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Exploring fungal-bacterial interactions for consortia building to facilitate enhanced monocrotophos remediation and plant growth promotion

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ABSTRACT

Role of pesticides for enhancing crop production through pest control is a widely practiced agricultural management strategy, and given the demands of sustained agricultural productivity it is imperative that the use of pesticide be continued. Nevertheless, risks of pesticide surplus at every trophic level cannot be ignored. Monocrotophos (MCP) is a common organophosphate insecticide used in various Asian nations, including India. This study investigates the applicability of consortia based on fungal-bacterial interaction (C-FBI) to facilitate enhanced MCP remediation and plant growth promotion. An interaction-based consortia matrix was designed to test compatibility between the selected members of the bacteria (RB1, RB2, RB3, and RB4) and fungus (T103), and it revealed the highest levels of compatibility in consortia C-FBI1and C-FBI4, while members of consortia C-FBI3 showed complete antagonism. Timed and successive inoculation of fungus and bacterial cultures were done to degrade MCP. According to HPLC analysis, the highest performing C-FBI for MCP degradation were C-FBI1(70%) and C-FBI4 (68%) at 5 Days. C-FBI administration led to better root and shoot growth as compared to a single fungal isolate. C-FBI1and C-FBI4 were the two C-FBIs with the strongest plant growth promotion outcomes. When plants were treated with C-FBIs, their biomass increased by 42% relative to untreated plants in the case of C-FBI1 treatments and 74% in the case of C-FBI4 treatments. Plants treated with C-FBIs showed robust shoot system and a well-developed root system architecture. C-FBI4 had the highest level of chlorophyll (2.41mg/gfr.wt.) and carotenoids (0.36mg/gfr.wt.). The outcomes of this study represent a step towards the utilization of C-FBIs for MCP bioremediation and plant growth enhancement.

Key words : *MCP, Bioremediation, Consortia-fungal bacterial interaction (C-FBI), Plant growth*

Introduction

Organophosphate pesticides (OPP) are presently among the most extensively used pesticides due to their effectiveness in managing a wide variety of insect pests. MCP is a significant OPP used to control a broad array of insect pests (Fu *et al*., 2022). Asia is the largest user of MCP, with India being the major Asian consumer. Despite their significance in preventing harmful insect pests, excessive use of pesticides have a variety of detrimental consequences on the environment, including the deterioration of soil-health, increased toxicity, and poor crop quality with toxic loads (Akash *et al.*, 2022). Excessive use of pesticides lead to their build up in soil. These pesticide residues in the soil can linger for considerable period of time, long enough to interfere with the physiological and biochemical func-

tions of the host plants. MCP residues have been found in tea, fruits, and vegetables that exceed the MRL threshold (Khandelwal *et al.,* 2022). Rangaswamy *et al.,* 2019 reported that MCP is among the most prevalent pesticides discovered in tea samples from India. Pesticide residues in crops cultivated on such soils might pose a health risk. For instance, in a study with radish, the percentage value of acute reference dose (%ARfD) value for MCP was over 100 for toddlers which highlights that the acute health risk should be paid more attention, especially to toddlers (Jiang *et al*., 2021). According to a study cotton is highly contaminated with MCP (Yadav and Dutta, 2019). Nath *et al.,* 2019, in their study identified MCP residues in okra and insisted that its application should be limited since okra crops require harvesting every 4d, while the dissipation rate of MCP is slow. Pesticide residues in soil have a negative impact on plant growth and lead to the risk of pesticide accumulation in plants. As a result, sustainable techniques of lowering these harmful chemicals in soil should be investigated in order to offer a toxin-free environment for plant development (Alengebawy *et al.,* 2021). Using microorganisms that can remove or minimize the effects of harmful pesticides will restore the health of the soil. To decontaminate the toxic load in soil, pesticide degrading bacteria such as *Pseudomonas*, *Sphingobium*, *Arthrobacter*, and pesticide degrading fungus such as *Trichoderma*, *Aspergillus*, and *Penicillium* can be used (Kumar *et al.,* 2021). In this context, C-FBI application could be even more advantageous because C-FBI fully utilises useful fungi traits (degradation of complex substrate at high concentration) and useful bacteria traits (faster degradation of pesticide intermediates and end products). In the soil, fungi build a fungal mat called the hyphosphere, which allows beneficial bacterial interactions. Fungal exudates provide nutrition and attract beneficial rhizobacteria. In case of C-FBI, mycophagy of the fungi is often seen. Hence, while making interkingdom C-FBI with fungi and bacteria, it is crucial to check their compatibility or antagonism (Vives-Peris *et al.,* 2020). The concepts of C-FBI are comparatively less explored for MCP degradation. Currently, market does not have any commercial consortia that can degrade MCP and give plant growth support simultaneously. The study aimed to design and select compatible C-FBIs that can degrade MCP and give holistic growth support to plants.

Materials and Methods

Study material and growth condition

Market available MCP formulation Monorin (36% SL) was used for the experiments. *S. bicolor* seeds were procured from the IIMR, Hyderabad. Surface sterilization was done with 0.1% $HgCl_2$. Details of fungal and bacterial isolates used in the study are given in Table 1. Plants were cultivated in a greenhouse for 30d, at temperatures of 28 ± 2^0C and watered every second day.

In-vitro compatibility assay

Consortia matrix of fungal and bacterial isolates was designed (Table 2). To check the compatibility 100 µl of 10^{-3} dilution overnight grown RB cultures were spread out on MDA media and a plug of T103 were placed as single central inoculation and incubated for 5d at 30 ± 2^0C.

Biodegradation of MCP by C-FBI

Fungal spores ($1X10^8$) were added to 30 ml of MDB media. Stage wise inoculations of fungal and bacterial cultures (On 3rd Day) were done and incubated at 30 °C at 120 RPM. On 5th Day, supernatant extracted with an equivalent volume of ethyl acetate twice and pooled in 1 ml. MCP breakdown ability of all C-FBI was investigated using HPLC technique (model LC-2010 Shimadzu Corp. Japan).

Plant growth assessment with C-FBI under MCP stress

Compatible C-FBIs were selected for plant growth impact analysis under MCP stress. On the 30th day the plants were harvested and plant growth parameters were recorded and estimated as per the method given by Kumari and Sattiraju, 2022. The study data were statistically analyzed using a data analysis tool in Microsoft Office Excel to find significant differences between treatments.

Results and Discussion

Compatibility among microbial members of consortia

C-FBIs were designed for holistic plant growth development under MCP stress. Genetic details of the cultures and design of consortia are given in Table 1 and 2 respectively. In the present study, two out of four C-FBIs, namely C-FBI1and C-FBI4 were show-

ing better compatibility, C-FBI2 showed weaker compatibility, while C-FBI3 showed complete antagonism (Figure 1). Microbes grown together may have positive, neutral or negative influence on each other, therefore it is important to check their compatibility (Santoyo *et al.,* 2021). To gain complete benefit from the consortia all the interacting members should grow well in presence of each other. Previously microbial consortia were employed for plant growth promotion, abiotic and biotic stress tolerance (Balkrishna *et al.,* 2022). Novelty of the paper lies where C-FBIs are employed for MCP bioremediation. Interkingdom C-FBIs may have multiple advantages for instance; fungi help in making soil hyphal network which facilitates interaction and dispersal of bacterial partners (Pierce *et al.* 2022). Time taken for pesticide degradation is faster for bacteria, while the range for pesticide degradation is higher for fungi. Bacteria are also known to metabolize the pesticide degradation metabolites (Kumar *et al.,* 2021). C-FBIs have an edge over individuals as they use the beneficial attributes of all interacting partners.

Table 1. Isolates employed in the study

S. No.	Lab isolates	Isolate's molecular characterization
1	RB1	*Sphingobacterium spp.*
2	RB2	*Brevundimonas spp.*
3	RB3	*Pseudomonas spp.*
4	RB4	*Pseudomonas monteilii*
5	T103	*Trichoderma harzianum*

*Source: Nivedita, 2016, Yadav 2021

MCP biodegradation studies with C-FBI

The biodegradation studies were carried out with all the three chosen C-FBIs, i.e. C-FBI1, C-FBI2, and C-FBI4. Figure 2 clearly depict a comparative HPLC profile of control (900 ppm) and degraded MCP pesticides by C-FBIs. Results confirmed that the peak area observed in control at retention time 4.40 min significantly decreased in biodegraded experiments. Among all the C-FBI, C-FBI1 showed the maximum MCP degradation of 70%, followed by C-FBI4 68%. The degradation of MCP by bacterial consortia was also studied by Singh *et al.,* 2021. *Aspergillus* and *Penicillium sp*. degraded 150 ppm and 100 ppm of MCP in 4 days. *A. oryzae* (ARIFCC 1054) effectively breakdown MCP (100-500 ppm) in 7 days, but no plant protective properties were examined, and the study did not cover concentrations beyond 500 ppm, as in the current study (900 ppm) (Nayak *et al.,* 2020). Present study suggests that the C-FBIs could be efficiently utilized for degradation of MCP.

Enhanced tolerance to MCP in *Sorghum bicolor* upon C-FBI treatment

Plant growth promotion was assessed for one week (the same time period for which MCP degradation study was performed) and for a 30-day period as well. C-FBI treatment resulted in greater root and shoot development compared to the control individual fungal isolate for both durations. Figure 4 shows the data from 30 days study. Among all three C-FBI, C-FBI1and C-FBI4 helped in better plant growth promotion. Upon C-FBI treatment, biomass of plant increased by 42 % in case of C-FBI1 and 74%

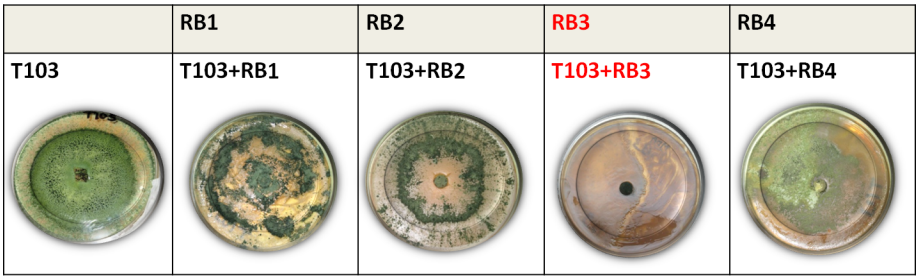

Fig. 1. *In-vitro* compatibility assay with chosen fungal and bacterial isolates, CFBI1 (T103+RB1), CFBI2 (T103+RB2), CFBI3 (T103+RB3), CFBI4 (T103+RB4)

Table 2. Consortia matrix of fungal and bacterial isolates

Fungal Isolates	Bacterial isolates			
	RB1	RB2	RB3	RB4
T103	T103+RB1 (C-FBI1)	T103+RB2 (C-FBI2)	T103+RB3 (C-FBI3)	T103+RB4(C-FBI4)

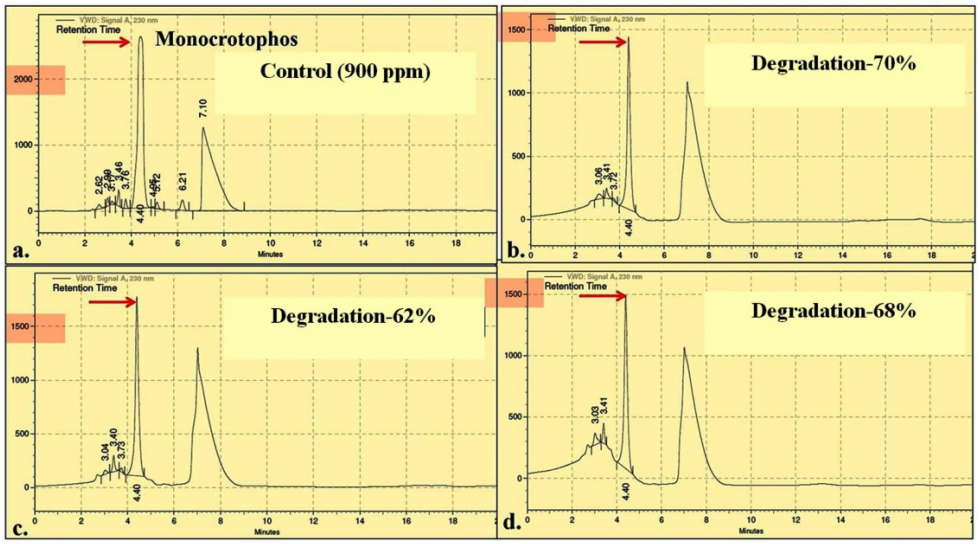

Fig. 2. Analysis of MCP biodegradation potential of fungal-bacterial consortia (C-FBI) by HPLC, a. MCP Control (900 ppm), b. C-FBI1, c. C-FBI2, d. C-FBI4, red arrows indicate the peak corresponding to MCP (RT= 4.40 minute).

in case of C-FBI4. The root architecture of C-FBI1and C-FBI4 were well developed and leaves were healthy and broader (Figure 3). Dash and Osborne, 2019 reported that *Bacillus aryabhattai* (VITNNDJ5) rhizoremediation enhanced root and shoot length at 50, 100, and 150 ppm MCP. The current investigation provides conclusive evidence that C-FBI treatment may promote plant development even at MCP concentrations of 300 ppm. Photosynthetic pigments take part in the formation of organic molecules, such as carbohydrates and proteins. Plant photosynthetic

Fig. 3. Plant growth promotion by selected fungal-bacterial consortia under MCP stress

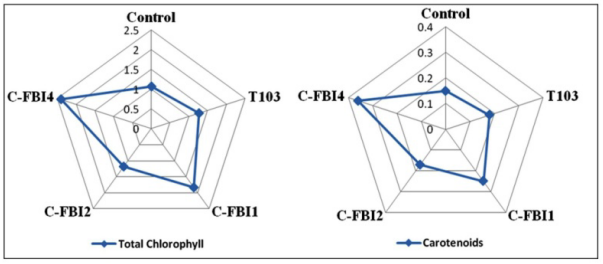

Fig. 5. Effect of seed treatment with T103 and its consortia on photosynthetic pigments of *Sorghum bicolor* at 300 ppm MCP

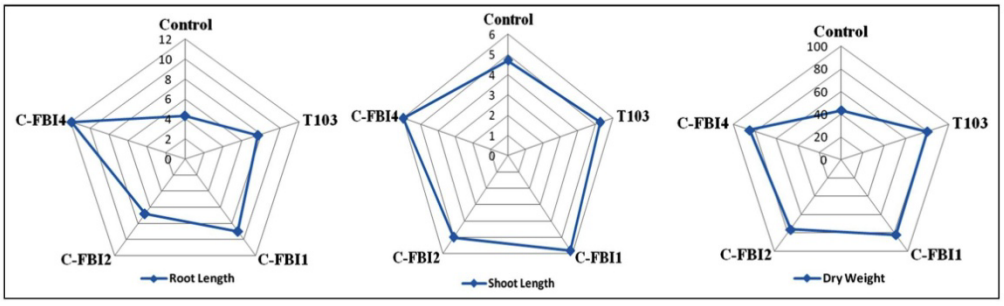

Fig. 4. Effect of seed treatment with T103 and C-FBIs on morphological parameters of *Sorghum bicolor* at 300 ppm MCP

pigments typically decrease under stress (Yang *et al.*, 2023). In our study, we have found that under MCP stress, C-FBI4 showed the maximum chlorophyll (2.41mg/gr.fr.wt.) and carotenoids content (0.36 mg/gr.fr.wt.) followed by C-FBI1 (Figure 5).

Conclusion

Monocrotophos accumulates in soil and translocates to plants and all forms of biotic life that consumes plants. C-FBI1and C-FBI4 are two multicompetent interkingdom consortia developed in this work, that have substantial MCP degradation and plant growth promotion abilities can potentially be exploited for MCP remediation and plant growth support.

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Conflict of interest: The authors declare that they have no conflict of interest in the publication.

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