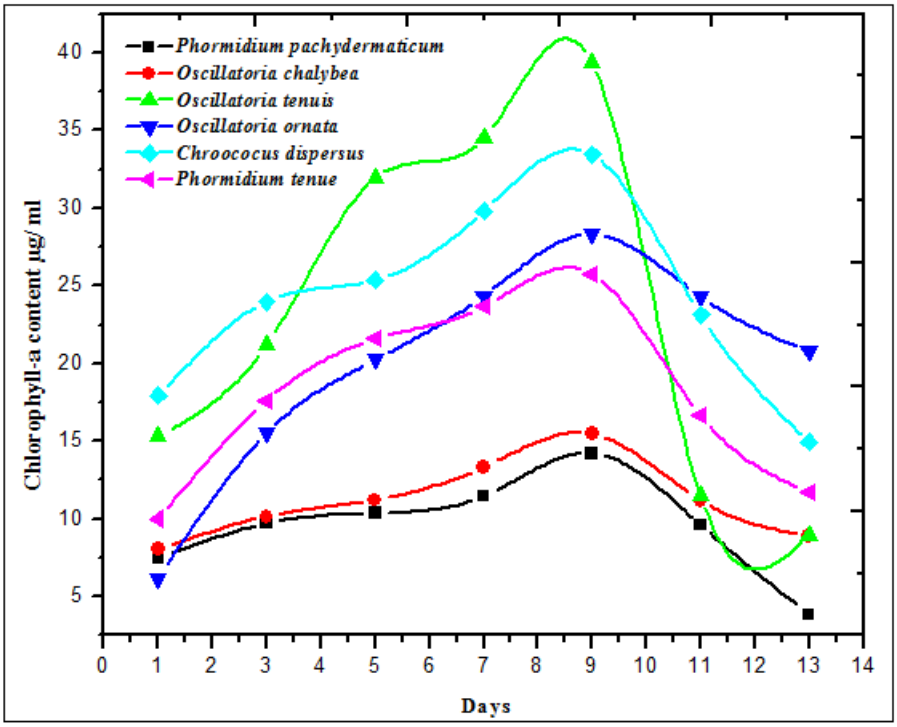

Fig-2: Effect of Lamdacyhalothrin on chlorophyll-a contents

This result indirectly proved that the strain has been capability to degrade the Lamdacyhalothrin for their sole source of carbon as well as nitrogen source. Contrastingly the 13th day of sample exhibited a decreased level of chlorophyll-a content with the following order, the strains JMCTTKC5-20.31914µg/mL, JMCTTKC6-13.96373µg/mL, JMCTTKC7-11.31648µg/mL, JMCTTKC4-9.912024µg/mL, JMCTTKC3-8.305488µg/mL and JMCTTKC1-3.758688µg/mL. Balakrishna Tiwari *et al.*, [37] isolated *Fischerella sp* from the paddy field which as the capacity to degrade and utilizing the organophosphorous pesticide methyl parathion as a phosphate source through the biosorption followed by the simultaneous bioaccumulation process. This organism is a filamentous, branched, heterocystous strain in the stigonematales order. The 5mg^{-1} methyl parathion concentration supported the cyanobacterial growth. At the same time 20mg $^{-1}$ and 30 mg $^{-1}$ methyl parathion reduced the chlorophyll-a content. The chlorophyll-a contents were decreased significantly with increased concentration of Lamdacyhalothrin. However in 20ppm treatment a slight insignificant increased was observed (13%, P>0.05, ns). However in 40, 80 and 160ppm of Lamdacyhalothrin the chlorophyll-a content was reduced 14%, 50% and 68% respectively. The 160ppm is the highest inhibitory grade reduced the chlorophyll-a content upto 68% on 8th day. Which further decreased of the 78% on 20th day was reported [38]. Muthukannan Satheesh *et al.* [39] pointed out that different concentrations of (1, 5, 10, 15, 20 and 25 mg/L) acephate and imidacloprid exhibited varying the growth of *C. mexicana*. The dry cell weight of the *C. mexicana* increasing the concentrations of the both insecticide 15, 20 and 25 mg/L declined inhibited the growth of the *C. mexicana*, which might be due to toxicity at high concentration. The 15mg/L of both insecticides which affect the chlorophyll-a and carotenoid content of the *C. mexicana*. The atrazine and endosulfan were decreased the accumulation of chlorophyll-a exposed to different microalgal species [40, 41]. Chlorpyrifos, endosulfan and tebuconazole were also decreased the chlorophyll-a and carotenoid content of cyanobacterial species [42, 43]. Thengodkar and Sivakami [44] clearly pointed out that the intensity of the pigment colour of the culture are inversely proportional the pesticide content. At the same time, [45] observed that organophosphorous compounds do not adversely affect bacteria because it doesn't have acetylcholine esterase that can be inhibited by organophosphorous compounds. Akhil *et al.* [46] revealed that 10µg/L had no effect on the microalgae. At the same time 25, 50 and 100µg/L exhibited toxicity which effects the cell growth and chlorophyll-a content 22%, 33% 36% and 13%, 24% and 27% respectively, and also pointed out that carbohydrate content was increased in atrazine concentration up to 15%.

Protein content

The protein content of the cyanobacterial strains JMCTTKC1, JMCTTKC3, JMCTTKC4, JMCTTKC5, JMCTTKC6 and JMCTTKC7 in mineral medium with 0.05% Lamdacyhalothrin with various time intervals such as 1st, 3rd, 5th, 7th, 9th, 11th and 13th days was analysed. Out of which, the highest protein content was observed at the 9th day sample compared to 3rd, 5th, 7th, day sample. However the percentage of the protein contents were varied with the respective

cyanobacterial strains JMCTTKC6 -99.9µg/mL, followed by JMCTTKC4-74.2µg/mL, JMCTTKC5-57.7µg/mL, JMCTTKC1-47.8333µg/mL, JMCTTKC7-45.8666µg/mL, JMCTTKC3-32.3µg/mL (Fig-3). At the same time the 13th day samples exhibited a decreased level of protein content, which also depends the respective cyanobacterial strains, when compared to 9th day sample. The protein content of JMCTTKC4-38.833µg/mL, JMCTTKC6-37.1µg/mL, JMCTTKC5-35.5333µg/mL, JMCTTKC1-32.1µg/mL, JMCTTKC7-30.666µg/mL and JMCTTKC3-24.73µg/mL. Our results are similarly coherence with the following workers. Muthukannan Satheesh *et al*. [39] studied the total protein content of *C. mexicana* on exposure 15mg/L of acephate and imidacloprid was observed that decreased protein content 271 and 334mg/g compared to control 358mg/g.

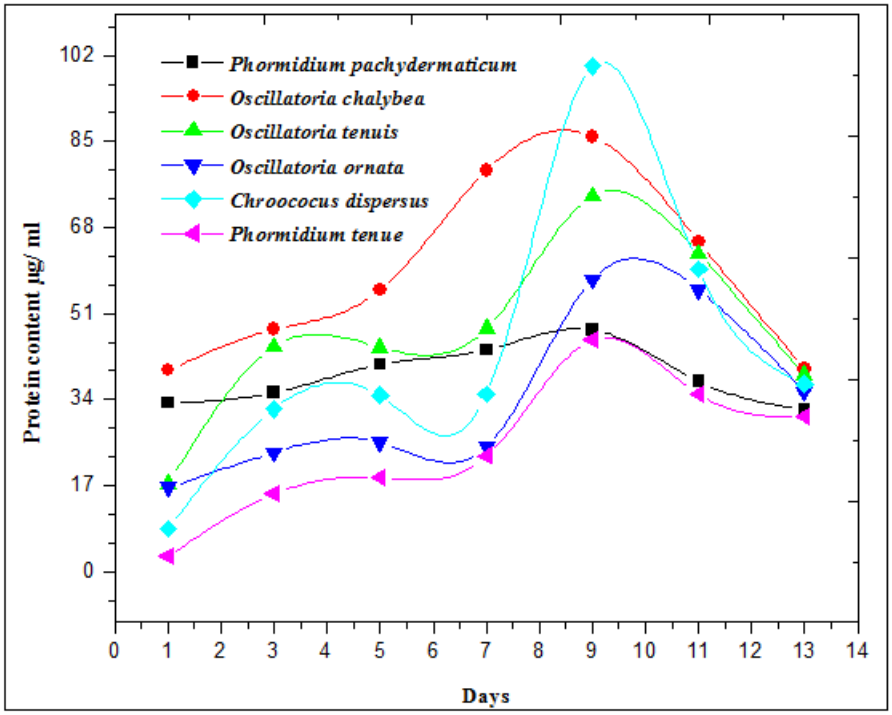

Fig-3: Effect of Lamdacyhalothrin on protien contents

The decrease in protein content may be due to toxicity of insecticides [47]. Kumar *et al.*, [48] explained lower concentration of pesticide stimulate synthesis of stress retarding proteins in *Aulosira fertilissima* 43% of protein enhancement was noted at 7.5µg/ml pesticide concentration. At the same time the decrease in protein content was beyond 7.5µg/ml endosulfan. This decrease in protein content may be due to exposure of pesticide beyond the tolerance range. The 50ppm and 100ppm of Malathion boost the protein content of *A.oryzea* and *N. muscorum*, at the same time 0.2ppm and 20ppm Malathion concentration increased the protein content of *S. platensis*, beyond the 100ppm concentration of Malathion caused gradual decrease in protein content [49].

CONCLUSION

In this paper we have reported the *invitro* finding of the effect of 0.05% Lamdacyhalothrin in mineral medium on the growth parameters of chlorophyll-a and protein content of an indigenous selected cyanobacterium JMCTTKC1-*Phormidium pachydermaticum*, JMCTTKC3-*Oscillatoria chalybea*, JMCTTKC3-*Oscillatoria tenuis*, JMCTTKC5-*Oscillatoria ornata*, JMCTTKC6-*Chroococcus dispersus* and JMCTTKC7-*Phormidium tenue* on different time intervals 1st, 3rd, 5th, 7th, 9th, 11th and 13th days intervals. The maximum amount of chlorophyll-a and protein content was observed in 9th day, in all the selected cyanobacterial strains. Contrastingly the 13th day sample of the cyanobacterial isolates exhibited a decreased level of chlorophyll-a and protein content by this observation is clearly observed that the isolates totally utilized the 0.05% of Lamdacyhalothrin in 9th day itself. After that the medium doesn't contained the carbon and nitrogen source for their chlorophyll-a and protein synthesis. By these preliminary investigations, it is clear that these cyanobacterial isolates might be used for biodegradation of Lamdacyhalothrin pesticides as well as for the biotransformation studies.

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REFERENCES

1. Housset, P., & Dickman, R. (2009). A promise fulfilled pyrethroid development and the benefits for agriculture and human health. *Bayer Crop Science Journal*, *62*, 135–144.
2. Hintzen, E. P., Lydy, M. J., & Belden, J. B. (2009). Occurrence and potential toxicity of pyrethroids and other insecticides in bed sediments of urban streams in central Texas. *Environmental Pollution*, *157*(1), 110-116.
3. Lubick, N. (2008). Pyrethroids are ubiquitous in California's urban streams. *Environmental Science Technology*, *42*, 8622–8628.
4. Delgado-Moreno, L., Lin, K., Veiga-Nascimento, R., & Gan, J. (2011). Occurrence and toxicity of three classes of insecticides in water and sediment in two southern California coastal watersheds. *Journal of agricultural and food chemistry*, *59*(17), 9448-9456.
5. Weston, D. P., Asbell, A. M., Hecht, S. A., Scholz, N. L., & Lydy, M. J. (2011). Pyrethroid insecticides in urban salmon streams of the Pacific Northwest. *Environmental pollution*, *159*(10), 3051-3056.
6. Kuivila, K. M., Hladik, M. L., Ingersoll, C. G., Kemble, N. E., Moran, P. W., Calhoun, D. L., & Gilliom, R. J. (2012). Occurrence and potential sources of pyrethroid insecticides in stream sediments from seven US metropolitan areas. *Environmental science and technology*, *46*(8), 4297-4303.
7. Markle, J. C., van Buuren, B. H., Moran, K., & Barefoot, A. C. (2014). Pyrethroid pesticides in municipal wastewater: a baseline survey of publicly owned treatment works facilities in California in 2013, In Describing the behavior and effects of pesticides in urban and agricultural settings (pp. 177-194). American Chemical Society.
8. Trunnelle, K. J., Bennett, D. H., Tulve, N. S., Clifton, M. S., Davis, M. D., Calafat, A. M., Moran, R., Tancredi, D. J., & Hertz-Picciotto, I. (2014). Urinary pyrethroid and chlorpyrifos metabolite concentrations in Northern California families and their relationship to indoor residential insecticide levels, part of the Study of Use of Products and Exposure Related Behavior (SUPERB). *Environmental science & technology*, *48*(3), 1931-1939.
9. Morgan, M. K. (2012). Children's exposures to pyrethroid insecticides at home: a review of data collected in published exposure measurement studies conducted in the United States. *International Journal of Environmental Research and Public health*, *9*(8), 2964-2985.
10. Lu, C., Adamkiewicz, G., Attfield, K. R., Kapp, M., Spengler, J. D., Tao, L., & Xie, S. H. (2013). Household pesticide contamination from indoor pest control applications in urban low-income public housing dwellings: a community-based participatory research. *Environmental Science and Technology*, *47*(4), 2018-2025.
11. Kaneko, H. (2010). Pyrethroids: mammalian metabolism and toxicity. *Journal of agricultural and food chemistry*, *59*(7), 2786-2791.
12. Soderlund, D. M. (2012). Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. *Archives of toxicology*, *86*(2), 165-181.
13. Fetoui, H., Feki, A., Salah, G. B., Kamoun, H., Fakhfakh, F., & Gdoura, R. (2014). Exposure to lambda-cyhalothrin, a synthetic pyrethroid, increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. *Toxicology and Industrial Health*, *31*(5), 433-441.
14. Muranli, F. D. G. (2013). Genotoxic and cytotoxic evaluation of pyrethroid insecticides lamdacyhalothrin and a-cypermethrin on human blood lymphocyte culture. *Bulletin of Environment Contamination Toxicology*, *90*(3), 357–363.
15. Zhang, Q., Wang, C., Sun, L., Li. L., & Zhao, M. (2010). Cytotoxicity of lambda-cyhalothrin on the macrophage cell line RAW 264.7. *Journal of Environmetnal Science*, *22*(3), 428–432.
16. Yousef, M. I. (2010). Vitamin E modulates reproductive toxicity of pyrethroid lambdacyhalothrin in male rabbits. *Food Chemistry Toxicology*, *48*(5), 1152–1159.
17. Velmurugan, B., Selvanayagam, M., Cengiz, E. I., & Unlu, E. (2007). Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. *Environmental Toxicology Pharmacology*, *24*(3), 286–291.
18. Fetoui, H., Makni, M., Garoui, E. M., & Zeghal, N. (2010). Toxic effects of lambdacyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: involvement of oxidative stress and protective role of ascorbic acid. *Experimental Toxicological Pathology*, *62*(6), 593–599.
19. Saleem, U., Ejaz, S., Ashraf, M., Omer, M. O., Altaf, I., Batool, Z., & Afzal, M. (2014). Mutagenic and cytotoxic potential of endosulfan and lambda-cyhalothrin—in vitro study describing individual and combined effects of pesticides. *Journal of Environmental Sciences*, *26*(7), 1471-1479.
20. Fortes, C., Mastroeni, S., Melchi, F., Pilla, M. A., Alotto, M., Antonelli, G., & Pasquini, P. (2007). The association between residential pesticide use and cutaneous melanoma. *European Journal of Cancer*, *43*(6), 1066-1075.
21. Ding, G., Shi, R., Gao, Y., Zhang, Y., Kamijima, M., Sakai, K., & Tian, Y. (2012). Pyrethroid pesticide exposure and risk of childhood acute lymphocytic leukemia in Shanghai. *Environmental science and technology*, *46*(24), 13480-13487.
22. Kumar, A., Sharma, B., & Pandey, R. S. (2012). Assessment of stress in effect to pyrethroid insecticides, l-cyhalothrin and cypermethrin, in a freshwater fish, *Channa punctatus* (Bloch). *Cell Molecular Biology (Noisy-le-grand)*, *58*(1), 153–159.

23. Laskowski, D. A. (2002). Physical and chemical properties of pyrethroids. *Rev Environmental Contamination Toxicology*, *174*(1), 49–170.
24. Alonso, M. B., Feo, M. L., Corcellas, C., Vidal, L. G., Bertozzi, C. P., Marigo, J., & Torres, J. P. M. (2012). Pyrethroids: A new threat to marine mammals?. *Environment International*, *47*, 99-106.
25. Yadav, G. S., Kumar, D., Shivay, Y. S., & Singh, H. (2010). Zn-enriched urea improves grain yield and quality of aromatic rice. *Better Crops*, *3*(1), 4–5.
26. Perucci, P., Dumontet, S., Bufo, S. A., Mazzatura, A., & Casucci, C. (2000). Effect of organic amendment and herbicide treatment on soil microbial biomass. *Biology Fertility Soils*, *32*(1), 17–23.
27. Schuster, E., & Schroder, D. (1990). Side effects of sequentially and simultaneously applied pesticides on non target soil microorganism: laboratory experiments. *Soil Biology Biochemistry 22*(3), 375-383.
28. Roger, P. A., Simpson, I., Oficial, R., Ardales, S., & Jimenez, R. (1994). Effects of pesticides on soil and water microflora and mesofauna in wetland rice fields: a summary of current knowledge and extrapolation to temperate environments. *Australian Journal of Experimental Agriculture*, *34*(7), 1057–1068.
29. Da Silva, E. J., Henricksson, L. E., & Henricksson, E. (1975). Effect of pesticides on blue green algae and nitrogen fixation. *Archives of Environmental Contamination Toxicology*, *3*(2), 193–204.
30. Song, T., Mårtensson, L., Eriksson, T., Zheng, W., & Rasmussen, U. (2005). Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. *FEMS Microbiology Ecology*, *54*(1), 131-140.
31. Frank, I. B., Lundgren, P., & Falkowski, P. (2003). Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research Microbiology*, *154*(3), 157–164.
32. Ohki, K., Zehr, P. J., & Fujita, Y. (1992). Regulation of nitrogenase activity in relation to the light dark regime in the filamentous non-heterocystous cyanobacterium *Trichodesmium sp*. NIBB 1067. *Journal of General Microbiology*, *13*(12), 2679–2685.
33. Stanier, R. Y., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue green algae (order Chroococcales). *Bacteriological Review*, *5*(2), 171–205.
34. Desikachary, T. V. (1959). Cyanophyta. Indian Council of Agricultural Research, New Delhi, p 686.
35. Parson, T. R., & Strickland, J. D. H., (1965). Particulate organic matter III. I. pigment analysis III, I.I. Determination of phytoplankton pigments. *Journal of Fisheries Research Board of Canada*, (18), 117-127.
36. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, *193*(1), 265-275.
37. Tiwari, B., Verma, E., Chakraborty, S., Srivastava, A. K., & Mishra, A. K. (2017). Tolerance strategies in cyanobacterium *Fischerella* sp. under pesticide stress and possible role of a carbohydrate-binding protein in the metabolism of methyl parathion (MP). *International Biodeterioration & Biodegradation*, *127*, 217–226.
38. Kiran Gupta, P. & Baruah, P. (2015). Effect of lambdacyhalothrin on *Calothrix sp.* (GUEco 1001), an autochthonous cyanobacterium of rice fields of Brahmaputra flood plain. *Environmental Science Pollution Research*, *22*, 18554–18560.
39. Muthukannan Satheesh, K., Akhil, N., Kabra Booki, M., Marwa, M., El-Dalatony, Jiuqiang, X., Thajuddin, N., Dae Sung, L., & Byong-Hun, J. (2015). Insecticides induced biochemical changes in freshwater microalga *Chlamydomonas mexicana*. *Environmental Science Pollution Research*, DOI 10.1007/s11356-015-4681-6.
40. Kumar, S., Habib, K., & Fatma, T. (2008). Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. *Science Total Environment*, *403*, 130–138.
41. Kabra, A. N., Ji, M. K., Choi, J., Kim, J. R., Govindwar, S. P., & Jeon, B. H. (2014). Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*. *Environmental Science Pollution Research*, *21*, 12270–12278.
42. Kumar, N., Bora, A., Kumar, R., & Amb, M. K. (2012b). Differential Effects of Agricultural Pesticides Endosulfan and Tebuconazole on Photosynthetic pigments, Metabolism and Assimilating Enzymes of Three Heterotrophic, Filamentous Cyanobacteria. *Journal of Biological Environmental Science*, *6*, 67–75.
43. Kumar, M. S., Praveenkumar, R., Jeon, B. H., & Thajuddin, N. (2014). Chlorpyrifos induced changes in the antioxidants and fatty acid compositions of *Chroococcus turgidus* NTMS12. *Letters Applied Microbiology*, *59*(5), 535– 541.
44. Thengodkar, R. M. M., & Sivakami, S. (2010). Degradation of Chlorpyrifos by an alkaline phosphatase from the cyanobacterium *Spirulina platensis*. Biodegradation, 21, 637–644.
45. Singh, B. K. (2009). Organophosphorous degrading bacteria: ecology and industrial applications. Nature Review Microbiology, (7), 156–164.
46. Akhil, N., Kabra, M. J., Jaewon, C., Jung Rae, K., Sanjay, P., Govindwar, B-H. J. (2014). Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*. Environmental Science Pollution Research, DOI 10.1007/s11356-014-3157-4.

47. Leitao, M. A. S., Cardozo, K. H. M., Pinto, E., Colepicolo, P., (2003). PCB-Induced oxidative stress in the unicellular marine dinoflagellate *Lingulodinium polyedrum*. Archives of Environmental Toxicology, 45, 59–65.
48. Kumar, S., Habib, K., & Fatma, T. (2008). Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. *Science of the total environment*, *403*(1), 130-138.
49. Ibrahim, W. M., Karam, M. A., El-Shahat, R. M., & Adway, A. A. (2014). Biodegradation and utilization of organophosphorus pesticide malathion by cyanobacteria. *BioMed research international*, *2014*.