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Efficacy of Cereal Straw and its Conjoint use with Microbial consortium in Reducing the Leaching of Chlorpyrifos: A soil Column study

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Abstract

The efficacies of cereal straw and conjoint use of cereal straw with microbial consortium (Pseudomonas sp. HY8N with other bacterial isolates) were evaluated in reducing the leaching of chlorpyrifos using soil columns under laboratory conditions. Application of cereal straw @ 5 t ha^{-1} decreased the leaching of chlorpyrifos by 22 percent whereas the conjoint use of cereal straw @ 5 t ha^{-1} and microbial consortium was more effective than cereal straw alone and reduced the leaching of chlorpyrifos by 44 percent. The amount of chlorpyrifos residue in the surface soil (0-15) was also reduced by the application of microbial consortium in addition to cereal straw.

Keywords: Cereal straw, microbial consortium, leaching, soil column.

Introduction

An insecticide is a substance used to control insects either by destroying, preventing, mitigating or repelling them. Insecticides come under the category of pesticides and usually include ovicides and larvicides used against insect eggs and larvae, respectively. These are generally used in agriculture, environmental health, medicine, industry, human and animal health.

Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothionate) is an organophosphorus insecticide which is used as a termiticide in households and for controlling pest in agriculture[1]. It is widely used to control pests such as termites, lice, cockroaches, fleas, mosquitoes, fire ants and ornamental plant insects[2]. The different trade names of the insecticide include Dursban, Dorson, Lorsban, Dhanwan etc. The mode of action of chlorpyrifos involves interference with the normal functioning of the central nervous system with same effects in insects and humans[3].

It has low solubility in water and has high hydrophobicity. Thus it is said to partition easily onto aquatic sediments and macrophytes and can possess harm to benthic organisms. It is a volatile toxicant and its residues have been detected in air and rainwater also[4]. Chlorpyrifos has been classified as Class II (moderately hazardous pesticides) by WHO, following acute oral, dermal and inhalation exposures. It is an endocrine disruptor and has been found to have various detrimental effects on animals and humans[5]. It is toxic to amphipods[6] and earthworms[7].

A large variation in the half life of chlorpyrifos in soil (10 to 120 days) has been observed which has been attributed to the changing soil pH, moisture content, temperature, organic carbon content and pesticide formulation[8]. The degradation of chlorpyrifos includes both biotic and abiotic processes. One of the key process includes enzymatic or clay-metal-catalysed hydrolysis which increases with increase in pH and temperature. Photodegradation is another pathway involved in the degradation of the insecticide[9]. Although aerobic and anaerobic metabolism are the main route for the degradation of chlorpyrifos but the degradation in soil is slow under these conditions. 3, 5, 6-trichloropyridinol (TCP) is the major metabolite formed during the degradation. It is mobile in soil and is persistent in absence of sunlight[10]. In water chlorpyrifos is degraded by abiotic hydrolysis and photosensitized oxidation. Here pH plays a major role and neutral hydrolysis is favoured below pH 9, whereas alkaline hydrolysis dominates above pH 9[11]. In alkaline soils under low moisture conditions, hydrolytic degradation is the major route of degradation which is inhibited when the concentration of the insecticide is high (1000 μg/g) [12].

Leaching studies have shown that chlorpyrifos has moderate mobility in soil whereas presence of chlorpyrifos in ground water has been reported[13]. The major degradate of the insecticide, TCP, is more mobile than chlorpyrifos itself and has the tendency to leach to ground water. Chlorpyrifos and its metabolites have been detected in subsoils upto 50 cm of soil depth. The maximum concentrations (2.3 mg kg^{-1}) were observed at a depth of 10 to 20 cm[14].

Organic amendments are being used in agriculture to improve physicochemical properties of soil at low cost, and are regarded as one of the most suitable technologies for sustainable agriculture. They not only enhance the content of organic matter but also improve soil aeration, water infiltration, enhancing plants nutrient holding capacity, and adjustment of soil pH. These changes also influence biodegradation, retention and transport of pesticides in soil. It has been demonstrated that organic amendments can significantly change pesticide adsorption-desorption and leaching behaviour in soils[15]. Organic manures have also been reported to provide significant amount of residual nutrients for cultivation of a short durational succeeding crop. However the residual benefits depend on the initial nutrient content of the manure[16]. Organic amendments are being used widely in agriculture nowadays. Cowdung slurry has been used for the decomposition of teak and bamboo leaf litter[17]. Compost has been reported to be an excellent vehicle for carrying nutrients to soil and plants[18].

Organic amendments were also found to increase the microbial activity in the chlorpyrifos contaminated soil as compared to the unamended soil. The soil amendments vermi compost and mushroom spent compost have been reported to act as biostimulation agents to sustain microbial activity in chlorpyrifos contaminated soil[19]. Dissolved Organic Matter (DOM) used as a soil amendment was found to reduce the acute and chronic toxicity of Chlorpyrifos[20].

Since chlorpyrifos sorbs strongly to soil organic matter[14] and application of organic amendments is associated with an increase in the microbial activity in chlorpyrifos contaminated soils, the present investigation was directed to determine the potential of cereal straw and a mixture of cereal straw with microbial consortium as a sorbent in reducing the leaching of chlorpyrifos and enhancing its degradation in soil using soil packed columns.

Material and Methods

The soil column study of chlorpyrifos was done under laboratory conditions in the Agricultural Chemicals laboratory, Department of Chemistry, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, using Polyvinyl chloride (PVC) columns (60 cm long x 6 cm diameter). The soil (Typic Hapludoll) of four different depths viz. 0-15, 15-30, 30-45, 45-60 cm was collected in bulk from the E_1 field of Crop Research Centre (CRC) of the university. Firstly, the soil samples were dried in shade and crushed by a wooden roller after which they were passed through a sieve with openings of 2 mm diameter. As the physicochemical parameters of the soil determine its adaptability to cultivation and the level of biological activity it can support[21], therefore some of the physicochemical properties of the experimental soils like mechanical analysis, pH, electrical conductivity and percentage of organic carbon were analyzed by standard analytical methods[22]. The analytical grade Chlorpyrifos (99% purity) was obtained from Sigma-Aldrich, India. Cereal Straw was collected from Pantnagar farm and the microbial consortium used in the experiment was prepared in the laboratory in Department of Microbiology, of the university. Triple distilled water used in the study was prepared in the laboratory and all the other chemicals used in analysis were of analytical grade.

Preparation of Microbial consortia: The bacterial strains used in the study were isolated from the agricultural fields of Udham Singh Nagar. The ability of the bacteria to grow and degrade pesticides was checked before preparation of the consortium and the bacterial isolate of *Pseudomonas sp. HY8N*, showing highest tolerance for chlorpyrifos was used for the preparation of consortium with other bacterial isolates (*Bacillus* sp. and *Pseudomonas* sp.).

Preparation of amendment mixed soil (0-15): Cereal straw was mixed with soil of 0-15 cm depth @ 5 t ha^{-1} which is its usual field application rate. The amended soil was kept in separate poly bag and was moistened to near field capacity moisture regime (15% w/w) and incubated at room temperature (27°C) for one week. The treatment, cereal straw with microbial consortium was prepared by adding 50mL of nutrient broth to the amended soil once it was incubated.

Filling of soil columns: The PVC columns were cut longitudinally into three parts for proper filling of soil in the column and were rejoined using cellophane tape. The bottom of the last column was covered by perforated polythene and a uniform layer of 1cm glass wool was placed at the bottom to avoid seepage of soil in the leachate. Above the glass wool padding, a layer (5 – 6 cm) of acid washed river bank sand was packed. Depth-wise moist (15% on weight basis) soil samples were filled in the columns and gently tapped by a wooden plunger to maintain natural bulk density. Six columns were prepared out of which two were control (receiving no amendment), two columns were filled with cereal straw mixed soil at the top of the column whereas in the other two columns the top of the column was filled with the amended soil plus microbial consortium. The column joints were covered by cellophane tape and it was clamped in stands for support.

A solution of the pesticide in methanol containing 1 mg chlorpyrifos was mixed with 10 g of amended or control soil and was applied uniformly at the top of the column. The top soil column was saturated with distilled water. On the third day the leachate was obtained. A continuous flow of water at the rate of about 4 drops per minute from the top of the column was maintained throughout the leaching process. The leachates were collected daily, filtered and extracted for chlorpyrifos residues and the study was continued for 10 days.

Extraction of chlorpyrifos from the leachate: Chlorpyrifos was extracted from the leachate following a modified QuEChER's method, which is a highly versatile sample preparation method with excellent results and can be used for the analysis of a wide range of pesticides[23]. Four mL of the aliquot of the leachate was taken in a centrifuge tube and 4 mL of hexane, 3 g of anhydrous $MgSO_4$ and 2 g of NaCl were added to it. The mixture was vortexed until fully dissolved. Thereafter, 150 mg PSA (primary secondary amine) reagent and 1 g $MgSO_4$ were added to the tube and the mixture was centrifuged for 5 minutes at 3000 rpm. After phase separation the upper organic layer was retained for analysis. Prior to analysis, the samples were filtered through 0.2 µm PTFE disc filter.

Extraction of chlorpyrifos from the soil: At the end of leaching period, soil columns were separated and soil mass of each depth was removed from the column and spread over a clean plastic sheet under shade and mixed thoroughly. An aliquot of soil was drawn and extracted for chlorpyrifos residues following the simplified QuEChER's method. Three g soil was taken into a 15 mL centrifuge tube and after addition of 4 mL of hexane and 5 mL of distilled water it was vortexed for 2 minutes. The contents of the tube were allowed to stand for 10 minutes after which 3 g of anhydrous $MgSO_4$ and 2 g of NaCl were added. The mixture was vortexed for 2 minutes more. The contents were then centrifuged for 5 minutes at 3000 rpm and in the aliquot obtained 150mg PSA reagent and 1 g $MgSO_4$ were added. The mixture in the tube was centrifuged for 5 minutes more which led to the separation of the two layers. The upper organic layer was decanted off and filtered through 0.2 µm PTFE disc filter for the analysis of chlorpyrifos by GC.

Analysis of the samples using GC: The insecticide chlorpyrifos was analyzed using a Gas Chromatograph (GC), model Chemito (Ceres 800 plus) containing a capillary column and an Electron Capture Detector with the following temperature conditions: Injector Temperature: 280°C, Oven Temperature: 270°C and Detector Temperature: 300°C. Nitrogen was used as the carrier gas at a flow rate of 30 mL /min. The retention time for chlorpyrifos was 5.24 minutes. The chromatogram of 10 ppm standard of chlorpyrifos is shown in figure-1.

Results and Discussion

Some general properties of the soils used in the experiment are listed in table-1. The experimental soil was a coarse textured sandy loam soil of slightly alkaline reaction. The proportion of soil clay, soil organic carbon and electrical conductivity was found to decrease with depth. The dynamic soil properties of surface (0-15 cm) sample which was treated with CS@ 5 t ha^{-1} compared to the control are presented in table-2. In general, in comparison to the control the application of CS decreased the soil pH and increased the electrical conductivity of soil due to mineralization of organic amendment. The content of soil organic C was also increased by the application of CS.

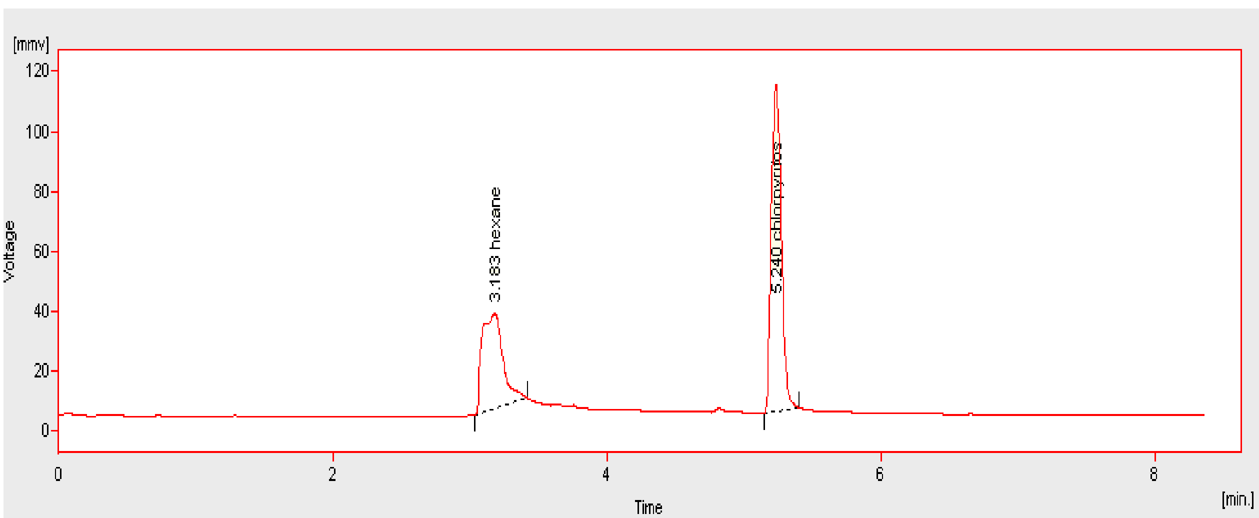

Figure-1
Standard chromatogram of Chlorpyrifos

Table-1
General Properties of Depth-wise drawn Soil Samples Used for packing the columns

Soil Properties						
Soil Depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH (1:2, soil Water suspension)	EC (mSm-1, 1:2, soil water suspension)	Organic C (g kg^{-1})
0-15	64.84	20.0	15.16	7.80	0.086	6.11
15-30	70.84	18.0	11.16	8.12	0.042	5.73
30-45	76.84	14.0	9.16	7.55	0.048	4.20
45-60	80.84	10.0	9.16	8.21	0.035	3.25

Table-3 shows the total amount of chlorpyrifos leached from the columns and that retained in soil at different depths after ten days leaching event. It is clearly evident from the data that CS applied @ 5 t ha^{-1} reduced the levels of chlorpyrifos leached, from 27.53 to 21.349 (µg/column) during the ten days leaching event. The leaching pattern of chlorpyrifos in the two treatments and the control during the 10 day leaching event is presented in figure-2. As shown in the figure, the leaching of chlorpyrifos was slow in the initial days and increased after 4^{th} day, whereas the leaching was almost constant during the last four days of the experiment. The total amount of chlorpyrifos bound to the soil at different depths in the columns receiving different treatments is shown in figure-3. The major amount of chlorpyrifos in the soil column was present in the (15-30cm) of soil in control whereas in the soil columns treated with amendments major part of the retained insecticide was present in the surface soil (0-15cm). This indicates higher mobility of chlorpyrifos in control as compared to the other treated soil columns.

Table-2
Effect of Cereal Straw on some dynamic properties of surface soil (0-15cm)

Soil Properties			
Soil	pH (1:2, soil Water suspension)	EC(mSm-1, 1:2, soil water suspension)	Organic C (g kg^{-1} soil)
Control (0-15 cm)	7.34	0.087	5.13
CS @ 5 t ha^{-1}	6.74	0.158	6.29

Table-3
Chlorpyrifos residues in leached water and soils of different depths under different treatments applied to the columns

Parameters	Control	CS	CS + Microbial consortium
Total amount of chlorpyrifos leached after 10 days (µg/column)	27.53	21.35 (22.45%)	15.48 (43.78%)
Chlorpyrifos residue in 0-15 cm soil (µgg^{-1} of soil)	0.049 ± 0.001	0.073 ± 0.009	0.042 ± 0.027
Chlorpyrifos residue in 15-30 cm soil (µg g^{-1} of soil)	0.077 ± 0.016	0.053 ± 0.005	0.048 ± 0.015
Chlorpyrifos residue in 30-45 cm soil (µg g^{-1} of soil)	0.036 ± 0.023	0.037 ± 0.008	0.024 ± 0.004
Chlorpyrifos residue in 45-60 cm soil (µg g^{-1} of soil)	0.027 ± 0.001	0.016 ± 0.007	0.10 ± 0.004

* Values in the parenthesis indicate the percentage of chlorpyrifos leached in comparison to control

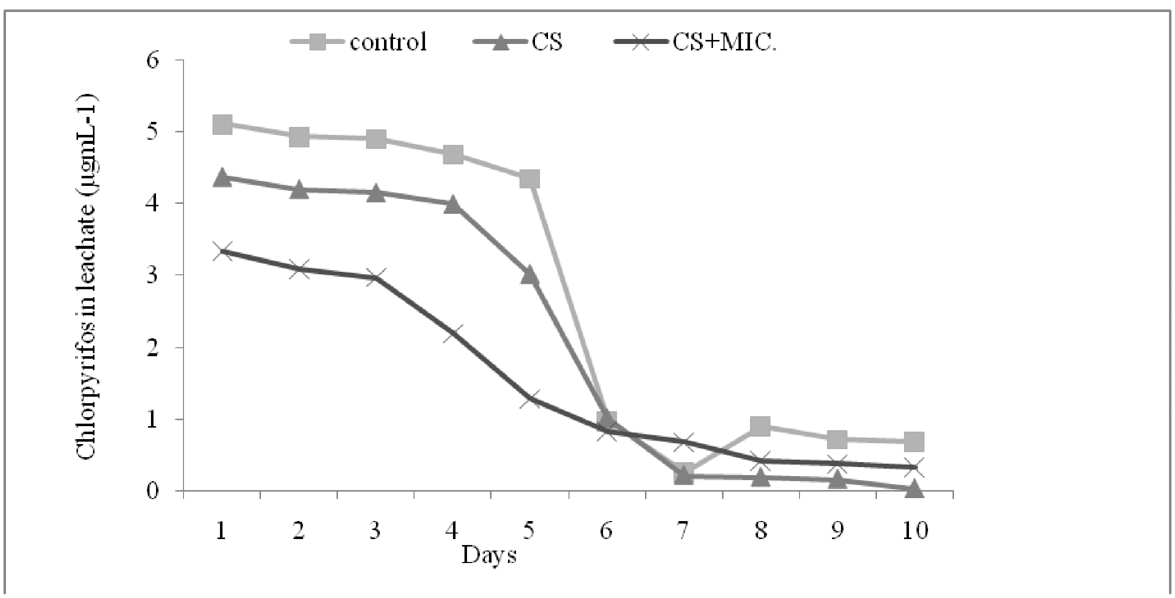

Figure-2
Leaching of chlorpyrifos in soil columns with different treatments along with control

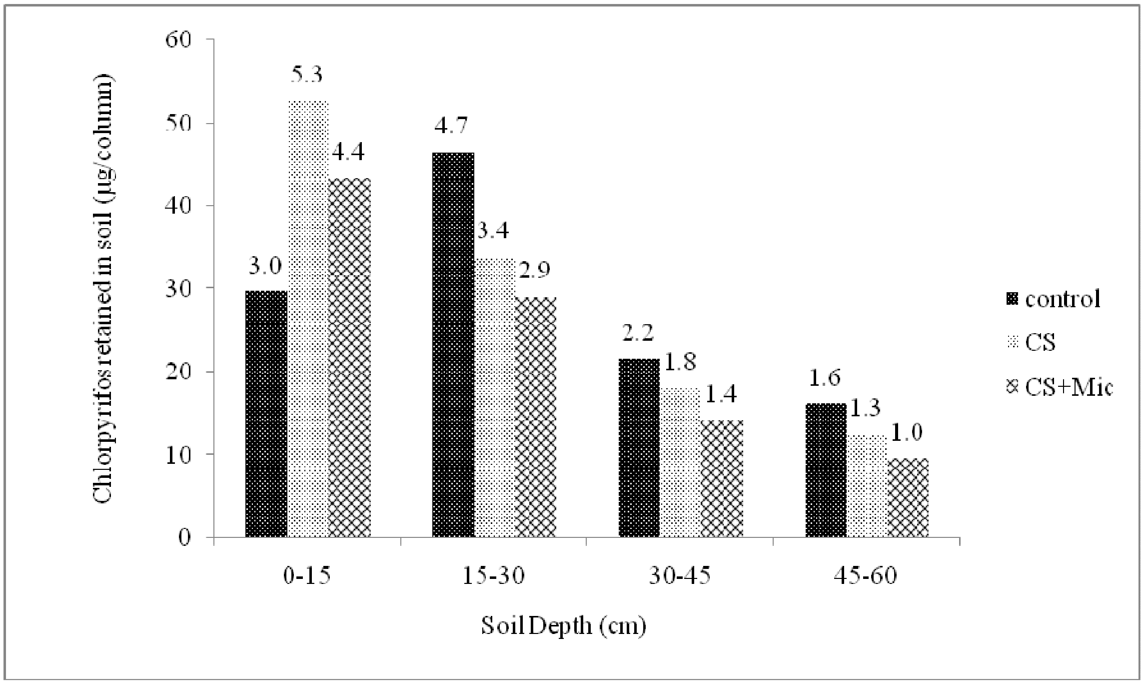

Figure-3

Effect of CS and the mixture of CS with microbial consortium on chlorpyrifos residue at different depths of soil in the column. The values above the vertical bars indicate the percentage of initially applied chlorpyrifos retained in the soil at different depths

The amount of chlorpyrifos retained in the surface soil (0-15cm) was increased from 0.049 to 0.073 ($\mu g g^{-1}$of soil) by application of CS, as chlorpyrifos sorbs strongly to soil organic matter[14]. However, the amount of pesticide in the subsurface soil (15-30) was decreased from 0.077 to 0.053 ($\mu g g^{-1}$of soil), which may be due to the adsorption of chlorpyrifos in the soil amended with cereal straw leading to controlled leaching of chlorpyrifos after application of CS. The amount of chlorpyrifos in the 30-45 cm soil was almost similar in both the soil columns whereas there was a decrease in the chlorpyrifos content from 0.027 to 0.016 ($\mu g g^{-1}$of soil) in the soil at 45-60cm of depth.

Further, conjoint use of CS @ 5 t ha^{-1} and microbial consortium of *Pseudomonas sp. HY8N* with other bacterial isolates was more effective in reducing the residue levels of chlorpyrifos in leached water and surface soil (0–15cm). The residue level of chlorpyrifos in the percolation water under conjoint application of CS @ 5 t ha^{-1} and microbial consortium was reduced to 15.475 (µg/column). The amount of chlorpyrifos present in the surface (0-15cm) and sub surface (15-30) soil was further reduced in comparison to CS applied alone, which may be due to the degradation of pesticide by the microbial activity. CS @ 5 t ha^{-1} reduced the leaching of chlorpyrifos by 22 percent whereas CS along with microbial consortium was more effective and was capable of reducing the amount of chlorpyrifos in leachate by 44 percent i.e. just double of the CS alone. A large fraction of chlorpyrifos initially applied was found to dissipate in all the columns after the 10d study, which may be attributed to the high moisture content of the soil in the column under saturated flow conditions which is likely to increase the degradation of chlorpyrifos due to increased microbial activity[24]. The fast decay of chlorpyrifos due to the enhanced activity of microorganisms in soil, with a dissipation time of 10-14 days, has also been reported [25].

Conclusion

The results obtained from the present investigation demonstrated that the leaching of chlorpyrifos was reduced by the application of CS @ 5 t ha^{-1}. Further application of microbial consortium along with CS was more effective in retaining the pesticide and their degradation in soil due to the increased microbial activity. Therefore, cereal straw which is a cost effective organic material and is easily available to farmers may be used in combination with the microbial consortium containing the soil bacteria *(Pseudomonas sp. HY8N with other bacterial isolates)* for controlling the leaching of chlorpyrifos.

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