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Biodegradation of Quinolphos by Defined Microbial Consortium

Radha S[1], Devi Prasad AG[1*] and Manonmani HK[2]

[1]*Department of studies in Environmental Science, University of Mysore, Mysore, India*

[2]*Food Protectants and Infestation Control Department, CSIR-Central Food Technological Research Institute, Mysore – 570 020, Karnataka, India*

ABSTRACT

Quinolphos (O,O-diethyl O-quinoxalin-2-yl phosphorothiate) is a synthetic organo-phosphate, non-systemic, broad spectrum insecticide and acaricide, acting as a cholinesterase inhibitor in contact with stomach and respiratory system. A microbial consortium that can utilize quinolphos as a sole source of carbon and energy was isolated from soil through a novel technique involving an initial enrichment in a column reactor followed by enrichment in a shake flask. Exposure to Quinolphos and its metabolites can affect the human health. A diverse range of microorganisms responsible for Quinolphos degradation has been reported. In the present investigation, a new microbial consortium degrading Quinolphos has been developed. Factors such induction, inoculum level, concentration of the substrate, soil pH, soil moisture, etc., affecting degradation were also studied. Inoculum level of 500 µg protein/mL and a pH 7.5 at ambient temperature (26–28°C) resulted in degradation of Quinolphos upto 50 ppm.

Keywords: Quinolphos; Consortium; Soil; Inoculum

INTRODUCTION

Pesticides were applied to control the damages caused by the pest's important crop in agriculture field. The rapidly growing industrialization along with increasing population has resulted in the accumulation of a wide variety of chemicals. The pesticides that are exhaustively applied to the land surface travel long distances and can move downward reaching the water table at detectable concentrations reaching aquatic environments at significantly longer distances. Therefore, the fate of pesticides is often uncertain; they contaminate other areas that are distant from where they were originally used. Thus, decontaminating pesticide polluted areas is very complex task. Biological and biochemically mediated processes in soil and water are significant for ecosystem functions [1].

Quinolphos is a broad-spectrum organo phosphorous insecticide (acaricide), which is widely used in Indian agriculture for control of pests of various crops such as sugarcane, cotton, groundnut, rice, etc. [2-4]. It is reddish brown liquid. The chemical formula $C_{12}H_{15}N_2O_3P_5$ and IUPAC name 0,0 diethyl 0-quinoxalin-2yl-phosphorothioate. It is an inhibitor of acetyl cholinesterase (AChE) its toxicity increases with temperature [5,6]. Some of its degradation products are stronger inhibitor of AChE than the parent compound [7]. Ranked 'moderately hazardous' in WHO acute hazard ranking, use of quinolphos is either banned or restricted in most nations. The mammalian toxicity which is labeled in terms of LD50 (oral) to rats is 66 mg/kg [8]. Common formulations of Quinolphos are Emulsion concentrate, Granulars, Wettable powder, Dusting Powder, Ultra Low Volume sprays, encapsulated granulars etc.

Residues of Quinolphos in soil have been observed up to 40 days [9,10]. Photodegradation of quinolphos under sunlight and UV light have been reported by [11].

Quinolphos has been shown to be degraded by Pseudomonas strain up to 90.4% in the glucose, whereas up to 38.2% in the absence of glucose [12]. However, not much information is available on the accelerated biodegradation of

Quinolphos by the soil micro-organisms, which play a significant role in detoxifying pesticides in the environment. Hence the present study was undertaken to study the biodegradation of Quinolphos in soil by a defined microbial consortium.

MATERIALS AND METHODS

Substrate

Quinolphos (72% pure) was obtained from Rallis India, Bangalore, India. Other chemicals and the reagents used in this study were of analytical grade and were purchased from standard chemical companies.

Soil

Soil used for bioremediation studies was a red loamy soil collected from Mandya District, Karnataka with and without history of QP application with good water holding capacity and 1.0–1.5% organic matter. The soil was sieved to 2.00 mm size before use.

Microbial consortium

The microbial consortium capable of degrading Quinolphos was developed in our laboratory by long term enrichment of Quinolphos contaminated soil (having history of QP application) according to Manonmani et al. [13]. The Quinolphos degrading consortium that got enriched was acclimated with increase in concentration of Quinolphos from 5 to 25 ppm. The consortium thus obtained was maintained as liquid culture in minimal medium containing 10 ppm of Quinolphos and in minimal agar medium containing 10 ppm of Quinolphos.

Isolation and selection of Quinolphos degrading bacterial strains

The isolated consortium was grown in nutrient broth and individual cultures were isolated by dilution plating technique on nutrient agar. Then cultures were purified by repeated sub culturing on nutrient agar.

Taxonomic identification of the isolates

The isolates were identified based on biochemical and 16srRNA techniques.Initially, Quinolphos utilization by the individual isolates was monitored on minimal salt medium (MSM) agar plates containing Quinolphos (100 mg/L). Growth and Quinolphos degradation potential of the individual isolates was also observed in liquid cultures minimal medium supplemented with 100 mg/L Quinolphos is the only source of carbon and energy.

Preparation of defined microbial consortium

A bacterial isolates capable of growing on Quinolphos were grown in nutrient broth for 24 h, harvested by centrifugation, induced with 100 mg/L Quinolphos in minimal medium for 24- 48 h. The harvested cells were washed twice with sterile minimal medium and resuspended in minimal medium. The consortium was reconstituted by mixing the individual isolates at equal OD600. This was used as inoculums for bioremediation studies. The consortium was induced with 100 mg/L Quinolphos in mineral medium for 24 h in a rotary shaker (150 rpm) at ambient temperature (26–28°C). Minimal medium used in induction studies contained (g/L of distilled water), KH_2PO_4, 0.675; Na_2 HPO_4, 5.455; NH_4NO_3, 0.25. pH was maintained at 7.2.

BIOREMEDIATION OF QUINOLPHOS SPIKED SOIL

In plastic cups, 100 g sterile soil was taken (15%moisture) and spiked with 5, 10, 25 and 50 ppm of Quinolphos. This was inoculated with 24 h Quinolphos induced microbial consortium containing 10^8 cells of each organism. The cups were incubated at ambient temperature for five days and to maintain moisture 5 ml sterile water was added to each cup every alternate day. Samples were analyzed at regular intervals for residual substrate and to measure the growth of microbial biomass (colony forming unit). Soil without Quinolphos was used as control.

EFFECT OF SOIL pH ON DEGRADATION

In plastic cups, 10 µg mL^{-1} Quinolphos in minimal medium, added to 100 g sterile soil. The pH of the medium was adjusted by altering the ratio of KH_2PO_4 and Na_2HPO_4 (pH 5.0-8.0) or by the addition of 1 N HNO_3 or 1 N NaOH (to obtain pH 8.0, respectively). This was inoculated with 24 h Quinolphos- induced microbial consortium containing 10^8 cells of each organism. The cups were incubated at ambient temperature for five days and to maintain moisture 5 ml sterile water was added to each cup every alternate day. Samples were analysed at regular intervals for residual

substrate and to measure the growth of microbial biomass (colony forming unit). Soil without Quinolphos was used as control.

EFFECT OF SOIL MOISTURE ON DEGRADATION

Effect of soil moisture on degradation of Quinolphos was tested in sterile soil. Quinolphos was spiked at 10ppm concentration. The moisture of soil was adjusted separately to 5, 10, 15 and 20% with minimal medium. 24 h Quinolphos - induced microbial consortium containing 10^8 cells of each organism. The cups were incubated at ambient temperature for five days and to maintain moisture 5 ml sterile water was added to each cup every alternate day. Samples were analysed at regular intervals for residual substrate and to measure the growth of microbial biomass (colony forming unit). Soil without Quinolphos was used as control.

ANALYTICAL

Determination of growth

The survival and growth of the individual members of the consortial community was determined by estimating viable counts. Viable counts were made by plating suitably diluted soil suspensions on nutrient agar medium. The plates were incubated at 30°C for 72 h and the colonies of individual members of the consortium were counted [14].

Determination of residual substrate

The residual Quinolphos in soil was quantified by gas chromatography. After required incubation time, Soil (whole cup) was mixed well and air-dried. Soil was extracted with acetone followed by acetone: Ethyl acetate (1:1) and then by acetone: Ethyl acetate (5:95) and ethyl acetate alone. The solvent fractions were pooled, passed through florisil, evaporated and re-suspended in known volume of acetone. The recovery of Quinolphos was 95 ± 2%. Appropriately diluted acetone solution of residual Quinolphos was injected into gas chromatograph (Chemito100l) equipped with FID detector and glass column (1 mm• 3 mm) packed with 3% OV–17 plus 1.95 QF-1 on chromosor bW, 80–100 mesh. The column, injector and detector were maintained at 210, 250, 250°C respectively with a flow rate of carrier gas nitrogen at 30 ml min^{-1}. Under these conditions, the retention time of Quinolphos was 14.56 min.

RESULTS AND DISCUSSION

Development and acclimation of Quinolphos degrading consortium

The broth samples collected after 15 days from the column reactor were enriched in shake flasks for 2 weeks with transfers to fresh medium once in two days and a microbial consortium was established that could degrade Quinolphos. With this consortium, 5 and 10 µg mL^{-1} of Quinolphos disappeared in 3 days, 25 and 50 µg mL^{-1} of Quinolphos disappeared by 4 and 6 days (Figure 1). Growth increased steadily and reached maxima on days 2, 4, and 6 with the substrate concentrations of 5, 10, and 25 µg mL^{-1}, respectively. More than 95% of the added Quinolphos (10 µg mL^{-1}) was recovered from the un-inoculated control flasks, and, this indicated clearly that auto degradation did not occur under the culture conditions followed.

One milliliter of washed cell suspension (~5 µg of protein) was added to 25 mL of minimal medium containing 5, 10 (b), 25, and 50 µg mL^{-1} of Quinolphos and incubated at 30°C on a rotary shaker (150 rpm).

The above consortium was acclimated to increasing concentrations of Quinolphos by repeated transfers, at least two to three times, to fresh medium containing the same concentration of Quinolphos and then going to higher concentrations. The total cell biomass obtained was pelleted and washed with sterile phosphate buffer solution followed by a wash with minimal medium and used as the inoculum for the next culture. After a serial passage through different concentrations of Quinolphos, namely, 10, 25, 50 and 100 µg mL^{-1}, an acclimated microbial consortium was obtained. This consortium was then used to obtain defined microbial consortium.

The acclimated consortium consistently showed the presence of five distinct bacterial types. The bacterial members, *Pseudomonas stutzeri* (Chlc), *Pseudomonas aeruginosa* (Chlp), *Pseudomonas flurosences* (MCP), *Burkholderia pseudomallei* (Qlp), *Serratia spp.* (Qlpr) and their relative predominance was 15.8, 16.4, 18.3, 7.6 and 10.9% of the total bacterial counts, respectively.

Degradation of Quinolphos by pure cultures

All of the individual strains of bacteria from the consortium were found to utilize up to 5 µg mL^{-1} - Quinolphos as the sole source of carbon and energy. All of these pure cultures and even different combinations of them failed to completely degrade 25 µg mL^{-1} of Quinolphos even after induction. However, the consortium reconstituted by inclusion of all five cultures could degrade Quinolphos but needed acclimation for a long period to attain the ability to degrade higher concentrations. On the other hand, the acclimated consortium maintained as liquid culture in minimal medium containing Quinolphos (25 µg mL^{-1}) could adapt itself easily to higher concentrations of Quinolphos (data not shown).

Effect of soil pH on degradation

The soil pH is one of the important factor that affect pesticide adsorption, abiotic and biotic degradation Processes. The acclimated defined consortium was able to grow and degrade Quinolphos in all the pH values studied (pH 5.0-8.0). The degradation was slow at lower pH levels , i.e., at pH 5.0 and 6.0 degradation of Quinolphos took 72 h. At pH 7.0 and 8.0, 50 ppm of Quinolphos was degraded by 36 h (Figure 2). The bacterial isolates grew well in pH values 7 and 8 (Table 1).

Bioremediation of Quinolphos contaminated soil

Sterile soil spiked with different concentrations of Quinolphos was treated with Quinolphos degrading defined microbial consortium. At low concentrations of 5 µg Quinolphos g^{-1} soil, the degradation was complete by 24 h. At 10 ppm Quinolphos there was initial slow rate of degradation by the degradation picked up and 10ppm was completely degraded by 24 h. At higher concentrations of Quinolphos, i.e., at 25 µg Quinolphos g^{-1} soil, the time taken for degradation increased (Figure 3) to 36 h. There was a gradual reduction in the concentration of Quinolphos after the addition of the microbial consortium. At 50 µg Quinolphos g^{-1} soil, the degradation took still more time, i.e., 72 h was required for degradation of Quinolphos, There was no degradation of Quinolphos in uninoculated soil with increase in incubation period.

The cell population increased indicating the utilization of Quinolphos as a source of carbon and energy. In the inoculated soil without Quinolphos practically no growth was observed (Table 2).

Degradation of Quinolphos by microbial consortium in native soil

The degradation of Quinolphos was very low by native organisms (uninoculated non-sterile control soils). More than

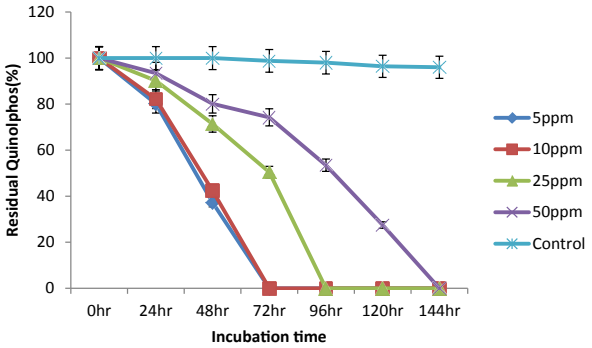

Figure 1: Degradation of different concentrations of Quinolphos by the unacclimated microbial consortium

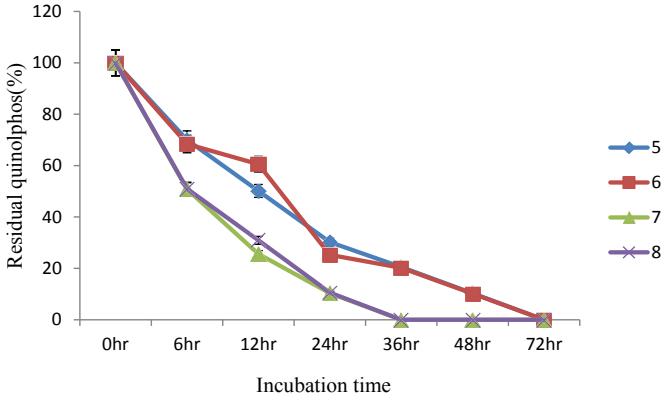

Figure 2: Effect of soil pH on the degradation of quinolphos

Table 1: Survivability of individual members of the consortium during degradation of Quinolphos

	Log of CFU at pH values			
	5	6	7	8
0 h	10.13 ± 0.21	9.33 ± 0.12	11.21 ± 0.20	10.65 ± 0.21
6 h	13.45 ± 0.11	12.32 ± 0.16	15.22 ± 0.19	13.29 ± 0.17
12 h	19.32 ± 0.18	17.35 ± 0.23	19.24 ± 0.07	17.57 ± 0.36
24 h	21.21 ± 0.13	24.36 ± 0.18	27.46 ± 0.36	23.46 ± 0.16

Table 2: Survivability of individual members of the consortium during degradation of different concentrations of Quinolphos

	Log of CFU at different concentration of Quinolphos			
	5 ppm	10 ppm	25 ppm	50 ppm
0 h	9.42 ± 0.09	10.18 ± 0.16	12.17 ± 0.16	11.46 ± 0.21
6 h	13.42 ± 0.33	13.79 ± 0.18	15.21 ± 0.16	13.52 ± 0.16
12 h	18.41 ± 0.25	19.41 ± 0.17	18.47 ± 0.08	17.69 ± 0.17
24 h	21.38 ± 0.18	23.34 ± 0.19	26.58 ± 0.16	25.56 ± 0.23

Table 3: Effect of soil moisture on cell viability during Quinolphos degradation

	Log of CFU at different moisture values			
	5%	10%	15%	20%
0 h	9.62 ± 0.27	9.68 ± 0.27	10.67 ± 0.14	11.89 ± 0.46
6 h	13.81 ± 0.44	11.89 ± 0.12	13.27 ± 0.16	15.69 ± 0.29
12 h	15.40 ± 0.15	14.58 ± 0.20	17.67 ± 0.17	19.41 ± 0.27
24 h	18.58 ± 0.07	20.36 ± 0.19	27.78 ± 0.13	24.37 ± 0.21

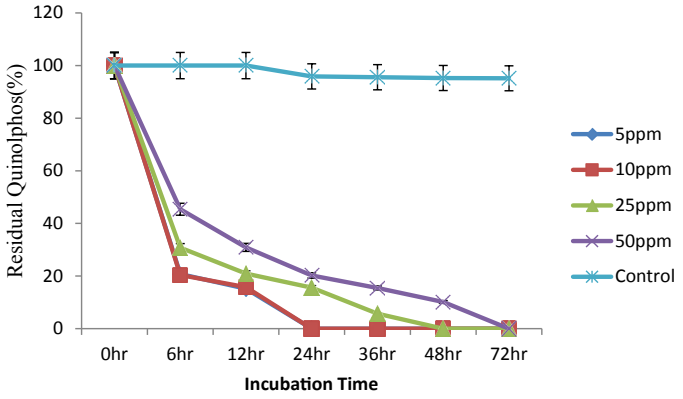

Figure 3: Degradation of Quinolphos by microbial consortium in sterile soil

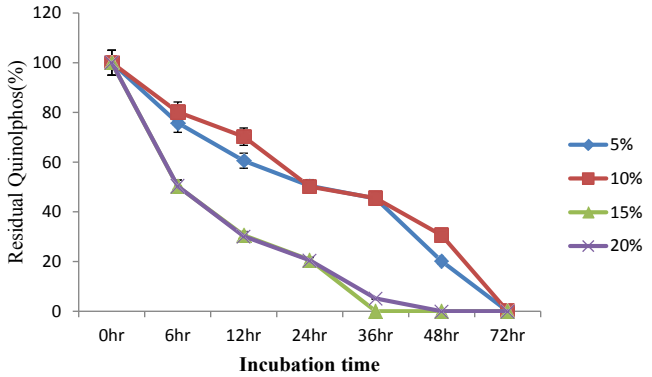

Figure 4: Effect of soil moisture on the degradation of Quinolphos

80% of the substrate was found to be remaining even after 72 h of incubation. Addition of the microbial consortium enhanced the degradation of Quinolphos . Degradation of Quinolphos in non-sterile soil inoculated with consortium was complete in par with sterile soil (data not shown).

Effect of soil moisture on degradation

The moisture content in soil acts as solvent for pesticide movement and is essential for microbial functioning. Effect

of soil moisture on degradation of Quinolphos was tested in sterile soil. At lower moisture level of 5%, the degradation was by 72 h of incubation. With increase in moisture content degradation appeared to be complete. At 10% moisture content added Quinolphos was degraded by 72 h of incubation. Only 36 and 48 h were needed for the degradation of added Quinolphos at 15 and 20% soil moisture levels (Figure 4).

The cell population increased indicating the utilization of Quinolphos as a source of carbon and energy. The cell population also showed the same trend. At lower moisture levels, the growth was low. It increased with increase in moisture content (Table 3).

CONCLUSION

The biodegradation of Quinolphos is strongly related to soil pH, soil moisture and degradation is microbial and not due to abiotic hydrolysis. In our studies, bacterial isolates capable of degrading Quinolphos was isolated from the soil, acclimated and complete degradation was shown to occur. These studies suggested that further experiments are required to determine the extent of degradation of Quinolphos in actual field.

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