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SCREENING OF CHLORPYRIFOS (CPF) TOLERANT CYANOBACTERIA FROM PADDY FIELD SOIL OF LUCKNOW, INDIA

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Abstract:

In this study, paddy field soil, collected from different locationsof Lucknow, India i.e.BBAU campus, Mohanlalganj, Barabanki, BakshikaTalab and Malihabad. The cyanobacterial populations in soil samples of selected sites were maximum at BakshikaTalab(Indefinite number g^{-1} soil) and minimum at Mohanlalganj (430 g^{-1} soil). Total 10 cyanobacteria *Synechococcus* sp., *Gloeocapsa* sp., *Anabaena* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoelus* sp., *Gloeothece* sp., *Oscillotoria* sp., *Gleotrichia* sp.and *Aphanothece* sp. were isolated from paddy field soil. For the screening of Chlorpyrifos (CPF)pesticide tolerant cyanobacteria, all the isolated cyanobacterial strains were grownonBG-11 media containing 5 ppm of CPF concentration. Among 10 cyanobacteria,only *Synechococcus* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoelus* sp. and *Oscillotoria*sp were able to tolerateCPF. These CPF tolerant cyanobacteria will be used further for the studyrelated to efficacy of the strains to tolerate CPF.

Key Words: Paddy Field Soil, Chlorpyrifos, Cyanobacteria, Pesticide Stress&Pesticide Tolerance.

Abbreviations: CPF-Chlorpyrifos; MPN- Most Probable Number; WHO-World Health Organization; FAO-Food and Agriculture Organization

Introduction:

Paddy is a major cereal crop in the world and rice is a staple food for nearly 3.5 billion peoples worldwide (IRRI, 2013). Currently worldwide rice production has been estimated about 481.04 million tonnes which is 2.06 million tonnes (0.43%) less than previous year's rice production 483.1 million tonnes (USDA, 2017). However, the present rate of rice production would not be enough to satisfy the food demand coming generation population. Apart from pressure of increasing rice production, paddy cultivation is affected by several pests mediated diseases that are responsible for almost 37 % crop yield losses (Sparks et al., 2012).To deal with this situation of pest mediated paddy yield loss, the farmersare using extensivelythe chemicals likepesticides.These pesticides not only pollute soils and food grains but also harmful for the loss of beneficial micro-flora including cyanobacteria (Kumar and Singh, 2017).

In paddy fields, cyanobacteria contributes in enhancing the soil fertility by fixing atmospheric nitrogen and releasing some plant growth promoting substances, extra cellular polysaccharides and also solubilizing phosphates (Singh et al., 2016). A number of reports are available related to pesticide-induced inhibitory effects on cyanobacteriaandstated that it influence their growth, photosynthesis, nitrogen fixation, biochemical composition and metabolic activities (Singh et al., 2011, Singh et al., 2013, Kumar et al., 2013; Kumar et al., 2014; Kumar and Singh, 2017).However, only few studydemonstrated the capability of cyanobacteria for the degradation of pesticides(Lee et al., 2003; Barton et al., 2004; El-Bestawy et al. 2007; Cáceres et al., 2008;Singh et al., 2011; Singh et al., 2013). Therefore, it is need of the hour to investigate the role of cyanobacteria regarding its tolerance level and degradation of insecticides/pesticides used in the paddy agriculture.

Chlorpyrifos (CPF), a broad spectrum insecticide, is extensively used for the control of foliar insects in paddy fields (Lee et al., 2012). It is moderately persistent in nature and could be remains active in soil for 20-90 days along with 10-60 days of half-life (Lakshmi et al., 2008; Singh et al., 2011).It has been reported thatCPF degradation in the soil is affected by some factors such as soil pH, moisture, temperature and initial pesticide concentration.It is well known that volatilization, hydrolysis and microbial degradation are the common routes by which CPF is assimilated in soil or environment.Aysal et al. (2004) during an investigation found that the CPF residues entered in significant quantity in food chain. In 2004, WHO and FAO conducted good agricultural practices in Columbia, Philippines, Thailand, Vietnam and India to estimate the maximum residue level of CPF in rice grains through supervised trials using CPF median residue ranged from 0.12 to 0.28 mg/kg. The data showed the maximum CPF residue level of 0.5 mg/kg in the rice. It is also reported that CPF residue in some vegetables with the range of 0.024–0.07 mg/kg and 0.018–0.021 mg/kg in Cauliflower and Brinjal (Chandra et al., 2010). The LD50 for CPF in rat has been reported to be 82–270 mg/kg body weight (Berg 1986; US Environment Protection Society, 1984).Sun and Chen (2008) found CPF residue in the farmed fish because of existence in fish feed and they also confirmed dietary accumulation of CPF by indoor carp study.

Apart from this, human health also may have serious threats by consuming cereals, vegetables and animal products contaminated withCPF residues. So there is growing concern about the toxicological and environmental risks associated with CPF residues magnification in the food chain. Therefore,there is a need to detoxify this lethal moiety from the contaminated agriculture soils and environment (Mukherjee et al., 2004).Singh et al. (2011) reported the CPF degradation by *Synechocystis* sp. strain PUPCCC 64.It is also investigated CPF exposure with marine cyanobacterium *Phormidiumvalderianum* BDU 20041 (Palanisami et al., 2009). Kumar et al.(2014) also showed that *Chroococcusturgidus* NTMS12 successfully resisted the CPF exposure.

Hence the main objective of this study to find whether cyanobacterial strains isolated from paddy fields is able to tolerate CPF pesticide and based on this study to specify the efficient cyanobacterial strainfor further degradation experiments. This strain can be recommended for inoculation in rice fields to minimize deleterious effects of CPF and also to improve soil fertility.

Materials and Methods:

Chemicals:

The media (BG-11 Broth) used for cultivation and maintenance for cyanobacteria purchased from HiMedia Laboratories Pvt. Ltd. commercial grade CPF (Eldrin TC) (Chlorvip 20%, w/v) used during the present study was manufactured by Crystal Crop Protection Pvt. Ltd. and purchased from the local market.

Soil Sample Collection and Physico-chemical Analysis:

Soil samples were collected from paddy fields from five different selected sites namely:(1) BBAU campus, (2)Mohanlalganj, (3) Barabanki,(4) BakshikaTalab, (5) Malihabad from theadjoining areas of Lucknow (Figure 1). Soil samples from each site were collected from the subsurface horizon (2–15 cm) at three different points and mixed thoroughly. The collected soil samples were brought to the laboratory in polythene bags, air-dried and sieved before analyses. The soil texture of all the sites was sandy-clay in nature.

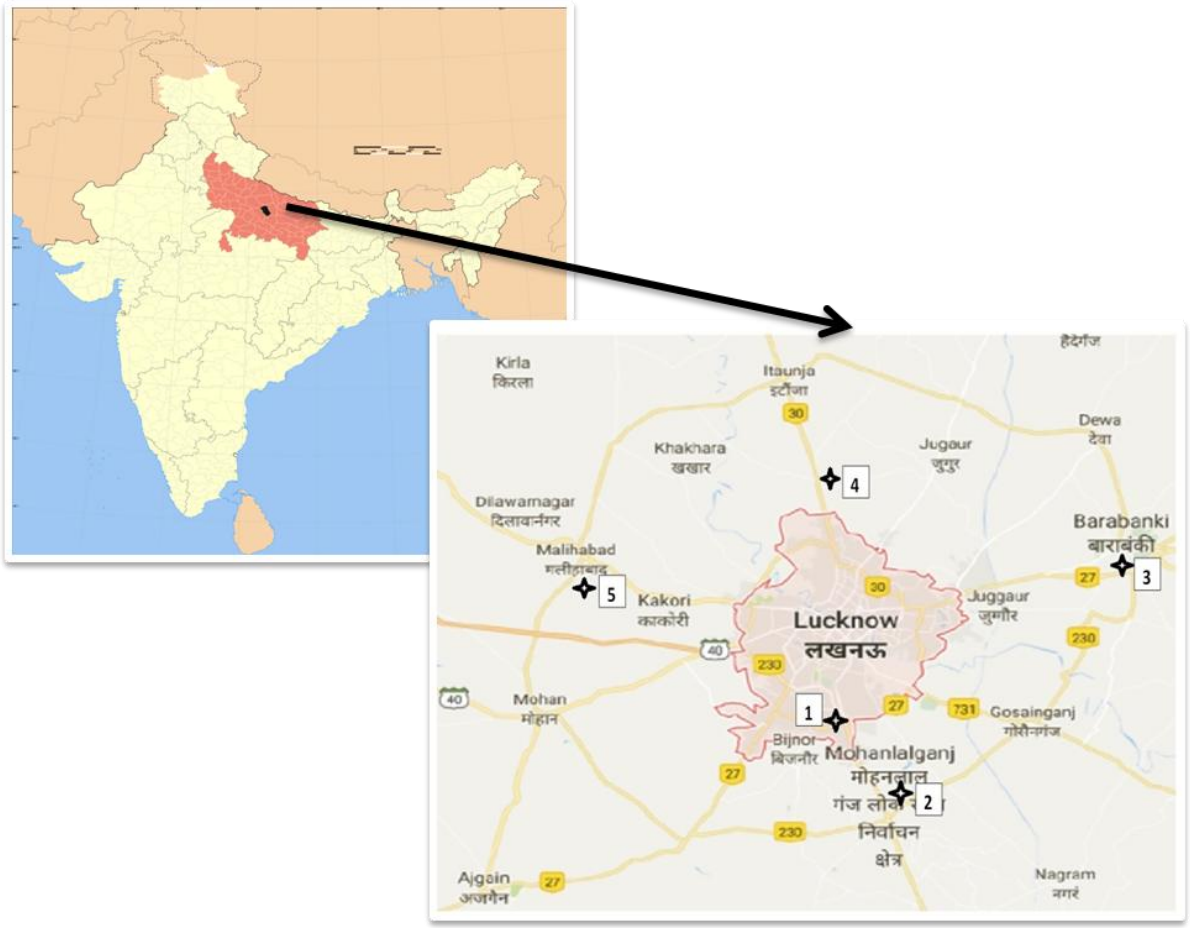

Figure 1: Map showing study sites for soil sample collection (curtsey: Google Maps)

The physico-chemical properties of soil such as pH, temperature, conductivity,total-N, total-P and Organic-C content were analysed as per standard procedures.

Assessment of Cyanobacterial Population (MPN Method):

The cyanobacterial population was assessed through MPN method (Pepper et al., 1995). In brief 10g of soil sample was suspended in 95 mL of BG-11 medium and a serial dilution of 10^{-2}, and 10^{-6} was prepared. From dilution 10^{-3}, 10^{-4} and 10^{-5}, l.0 mL was transferred to 5 replicate tubes containing 10 mL BG-11 medium.

These tubes were incubated for 4 weeks in culture racks with 14/10 photoperiod and the number of cyanobacteria was enumerated.

Figure 2: Cyanobacteria growth in paddy fields

Isolation, Purification and Identification of Cyanobacteria Strains:

Cyanobacteria were isolated by serial dilution and plating method (Stanier et al., 1971). The organism was grown photoautotrophically in batch cultures in BG-11 medium in 250 mL Erlenmeyer flasks. The cultures were maintained in a culture room at 28°C±2°C. The surface of culture vessels was illuminated with fluorescent tubes giving photon flux of 40 W CFL with light/dark cycle of 14/10 h. The culture vessels were hand-shaken four to five times daily to keep the cultures in homogenous state.Identification of purified cyanobacterial strains were done based on their morphology as per the methods of Desikachary(1959),Komarek and Anagnostidis (1998, 2005).

Growth of Cyanobacterial Strains in Chlorpyrifos:

CPF uptake experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL cultures supplemented with 5 mg/L CPF (double the recommended dose of field application). At this concentration, there is 20% inhibition in the growth of the organism. So the selected concentration, at one hand, is more than the recommended field application dose, and on the other hand it is not causing profound effect on growth of the cyanobacteria. Exponentially growing cultures were inoculated to get initial absorbance 0.010 at 680 nm (12.5 µgchl/mL culture). At regular intervals of 2 days, extending up to 14 days, 5 mL samples were withdrawn and growth was measured spectrophotometrically as the increase in absorbance of the cultures with a Thermo ScientificEvolution 201 UV-Visible spectrophotometer (Thermo Fisher Scientific, USA).

Statistics:

Data in figures and tables are expressed as mean ± standard error (SE) for three independent experiments with triplicate samples within each experiment.

Results:

Physiochemical Analysis:

Collection of soil samples was done during September-October, which is why temperature was not so high and it was suitable for cyanobacterial growth. In the collected soil samples, temperature varied from 28.4°C (BBAU campus) to 30.4°C (Malihabad). The lowest pH (7.5) was recorded atBarabanki site, while highestpH (8.7) was notedfor the Mohanlalganj site. The conductivity of soil samples was ranges from 0.112 ms/cm (Malhiabad) to 0.295 ms/cm (BBAU campus).

Among nutritional status of collected soil samples (Table1), Total-Nranged from 0.11 % (Mohanlalganj) to 0.22 % (Bakshikatalab). Total-P was recorded from 5.9 ppm (Mohanlalganj) to 7.7 ppm (Bakshikatalab). Total Organic-C was noted from 0.43 %(Mohanlalganj) to 0.81 % (Bakshikatalab).

Table 1: Physicochemical analysis of soil samples of paddy fields

Parameters	BBAU campus	Mohanlalganj	Barabanki	Bakshikatalab	Malhiabad
Temperature (°C)	28.4 ± 0.04	28.5 ± 0.04	29.4 ± 0.04	30.1 ± 0.04	30.4 ± 0.04
Conductivity (ms/cm)	0.29 ± 0.004	0.15 ± 0.004	0.25 ± 0.004	0.12 ± 0.004	0.11 ± 0.004
pH	8.4 ± 0.04	8.7 ± 0.03	7.5 ± 0.04	8.1 ± 0.05	7.9 ± 0.04
Total-N (%)	0.17 ± 0.001	0.11 ± 0.003	0.12 ± 0.005	0.22 ± 0.002	0.14 ± 0.004
Total-P (ppm)	7.3 ± 0.04	5.2 ± 0.04	5.9 ± 0.04	7.7 ± 0.04	6.8 ± 0.04
Organic-C (%)	0.69 ± 0.003	0.43 ± 0.006	0.60 ± 0.002	0.81 ± 0.007	0.64 ± 0.003

Assessment of Cyanobacterial Population (MPN Method):

In the paddy field soil, the cyanobacterial counts varied from 430 g^{-1}(Mohanlalganj) to infinite (Bakshikatalab) (Table 2). On the basis of nutritional status of soil samples, better Nitrogen, phosphorous and carbon content favours the cyanobacterial growth. The paddy field soil of Mohanlalganj was poor in Nitrogen,

phosphorous and carbon content; had the lowest cyanobacterial growth (430 g^{-1}), while paddy field soil of Bakshikatalab was good in Nitrogen, phosphorous and carbon content; had infinite cyanobacterial growth.

Table 2: Number of Cyanobacteria estimated by MPN method

SNo.	Study Sites	MPN (g^{-1} soil)
1.	BBAUCampus	2100
2.	Mohanlalganj	430
3.	Barabanki	930
4.	Bakshikatalab	Indefinite
5.	Malhiabad	1500

Identificationand Distribution of Cyanobacterial Strains:

Isolated cyanobacterial strains were identified as *Synechococcus* sp., *Gloeocapsa* sp., *Anabaena* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoelus* sp., *Gloeothece* sp., *Oscillotoria* sp., *Gleotrichia* sp., *Aphanothece* sp.(Figure 3). The distribution of cyanobacterial strains were in Table 3.

Table 3: Cyanobacteria isolated from paddy field soil of different sites of Lucknow, India

Cyanobacteria	BBAU campus	Mohanlalganj	Barabanki	Bakshikatalab	Malhiabad
Synechococcus sp.			+	+	+
Gloeocapsa sp.	+			+	
Anabaena sp.	+	+	+		+
Aphanocapsa sp.		+		+	
Chroococcus sp.			+	+	
Microcoelus sp.				+	
Gloeothece sp.	+				+
Oscillotoria sp.			+	+	
Gloeotrichia sp.				+	
Aphanothece sp.	+	+	+		+

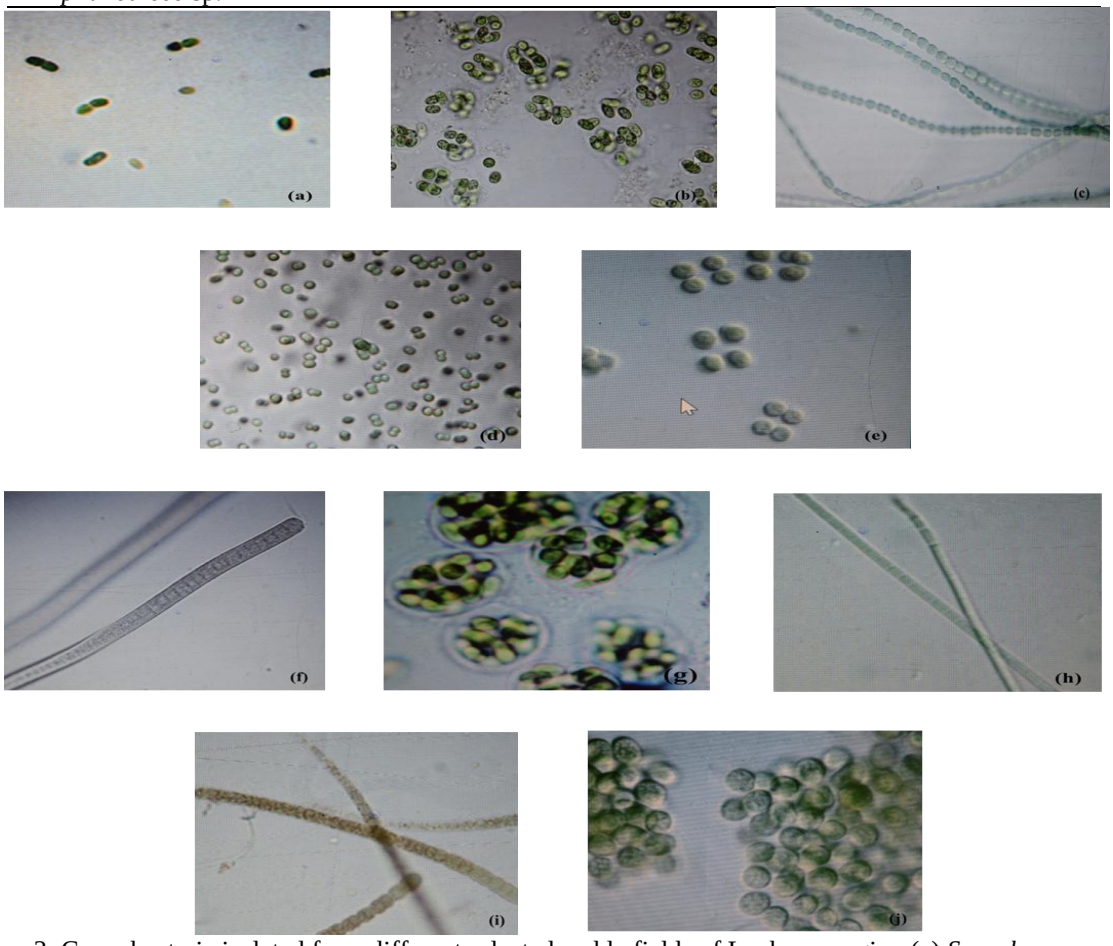

Figure 3: Cyanobacteria isolated from different selected paddy fields of Lucknow region (a) *Synechococcus* sp., (b) *Gloeocapsa* sp., (c) *Anabaena* sp., (d) *Aphanocapsa* sp., (e) *Chroococcus* sp., (f) *Microcoelus* sp., (g) *Gloeothece* sp., (h) *Oscillotoria* sp., (i) *Gleotrichia* sp., (j) *Aphanothece* sp.

Growth of Cyanobacterial Strains in Chlorpyrifos:

The growth of cyanobacterial strains in concentration 5 mg L^{-1} of CPF is shown in Table 4 &Figure 4. The growth of cyanobacterial strains*Synechococcus*sp.,*Aphanocapsa* sp., *Chroococcus* sp., *Microcoelus* sp. and*Oscillotoria* sp.increased regularly from 0.010 on 0 day to 0.340, 0.408, 0.433 0.511 and 0.421 on 12 day and after 12 day was showing stationary stage, while some cyanobacterial strains*Gloeocapsa* sp., *Anabaena* sp.,*Gloeothece* sp., *Gloeotrichia* sp. and *Aphanothece* sp. increased from 0.010 on 0 day to 0.097, 0.101, 0.103, 0.121 and 0.104 on 8 day and after 8 day was showing declining in growth.

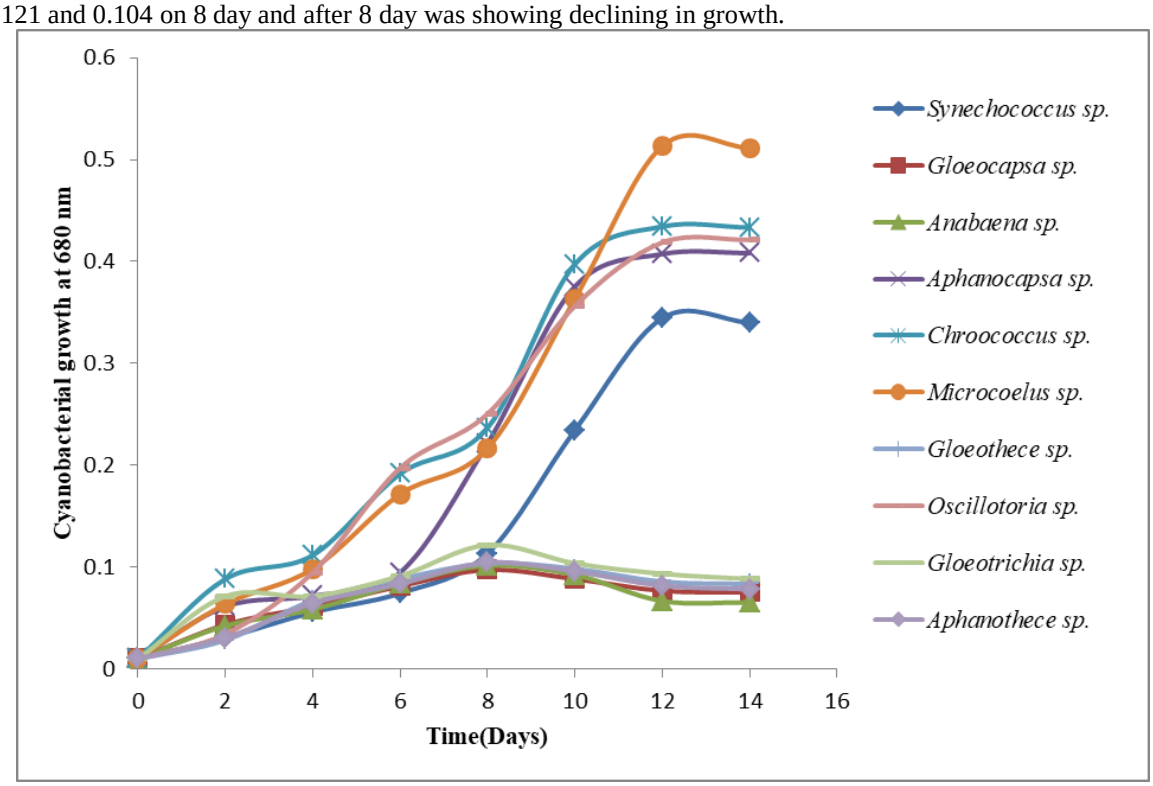

Figure 4:Growth of different isolated cyanobacterial strainsat 5 ppm CPF concentration

Discussion and Conclusions:

Paddy fields, wet soil habitats;provides conditions that suitable for cyanobacteria to flourish (Prasanna et al., 2009).Nayak and Prasanna (2007) stated that cyanobacteria alsocontribute to soil fertility of paddy field in terms of the physico-chemical, biological and soil-water relations. It is also evident that they can act as soil conditioner and bio-indicators (Zancan et al., 2006; Choudhary and Bimal, 2010). In this study, the physico-chemical analysis also shows that BakshikaTalab which had better soil parameters having maximum cyanobacteria among all the sampling sites. It is also confirmed by MPN number of BakshikaTalab site.

The pesticides are main component of current agriculture practices especially in paddy fields to prevent the crops from pest and diseases. Apart from the role of pesticides to improve yield of crops; it has negative impact on every organism of paddy ecosystem including beneficial cyanobacteria. Although it is also reported that cyanobacteria are able to survive in environments having pesticide contamination. In paddy fields, CPF is the widely used insecticide for the control of foliar insects. In present study five cyanobacteria *Synechococcus* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Microcoelus* sp. and *Oscillotoria* sp.are successfully tolerate the CPF.

These CPF tolerant cyanobacteria will be used for further studies to (a) determine the efficacy of these cyanobacteria to tolerate CPF, (b) the impact of CPF on the physiology of cyanobacteria and, (c) degradation of CPF.

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Conflict of Interest:

The authors declare that there are no conflicts of interest associated with this work.

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