International Journal of Environment and Climate Change

Volume 13, Issue 11, Page 3096-3103, 2023; Article no.IJECC.108728
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Analysis of Soil Microbial Activity and Population in Rhizosphere Soil Exposed to Chlorpyrifos

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i113480

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/108728

Original Research Article

Received: 03/09/2023
Accepted: 09/11/2023
Published: 15/11/2023

ABSTRACT

Chlorpyrifos (CPF) is a popular organophosphorus pesticide that is widely employed in agricultural activities. However, we lack information regarding the relationship between soil microbial activity and population under CPF influence, which prohibits us from measuring CPF's actual impact on soil. In the present study, the effect of chlorpyrifos on soil microbial activity was assessed in an indoor pot experiment. The fertile soil was treated with 500 ppm of commercial-grade chlorpyrifos and paddy seedlings were transplanted. The soils treated with bacterial inoculants showed an increased microbial population on the 30th day after inoculation compared to soil receiving sole chlorpyrifos treatment. The same trend was followed on the 60th & 90th day as well. The

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combination of inoculants resulted in a maximum increase in the population compared to the single inoculants. As a result, there was an increase in soil enzymatic activities viz. dehydrogenase and phosphatase. Thus, inoculating the pesticide-degrading bacteria would lessen the detrimental effects of pesticides on the soil health.

Keywords: Chlorpyrifos; microbial population; plate count; soil enzymes; paddy.

1. INTRODUCTION

The pesticides are aimed to prevent, destroy, repel, or mitigate pests. They are widespread environmental chemicals found in food, water, air, dust, and soil. These are called xenobiotics and recalcitrant due to resistance to biodegradation in nature for a long period. The problem with pesticides is that they are environmental contaminants that extend their effect on various other sites beyond the site of application [1]. The pesticides extensively used for agricultural purposes on the land surface percolate down the groundwater and contaminate it. Since pesticides are detrimental to the soil environment, they decrease fertility, productivity, and biological health. They cause eutrophication of the water bodies by joining into lakes, and ponds from the agricultural fields and making the water unfit for human consumption [2,3].

The pesticides also harm human health by entering the food chain and hampering the reproductive capabilities of both males and females causing infertility problems as well as delayed conceptions and multiple miscarriages in females [4,5]. They are carcinogenic and also damage the central nervous system [6]. As these pesticides remain in the soil for a very long time the risk of exposure increases [7]; Gireeshkumar et al. 2016.

Organophosphorus pesticides are organic compounds that are used to manage weeds, insects, and plant diseases to increase crop productivity and improve the quality of agricultural products [8,9]. Chlorpyrifos is a broad−spectrum organophosphorus insecticide that can be used in crops like maize, wheat, and rice to control a variety of pests including aphides, leaf folder, cutworms, cockroaches, grubs, flies, mosquitoes' larvae, and adults [10-12]. While the majority of pesticides remain in the environment because of their tenacity and persistence, only 0.1% of them reach their intended target after application [13].

Chlorpyrifos causes acute toxicity and has been commonly considered a better alternative to highly toxic organophosphate pesticides, such as methamidophos, parathion, and methyl parathion. Since 1965, CPF has been widely used to combat plant pests as well as urban pests to public health [9]. The mode of action is by suppressing an essential enzyme in the nervous system called acetylcholine esterase. Upon continuous exposure to these kinds of pesticides, the enzyme loses its activity, thus resulting in the elevated concentrations of acetylcholine, that intervene with the transfer of the nerve impulse at the nerve end. It causes several ill effects on humans which include-general weakness, headache, salivation, nausea, vomiting, diarrhea, abdominal cramps, and tumors. It also hampers the reproductive system of humans [2,5].

The environmental fate of CPF is linked to both abiotic and biotic processes, such as photolysis, chemical utilization methods, and microbial degradation [14]. Microbial degradation is the prominent mechanism for determining CPF's fate and actions. But, at higher concentrations, it is fatal to many of the microorganisms which might change overall microbial composition [15]. Thus, to understand the effects of chlorpyrifos on soil microbes, we applied the most widely used chlorpyrifos formulation to the pots with rice as a standing crop. We assessed soil microbial population and soil enzyme activities which would contribute brief knowledge about CPF effect on soil health.

2. MATERIALS AND METHODS

The pots were filled with 6 kg sterilized paddy field soil and chlorpyrifos at the conc. of 500 ppm was applied. The uninoculated pots were used as controls. The seedlings treated with efficient isolates were transplanted in pots. The pots were arranged as a completely randomized block design with three replicates. Irrigation, manuring, and weeding were followed as per standard method. The soil samples were drawn at 30-day intervals for the estimation.

2.1 Determination of Enzymatic Activity in the Soil

2.1.1 Dehydrogenase

2-3-5-Triphenyl Tetrazolium Chloride (TTC) reduction technique was used for the estimation of dehydrogenase activity in soil. For this, one gram of fresh soil was taken in a test tube and then mixed with 0.1 g of calcium carbonate ($CaCO_3$) and 1 ml of 1 % TTC solution. The mixture was then shaken and plugged with a rubber stopper and incubated at 30 °C for 24 hours in an incubator. The resulting slurry was transferred to Whatman filter paper No.1 and extracted with successive aliquots of concentrated methanol. The volume of the filtrate was made to 50 ml by adding methanol. The optical density of the filtrate was read at 485 nm using a spectrophotometer. The activity was represented in terms of concentration of TPF, as calculated by a standard curve of triphenyl formazan in methanol. Dehydrogenase activity per gram of dry soil was expressed in terms of microgram formazan per gram of dry soil per 24 hours [16].

2.1.2 Phosphatase

Air-dried soil was weighed to 0.1 g and placed in a 50 ml conical flask. 4 ml of modified universal buffer (pH 6.5), 0.25 ml of toluene, and 1 ml of 0.115 M p-nitrophenyl phosphate (PNP) solutions were added. The flask was swirled for a few seconds and incubated at 37 °C for 1 hour. Later, 1 ml of 0.5 M calcium chloride and 4 ml of 0.5 M sodium hydroxide were added to the mixture. The soil suspension was filtered through Whatman filter paper no. 1. The optical density of the filtrate was measured at 430 nm using a spectrophotometer. The phosphatase activity in terms of concentration of p-nitrophenyl in each sample was calculated by a standard curve of p-nitrophenol in water and was expressed as moles of p-nitrophenol released per gram of soil per hour.

2.2 Determination of Microbial Population in the Soil

The soil samples were weighed to one gram and then serially diluted to 10^{-5} to 10^{-6} in 9 ml sterile water blanks. 0.1 ml of suspensions from final dilutions were inoculated onto nutrient agar plates and incubated at 37 °C for 24 hours in a BOD incubator for the enumeration of bacteria. Similarly, 0.1 ml of suspension from 10^{-3} dilution was plated on Martin Rose Bengal Agar for isolation of fungi and 0.1 ml of suspension from 10^{-4} was plated on starch casein agar for determination of actinomycetes population. The plates were incubated at 37 °C for 4 days in BOD. The observations were taken at the 30th, 60^{th,} and 90th DAT and represented as CFU per gram of soil.

2.3 Statistical Analysis

For the analysis of data, Completely Randomized Design with 3 replications was followed. The data was subjected to a one-way analysis of variance (ANOVA) by Snedecor and Cochran (1969).

3. RESULTS

3.1 Dehydrogenase Activity

Individual inoculation enhanced the dehydrogenase activity but inoculating these isolates in combination has proved much more effective in increasing dehydrogenase activity under the influence of chlorpyrifos in the rice rhizosphere. The dehydrogenase activity of the soil was highest on the 60th DAT in T_9 among all. At the 30th, 60th, and 90th DAT, T_9 (CPF + CDB-6 + CDB-11 + CDB-18) recorded 31.3, 54.0, and 44.7 µg TPF g^{-1} of soil d^{-1}, respectively. Individual inoculation in T_4 (CPF + CDB-11) also increased activity to 24.6, 44.6 and 36.6 µg TPF g^{-1} of soil d^{-1}. The combination of two inoculants showed better results than individual inoculation as T_6 (CPF + CDB-6 + CDB-11) recorded 26.6, 47.6, and 40.3 µg TPF g^{-1} of soil d^{-1}. All these treatments were significantly higher than T_2 (CPF) and T_1 (control) at their respective time of recording (Fig. 1).

3.2 Phosphatase Activity

The phosphatase activity in the chlorpyrifos-treated soils was recorded as highest on the 60th DAT in T_9 among all. Phosphatase activity at 30th, 60th and 90th DAT in T_9 (CPF + CDB-6 + CDB-11 + CDB-18) was 31.4, 41.8, and 35.9 µg PNP g^{-1} of soil h^{-1}, respectively. Individual inoculations also significantly increased the activity where, T_4 (CPF + CDB-11) recorded 25.8, 35.7, and 31.8 µg PNP g^{-1} of soil h^{-1}. The combination of two inoculants showed an increased effect compared to individual treatments where, T_7 (CPF + CDB-11 + CDB-18) recorded 28.9, 39.9, and 34.9 µg PNP g^{-1} of soil h^{-1}. These results were significantly superior to T_2

and T_1 (control) at their respective time of recording. T_2 recorded 15.9, 25.7, and 22.7 µg PNP g^{-1} of soil h^{-1} at the 30th, 60th and 90th DAT, respectively (Fig. 2).

3.3 Enumeration of Bacteria

The bacterial population varied significantly in different treatments owing to the effect of chlorpyrifos. The triple inoculation in T_9 recorded 43.3 × 10^6, 84.6 × 10^6, and 70.7 × 10^6 CFU g^{-1} of soil, at the 30th, 60th and 90th DAT respectively. Individual inoculation also enhanced the bacterial population where, T_4 recorded 32.6 × 10^6, 71.8 × 10^6, and 60.2 × 10^6 CFU g^{-1} of soil; while, T_5 recorded 30.6 × 10^6, 70.0 × 10^6, and 55.7 × 10^6 CFU g^{-1} of soil. Dual inoculation in T_7 recorded increased results compared to single inoculations (38.6 × 10^6, 79.8 × 10^6, and 68.2 × 10^6 CFU g^{-1} soil). These observations were significantly higher than T_2 (CPF) which recorded 22.0 × 10^6, 55.0 × 10^6, and 38.3 × 10^6 CFU g^{-1} of soil at the 30th, 60th, and 90th DAT, respectively (Fig. 3).

3.4 Enumeration of Fungi

Inoculation of chlorpyrifos degrading bacteria, either singly or in combination increased the fungal population even under an elevated chlorpyrifos concentration. The fungal population at 30th, 60th, and 90th DAT in T_9 was significantly superior over other treatments (17.3 × 10^3, 24.3 × 10^3, and 21.0 × 10^3 CFU g^{-1} of soil, respectively). Individual inoculation in treatment T_4 recorded 12.7 × 10^3, 20.3 × 10^3, and 16.6 × 10^3 CFU g^{-1} of soil, while, T_5 recorded 11.3 × 10^3, 18.6 × 10^3 and 14.6 × 10^3 CFU g^{-1} of soil at 30th, 60th and 90th DAT, respectively. The combination of two inoculants in T_7 recorded 15.6 × 10^3, 22.3 × 10^3, and 18.3 × 10^3 CFU g^{-1} soil. These results were significant to T_2 (CPF) and T_1 (control) at their respective time of recording (Fig. 4).

3.5 Enumeration of Actinomycetes

Inoculation of chlorpyrifos degrading bacteria significantly enhanced the population of actinomycetes under the elevated chlorpyrifos level. Individual inoculation in T_4 recorded 22.9 × 10^4, 35.8 × 10^4, and 33.8 × 10^4 CFU g^{-1} of soil but combined inoculation of two bacteria in T_7 recorded 25.8 × 10^4, 41.8 × 10^4 and 37.8 × 10^4 CFU g^{-1}. These were significant to T_2 (CPF) and T_1 (control). T_2 recorded 11.8 × 10^4, 17.5 × 10^4, and 14.8 × 10^4 CFU g^{-1} of soil. However, triple inoculation at the 30th, 60th, and 90th DAT in T_9 recorded the highest population of actinomycetes 30.4 × 10^4, 45.5 × 10^4, and 40.4 × 10^4 CFU g^{-1} of soil respectively (Fig. 5).

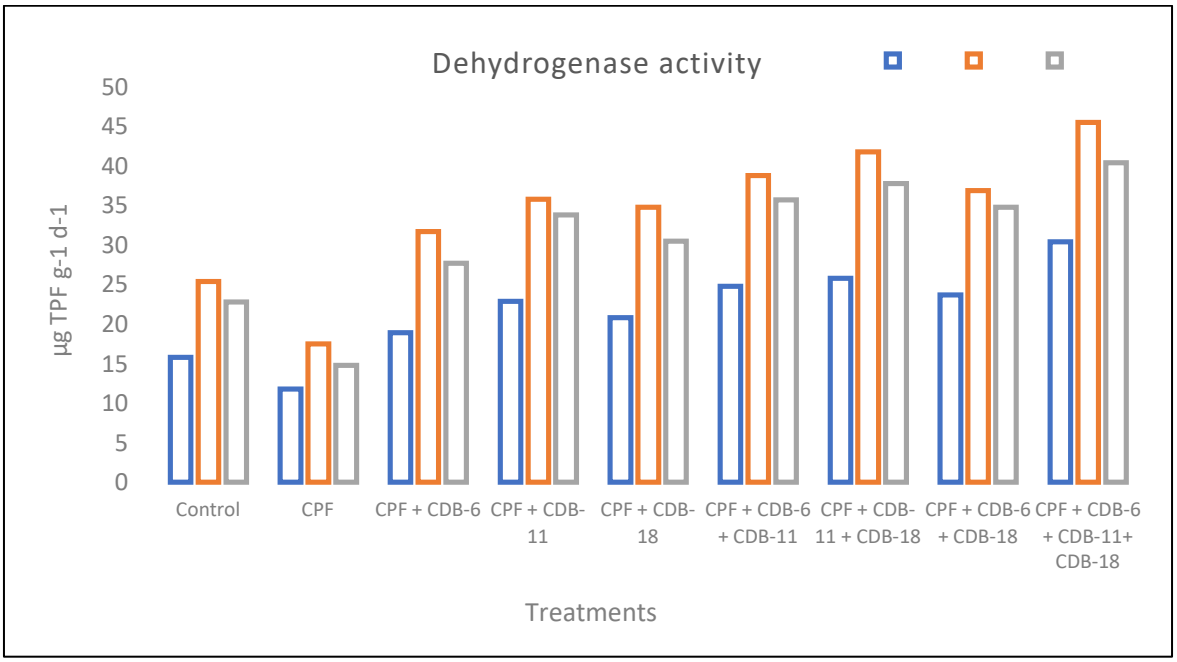

Fig. 1. Dehydrogenase activity of the soil as influenced by the application of efficient chlorpyrifos degrading bacterial inoculants

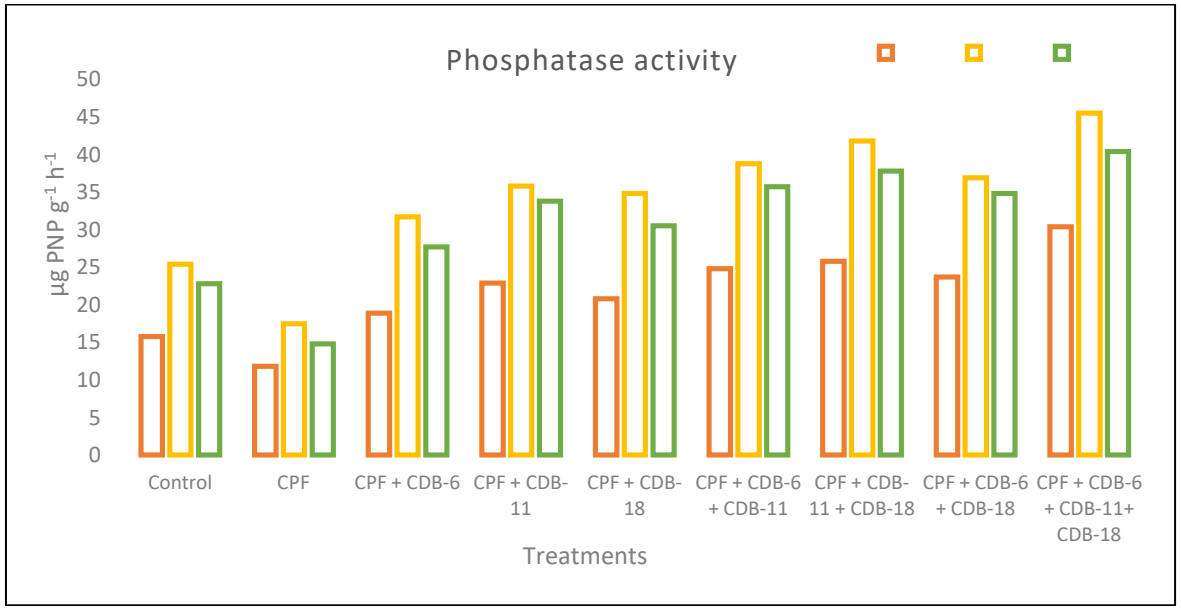

Fig. 2. Phosphatase activity of the soil as influenced by the application of efficient chlorpyrifos degrading bacterial inoculants

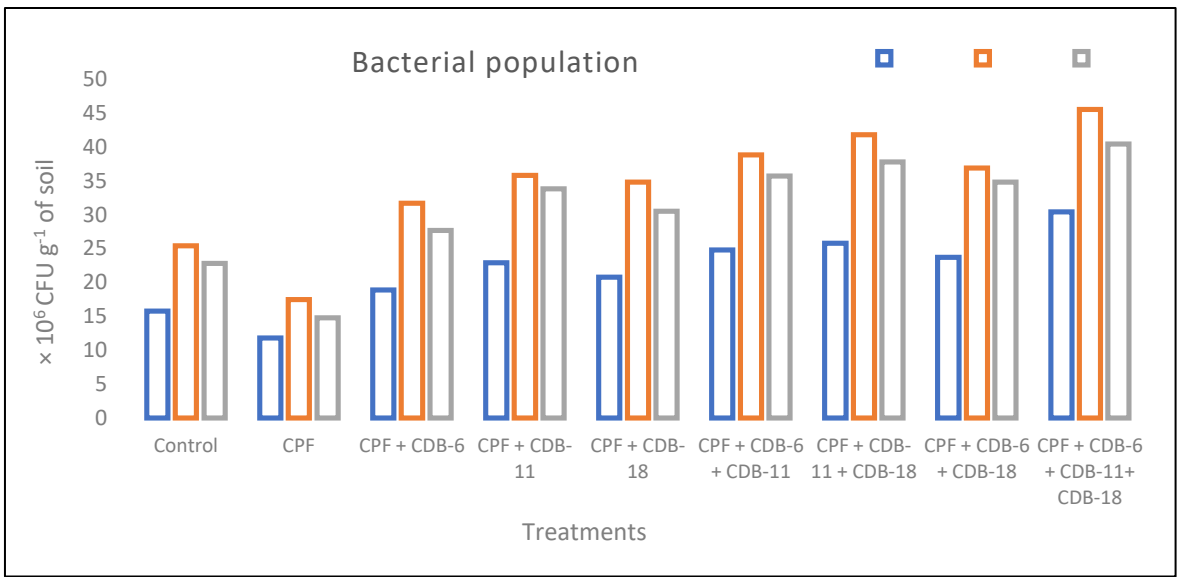

Fig. 3. Bacterial population of the soil as influenced by the application of efficient chlorpyrifos degrading bacterial inoculants

4. DISCUSSION

The applied insecticide persists in the soil for long periods and has negative impacts on soil microbial flora, killing or inhibiting certain specific groups of microorganisms [17]. The present work indicates that the untreated soil with chlorpyrifos recorded higher enzymatic activity as compared to the soil treated with the pesticide. This was because pesticide application to the soil inhibits the activities of different soil microorganisms [18]. This agrees with the results obtained by Lan et al. [19]; Supreeth et al. [20]. The pesticide application to the soil harmed microbial populations and consequently, the microbial enzyme activities were decreased [21]. In addition, the soil treated with chlorpyrifos and inoculated with the mixture of the tested bacteria showed higher enzymatic activity than the soil inoculated with each one individually. Higher

values of enzymatic activity in the case of the soil inoculated with the mixture of the strains are likely due to the synergistic effect between the strains. Similar results were observed by Gilani et al. [22]. Some pesticides are readily degraded by microorganisms including members of genera *Alcaligenes, Bacillus, Flavobacterium, Pseudomonas, Streptomyces,* and *Rhodococcus* (Kumar *et al.,* 2020). The soils treated with bacterial inoculants showed an increased microbial population on the 30th day after inoculation compared to soil that was solely treated with chlorpyrifos which also agrees with Shan et al. [23]. The combination of inoculants resulted in a maximum increase in the population compared to the single inoculants. The pot soils with chlorpyrifos as the sole treatment resulted in a lesser population of all three microflorae

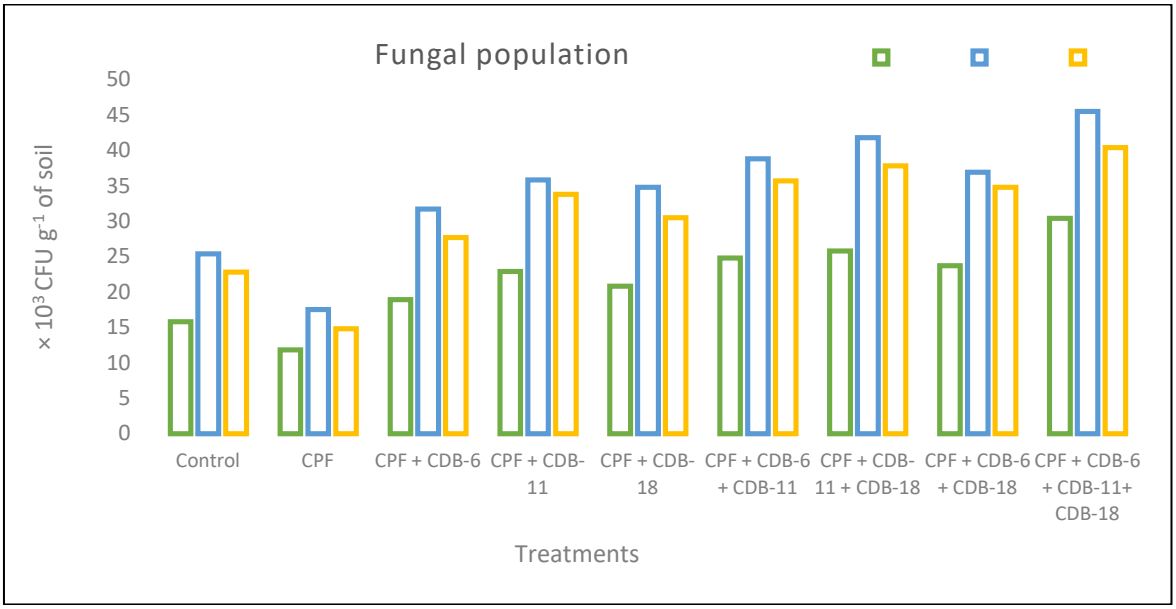

Fig. 4. Fungal population of the soil as influenced by the application of efficient chlorpyrifos degrading bacterial inoculants

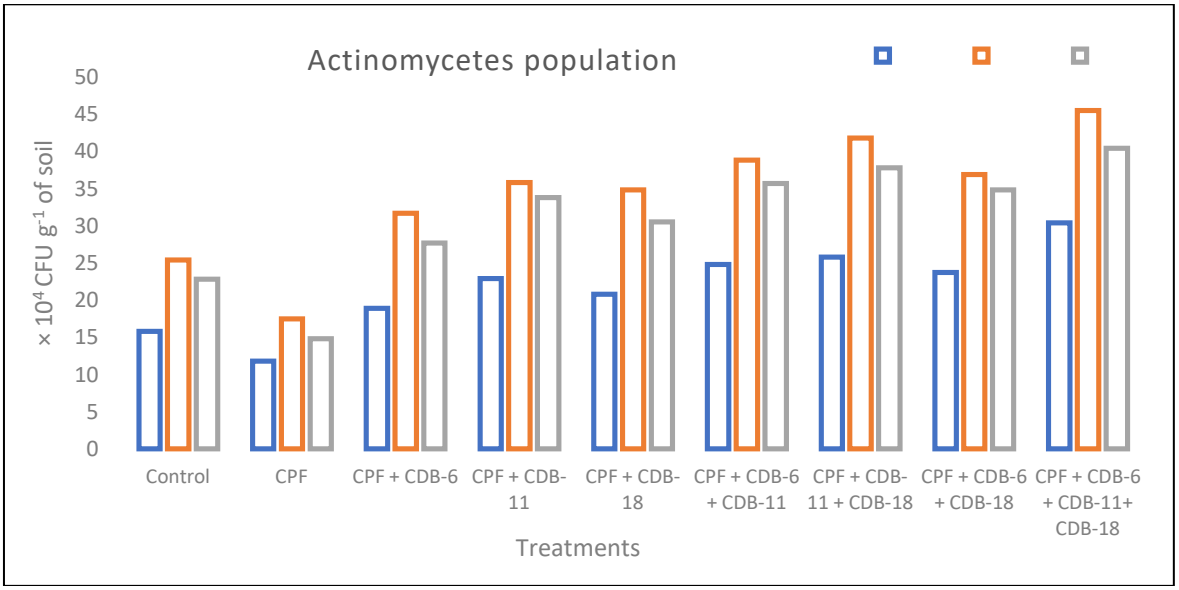

Fig. 5. Population of actinomycetes in the soil as influenced by the application of efficient chlorpyrifos degrading bacterial inoculants

Note: CPF: Chlorpyrifos; CDB: Chlorpyrifos degrading bacteria; DAT: Days after transplanting; Values are mean of three replications

viz., bacteria, fungi, and actinomycetes compared to the control. The population of the microbes in the control is due to the extraneous inoculation through the irrigation water, FYM, *etc.* There have been many contradictory reasons for the change in the microbial population due to chlorpyrifos application. Researchers have reported short-term inhibitory effects on the total bacterial population [24,25]. On the other hand, some studies showed a significant increase in the same after chlorpyrifos treatment due to the application of chlorpyrifos-degrading bacteria [26-28].

5. CONCLUSION

The applied insecticide persists in the soil for a long period and has negative impacts on soil microbial flora, resulting in decreased microbial activity. Several microorganisms were inhibited by chlorpyrifos when applied more than the recommended dosage. A significant decrease in the population of microbes was observed in the CPF-inoculated soils. However, the inoculation of chlorpyrifos-degrading bacteria either singly or in combination helped to minimize the detrimental effects of CPF.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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