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Original Research Article

Rhizosphere Bioremediation of Pesticides by microbial consortium and potential microorganism

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A B S T R A C T

Rhizoremediation is the most evolved process of bioremediation which involve the remediation of contaminants by interaction of microflora associated with plant roots in mycorrhizosphere. In the present study mycorrhizal soil has been developed in a pot culture using *Sorghum* as a host plant. The plant *Pennisetum pedicellatum* and *Cenchrus setigerus* have been selected for rhizosphere remediation of selected pesticides Chlorpyrifos, Cypermethrin and Fenvalerate in developed mycorrhizal soil using pot culture in green house. Each pesticide at a concentration of 10, 25, 50, 75 and 100 ppm were amended in mycorrhizal soil separately in triplicate set for experimental set up and *Pennisetum pedicellatum* and *Cenchrus setigerus* were planted. At regular time interval of 7, 15, 30, 45 and 60 days periodical evaluation of soil was done. Samples were analyzed for physico-chemical, microbial and degradation of pesticides. The pesticide degradation was studied by HPLC. The consortium survived in pesticides environment were developed and used for degradation of pesticides at a concentration of 25, 50, 75 and 100 mg/L concentration using flask shake method. The potential microorganism for Chlorpyrifos, Cypermethrin and Fenvalerate were identified by exposing the microbial consortium from lower to higher concentration in MSM under controlled condition. The microorganisms survived at higher concentration of each pesticide were identified using 16 S rRNA technique. Potential microorganism identified were *Stenotrophomonas maltophila* MHF ENV20, *Stenotrophomonas maltophila* MHF ENV22 and *Sphingobacterium thalpophilum* MHF ENV 23. The results of bioremediation of pesticides chlorpyrifos, Cypermethrin and Fenvalerate by consortium as well as by potential microorganism were compared. The results have shown that complete bioremediation was found in rhizosphere.

Introduction

Growth in industrialization, urbanization, modern agricultural development and energy generation has resulted indiscriminate exploitation of natural resources for

fulfilling the human desires and need, which have contributed in disturbing the ecological balance on which the quality of our environment depends. Contamination of soils, ground water, sediments, surface water and air with hazardous and toxic chemicals is one of the major problems which the industrialized world is facing today. Over the years intentional, unintentional and indiscriminate use of the synthetic chemicals has released several such hazardous organic contaminants in the environment. Hazardous waste in particular pesticide is a massive environmental and health problem. It has been observed that the pesticides exposures are increasingly linked to immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (EPA, 1986). Chemical pesticides have contributed greatly to the increase of yields in agriculture by controlling pests and diseases and also towards checking the insect-borne diseases (malaria, dengue, encephalitis, filariasis, etc.) in the human health sector (Bhatnagar, 2001; Rekha and Naik, 2006). The World Health Organization estimates that there are 3 million cases of pesticide poisoning each year and up to 220,000 deaths, primarily in developing countries.

The conventional techniques used for remediation have been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. It uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. Bioremediation and phytoremediation are innovative technologies that have the potential to alleviate pesticide contamination. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water (EPA, 2005; Raskin and Ensley, 2000). The use of microbial metabolic potential for eliminating soil pollutants provides a safe and economic alternative to other commonly used physico-chemical strategies (Vidali, 2001).

Phytodegradation or rhizoremediation is the breakdown of contaminants through the activity existing in the rhizosphere. This activity is due to the presence of proteins and enzymes produced by the plants or by soil organisms such as bacteria, yeast, and fungi. Rhizodegradation is a symbiotic relationship that has evolved between plants and microbes. Plants provide nutrients necessary for the microbes to thrive, while microbes provide a healthier soil environment. Plant enzymes establish the degradation of pollutants during phytoremediation; whereas, during natural attenuation or bio-augmentation, the (indigenous) microbial population performs the degradation. The first studies toward degradation of compounds in the rhizosphere mainly focused on the degradation of herbicides and pesticides (Hoagland et al., 1994; Jacobsen 1997; Zablotowicz et al., 1994). Today, many reports deal with degradation of hazardous organic compounds). Studies of the most suitable plant species for rhizoremediation showed that various grass varieties and leguminous plants such as alfalfa are suitable (Kuiper et al., 2001; Qiu et al., 1994; Shann and Boyle 1994). Vesicular arbuscular mycorrhiza (VAM) has been reported to modify the quality and abundance of rhizosphere microflora and alter overall rhizosphere microbial activity, which affects bioremediation in the

contaminated soil (Khan, 2006). Mycorrhiza not only provide the plants with water and mineral compounds and help to improve the structure of soil, but have also been shown to act as filters, blocking toxic compounds within their mycelium resulting into reduced toxicity to the plants. Mycorrhiza fungal strains have shown improved tolerance to toxic compounds, and hence could increase the success of phytoremediation (Turnau et al., 2002). The bacterial species that are known to be the most useful in bioremediation belong to the genera *Flavobacterium*, *Arthrobacter*, *Azotobacter*, *Pseudomonas* and *Burkholderia* (Chaudhry et al., 2005). Indeed, studies have shown that a number of *Burkholderia* species exist naturally in the rhizosphere of several crop plants. The inoculation of rhizospheres with (a) particular pollutant-degrading microorganism(s) has been shown to lead to an enhancement of biological remediation (Chaudhry et al., 2005).

Keeping in view the advantages of phytoremediation and bioremediation technology the present research study has developed the methods and advancement for the biodegradation of pesticides such as chlorpyrifos, cypermethrin and fenvalerate using *Pennisetum pedicellatum* for rhizoremediation strategies and using rhizosphere soil as source of potential microorganism from developed microbial consortium and assessment of their degrading ability in liquid media for their potential use in pesticide contaminated soil and water environment.

Materials and Methods

Soils collection

Soil was collected from a depth of about 0 - 15 cm along the banks of Surya River, Palghar (located 100 km, north of Mumbai). The soil was screened through 2 mm stainless steel sieve, and used for the rhizosphere bioremediation.

Spiking of soil mix

Experimental soil mix was spiked with each pesticide (chlorpyrifos, cypermethrin, fenvalerate) at the concentrations viz. 100, 75, 50, 25, and 10 mg/kg separately. All samples were thoroughly mixed with metal spatula to obtain final pesticide concentrations of dry weight of soil mix; unspiked soil mix was taken as control.

Pot culture green house experiment

Time course pot culture experiments were conducted in a green house, where pots were filled with soil mixture spiked at various concentrations of each pesticide (chlorpyrifos, cypermethrin, fenvalerate) separately. The seeds of *Pennisetum pedicellatum* (procured from Indian grassland and fodder research institute, Zhansi) were surface sterilized with 70% ethanol for 30 seconds followed by sterilization with 0.1% mercuric chloride for 5 min. Twenty pre-germinated seeds were transferred to plastic pots (1 kg, 11 cm diameter, 11.5 cm height) containing spiked and control soil mix. Three replicates were done for this experiment and pots were kept in green house with temperature 24-26°C at day, 22-23°C at night with the natural light.

The rhizosphere bioremediation of pesticide was carried out up to the period of 60 days. Rhizosphere soil samples were collected at five intervals of 7, 15, 30 and 45 and 60 days to evaluate rhizospheric degradation of pesticide. The rhizospheric and bulk soil samples were also microbial enumerated (immediately within 24hrs of the final harvests). The plate counts of bacteria were taken by spread plate method and compared.

Extraction and clean up of soil samples

For the analysis, soil from planted pots was carefully collected, mixed, and air dried. Each replicated soil sample (10g) was added with activated charcoal (0.05g), florisil (0.05g) and anhydrous sodium sulphate (1g) and mixed well. The mixture was packed in filter paper to fit in a soxhlet apparatus for extraction with 150 ml of hexane acetone solvent mixture (11 v/v) for 6 h. The extracted solvent was then passed through anhydrous sodium sulphate and evaporated to dryness. The residue was then dissolved in suitable solvent for final estimation of Pesticides.

Development of microbial consortia from Rhizosphere at higher rate of degradation of each pesticide (chlorpyrifos, cypermethrin, fenvalerate)

The remediated rhizosphere soil initially amended with highest concentration of each pesticide chlorpyrifos (100 mg/kg), cypermethrin (100 mg/kg) and fenvalerate (100 mg/kg) obtained from the first set of experiment was further characterized and used for isolation of respective developed consortia and potential degrader for each pesticide.

Isolation of Bacterial consortium from pesticide rhizoremediated soil

A 10g soil sample was homogenized in 10 ml of 0.85% saline solution on a rotary shaker at 29ºC and 150 rpm. A tenfold dilution series was prepared and 0.1 ml of each dilution was inoculated into test tubes containing 3 ml of MSM supplemented with 50µg/ml for chlorpyrifos, 25µg/ml for cypermethrin and 25 µg/ml for fenvalerate separately. The tubes were incubated on a rotary shaker at 29ºC for 2 weeks at 150 rpm. The culture of the terminal positive tube showing the growth was enriched by two serial transfers into fresh media. Developed consortia were serially diluted and plated on basal agar medium containing chlorpyrifos, cypermethrin and fenvalerate (50 mg/L) seperately. For isolation of potential degrader same method as section 4.4.1.1 was followed.

Pesticides bioremediation by isolated potential degrader in shake flask bioreactor

Pure culture of cypermethrin potential degrader isolated from rhizoremediated soil was further used for bioremediation studies in shake flask bioreactor. The mineral salt medium comprised of (in gm/l) K2HPO4-0.255, KH2PO4-0.255, (NH4)2SO4-0.255, MgSO4.7H2O-0.05, CaCO3- 0.005 and FeCl2.4H2O-0.005 blended with 1 ml of trace elements solution (Focht, 1994). The Focht trace element solution contained (in mg/l) MgSO4.H2O-169, ZnSO4.7H2O-288, CuSO4.5H2O-250, NiSO4.6H2O-26, CoSO4-28 and Na2.MoO4.2H2O-24. Erlenmeyer flasks (250 ml) and nutrient culture media were autoclaved for 20 minutes at 121ºC. A 500µl acetone containing each pesticide separately was aseptically added to autoclaved dried Erlenmeyer flasks allowing the acetone to evaporate. After complete evaporation of acetone from the Erlenmeyer flasks, 100ml culture media was added under laminar flow hood so as to reach the desired pesticide final concentration. 1ml sub cultured potential degrader (in nutrient broth) was inoculated into Erlenmeyer flasks (250 ml) containing nutrient culture media with a pesticide concentration of 10mg/l. the inoculated flasks were kept in orbital shaker incubator at 160 rpm, 30ºC for 14 days. After 14 days, 10 ml of this culture media with a pesticide concentration of 25mg/l, the flasks were again kept on orbital shaker

incubator at 160 rpm, 30ºC for 14 days. Likewise, the microbial culture was sub cultured into nutrient culture media with a pesticide concentration of 50mg/l and 75mg/l and was kept on orbital shaker incubator at 160 rpm, 30ºC for 14 days. Optical density at 600nm for determining colony forming unit (CFU/ml) was performed to studying cell density.

Analytical procedure

The samples were centrifuged (4oC, 5 min, 10,000 rpm, Plasto crafts, Rota 6R- V/Fm) to remove the suspended microbial cell mass. The supernatant was extracted in n-hexane using the separating funnel. 5 ml of sample was extracted with equal volume of n-hexane. To ensure the maximum recovery of organic contaminant from samples, the extraction was performed thrice using the aliquots of 5 ml n-hexane. The extracted samples were analyzed on HPLC and MS.

Results and Discussion

The present study was done with the aim of establishment of highly effective remediation method using plant with degradative rhizosphere and isolation of naturally occurring rhizosphere associated potential degrader providing the possibility of both environmental and insitu detoxification of pesticide contamination. The remediation efficacy of *Pennisetum pedicellatum* was investigated using green house pot culture experiments in pesticide (Chlorpyrifos, cypermethrin and fenvalerate) amended soil (10, 25, 50, 75 and 100 mg/kg) for periodic evaluation of changes in concentration. Total proportion of pesticide degraders were found to be higher in rhizosphere soil compared to bulk soil. Pesticides (chlorpyrifos, cypermethrin and fenvalerate) degrading strain associated with rhizosphere capable of surviving at higher concentrations were designated as potential degrader. On the basis of morphological characteristics, biochemical tests and 16S rDNA analysis, isolates were identified and further taken for bioremediation studies purpose in liquid MSM in laboratory at bench scale in shake flask bioreactors. Rhizoremediation strategy will be of immense importance in remediation of pesticides residues to a level permissible for technogenic and natural environment.

Rhizoremediation experiment using *Pennisetum pedicellatum*

Pot culture experiments were conducted in the green house where *Pennisetum pedicellatum* was grown in soil amended with chlorpyrifos, cypermethrin and fenvalerate. The dissipation of the three pesticides in *Pennisetum pedicellatum* rhizosphere soils at different concentrations was quantified using HPLC. Time course pot culture experiments were conducted in a green house, where pots were filled with soil mixture spiked at various concentrations of pesticides viz. T1 (100 mg/kg), T2 (75 mg/kg), T3 (50 mg/kg), T4 (25 mg/kg), T5 (10 mg/kg). Three replicates were done for this experiment and pots were kept in green house with temperature 24-26°C at day, 22-23°C at night with the natural light. There were six treatments in this trial, five contaminated soil spiked with pesticide and one control. The rhizosphere bioremediation of pesticide was carried out up to the period of 60 days. Rhizosphere soil samples were collected at five intervals of 7, 15, 30 and 45 and 60 days to evaluate rhizospheric degradation of pesticide. The rhizospheric and bulk soil samples were also microbial enumerated (immediately within 24hrs of the final harvests). The plate counts of bacteria were taken by spread plate method and compared. The mean extraction recovery of chlorpyrifos, cypermethrin and

fenvalerate achieved from spiked pot soil is reported.

The initial recovered concentrations of chlorpyrifos were found to be 78.15, 58.4, 39.0, 18.52 and 7.6 mg/kg for T1, T2, T3, T4, and T5 doses respectively. T1 degraded to 31.28% and 84.94% at the harvest period of 15 and 45 days and NDL (non-detectable limit) 60th day after application. The initial deposit in T2 was degraded by 41.6% 15th day that progressively increased to 82.5% on 45th day and NDL after 60 days. The corresponding values were about 27.7%, 47.95%, 75.52% and NDL in case of T3. The percentage degradation was 34.7%, 53.03% and NDL in T4 on 7th, 15th and 30th days. T5 was found to be completely degraded at the first harvest period of 7 days (Table 1). The results indicated lower degradation percentage in higher doses T1, T2 and T3 doses in comparison to T4 and T5 in the order of T5 > T4 > T3> T2 > T1. Chlorpyrifos was found to degrade in soil to different metabolites of which 3, 5, 6 trichloro -2- pyridinol (TCP) was the main biodegradation product of chlorpyrifos. Khanna et al., (2008) also reported TCP as degradation product of chlorpyrifos in soil.TCP was majorly detected in the rhizosphere soils initially spiked at 75 and 100 mg/kg (higher) concentrations suggesting that TCP formed in the soils contaminated at lower levels dissipated quickly into simpler compounds below detectable limits. This might be due to the enhancement of a soil microflora population capable of degrading chlorpyrifos (Singh et al., 2004; Menon et al., 2005).

The initial recovered concentrations of cypermethrin were 92.6, 68.9, 45.0, 22.10 and 8.10 mg/kg for 100 mg/kg, 75 mg/kg, 50 mg/kg, 25 mg/kg, and 10 mg/kg doses respectively, which dissipated to 4.42% and 62.21% at the harvest period of 15 and 45 days respectively in case of 100 mg/kg, and NDL (non-detectable limit) on 60th day after application. The initial deposit in 75 mg/kg was dissipated by 41.6% on 15th day that progressively increased to 82.5% on 45th day and NDL after 60 days. The corresponding values were about 27.7%, 47.95% and 75.52% and NDL respectively on 7th, 15th and 30th day in 50 mg/kg. The percentage dissipation was 34.7%, 53.03% and NDL in 50 mg/kg on 7th, 15th and 30th days respectively, 25 mg/kg was found to be completely degraded at the first harvest period of 7 days (Table 1). Microbial degradation is known to be one of the most important factors determining the environmental fate of pesticide in the soil (Arnold et al 1990). Standen et al., (1997) studied the metabolic fate of cypermethrin in the aerobic soil and the half life was found to vary from 2-16 weeks. Wauchope et al., (1982) also reported the half life of cypermethrin to vary from 4 days to 8 weeks. Degradation of cypermethrin in soil significantly depends on either soil characteristics or microbial activity (Miyamato et al., 1981).

The initial recovered concentrations of fenvalerate were found to be 90, 71.1, 45.0, 22.0 and 9.2 mg/kg for T1, T2, T3, T4, and T5 doses respectively. Rhizospheric degradation of fenvalerate results indicated lower degradation percentage at higher doses 100 mg/kg,75 mg/kg and 50 mg/kg in comparison to 25 mg/kg and 10 mg/kg in the order of 10 mg/kg > 25 mg/kg > 50 mg/kg > 75 mg/kg > 100 mg/kg . The residue data were statistically interpreted for computation of regression equation and residual half life values (fig. 5.44).100 mg/kg, 75 mg/kg, 50 mg/kg, 50 mg/kg, and 25 mg/kg doses respectively, which dissipated to 5.1% and 44.7% at the harvest period of 15 and 45 days respectively in case of 100 mg/kg, and 72.4% degradation on 60th day after

application. The initial deposit in 75 mg/kg was dissipated by 46.5 % on 15th day that progressively increased to 82.9% on 30th day and NDL (Non detection limit) after 45 days (Table 1). The corresponding values were about 50.4%, 77.7 % and NDL respectively on 7th, 15th and 30th day in 50 mg/kg. The percentage dissipation was 55.4% and NDL in 25 mg/kg on 7th and 15th days respectively, 25 mg/kg was found to be completely degraded at the first harvest period of 7 days. This may be due to selective enrichment of fenvalerate degraders in the rhizospheric zone. The soil in the rhizospheric zone generally consists of 10-100 times greater number of indigenous microorganisms than in bulk soil, this might be due to carbon containing compounds exudated from plant root such as sugars, carbohydrate, alcohol and amino acids, which could stimulate the microbial activities and increase the number of rhizosphere microorganisms (Schnoor et al., 1995; Stottmeister et al., 2003). The pesticide degradation ability was found to be increased in this soil (Anderson et al., 1994).

Bioremediation-Chlorpyrifos, Cypermethrin and Fenvalerate by identified potential microorganism

The naturally occurring bacterial isolates capable of metabolizing pesticides were isolated from pesticides rhizoremediated soil. Isolated degraders were then identified by various biochemical tests and 16 S rDNA characterization. Bioremediation potential of identified degraders was then evaluated in liquid MSM medium supplemented with pesticides at various concentrations. Three different potential degraders *Stenotrophomonas maltophilia* MHF ENV 20, *Stenotrophomonas maltophilia* MHF ENV 22 and *Sphingobacterium thalpophilum* MHF ENV 23 were taken for bioremediation of Chlorpyrifos, Cypermethrin and Fenvalerate respectively. In case of 50 mg/L of concentration cypermethrin bioremediation by *Stenotrophomonas maltophilia* MHF ENV 22 was found to be 12%, 0% and 0% at the concentration of 25, 50 and 100 mg/L respectively. Fenvalerate was degraded by *Sphingobacterium thalpophilum* MHF ENV 23 and degradation was 20%, 20% and 10% in case of 25, 50 and 100 mg/L respectively at the end of 4th hour. At the end of 24th hour i.e day 1 degradation was 100%, 40% and 100% at the concentration of 25 mg/L in case of chlorpyrifos, cypermethrin and fenvalerate. At the end of bioremediation experiment in MSM the chlorpyrifos degradation was found to be 100% at the concentration of 25 and 50 mg/L and was 95% at 100 mg/L (Table 5.23). Cypermethrin biodegradation by *Stenotrophomonas maltophilia* MHF ENV 22 was found to be 100% at 25, 50 mg/L and 77% at 100 mg/L at 7th day while in case of fenvalerate bioremediation by *Sphingobacterium thalpophilum* MHF ENV 23 degradation was found to be 100% at 25, 50 mg/L and 94.7% at 100 mg/L at 7th day (Fig 1).

Environmental parameters- Variation during bioremediation of pesticides by identified potential microorganisms

Bioremediation of pesticides (Chlorpyrifos, Cypermethrin and Fenvalerate) was carried out at the selected concentrations 25, 50 and 100 mg/L by identified potential degraders *Stenotrophomonas maltophilia* MHF ENV 20, *Stenotrophomonas maltophilia* MHF ENV 22 and *Sphingobacterium thalpophilum* MHF ENV 23 in shake flask bioreactor under controlled environmental conditions.

The environmental parameters monitored and assessed were pH, temperature, CFU,

COD and BOD for each of the pesticides. The bioremediation parameters studied during bioremediation of each pesticide are represented in the table 3. The growth and proliferation was found to be maximum at the concentration of 100 mg/L in case of chlorpyrifos and fenvalerate, while in case of cypermethrin it was highest at 50 mg/L of concentration. pH was found to be fluctuating from 7.3 to 6.6 at 25 mg/L and 7.0 to 7.45 at 50 mg/L of chlorpyrifos. Initial and final pH was found to be 7.2 in case of 100 mg/L chlorpyrifos concentration. During bioremediation biological oxygen demand (BOD) and chemical oxygen demand (COD) were also monitored as indicators for microbial growth and bioremediation. The decrease in BOD values indicates the growth of microorganisms in the varying concentration of pesticides. The basis for the COD test is that nearly all organic compounds can be fully oxidized to carbon dioxide with a strong oxidizing agent under acidic conditions. Table 2 demonstrates the decrease in COD levels at the pesticide concentration of 25, 50 and 100 mg/L over a period of bioremediation, which indicates the degradation of chlorpyrifos by *Stenotrophomonas maltophilia* MHF ENV 20, cypermethrin by strain MHF ENV 22 and fenvalerate by *Sphingobacterium thalpophilum* MHF ENV 23.

Comparison of Bioremediation-Chlorpyrifos, Cypermethrin and Fenvalerate by identified potential microorganism

In the present research study naturally occurring bacterial isolates capable of metabolizing pesticides were isolated from pesticides rhizoremediated soil. Isolated degraders were then identified by various biochemical tests and 16 S rDNA characterization. Three different potential degraders *Stenotrophomonas maltophilia* MHF ENV 20, *Stenotrophomonas maltophilia* MHF ENV 22 and *Sphingobacterium thalpophilum* MHF ENV 23 were taken for bioremediation of Chlorpyrifos, Cypermethrin and Fenvalerate respectively. Bioremediation of pesticides was carried out at the selected concentrations 25, 50 and 100 mg/L by identified potential degraders *Stenotrophomonas maltophilia* MHF ENV 20, *Stenotrophomonas maltophilia* MHF ENV 22 and *Sphingobacterium thalpophilum* MHF ENV 23 in shake flask bioreactor under controlled environmental conditions.

The bioremediation studies carried out for selected organic pesticides viz. chlorpyrifos, cypermethrin and fenvalerate by developed microbial consortium in *Pennisetum* rhizosphere and as well by their respective potential microorganism has been compared. The comparison of bioremediation by microbial consortium and potential microorganism for chlorpyrifos, cypermethrin and fenvalerate at 25 ppm, 50 ppm and 100 ppm are represented in table 3-5. The bioremediation results showed that the consortium was able to biodegrade the selected pesticides efficiently upto 100 ppm. For 25 ppm of chlorpyrifos, the MHF ENV 20 degraded it completely within 24 hours while only 8.3% of chlorpyrifos was degraded within 24 hours by microbial consortium in *Pennisetum* mycorrrhizosphere soil. Similarly for 50 ppm of chlorpyrifos 4.9% degradation was observed at the end of 1st day which increased to 18.7% at the end of 5th day by microbial consortium whereas potential degrader was reported to degrade 80% at the end of 1st day and 100% at the end of 3rd day. For higher concentration like 100 ppm 20.29% of chlorpyrifos was degraded by consortium and 95% degradation was

observed in case of potential degrader (fig. 2 and table3).

In similar manner bioremediation results for cypermethrin shows that for 25 ppm the degradation was 11.3%, 21.1% and 45% at the end of 1st, 3rd and 7th day respectively for microbial consortium, whereas potential degrader MHF ENV 22 was reported to degrade it 80% at the end of 1st day and 100% at the end of 3rd day. For higher concentration like 100 ppm 5.7 % of cypermethrin was degraded by consortium and 77% degradation was observed in case of potential degrader at the end of 7th day (fig. 2, table 4).

The biodegradation studies for fenvalerate illustrated that for 25 ppm concentration 12, 25.6 and 41.9% was degraded at the end of 1st, 5th and 7th day respectively, whereas potential degrader was reported to degrade it 7.0% at the end of 0 day and 100% at the end of 1st day. For 50 ppm concentration 6.8%, 10.9% and 50% of chlorpyrifos was degraded and MHF ENV 23 was found to degrade 30, 80.3 and 100 % at the end of 1st, 5th and 7th day. For higher concentration like 100 ppm 8 % of cypermethrin was degraded by consortium and 94.7% degradation was observed in case of potential degrader at the end of 7th day (fig. 2; table 5).

Rhizoremediation of selected pesticides has been studied using developed rhizospheric microbial consortium in *Pennisetum pedicellatum* grass rhizosphere in soil and by potential degraders in liquid MSM. The rhizoremediation of pesticide in the rhizosphere varies with the characteristics of plants, growth media, types of pesticides, concentration of pesticides, mobility of pesticides and behavior of pesticides in the respective media and environmental conditions. In liquid medium microorganism are in direct contact with the pesticides contaminated environment. The degradation of pesticides in liquid MSM takes place by the utilization of pesticides as a sole source of carbon and energy. The removal of pesticides from MSM correlated with their degradation by microorganisms during bioremediation experiment in shake flask bioreactor. The rhizoremediation of pesticides in plant rhizosphere differs due to their different characteristics and properties. *Pennisetum pedicellatum* was found to be efficient and effective for the rhizoremediation of pesticides from the soil environment. In pot culture experiment, *Pennisetum pedicellatum* was used for the rhizoremediation of pesticides in rhizosphere have different environmental conditions. Root morphology and secreted exudates have fundamental impacts on the rhizosphere environment and its microbial community. In the rhizosphere, plant root exude compounds that can serve as co-metabolites in microbial pollutant degradation (Hedge and Fletcher., 1996). Apart from the direct release of degradative enzymes, the susceptibility of organic pollutants to bacterial metabolism and enzymatic attack differs widely and is related to molecular structure. Plants are able to stimulate the activities of microbial degrader organism/communities. Mechanism of nonspecific stimulation potentially involved in rhizodegradation includes exudates that serve as analogues or co-metabolites of organic pollutants (Siciliano and Germida., 1998). Metabolites formed in the soils contaminated at lower levels of pesticides dissipated quickly into simpler compounds below detectable limits. This might be due to the enhancement of a soil microflora population capable of degrading chlorpyrifos (Singh et al., 2004; Menon et al., 2005). The potential microorganism existing in rhizospheric soil which have capacity to survive and multiply

at higher concentration of pesticides were selected, identified and characterized for bioremediation of hazardous pesticides in MSM. Therefore selective stimulation offered by addition of pesticides to MSM offers selection of more competent potential degrader possessing higher pesticides degrading ability in liquid medium. In liquid medium microorganisms are in direct contact with pesticides in controlled environmental conditions responsible for higher pesticide degradation in case of all the three pesticides chlorpyrifos, cypermethrin and fenvalerate.

Table.1 Rhizosphere Bioremediation of Pesticides

Pesticides	Dose in mg/kg	7 days	15 days	30 days	45 days	60 days
Chlorpyrifos	10	7.6	100	100	100	100
	25	82.15	89.94	100	100	100
	50	65.73	69.94	75.52	100	100
	75	31.28	41.6	47.45	53.63	100
	100	20.29	25.3	27.7	34.7	76
Cypermethrin	10	18.1	100	100	100	100
	25	11.6	28	64	100	100
	50	23.3	33	54.1	71.3	100
	75	22.35	43.39	59.36	66.15	71.26
	100	4.42	17.4	40.9	42.9	62.21
Fenvalerate	10	63.1	100	100	100	100
	25	55.45	100	100	100	100
	50	50.4	71.7	100	100	100
	75	46.5	66.38	82.9	100	100
	100	5.1	19.4	32.4	44.7	72.4

Figure.1 Pesticide degradation comparison on initial and final day of bioremediation by potential degrader

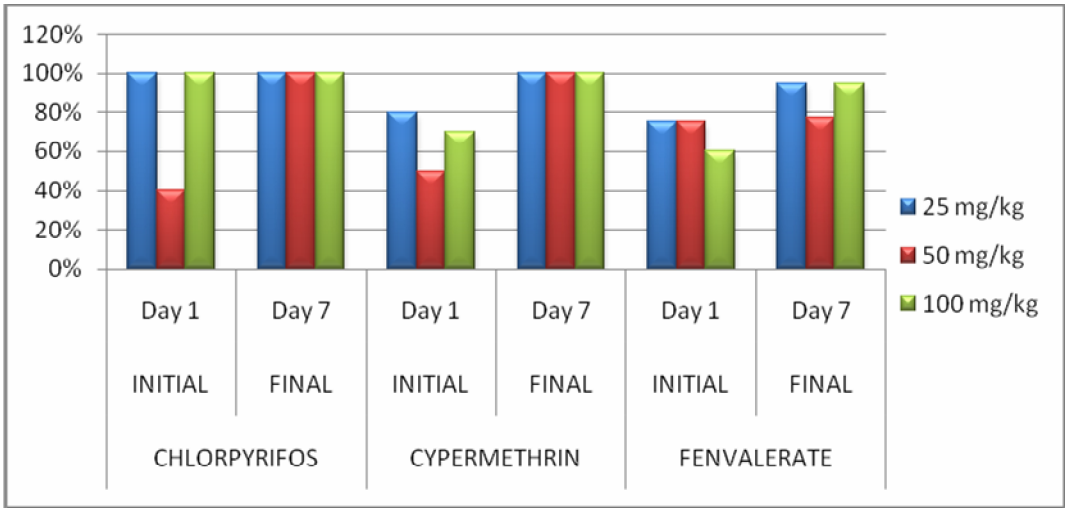

Table.2 Variation in environmental parameters during bioremediation of chlorpyrifos, cypermethrin and fenvalerate by potential degrader

Pesticides	Parameters	25 mg/l		50 mg/l		100 mg/l	
		Initial	Final	Initial	Final	Initial	Final
Chlorpyrifos	**pH**	7.3	6.6	7.45	7.0	7.2	7.2
	Temperature	28□C	28□C	28□C	28□C	28□C	28□C
	Growth curve (OD)	0.0081	0.006	0.0081	0.04	0.0081	0.047
	CFU/ml x 10^7	4.86	3.6	4.86	24	4.86	28.2
	COD	125	70	150	30	289	15
	BOD	22	8	22	7	22.5	2
Cypermethrin	**pH**	7.2	7.6	7.2	7.2	7.2	7.4
	Temperature	28□C	28□C	28□C	28□C	28□C	28□C
	Growth curve (OD)	0.008	0.04	0.008	0.047	0.008	0.035
	CFU/ml x 10^7	4.86	42.27	4.86	29.16	4.86	20.0
	COD	48	25	98	33	200	132

	BOD	10.2	3.8	10.2	4.3	10.2	5.23
Fenvalerate	**pH**	7.0	7.6	6.92	7.0	7.2	7.4
	Temperature	28□C	28□C	28□C	28□C	28□C	28□C
	Growth curve (OD)	0.008	0.048	0.008	0.05	0.008	0.082
	CFU/ml x 10^7	4.8	21.3	4.8	21.1	4.8	33.6
	COD	550	400	667	385	688	390
	BOD	26.0	8.0	25.9	6.0	26.0	6.0

Table.3 Comparison of Chlorpyrifos Degradation using microbial consortium and potential degrader *Stenotrophomonas maltophilia* MHF ENV 20

Chlorpyrifos	**0 day**		**1 day**		**3 day**		**5 day**		**7 day**	
	Microbial Consortium	**MHF ENV 20**	**Microbial consortium**	**MHF ENV 20**	**Microbial consortium**	**MHF ENV 20**	**Microbial consortium**	**MHF ENV 20**	**Microbial consortium**	**MHF ENV 20**
25 ppm	5.6	60	8.3	100	16.7	100	20.5	100	34.7	100
50 ppm	2.3	50	4.9	80	8.9	100	18.7	100	27.7	100
100 ppm	1.2	30	3.6	50	7.1	60	13.4	75	20.29	95

Table.4 Comparison of Cypermethrin Degradation using microbial consortium and potential degrader *Stenotrophomonas maltophilia* MHF ENV 2

Cypermethrin	**0 day**		**1 day**		**3 day**		**5 day**		**7 day**	
	Microbial Consortium	**MHF ENV 22**	**Microbial consortium**	**MHF ENV 22**	**Microbial consortium**	**MHF ENV 22**	**Microbial consortium**	**MHF ENV 22**	**Microbial consortium**	**MHF ENV 22**
25 ppm	3.8	40	11.3	60	21.1	100	38.7	100	45.0	100
50 ppm	2.16	30	9.8	50	16.7	80	20.2	100	25.1	100
100 ppm	0.5	0	1.7	25	3.4	40	4.1	60	5.7	77

Table.5 Comparison of Fenvalerate Degradation using microbial consortium and potential degrader *Sphingobacterium thalpophilus* MHF ENV 23

Fenvalerate	**0 day**		**1 day**		**3 day**		**5 day**		**7 day**	
	Microbial Consortium	**MHF ENV 23**	**Microbial consortium**	**MHF ENV 23**	**Microbial consortium**	**MHF ENV 23**	**Microbial consortium**	**MHF ENV 23**	**Microbial consortium**	**MHF ENV 23**
25 ppm	6.5	7.0	12.0	100	25.6	100	41.9	100	57.2	100
50 ppm	6.0	6.5	6.8	30	10.9	48.0	23.5	81.3	50.0	100
100 ppm	2.5	2.5	3.1	40.0	4.3	72.0	6.7	85.4	8.0	94.7

The microbial consortium and identified potential have proved effective, efficient, novel and innovative for the bioremediation of pesticides which could be also applicable for the bioremediation of other hazardous toxic compounds belonging to organophosphorus and pyrethroid group. The rhizoremediation technology developed for the bioremediation of chlorpyrifos, cypermethrin and fenvalerate in *Pennisetum* rhizosphere by developed microbial consortium would be applicable for bioremediation of pesticide contaminated sites for the treatment of pesticide waste or effluents generated through pesticide manufacturing and formulation units and from their agricultural and domestic usages. The bioremediation technology developed for pesticides- chlorpyrifos, cypermethrin and fenvalerate using novel source of rhizosphere microbial consortium and identified potential microorganisms would be cost effective remedial measures to cleanup the environment.

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