Isolation and Characterization of Cyanobacteria from Paddy Field Soil
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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 tenuis. 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) Na₂HPO₄, 5.8; KH₂PO₄, 3.0; NaCl, 0.5; NH₄Cl, 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).
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.

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 1% 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

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

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.
This result indirectly proved that the strain has been capable 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\textsuperscript{-1} methyl parathion concentration supported the cyanobacterial growth. At the same time 20mg\textsuperscript{-1} and 30 mg\textsuperscript{-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 $C.\text{mexicana}$ increasing the concentrations of the both insecticide 15, 20 and 25 mg/L declined inhibited the growth of the $C.\text{mexicana}$, 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 $C.\text{mexicana}$. 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 - 47.833µg/mL, JMCTTKC7 - 45.866µg/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 - 35.533µg/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.

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, JMCTTKC5-Oscillatoria tenuis, JMCTTKC2-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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