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MICROCOSM STUDIES FOR THE BIODEGRADATION OF CARBARYL IN SOIL

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Abstract

A field-scale experiment with a complete randomized block design was performed to study the degradation of carbaryl in soil. Soil was treated with different types of treatments. Degradation was observed at two levels of soil after 7, 14 and 21 days of treatments. Soil bacteria have significant effect on the degradation of carbaryl in soil. Nutrient additions stimulate the biodegradation of carbaryl and the rate of degradation of carbaryl increase with respect to number of days of treatment. It has been found that after 21 days 85% carbaryl has been degraded from soil treated with nitrogen source.

Introduction

Pakistan is an agriculture based country where cotton is a major cash crop in which more than 80% of the pesticides is used (Tariq, *et al*., 2007). Carbamates like carbaryl, carbofuran, aldicarb etc., are extensively used for the control of white fly and other insect pest of cotton plants and other crops as well. Their extensive and irrational use cause environmental health hazards due to the persistence of their residues in soil and water, residues of carbaryl were observed from 0 ppm to 0.5 ppm in rice fields after one month of their application (Baig, *et al*., 1992). Ahmad *et al*., (2004) investigated bioavailability and biodegradation of carbaryl (1-naphthyl methylcarbamate) in a soil with a long history of pesticide contamination from a storage facility located at Mamoon Kanjan, Pakistan. Their results demonstrated that even a weakly sorbed and easily degradable pesticide, carbaryl, was effectively sequestrated in soil with time, rendering its partly inaccessible to microorganisms and affecting the bioavailability of the compound (Ahmad *et al*., 2004).

Carbamate insecticides have been extensively used all over the world to control wide range of insect species. These insecticides are potent inhibitors of acetylcholine esterase. A carbamate insecticide such as carbaryl is one of the broad spectrum insecticide used in agricultural industry (Hashimoto *et al*., 2002). Thus, extensive use of these insecticides results in pollution of soil and water system and constitutes potential environmental and human health hazards (Swetha & Phale, 2005). Carbamate pesticide such as carbaryl are highly toxic and widely used in agricultural practices. These compounds are considered hazardous because they potently inhibit acetyline esterase and the N-nitrosocarbamates formed are potent mutagens (Fahmy *et al*., 1970). Disturbances in the carbohydrate metabolism and protein synthesis and detoxification function of the liver in mammals have been reported. Carbaryl is a weak inducer of hepatic microsomal drug-metabolizing activity (WHO). There are number of bacteria in soil that have ability to degrade carbamate pesticides. Microbial degradation of pesticides in soil and aqueous system reduces their persistence and as a result reduces the risk of environmental contamination posed by the use of these compounds (Tam, *et al*., 1987). Microorganisms that have

capability to degrade Carbamate pesticides are thought to play a significant role in the break down and detoxification of pesticides from the environment. These microorganisms have received considerable attention because of their potential to use pesticides detoxification as well as on fate of Carbamate pesticide in the environment (Chaudhry *et al*., 1999; Larkin *et al*., 1986; Stalker *et al*., 1992; Hayatsu *et al*., 1999). Bacteria capable of degrading Carbamate pesticides have been isolated from soil (Chapalmadugu *et al*., 1993; Doddamani *et al*., 2001). There is serious need to develop remediation process to eliminate or minimize contamination of surface water and ground water by carbamates, other wise they will ultimately spoil the whole environment.

Present study is aimed to study the effect of selected bacterial consortium in soil for the treatment of carbaryl contaminated soil and to observe the effect of different types of nutrients on the degradation of carbaryl.

Materials and Methods

Chemicals: Analytical grade carbaryl (99.9%), other chemicals and media component were obtained from Sigma-Aldrich (USA). Commercial grade carbaryl that is Sevin dust was obtained from local distributors.

Microorganisms: Bacteria used in the study were isolated from pesticide contaminated soil by enrichment technique. Bacterial strains were used in consortia by growing them in broth and mixed them to make consortia. These were identified on basis of 16sRNA and identified as *Micrococcus arborescens* (X 77443.1), *Pseudomonas aeruginosa* (EU 344794.1), *Brachybacterium* sp., (AB 257583.1) and *Salsuginibacillus kocurii* (AM 492160.1).

Soil treatment: Soil was dried and sieved. For sterilization of soil it was autoclaved three times at temperature of 120°C and pressure of 760 mmHg. Then both types of soil that is sterilized and unsterilized were treated with nitrogen and phosphorus source.

Inoculum preparation: Inoculum was prepared by inoculating each strain in nutrient broth culture incubating it at temperature of 37°C. After 16 hours these cultures were mixed well for preparation of consortia. The soil was inoculated with approximately 10^6 cells per gram soil. Each soil sample was thoroughly mixed several times during the addition of cells.

Sampling: Experiments were carried out for three week and samples were collected from surface and 6 inch depth every week and analyzed for the carbaryl contents in acetonitrile extract.

Experimental field: A completely randomized block design was used, with three replicates, for each treatment (Table 1). Nine different treatments were performed in plastic pots; each pot was filled with 5 Kg soil. Each pot was treated with 30 gm of commercial grade carbaryl i.e., Sevin dust (10%), mixed it thoroughly to get the final concentration of 100 ppm carbaryl in soil. These pots were then placed randomly in field, in natural environment. The nitrogen and phosphorus source were applied one week after the treatment of carbaryl. The pots either treated with nitrogen and phosphorus source or one of them always has 1% nitrogen and 0.1% phosphorus source, for nitrogen source sodium nitrate was used and potassium dihydrogen phosphate was used as phosphorus source. Soil was sterilized, two times by sterilizing it at 120°C and 760 mmHg.

Table 1. Following treatments were performed during the field experiment.

Symbol	Treatments	Symbol	Treatments
Control	Sterilized soil treated with Carbaryl		
T1	Unsterilized soil treated with carbaryl	T2	Sterilized soil treated with carbaryl along with inoculum
T3	Unsterilized soil with carbaryl along Nitrogen and phosphorus source	T4	Sterilized soil with carbaryl along with nitrogen and Phosphorus source with inoculum
T5	Unsterilized soil with carbaryl along with phosphorus source	T6	Sterilized soil with carbaryl along with phosphorus source and with inoculum
T7	Unsterilized soil with carbaryl along with Nitrogen source	T8	Sterilized soil with carbaryl along with nitrogen source and with inoculum

Carbaryl extraction from soil: Carbaryl was extracted from soil by following EPA Method 8318. A sample 20 ± 0.1 gm was but into a 250 ml Erlenmeyer flask with Teflon lined screw cap. To this 50 ml of acetonitrile was added and shaken for two hours on a platform shaker. Mixture was allowed to settle for 5-10 minutes and then decanted the extract in 250 ml centrifuge tube. This step was repeated two more times with 20 ml acetonitrile and one hour shaking. Then all these extracts were combined and centrifuged at 200 rpm for 10 min., and decant it in 100 ml volumetric flask and diluted it to the volume with acetonitrile. Pippet 10 ml of the sample in a flask containing 100 µl of ethylene glycol and evaporated it by rotary evaporator until only the ethylene glycol keeper remains. Then methanol was added to the ethylene glycol residue, drop wise for make final volume of 1ml. The samples were then filtered through 0.45 um nylon filters and placed in vials at 4°C till analyzed.

HPLC analysis: The remaining concentration of carbaryl in soil after degradation was determined by HPLC with C-18 column and detection was based on absorption at 220 nm. The eluent was acetonitrile, methanol and water (30%, 30%, 40%) with a flow rate of 1.5 ml/min.

Statistical analysis: SPSS 13 was used for statistical analysis of data. ANOVA and Dunnett test was applied to compare different treatments with control.

Results

ANOVA shows that there is significant difference between different types of treatment with each other while non significant results were obtained in case of sub soil after 7 days of treatment (Table 2). Dennett's test revealed that the degradation was high as compared to control in all treated soils except treatment T1 and T2 after 7 days of treatment while most significant results were observed in unsterilized soil amended with nitrogen source (Table 3). After 14 days in both soils i.e., sub soil and top soil, highly significant results were obtained from treatment T6 (Sterilized soil with carbaryl along with phosphorus source and with inoculum) and T8 (Sterilized soil with carbaryl along with Nitrogen source and with inoculum) whereas least degradation was observed in soil amended with both phosphorous and nitrogen sources simultaneously although the degradation in this treatment is also high as compared to control (Table 3). Significantly positive results were observed after 21 days of treatment and found that various treatments have enhanced the rate of degradation of carbaryl as compared to control. The pattern of degradation after 21 days was very much similar to treatments after 14 days and the rate of degradation has been increased to 85% in case of treatments T6 and T8 (Table 3).

Table 2. ANOVA showing degree of significance between different types of treatments.

Treatments	Variables	df	Significance
After 7 days (Top soil)	Between groups within groups total	8 18 26	0.017
After 14 days (Top soil)	Between groups within groups total	8 18 26	0.00
After 21 days (Top soil)	Between groups within groups total	8 18 26	0.00
After 7 days (Sub soil)	Between groups within groups total	8 18 26	0.13
After 14 days (Sub soil)	Between groups within groups total	8 18 26	0.00
After 21 days (Sub soil)	Between groups within groups total	8 18 26	0.00

Table 3. Dennett's Test showing the comparison of different treatments with each other after different time intervals.

Treatments	After 7 days (Top soil) µg/g	After 14 days (Top soil) µg/g	After 21 days (Top soil)	After 7 days (Sub soil)	After 14 Days (Sub soil)	After 21 days (Sub soil)
Control	7.76c	7.76f	8.04d	4.01d	7.78e	8.09c
T1	8.56bc	34.07b	69.96bc	5.42bcd	36.78ab	70.09bc
T2	9.01abc	32.20c	70.08bc	6.67bc	32.06bc	73.30b
T3	9.23ab	29.98d	60.06c	6.70bc	29.96c	69.06bc
T4	9.90ab	24.07e	60.06c	6.70bc	30.03c	68.98bc
T5	9.64ab	33.26bc	80.07ab	8.88ab	34.08b	80.06ab
T6	9.45ab	41.02a	85.0a	10.4a	40.10a	85.67a
T7	10.01a	34.98b	83.01a	8.07b	34.87b	80.02ab
T8	9.66ab	40.56a	83.67a	10.04a	39.98a	84.30a

Table 4. Dunnett's Test showing the comparison of different treatments w.r.t number of days.

No. of days	Control	T1	T2	T3	T4	T5	T6	T7	T8
After 7 days (Top soil)	7.76d	8.56c	9.0d	9.23d	9.9e	9.64c	9.6c	10c	9.66c
After 7 days (Sub soil)	4.01c	5.42d	6.7e	6.7e	6.7f	8.88c	10.4c	8c	10.0c
After 14 days (Top soil)	7.76d	34.07bc	32.3c	29.9c	24.0d	33.2b	41.0b	34.98b	40.5b
After 14 days (Sub soil)	7.78d	36.78bc	32c	29.9c	30.3c	34.0b	40.1b	34.87b	39.98b
After 21 days (Top soil)	8.04d	69.96a	70b	60.6b	60b	80.1a	85a	83.1a	83.7a
After 21 days (Sub soil)	8.09d	70.09a	73a	69.06a	68.98a	80a	85.7a	80.02a	84.3a

Dunnett's test has clearly showed significant difference between number of days and indicates positive effect on the degradation of carbaryl (Table 4). In case of each treatment degradation was significantly increased with respect to number of days of treatment (Table 4). After 21 days of treatment the most significant increase in treatment T6 and T8 was observed where after 21 days 85% carbaryl has been degraded. After 7 days about 10% carbaryl was degraded while after 14 days it reached in the range of 40 to 60% and after 21 days about 40 to 85% carbaryl has been degraded in different types of treatments (Table 4).

Results have shown that there is significant difference between different types of nutrients on the rate of degradation of carbaryl (Table 3). Addition of nitrogen and phosphorus significantly increase the degradation rate as compare to control (T6 & T8) while addition of simultaneous amendments of both sources (T4) at a time showed less significant results as compared to them (Table 3).

Discussion

No degradation was observed in case of sterilized soil and the level of pesticide was maintained throughout the experiment while degradation was observed in unsterilized soil. This was also favored by previous studies that showed that the soil bacteria play vital role in pesticide degradation (Hernandez *et al*., 2001; Chaudhry & Ali, 1988). The results have shown that the sterilized soil inoculated with bacterial consortia has effectively degraded carbaryl (Table 3). A number of bacteria have been isolated from soil which has ability to degrade carbamate pesticides (Chaudhry & Ali, 1988; Larkin & Day, 1986).

Addition of nutrients has increased the rate of degradation of carbaryl and nitrogen amended soil has most significant effect on the degradation of carbaryl which was about more than 85%. Previous findings showed that the nutrient addition stimulate the degradation of different types of pollutants. The degradation of oil was observed in field scale and was observed that the fertilizers increased the rate of degradation of carbaryl in soil (Roling *et al*., 2004; Roling *et al*., 2002). The effect of phosphorus addition on degradation of benzylamine and caprolactum by means of selected microbes has been studied and found that phosphorus addition positively increase the rate of degradation of both compounds (Steffensen & Alexander, 1995). Young-Sook *et al*., (2001) reported that adding nutrients at higher dose had a positive effect on oil degradation rate, which was especially obvious during the early phase of treatment. It was observed that the simultaneous addition of both nitrogen and phosphorus has decreased the rate of degradation of carbaryl. Previously findings also favored our results that nutrient addition did not always have positive effect on degradation of xenobiotics as the addition of phosphate, nitrate, or sulfate decrease the acclimation period for the mineralization of low concentration of p-notrophenol and 2,4-dichlorphenoxyacetate (Jones & Alexander, 1988). Many reasons are there behind this phenomenon it may be due to easily available sources, bacteria utilize these compounds instead of test compound as some researchers reported that the presence of other bacteria or other substrates may reduce the degradation of test compounds (Fredricksen 1977; Hernandez *et al*., 1991; Lewis, *et al*., 1984).

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