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**ORIGINAL PAPER**

# Effect of pesticides on the Seed Germination of *Cenchrus setigerus* and *Pennisetum pedicellatum* as Monocropping and Co-cropping System: Implications for Rhizospheric Bioremediation

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## Abstract

*The present research study has been carried out to evaluate the potential use of two grass species Cenchrus setigerus, and Pennisetum pedicellatum as a monocropping and co-cropping system for the rhizospheric bioremediation of pesticides Chlorpyrifos, Cypermethrin and Fenvalerate .The effect of the three pesticides on the germination of grass seeds was investigated using pesticide spiked soil at the concentrations 10, 25, 50, 75 and 100 mg/kg, while unspiked soil has been taken as control. The heterotrophic microbial numbers were also enumerated in the developing rhizospheric zone and in the bulk soil in order to assess developing microbial associations for biodegeradation of pesticides in mycorrhizosphere. The research finding shows that Chlorpyrifos was more toxic than Cypermethrin and Fenvalerate at higher concentrations (75 and 100mg/kg) for the germination, survival and subsequent growth of Cenchrus setigerus, and Pennisetum pedicellatum. The heterotrophic microbial populations were found to be higher in the mycorrhizosphere soil of co-cropping system of Cenchrus setigerus and Pennisetum pedicellatum as compared to individual mycorrhizospheres of Cenchrus setigerus and Pennisetum pedicellatum, for all the three pesticides at each concentration ranging from 10 mg/kg to 100mg/kg. This study will help in selection plants for further investigation of the rhizospheric bioremediation of Chlorpyrifos, Cypermethrin and Fenvalerate contaminated soil.*

**Keywords:** *Cenchrus setigerus, Pennisetum pedicellatum,* Chlorpyrifos, Cypermethrin Fenvalerate, mycorrhizosphere, rhizospheric bioremediation.

## Introduction:

Phytoremediation has been recognized as an alternative for the removal of organic pollutants from soil in comparison with physico-chemical remediation technologies due to its potentially lower cost and suitability for applications that require sustenance and low maintenance [7]. As the search for remediation alternatives has intensified, the contaminant list has also grown to include the most commonly used pesticides taken for the experimental study are Chlorpyrifos, Cypermethrin, and Fenvalerate. The metabolic fate of pesticides is dependent on abiotic environmental conditions, microbial community, plant species (or both), pesticide characteristics and biological and chemical reactions.

The first step in optimizing the rhizospheric bioremediation of organic contaminants is finding the plant species from a vast array of species [17]. The selection of an appropriate set of plants that are adapted to the exact site conditions and most capable of increasing the contaminant-degradation potential of the soil microbial community is crucial for the successful application of this technology [24].The selected plant species should possess characteristics that enable them to grow on contaminated sites. Simultaneously, they should be able to establish microbial associations that facilitate the degradation of contaminants. A variety of plant species have been investigated and successfully used for the remediation of organic contaminants [9] [10]. Early indications of the potential use of plants for the managed

removal of organic contaminants from soil came from observations of the enhanced dissipation of pesticides under rhizosphere environments compared to dissipation in root-free environments [23]. Peterson et al.'s [20] exploration of the potential use of alfalfa (*Medicago sativa L.*) for TNT remediation points to a new focus upon the use of forage crops for addressing organic contamination in soil. Forage crops seem to be an ideal choice for phytoremediation because they have well-established cultural practices, which should facilitate their managed manipulation for accelerated contaminant destruction and removal. Schwab and Banks [3] also reported the enhanced degradation of pyrene in tall fescue- (*Festuca arundinacea*), Sudan grass- (*Sorghum bicolor L.*), Switch grass- (*Panicum virgatum L.*), and alfalfa-planted soils compared to unplanted soils. Plant selection was found to be important in the field study by Qiu et al. [21]. Verde Kleingrass, a selection of *Panicum coloratum*, proved to be superior to 11 other tested warm-season grasses at a location in Texas, giving final PAH levels one to two orders lower than those of the other plants at the end of the experiment. *Lolium multiforum* (rye grass) has been successfully used for rhizospheric bioremediation of Chlorpyrifos in mycorrhizal soil using green house pot experiment [14]. Chlorpyrifos, Cypermethrin and Fenvalerate are widespread and persistent contaminants in a wide range of ecosystems. Studies have demonstrated the long-term accumulation of contaminants in soils and sediments is related to their physico-chemical characteristics [13]. In contaminated soil, most of the Chlorpyrifos, Cypermethrin and Fenvalerate are more incurrence to enhanced rhizodegradation, because their distribution in soil is usually restricted to the plant root zone depths. However, little work has been done to determine which plant-microbe association might optimize the degradation of Chlorpyrifos, Cypermethrin and Fenvalerate.

The main aim of this study is to investigate the effect of three chemical pesticides Chlorpyrifos, Cypermethrin and Fenvalerate, on seed germination of two grass species *Cenchrus setigerus* and *Pennisetum pedicellatum*, and associated rhizospheric microbial biomass. The long term goal is to potential use of the previous two grass species in designing rhizospheric bioremediation strategies of the tested species.

## 2. Materials and methods

### 2.1. Plants:

Two plant species – *Cenchrus setigerus*, and *Pennisetum pedicellatum,* individually and in co-cropping systems, were evaluated in terms of their potential for rhizospheric bioremediation. The seeds of *Cenchrus setigerus* and *Pennisetum pedicellatum* were procured from IGFRI (Indian Grass Land and Fodder Research Institute), Zhansi, India.

### 2.2. Soils collection and characterization:

Soil was collected from a depth of about 0 - 15 cm along the banks of Surya River, Palghar (located 100 km, north of Mumbai). Plant tissues and stones were totally removed from the soil prior to drying under laboratory conditions. The soil was screened through 2 mm stainless steel sieve, and was characterized for its physico-chemical parameters. After 20 minutes of vigorously mixing the samples at 1:2.5 (W/V) in deionized water, the pH and electrical conductivity (EC) were measured using digital meters [Deluxe water and soil analysis kit, Model 191E]. Total nitrogen, sodium and potassium were determined according to APHA method [1]. Total phosphorus (Pt) was determined colorimetrically according to [16&18] after perchloric/sulfuric acid digestion. Collected experimental soil was also characterized for its microbial status.

**2.3. Spiking of soil:**

Experimental soil was treated with solvent acetone containing pesticides separately (Chlorpyrifos, Cypermethrin, Fenvalerate). In the treatment procedure, 25 ml of acetone containing pesticide was mixed to 25% of the soil sample (250 gms) in closed flask, the flasks were left for 5 minutes to let the solvent disperse. Thereafter the solvent was evaporated for 16 hours at room temperature, and the sub sample was mixed with the remaining 75% of the soil sample (750 gms). All samples were thoroughly mixed with a metal spatula [5]. Soil was spiked to reach final concentrations of pesticides at 10, 25, 50, 75 and 100 mg/kg dry soil, unspiked soil was taken as control.

**2.3. Seed germination test and pot experiments:**

A seed germination test was performed in order to investigate the stress tolerance of plants to the contaminant. Prior to their use in the test, the seeds were inspected and any damaged seeds were removed, then seeds of a similar size were selected for the experiments. After surface-sterilization, ten seeds were sown in a plastic pot as one replicate, containing uncontaminated soil or pesticide contaminated soil, and three such replicates were used for each plant for monoculture or co-cropping system. Uncontaminated soil was used for control. Before sowing, grass seeds were surface sterilized with 1% mercuric chloride solution for 1 minute and rinsed with sterile distilled water, seeds were placed into each plastic pots containing 500 grams of soil and sand mixture (3:1 ratio) and 50 grams of mycorrhizae inoculums (as the same composition was used for phytoremediation studies) [14]. Plants were grown under natural light in green house. After 7 days incubation, the number of germinated seeds was counted and the germination rate was calculated as

Germination rate (%) = number of germinated seeds/number of sowed seeds × 100.

In order to investigate the growth of plant species at the concentrations of 10, 25, 50, 75 and 100 mg/kg of Chlorpyrifos, Cypermethrin and Fenvalerate, germinated seeds were grown in pesticide spiked soil in plastic pots for 30 days. After 30 days of plant growth soil was collected and further investigation of heterotrophic numbers of bacteria in the rhizosphere and the bulk soil was done and compared. The dilution plate method was used to estimate the microbial number in the soil. Soils were sampled from rhizosphere and bulk soil from uncontaminated and pesticide-contaminated soil. 1 g soil (wet weight, i.e. freshly collected soil with moisture content) was ground in a mortar and serially diluted (10-fold dilution) in 0.85% saline solution. Diluted suspensions were spread onto three replicates of one-tenth-strength tryptic soy agar (TSA) (Himedia, India) and incubated at 30°C. Heterotrophic bacteria were counted after 72 h.

**2.5. Statistical analysis:**

The data presented in this study is represented as mean of samples with standard deviation (X±S.D.). In order to examine the significant differences among means, analysis of variance (ANOVA) was performed and a probability of 0.05 or lower was considered to be significant [15].

## 3. Results and discussion

The germination trials have several advantages, such as sensitivity, simplicity, and low cost. These advantages of germination trials have got importance in testing the acute toxicity of chemical substances and evaluate the use of plants for phytoremediation capabilities. The toxicity assessment of chemical substances serves as a tool for evaluating the stress tolerance of plants and is particularly relevant when phytotoxic contaminants are present in soil.

### 3.1 Soil analyses:

Soil properties like organic matter, pH, nutrients etc. effect the degradation of pesticides in the soil [12]. The role of soil in pesticide degradation is critical because it provides the environment for degradative microorganisms. Collected soil for seed germination experiment was analyzed for its physico-chemical properties (Table 1.) and microbial properties (Table 2.).

**Table 1.** Physico-chemical characteristics of experimental soil

| **Physico-chemical Characteristics of Soil** | |
|---|---|
| **Soil Parameters** | **Values** |
| pH | 6.4 |
| Moisture Content | 42.4 |
| Electrical Conductivity (meq/100 gm) | 0.38 |
| Organic Carbon(gm/kg) | 72 |
| Total Nitrogen (gm/kg) | 5.8 |
| Total Phosphorous(Pt) (gm/kg) | 0.72 |
| Organic phosphorus (Po)(gm/kg) | 0.19 |
| Potassium (mg/Kg) | 21 |
| Sodium (mg/Kg) | 23 |
| C/N | 12.41 |
| N/P | 8.06 |

**Table 2.** Microbiota of experimental soil

| **Bacterial genera** | **Fungal genera** | **Actinomycetes genera** |
|---|---|---|
| *Alcaligenes* spp. | *Aspergillus flavus* | *Micromonospora* spp. |
| *Bacillus* spp. | *Aspergillus fumigatus* | *Nocardia* spp |
| *Pseudomonas* spp. | *Aspergillus niger* | |
| *Sarcina* spp. | *Penicillium* spp. | |
| *Serratia* spp. | *Rhizopus* spp | |
| *Streptococcus* spp | *Mucor* spp | |

### 3.2 Seed Germination:

Seed germination trials were performed in order to investigate and compare the rhizospheric bioremediation potential of monocultures and co-cropping system of *Cenchrus setigerus* and *Pennisetum pedicellatum* in pesticide contaminated soil. Germination test was performed at concentrations: 10, 25, 50, 75 and 100 mg/kg of Chlorpyrifos, Cypermethrin and Fenvalerate and non-contaminated soil taken as control. In uncontaminated soil, the germination rate of monocultures of *Cenchrus setigerus* was 86% and for *Pennisetum pedicellatum* was of 88% after 7 days, whereas in co-cropping system it was 85% and 86%. In the Chlorpyrifos, Cypermethrin, and Fenvalerate -contaminated soil, the germination rates of grass seeds significantly showed a tendency to decline as compared to those in the uncontaminated soil. Although no significant difference was observed in the germination rate of *Cenchrus setigerus* and *Pennisetum pedicellatum* as monocropping (figure 1.) and co-cropping system(figure 2.) of grasses at different concentrations of Chlorpyrifos.

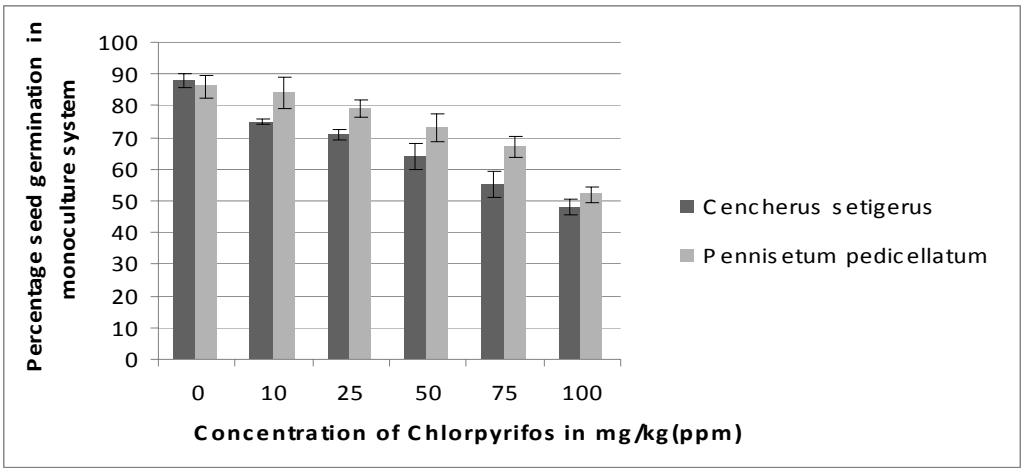


**Figure 1.** Germination of grass seeds as monocropping system in Chlorpyrifos contaminated soil

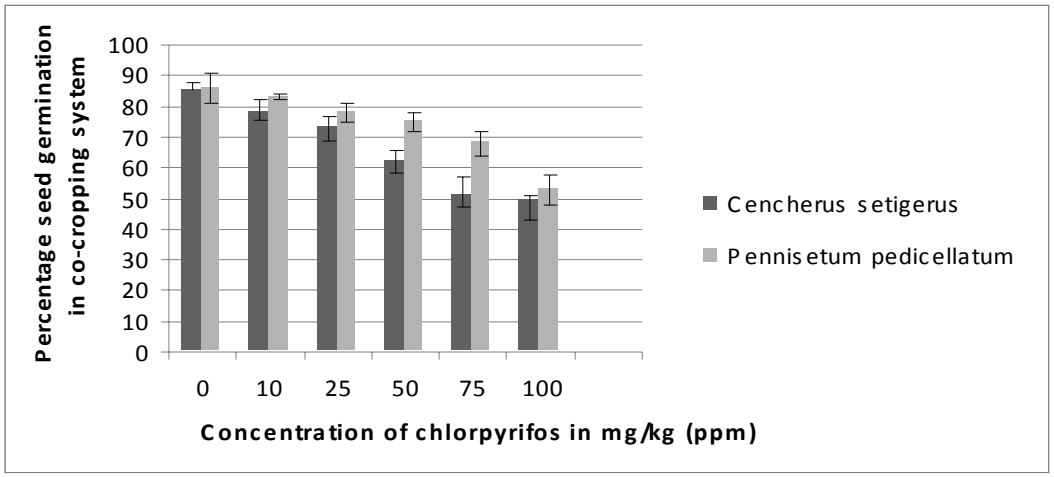


**Figure 2**. Germination of grass seeds as Co-cropping system in Chlorpyrifos contaminated soil

In the Cypermethrin contaminated soil, as the concentration was increased from 10 to 100 mg/kg the germination of grass seeds were reduced in monocropping (figure3.) and co-cropping (figure 4.) system.

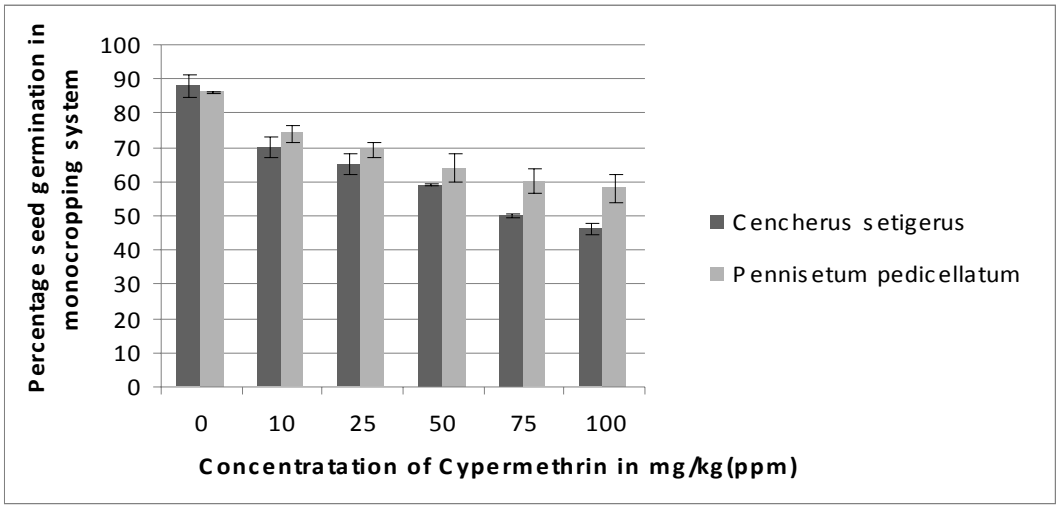


**Figure 3.** Germination of grass seeds as monocropping system in Cypermethrin contaminated soil

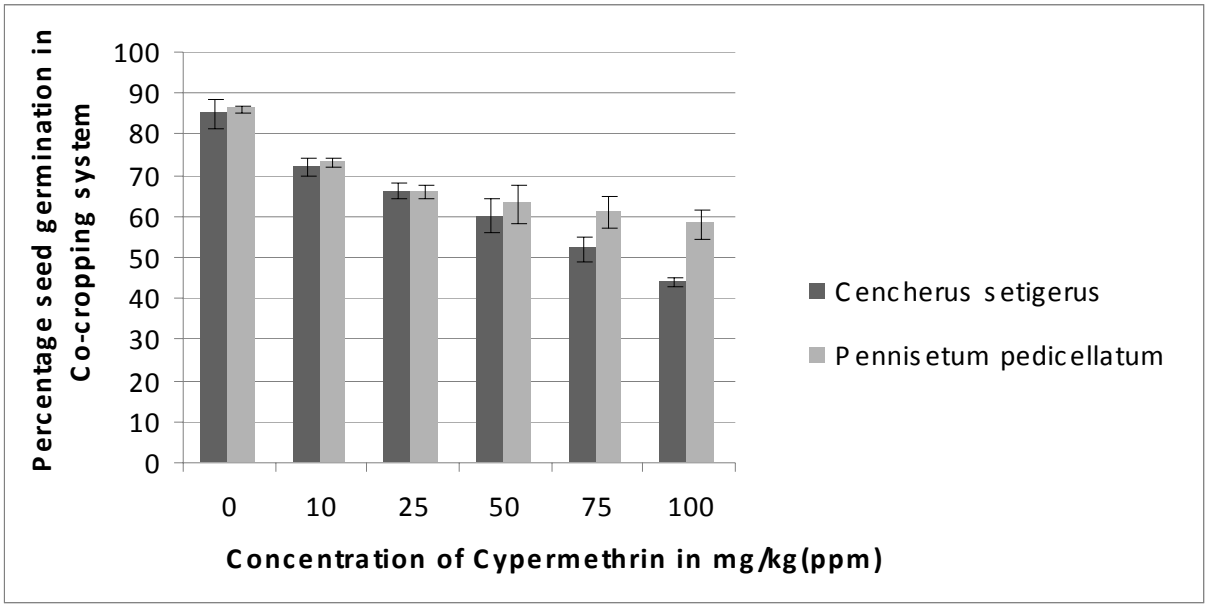


**Figure 4**. Germination of grass seeds as co-cropping system in Cypermethrin contaminated soil.

In control pots seed germination was above 80% which reduced to 60% and subsequently decreased with increase in concentration upto100mg/kg. Fenvalerate, which is a potent insecticide, was also found to have toxic effect on the seed germination of *Cenchrus setigerus* and *Pennisetum pedicellatum*. In control pots germination was 80 – 90% in monocropping and co-cropping system. As the concentration was increased in the pot, significant reduction in germination was observed at the higher concentration of the Fenvalerate (figure 5.).

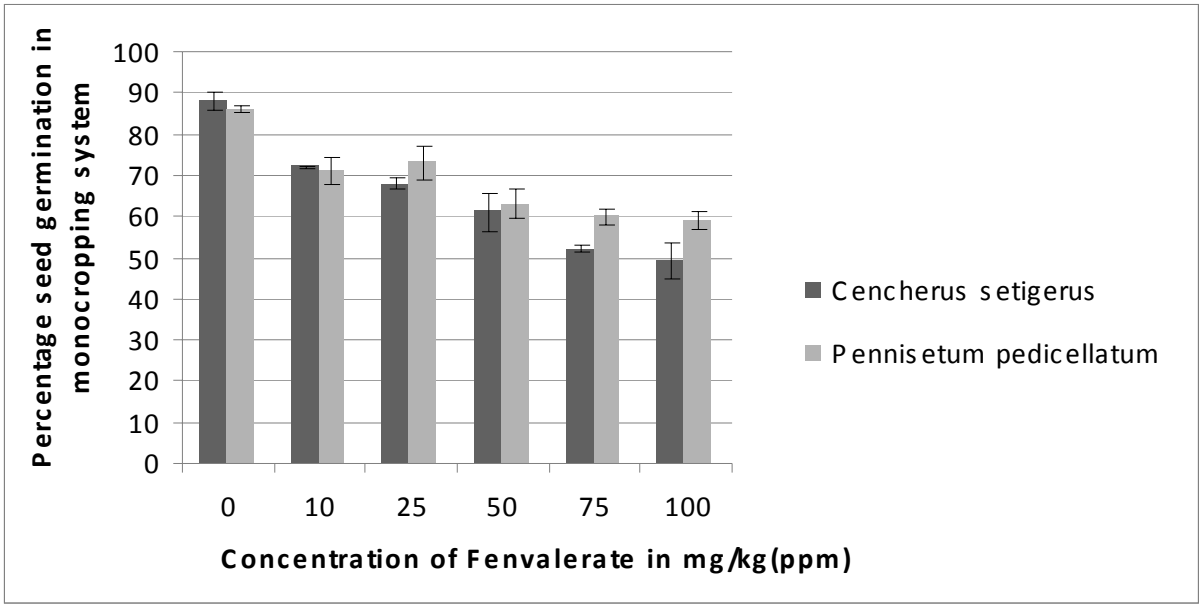


**Figure 5.** Germination of grass seeds as monocropping system in Fenvalerate contaminated soil.

Delayed and reduced germination of grass seeds were observed above 50mg/kg concentration of Fenvalerate in the soil. The highest concentration which seeds can survive was found to be 100mg/kg, at which germination was found to be around 30% lesser than that of control (figure 6.).

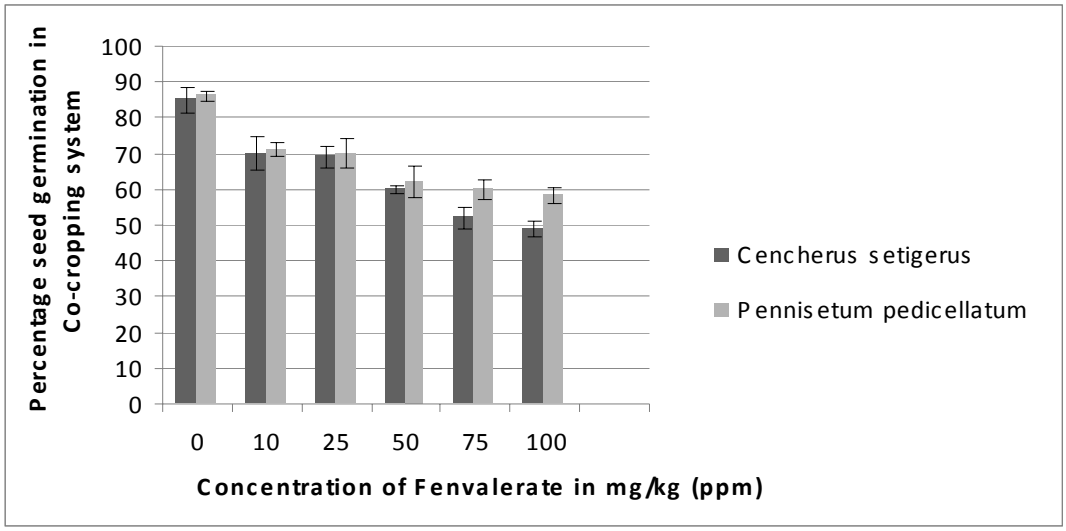


**Figure 6.** Germination of grass seeds as co-cropping system in Fenvalerate contaminated soil.

Germination of plant seed is an important stage in plant growth, and is particularly sensitive to contaminants [2]. Plants are direct recipient of agrotoxics, so they can be used for environmental monitoring of pesticides [6]. Gange et al. [11] studied the effect of three pesticides, Chlorpyrifos, (a contact insecticide); dimethoate (a systemic insectide) and iprodione (a contact fungicide) on seed germination of 20 weed species. Chlorpyrifos was found to reduce germination in the annual grass and one annual forb which is in accordance with our results. As a germination trial result, grass seeds were found to survive and germinate at the concentration 100mg/kg of Chlorpyrifos, Cypermethrin and Fenvalerate.

As the concentration of pesticide was increased in the soil, there was reduction and delay in seed germination of both the grass species *Cenchrus setigerus* and *Pennisetum pedicellatum* as monocropping and co-cropping system. These results suggest that cropping pattern has no effect on the seed germination properties of the grass seeds cultivated in contaminated soil with Chlorpyrifos, Cypermethrin and Fenvalerate.

### 3.3. Microbial enumeration:

Rhizosphere heterotrophic microbial numbers were enumerated after 30 days of growth to assess microbial associations that facilitate the degradation of contaminants. Both with and without the contamination of Chlorpyrifos, Cypermethrin and Fenvalerate, co-cropping system of *Cenchrus setigerus*, and *Pennisetum pedicellatum* had the higher rhizosphere microbial population compared with individual grass species system (Table 3).

**Table 3.** Microbial numbers in rhizosphere and bulk soils.

| **Pesticide** | **Concentration (mg/kg)** | ***Cenchrus setigerus* (monoculture) (log CFU/g soil)** | | ***Pennisetum pedicellatum* (monoculture) (log CFU/g soil)** | | ***Cenchrus setigerus* and *Pennisetum pedicellatum* (co-cropping system) (log CFU/g soil)** | |
|---|---|---|---|---|---|---|---|
| | | **Rhizosphere** | **Bulk** | **Rhizosphere** | **Bulk** | **Rhizosphere** | **Bulk** |
| **Control** | 0 | 11.13± 0.96[#] | 9.82±0.87 | 12.24±0.83 | 8.02±0.74 | 13.31±0.86 | 11.02±0.92 |
| **Chlorpyrifos** | 10 | 11.01± 0.90 (-1.08)[##] | 7.52±0.43 (-23.42) | 11.02±0.37 (-9.97) | 8.60±0.75 (7.23) | 12.24±0.83 (-8.04) | 8.02±0.78 (-27.22) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 25 | 7.74 ±1.82 (-30.46) | 6.82±0.81 (-30.55) | 8.52±0.77 (-30.99) | 5.26±0.41 (-34.41) | 11.93±0.86 (-10.37) | 6.81±0.71 (-38.2) |
| | 50 | 7.31 ±0.65 (-34.32) | 4.21±0.68 (-57.13) | 7.86±0.82 (-35.78) | 4.93±0.43 (-38.53) | 10.92±0.84 (-17.96) | 6.26±0.79 (-43.19) |
| | 75 | 6.79 ±0.44 (-38.99) | 3.08±1.38 (-68.64) | 6.01±0.34 (-50.9) | 4.12±0.42 (-48.63) | 10.02±0.95 (-24.72) | 5.80±0.90 (-47.37) |
| | 100 | 5.18 ±0.42 (-53.46) | 3.21±1.21 (-67.31) | 5.32±0.44 (-56.54) | 3.62±0.56 (-54.86) | 9.34±0.83 (-29.83) | 5.02±0.84 (-54.45) |
| **Cypermethrin** | 10 | 12.72±6.68 (14.29) | 10.00±0.99 (1.83) | 11.23±1.82 (-8.25) | 9.12±1.65 (13.72) | 13.97±1.57 (4.96) | 10.43±1.82 (-5.35) |
| | 25 | 11.36±0.72 (2.07) | 9.82±1.42 (0) | 11.03±1.02 (-9.89) | 8.31±1.85 (3.62) | 13.02±1.45 (-2.18) | 10.09±0.90 (-8.44) |
| | 50 | 9.27±0.79 (-16.71) | 9.02±1.42 (-8.15) | 10.30±1.34 (-15.85) | 8.02±1.53 (0) | 12.91±1.87 (-3.01) | 8.62±4.32 (-21.78) |
| | 75 | 8.63±1.02 (-22.46) | 8.60±1.31 (-12.42) | 9.38±1.54 (-23.37) | 7.21±1.29 (-10.1) | 11.06±1.86 (-16.9) | 4.31±4.66 (-60.89) |
| | 100 | 8.31±1.38 (-25.34) | 6.53±1.33 (-33.5) | 8.17±1.56 (-33.25) | 6.98±1.37 (-12.97) | 10.01±1.66 (-24.79) | 4.29±1.29 (-61.07) |
| **Fenvalerate** | 10 | 11.70±1.38 (5.12) | 9.10±0.98 (-7.33) | 11.86±0.62 (-3.1) | 7.76±1.35 (-3.24) | 16.52±1.90 (24.12) | 15.31±1.45 (38.93) |
| | 25 | 11.30±1.65 (1.53) | 8.34±0.49 (-15.07) | 11.23±0.91 (-8.25) | 7.26±1.57 (-9.48) | 14.97±1.97 (12.47) | 13.92±1.75 (26.32) |
| | 50 | 10.43±1.21 (-6.29) | 9.28±1.92 (-5.5) | 9.00±1.82 (-26.47) | 6.90±1.86 (-13.97) | 12.12±1.36 (-8.94) | 11.82±1.86 (7.26) |
| | 75 | 6.62±1.68 (-40.52) | 4.29±1.02 (-56.31) | 8.52±1.37 (-30.39) | 5.28±1.22 (-34.16) | 10.92±1.28 (-17.96) | 9.37±1.66 (-14.97) |
| | 100 | 6.21±1.66 (-44.2) | 4.11±1.63 (-58.15) | 6.11±1.37 (-50.08) | 5.13±1.40 (-36.03) | 9.98±1.08 (-25.02) | 6.65±1.26 (-39.66) |

[#] Values are mean ± S.D. of three replicates.
[##]Values in parantheses are % differences [(treated-control) ×100/control].

Bacterial population in Chlorpyrifos contaminated soil was found to be decreasing with increase in concentration from 10 to 100 mg/kg, which was significantly different from rhizosphere (11.13) and bulk (9.82). Minimum number of rhizospheric population was found at 100 mg/kg which was significantly higher from bulk soil (3.21).The bacterial population in monocropping of *Cenchrus setigerus*, and *Pennisetum pedicellatum* control was found to be not significantly different from that of co-cropping control. As the concentration increased from 10 to 100 mg/kg, The rhizospheric associations of co-cropping system was found to contain higher number of microbial population(9.34) which was found to be four magnitudes higher than that of *Cenchrus setigerus* (5.18) and *Pennisetum pedicellatum* (5.32).

Cypermethrin registered a reduction in bacterial population with increase in concentration compared to control. Significant difference in microbial population was observed in rhizospheric and bulk soil population in monocropping and co-cropping system of *Cenchrus setigerus* and *Pennisetum pedicellatum.* Cypermethrin showed similarity in population at 100 mg/kg of monocropping of *Cenchrus setigerus* (8.31) and *Pennisetum pedicellatum* (8.17) that was significantly different from rhizospheric population of co-cropping system of *Cenchrus setigerus* and *Pennisetum pedicellatum* (10.01). Fenvalerate treated soil showed significant changes in microbial populations over pretreatment counts in the monoculture and co-cropping of *Cenchrus setigerus* and *Pennisetum pedicellatum*. The rhizospheric microbial population of co-cropping system was found to be (9.98) three magnitudes higher than that of monocropping of *Cenchrus setigerus* (6.21) and *Pennisetum pedicellatum* (6.11). The result of present study revealed that Chlorpyrifos proved to be most

destructive on soil bacterial population. Short inhibitory effect on total bacterial population was observed after Chlorpyrifos and quinalfos application in groundnut seeds which recovered within 60 days after seed treatment and 45 days after soil treatment [19]. Cypermethrin had negative effect on bulk and rhizospheric microbial population, which is in conformity with Venkatashwarulu (1992) [22], who reported the above cited effect of Cypermethrin and Monocrotophos on bacteria. Our results do not support work of Binner et al. (1999) [4] who reported that Cypermethrin had no adverse effect on the soil microbes.

There was no significant difference observed in the microbial populations in the rhizospheric and bulk soil samples of monocropping system of *Cenchrus setigerus*, and *Pennisetum pedicellatum* with that of co-cropping system. As the concentration was increased the rhizospheric population of co-cropping system was found to contain the higher number of microbial population that may enhance overall capabilities of a phytoremediation system to explore the contaminated soil volume, and support differential microbial consortia in their shared rhizospheres. Consequently, the Rhizosphere microbial populations were stimulated. Further, in both uncontaminated soil and pesticide-contaminated soil, the heterotrophic microbial numbers in the rhizosphere soil of co-cropping of *Cenchrus setigerus*, and *Pennisetum pedicellatum* were of higher magnitude than those in bulk soil (Table 3). Higher microbial populations were found in the rhizosphere soil than in the bulk soil, presumably in response to the presence of readily available carbon sources and growth factors found in the form of root exudates and sloughed root cells [8]. A comparison of rhizospheric and bulk soil population at different concentrations of pesticides contaminated soil and the uncontaminated soil suggest that due to the addition of pesticide, the microbial population that could not degrade pesticide was inhibited and microorganisms which can resist higher concentrations of pesticides are able to survive. A vast number of species of microorganisms are present in the rhizosphere, and their numbers generally decrease as the distance from the root increases. The rhizosphere competence is the ability of a microorganism to colonize the rhizosphere. A microorganism with good rhizosphere competence is a good candidate for use as a microbial inoculant.

## 4. Conclusion

In conclusion, the relevance of seed germination trials of *Cenchrus setigerus* and *Pennisetum pedicellatum* were performed for their future potential use in mycorrhizospheric bioremediation of three pesticides Chlorpyrifos, Cypermethrin and Fenvalerate at the concentrations 10, 25, 50, 75 and 100mg/kg. There was significant reduction and delay in seed germination of grass seeds at higher concentrations (75 and 100 mg/kg) of Chlorpyrifos compared to Cypermethrin and Fenvalerate. There was no significant difference seen in germination percentage of grass seeds as monocroppimg and co-cropping system suggesting that cropping pattern has no effect on the germination of grass seeds. The heterotrophic microbial populations and associations were found to be higher in the rhizospheric soil samples of co-cropping system of *Cenchrus setigerus* and *Pennisetum pedicellatum* as compared to *Cenchrus setigerus* and *Pennisetum pedicellatum* monocropping system for all the three pesticides that may facilitate the degradation of pesticides in the soil. Therefore co-cropping system of *Cenchrus setigerus* and *Pennisetum pedicellatum* was selected for further investigation of the rhizospheric bioremediation of Chlorpyrifos, Cypermethrin and Fenvalerate which will provide an alternative approach for remediation of pesticide contaminated soil.

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