

Original Research Article

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## Elimination of Inhibitory Effects of Chlorpyrifos and Quinolphos on Radish and Green Gram Seed Germination by Bioremediation of Contaminated Soil: A Comparative Study

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### ABSTRACT

#### Keywords

Chlorpyrifos,  
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#### Article Info

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The effects of Chlorpyrifos and quinolphos on the germination of radish and green gram seeds were tested. Both radish and green gram showed marked reduction in germination percentage and seedling vigour index. The abnormalities and reduction in germination increased with increasing concentration of these pesticides studied. At 50 $\mu$ g of Chlorpyrifos and quinolphos level the germination of radish and green gram seeds were inhibited almost completely on moist filter paper and soil. Protease and amylase activities were reduced in seeds grown on soil spiked with Chlorpyrifos and quinolphos. Bioremediation of Chlorpyrifos and quinolphos-spiked soils with a Chlorpyrifos and quinolphos-degrading microbial consortium helped in eliminating the toxic effects of these pesticides towards seed germination. The degradation of 50 $\mu$ g Chlorpyrifos and quinolphos in soil was complete by 24 hrs. The seed germination and the activities of the assayed enzymes, amylase and protease, were same as before or better in bioremediated soils.

### Introduction

Application of the pesticide on agricultural crop has been a well known practice and is a vital aspect of integrated pest management (IPM) strategies. However it has adversely affected the characteristics of the soil by altering the pH of the soil that has indirectly effected the microbial activities of soil bacteria (Rahman and Motoyama, 2000; Malinowski, 2000).

Out of the pesticide sprayed in to the crop, approximately less than 0.1% of applied pesticide reaches the target pest, leaving the bulk to affect the environment (Ardley, 1999). One of the major environmental trepidations with pesticides is their capacity to affect soil, because of their persistence and mobility in the soil (Walker, 2003). Factors such as the mode of action of the

active ingredient of pesticide, the defense mechanism of the treated insects, the type of formulation, its systemic properties, mode of application, the moisture content and temperature of soil influence the efficacy of pesticide applied (Rehman and Motoyama, 2000).

Synthetic organophosphates (OPs) are the most widely used insecticides, accounting for an estimated 34% global insecticide sales (Singh and Walker 2006, Singh 2009). Most organophosphorus insecticides (OPs) share a similar structure and have acute neurotoxicity attributing to their ability to suppress acetylcholinesterase (AChE) (Anwar et al 2009). Chlorpyrifos, [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is one of the most extensively used broad-spectrum Ops and it is used worldwide to control a variety of chewing and sucking insect pests and mites on a range of economically important crops, including citrus fruit, bananas, vegetables, potatoes, coffee, cocoa, tea, cotton, wheat, rice, and soon (Thengodkar and Sivakami 2010). It is also used on lawns, ornamental plants, animals, domestic dwellings as well as commercial establishments (Racke 1993). Chlorpyrifos has been one of the pesticides most used worldwide since 1965. Contamination of soil by Chlorpyrifos could result from bulk handling in the farmyard, and rinsing of containers and accidental release may occasionally lead to the contamination of surface and groundwater. Reports from the Environmental Protection Agency suggest that a wide range of water and terrestrial ecosystems may be contaminated with Chlorpyrifos (Anonymous. 1998, EPA 1997). Chlorpyrifos, can easily enter the human food chain and has more victims to its credit than carcinogenic air pollutants. A strong link between prenatal exposure to chlorpyrifos and low birth weight and

smaller head size of infants has been reported (Bhagobaty et al 2006). The use of chlorpyrifos has been vastly restricted in US and some European countries, even for agricultural purposes. However, it is still widely used in developing countries like India, where in the year 2000, it was the fourth highest consumed pesticide after monocrotophos, acephate and endosulfan. (Ansaruddin and Vijayalakshmi 2003). It has been reported that repeated application of chlorpyrifos to the soil did not result in the development of a microbial population with the enhanced ability to degrade the pesticide (Singh *et al.*, 2003).

Microbial degradation of organophosphate pesticides is of particular interest because of the high mammalian toxicity of such compounds and their widespread and extensive use. Although chlorpyrifos has been widely used for agricultural and household pest control since 1965, it has been tricky isolating a degrading strain for this organophosphate. Several attempts to isolate a chlorpyrifos-degrading microbial system by repeated treatments or enrichment of soils and other media with chlorpyrifos have not been successful (Mallick et al 1999, Racke, et al 1990). The resistance of chlorpyrifos towards enhanced degradation in soil has been ascribed as a reasons for this failure. Co-metabolic degradation of chlorpyrifos by *Flavobacterium* sp. and by a clone of *Escherichia coli* (with an *opd* gene) has been reported in liquid media (Mallick et al 1999, Richnis et al 1997, Wang et al 2002). However these microbes did not utilize chlorpyrifos as a source of energy. Mallick et al. (Mallick et al 1999) reported degradation of chlorpyrifos by *Arthrobacter* species in mineral salt medium. However, the prevalence of degrading strains in soil and the pathway of degradation of chlorpyrifos by this bacterium in liquid culture are unknown.

Quinalphos (O,O-diethyl-O-quinoxalin-2-yl-phosphorothioate), also an organophosphate pesticide (OP), is used extensively in controlling the pests of a variety of crops including fruits and vegetables (Hayes and Laws 1991). The large scale use of quinalphos (QP) poses a health hazard to animals and humans because of its persistence in the soil and crops (Murphy 1980; Katti and Verma 1992).

Administration of QP to pregnant rats at doses of  $1.5 \text{ mg kg}^{-1}$  body weight produced inhibition of AchE activity in foetal brain and placenta, indicating the possible transfer of the pesticide from dams to fetuses (Srivastava et al. 1992). Quinalphos residues in soil have been observed up to 40 days. (Gajbhiye et al. 1995; Babu et al. 1998). Photodegradation of quinalphos under sunlight and UV light has been studied (Goncalves et al. 2006).

Megharaj et al. (1987) have studied the metabolism of quinalphos by soil algae but could not identify its metabolites. A strain of *Pseudomonas* has been shown to degrade quinalphos up to 90.4% in the presence of glucose, whereas up to 38.2% in the absence of glucose (Pawar and Mali 2014). However, not much information is available on the biodegradation of quinalphos by the soil micro-organisms, which play a significant role in detoxifying pesticides in the environment.

However, there are no reports on the effects of Chlorpyrifos and quinalphos on crop seed germination. Deleterious effects of Chlorpyrifos and quinalphos on the germination of few seeds were studied. Attempts were made to study the toxic effects of Chlorpyrifos and quinalphos on radish and green gram seeds and elimination of inhibitory effect of Chlorpyrifos and quinalphos on germination by microbial remediation of contaminated soil.

## **Materials and Methods**

### **Chemicals**

Chlorpyrifos and quinalphos was obtained from Rallis India, Bangalore, India and 2,3,5-triphenyltetrazoliumchloride (TTC) was obtained from SISCO Research Laboratories, India. All other chemicals used in the cultivation medium and reagents were of analytical grade and were purchased from standard companies.

### **Seeds and Soil**

Seeds of radish and green gram were obtained from Hind seeds Co., Faizabad, India. Soil was collected from Mandya Dist., Karnataka. The soil was a red loamy type with good waterholding capacity and 1.0–1.5% organic matter. The soil was sieved to 2.00 mm size before use.

### **Seed Germination Test by Filter Paper Method**

The seed germination test was carried out on moist filter paper (ISTA, 1985) as well as in soil. Seeds of radish and green gram, surface sterilized using 2% mercuric chloride, were placed on Whatman No. 1 filter paper discs (at 9.00 cm, sterile) kept in petriplate (10.0cm dia) and moistened with minimal medium containing Chlorpyrifos and quinalphos separately at different concentrations. In each petriplate 10 seeds were germinated at ambient temperature (26–28 °C) under 12 h–12 h cycles of light and darkness. For each concentration of Chlorpyrifos /quinalphos, 5 replicates of 10 seeds were taken. Controls were maintained on the filter paper moistened with minimal medium. To maintain moisture 1 ml of sterile distilled water was added every alternate day. The seedlings were

evaluated for germination and seedling vigour after five to seven days.

### **Seed Germination Test on Soil**

Germination on soil was carried out in plastic cups (8.0 cm dia) filled with 100 g sterile soil. To each cup different concentrations of Chlorpyrifos / quinolphos were added as acetone solution and mixed well to obtain uniform distribution. Then required quantity of mineral solution was added to obtain final moisture level of 20%. To the control cups same quantity of acetone solution was added and mixed well. The sides of the plastic cups were pricked with a needle to facilitate aeration and in each cup 10 seeds were sown at a depth of 0.5 cm. Ten replicates were taken for each concentration. The cups were kept at ambient temperature (26–28 °C) in a germinator. 5 ml sterile distilled water was added to each cup every alternate day to maintain moisture. After five to seven days seedlings were counted and their root and shoot lengths were measured. The seedling vigour was calculated as (mean root length + mean shoot length) · (percentage germination/10) (Bidlan et al 2003).

### **Seed Viability Test**

Seed viability tests were done in both soil and in suspension. For this 10 seeds were sown in sterile soil containing Chlorpyrifos and quinolphos separately at different concentrations, for different periods of time. The sown seeds were excised along the margin to expose the embryo and placed in 0.1% of TTC for 24 h at 37°C in darkness. The seeds were then removed from TTC solution, washed with distilled water and soaked in 10 ml of 95% ethyl alcohol until the entire colour was extracted. The absorbance of the extracted red colour (triphenylformazan) was determined at 480 nm. The studies for the viability tests were

also done in replicates of 10 cups (Bidlan et al., 2003). To study the viability of seeds in suspension condition, 10 seeds were soaked in petriplates in minimal medium containing different concentrations of Chlorpyrifos / quinolphos, from 0 to 50 µg ml<sup>-1</sup>. The viability of the seeds was tested as given above.

### **Preparation of Bacterial Inoculum**

The microbial consortium capable of degrading chlorpyrifos and quinolphos was isolated in the laboratory by long-term enrichment technique (Manonmani et al., 2000). Individual strains of Chlorpyrifos and quinolphos degrading consortium, *Pseudomonas stutzeri* (Chlc), *Pseudomonas aeruginosa* (Chlp), *Pseudomonas fluorescens* (MCP), *Burkholderia pseudomallei* (Qlp), *Serratia rubidieae* (Qlpr) were grown in nutrient broth, for 24 h in a rotary shaker at ambient temperature (26–28 °C). The cells were harvested by centrifugation at 10000 rpm for 10 min at 4°C. The consortium was reconstituted by mixing the individual isolates at equal OD<sub>600</sub> and the consortium was induced with 10 ppm of Chlorpyrifos and quinolphos separately in mineral medium for 24 h in a rotary shaker (150 rpm) at ambient temperature (26–28°C). The cells were harvested by centrifugation at 10000 rpm for 10 min at 4°C, resuspended in known volume of mineral medium and used in bioremediation studies. Minimal medium used in induction studies contained (g<sup>-1</sup> of distilled water), KH<sub>2</sub>PO<sub>4</sub>, 0.675; Na<sub>2</sub>HPO<sub>4</sub>, 5.455; NH<sub>4</sub>NO<sub>3</sub>, 0.25; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.20; Ca(NO<sub>3</sub>)<sub>2</sub>, 0.10.

### **Bioremediation of Chlorpyrifos and Quinolphos Spiked Soils**

In plastic cups, 100 g sterile soil was taken (15% moisture) and spiked with 50 µg Chlorpyrifos and quinolphos g<sup>-1</sup> soil

separately. This was inoculated with 24 h induced microbial consortium containing  $10^8$  cells of each organism. The cups were incubated at ambient temperature for Seven 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 (cfu). After seven days the seeds were sown as described above and germinated at ambient temperature. Seedlings were evaluated for germination and vigour after five to seven days. Soil without Chlorpyrifos and/or quinolphos was used as control.

### **Analytical**

#### **Assay for the Indicative Enzymes of Germination**

To get some insight into the mechanism of inhibition of germination of radish and green gram seeds by Chlorpyrifos and/or quinolphos, some representative enzymes such as amylases and proteases were assayed. These two enzymes were chosen as they are normally involved in the mobilization of stored nutrients of the seeds during germination (Koller et al., 1962). Studies were conducted with seeds sown in soil and also with seeds soaked in Chlorpyrifos and/or quinolphos. seeds in triplicates, 50 each for treated or untreated(control), were collected every 24 h for five days and ground well with sterile, acid washed sand in a mortar at 4°C (ice bath) for 15 min. The extract was prepared in 0.2 M acetate buffer (pH 5.2) and the debris was removed by centrifugation at 10000 rpm for 10 min at 4°C. The supernatant was made up to 5 mL.

Amylase activity in the seed extract was determined by measuring the release of reducing sugar from gelatinized soluble starch (1% in 0.1 M acetate buffer),

(Bernfeld, 1955). Enzyme activity was expressed as specific activity (activity  $\text{mg}^{-1}$  protein). Protease activity was measured using bovine serum albumin (BSA) ( $10 \text{ mg mL}^{-1}$ ) as substrate (Laskowsky, 1955). To BSA, equal volume of enzyme extract was added and reacted for 30 min at 30°C. Enzyme activity was measured at 660 nm after colour development of BSA hydrolysate with Folin-Ciocalteu reagent. Protease activity was expressed as activity  $\text{mg}^{-1}$  protein.

### **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 (Bidlan et al., 2004).

### **Determination of Residual Substrate**

The residual Chlorpyrifos and quinolphos in soil was quantified by gas chromatography. Soil collected at regular intervals was air-dried. One gram of soil was extracted with acetone followed by acetone : ethyl acetate (1:1) and then by acetone : ethyl acetate (10:90) and ethyl acetate alone. The solvent fractions were pooled, passed through florisil, evaporated and resuspended in known volume of acetone. The recovery of Chlorpyrifos and/or quinolphos was  $95 \pm 2\%$ . Appropriately diluted acetone solution of residual Chlorpyrifos and/or quinolphos was injected into gas chromatograph (Chemito10001) equipped with FID detector and glass column (1m $\times$  3 mm)packed with 3% OV-17 plus 1.95 QF-1 on chromosorb W, 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<sup>-1</sup>. Under these conditions, the retention time of Chlorpyrifos and quinolphos were 4.25 and 14.56 min respectively.

## **Results and Discussion**

### **Effect of Chlorpyrifos on Seeds of Radish and Green Gram**

Effect of different concentrations of Chlorpyrifos on the germination and seedling vigour of members of radish (Brassicaceae) and green gram (Leguminosae), were studied. The seeds of both the families tested were found to be effected by the addition of the insecticide. The seeds of radish and green gram showed more susceptibility.

### **Effect of Quinolphos on Seeds of Radish and Green Gram**

Effect of different concentrations of quinolphos on the germination and seedling vigour of members of radish (Brassicaceae) and green gram (Leguminosae), were also studied. The seeds of both the families tested were found to be effected by the addition of the quinolphos. The seeds of radish and green gram showed more susceptibility.

The effect of different concentrations of Chlorpyrifos on the germination and seedling vigour of radish and greengram seeds was studied by the filter paper method as well as in soil. Radish seeds were more susceptible towards toxicity of Chlorpyrifos towards germination. The inhibitory effect towards germination increased with increase in concentration of Chlorpyrifos. A low percentage of germination was observed at 50 µg chlorpyrifos level (Table 1) 50% and 0% germination was observed at 50 µg

Chlorpyrifos concentration with radish seeds by filter paper and soil methods respectively whereas, in green gram seeds the germination percentage was 100% and 100% by filter paper and soil methods respectively. A varying percentage of germination of these seeds was observed at different concentrations of chlorpyrifos under both paper and soil conditions. A considerable reduction in seedling vigour was observed in all concentrations Table1. With increasing concentration of Chlorpyrifos in soil the mean root lengths of the seedlings in radish was reduced by 81.20%, 77.52% and 41.9% of that of control seedlings from 10, 25 and 50µg Chlorpyrifos respectively.

Similarly, the shoot lengths were reduced by 55.3%, 49.9% and 36.29% respectively at concentrations from 2 to 50 µg Chlorpyrifos. Likewise in green gram seedlings also the reduced shoot and root lengths were observed. The reduction in root length observed was measured as 77.9%, 49.6% and 34.3% and shoot length, 78.7%, 64.5% and 56.01% respectively at 10, 25 and 50µg as compared to that of control. Reductions in growth of that of control were observed in green gram seeds with increase in Chlorpyrifos concentration from 10 to 50 µg respectively. The deleterious effects such as reduced shoot and root lengths, reduction in the formation of secondary roots, reduction in root hairs and delayed germination were observed at higher concentrations of Chlorpyrifos (Plates 1–4). Also negative geotropism, non-emergence of primary leaves from coleoptiles, thickening near the junction of root and shoot were observed.

The effect of different concentrations of quinolphos on the germination and seedling vigour of radish and greengram seeds was studied by the filter paper method as well as in soil. The inhibitory effect towards

germination increased with increase in concentration of quinolphos. A low percentage of germination was observed at 50 µg quinolphos level (Table 1). No germination was observed at 50 µg quinolphos concentration with radish seeds by filter paper and soil method respectively, whereas in green gram seeds the germination percentage was 100% and 66% respectively by filter paper and soil methods. A varying percentage of germination of these seeds was observed at different concentrations of quinolphos under both paper and soil conditions.

A considerable reduction in seedling vigour was observed in all concentrations (Table 1). With increasing concentration of quinolphos in soil the mean root lengths of the seedlings in radish was reduced by 87.6%, 24.2% and 0.00% of that of control seedlings from 10, 25 and 50µg quinolphos respectively. Similarly, the shoot lengths were reduced by 52.2%, 45.79% and 0.00% respectively at concentrations from 10 to 50 µg quinolphos. Likewise in green gram seedlings also the reduced shoot and root lengths were observed. The reduction in observed root length was measured as 38.19%, 34.5% and 24.9% and shoot length, 63.9%, 47.7% and 17.1% respectively as compared to that of control.

Reductions in growth of that of control were observed in green gram seeds with increase in quinolphos concentration from 10 to 50 µg respectively. The deleterious effects such as reduced shoot and root lengths, reduction in the formation of secondary roots, reduction in root hairs and delayed germination were observed at higher concentrations of quinolphos(Plates 5-8). Also negative geotropism, non-emergence of primary leaves from coleoptile, thickening near the junction of root and shoot were observed.

### **Effect of Chlorpyrifos on the Enzyme Activities of Germinating Radish and Green Gram Seeds**

The amylase activity was less in Chlorpyrifos treated seeds as compared to the control (untreated seeds) by the method used in this study. The activity decreased with increase in Chlorpyrifos concentration. Fairly good amylase activity was observed in control seeds. There was an increase in enzyme activity during the first 24 h after sowing and a further increase in activity up to 48 h for both radish and green gram seeds (Fig. 1a and 1b) and the activity decreased thereafter. The seeds sown in soil spiked with chlorpyrifos showed marked reduction in enzyme activity. Similarly there was marked decrease in protease activity in seeds sown in chlorpyrifos treated soil as compared to its control seeds (Fig. 2a and b). The activity was optimum at 48h of growth, which receded or remained same thereafter.

### **Effect of Quinolphos on the Enzyme Activities of Germinating Radish and Green Gram Seeds**

The amylase activity was less in quinolphos treated seeds as compared to the control (untreated seeds). The activity decreased with increase in quinolphos concentration. Fairly good amylase activity was observed in control seeds. There was an increase in enzyme activity during the first 24 h after sowing and a further increase in activity up to 48 h for both radish and green gram seeds (Fig. 1a and 1b) and the activity decreased thereafter. The seeds sown in soil spiked with quinolphos showed marked reduction in enzyme activity. Similarly there was marked decrease in protease activity in seeds sown in quinolphos treated soil as compared to its control seeds (Fig. 2a and 2b). The

activity was optimum at 48 h of growth, which receded or remained same thereafter.

### **Viability of Radish and Green Gram Seeds Exposed to Chlorpyrifos**

The viability of radish and green gram seeds after exposure to different concentrations of Chlorpyrifos was determined by TTC test. The losses in the viability with increasing amount of Chlorpyrifos are given in Fig. 3

There was a loss in viability with increase in concentration of Chlorpyrifos. The loss of viability was 43.38%, 51.43% and 64.6% at 10, 25 and 50  $\mu\text{g}$  Chlorpyrifos level respectively in greengram and 46.24%, 55.38% and 66.43% at 10, 25 and 50  $\mu\text{g}$  Chlorpyrifos level respectively in radish seeds. The loss of viability to convert TTC to triphenylformazan was observed at all concentrations studied. With the maximum effect being observed at 50  $\mu\text{g}$  chlorpyrifos  $\text{g}^{-1}$  soil.

### **Viability of Radish and Green Gram Seeds Exposed to Quinolphos**

The viability of radish and green gram seeds after exposure to different concentrations of quinolphos was determined by TTC test. The losses in the viability with increasing amount of quinolphos are given in Fig. 4. There was a loss in viability with increase in concentration of quinolphos.

The loss of viability was 59.51%, 85.1% and 99.89% at 10, 25 and 50  $\mu\text{g}$  quinolphos level respectively in greengram and 53.96%, 100% and 100% at 10, 25 and 50  $\mu\text{g}$  quinolphos level respectively in radish seeds. The loss of viability to convert TTC to triphenylformazan was observed at all concentrations studied with the maximum effect being observed at 50  $\mu\text{g}$  quinolphos  $\text{g}^{-1}$  soil.

### **Bioremediation of Chlorpyrifos and Quinolphos Contaminated Soil**

Sterile soil spiked with 100ppm of Chlorpyrifos and quinolphos separately was treated with Chlorpyrifos and/or quinolphos degrading microbial consortium. The degradation of Chlorpyrifos and quinolphos were almost complete with increase in incubation time. The degradation started without any lag. Around 10-12% degradation was observed in the early 2h of incubation. Degradation picked up with time and 50% of the added substrates were degraded by 6-8h of incubation. The added substrate was almost completely degraded by 72h of incubation. There was no degradation of both Chlorpyrifos and quinolphos in uninoculated soil with increase in incubation period. The cell population increased indicating the utilization of Chlorpyrifos and quinolphos as a source of carbon and energy. In the inoculated soil without Chlorpyrifos and quinolphos practically no growth was observed. The degradation of Chlorpyrifos and quinolphos was very low by native organisms (uninoculated non-sterile control soils). More than 80% of the substrate was found to be remaining after 120 h of incubation. Addition of the microbial consortium enhanced the degradation of Chlorpyrifos and quinolphos. Degradation of quinolphos in non-sterile soil inoculated with consortium was complete *in par* with sterile soil. In soils, bioremediated with the Chlorpyrifos degrading microbial consortium, normal germination and seedling vigour of both radish and green gram seeds was observed (Table 2). The amylase and protease activities were either same or slightly more than control seeds (Figs. 1 and 2). It is evident that the chlorpyrifos spiked in soil is degraded either completely or to non toxic metabolites by the chlorpyrifos degrading microbial consortium.



**Table.1** Effect of Chlorpyrifos and Quinolphos on the Germination and Seedling Vigour of Radish and Green Gram Seeds Tested in Soil as well as by Filter Paper Method Radish

Concentration ( $\mu\text{g g}^{-1}$ soil)	chlorpyrifos				Quinolphos			
	Germination (%)		Vigour index*		Germination (%)		Vigour index*	
	FP	Soil	FP	Soil	FP	Soil	FP	Soil
Control	100	<b>100</b>	114.4	81.86	100	<b>100</b>	119.4	68.56
10ppm	36	<b>36</b>	10.62	18.792	8	<b>28</b>	0.064	12.376
25ppm	6	<b>18</b>	0.12	8.676	0	<b>8.0</b>	0.006	2.316
50ppm	0	<b>8.0</b>	0	2.496	0	<b>0</b>	0	0

**Green Gram**

Concentration ( $\mu\text{g g}^{-1}$ soil)	chlorpyrifos				Quinolphos			
	Germination (%)		Vigour index*		Germination (%)		Vigour index*	
	FP	Soil	FP	Soil	FP	Soil	FP	Soil
Control	100	<b>100</b>	152.2	214.26	100	<b>100</b>	157.96	207.406
10ppm	100	<b>100</b>	81	169.08	100	<b>100</b>	68.2	117.3
25ppm	100	<b>100</b>	69.8	129.4	100	<b>94</b>	50.8	85.634
50ppm	100	<b>100</b>	50.4	106.98	100	<b>66</b>	35.4	26.532

FP=filter paper method.

Duncan's multiple range test (DMRT) was used to determine means, which differ significantly at  $P < 0.05$ .

\*Vigour index = (mean root length+mean shoot length) x (percent germination/10)

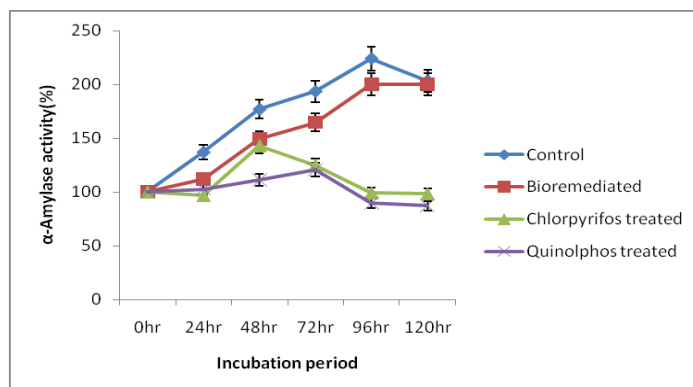
**Table 2** Seed Germination and Seedling Vigour of Radish and Green Gram Seeds in Chlorpyrifos Bioremediated Soil

Seed type	Radish			Green gram		
	Control	Treated	Bioremediated	Control	Treated	Bioremediated
Germination (%)	100	8	100	100	100	100
Vigour Index	81.86	2.49	109.18	214.26	106.98	251.68

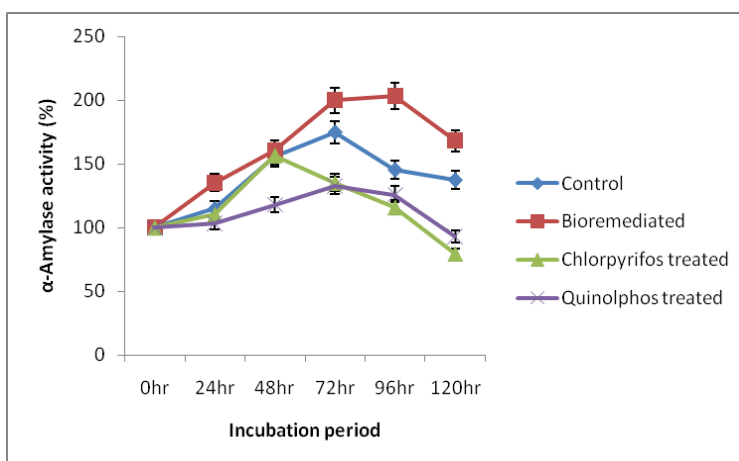
**Table 3** Seed Germination and Seedling Vigour of Radish and Green Gram Seeds in Quinolphos Bioremediated Soil

Seed type	Radish			Green gram		
	Control	Treated	Bioremediated	Control	Treated	Bioremediated
Germination (%)	100	8	100	100	66	100
Vigour Index	68.65	2.316	90.1	207.406	26.532	231.00

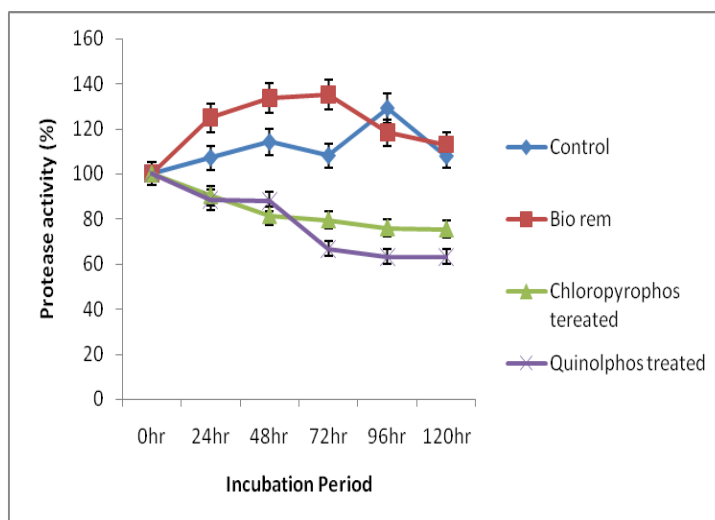
**Fig.1a** Effect of Chlorpyrifos and Quilophos on  $\alpha$ -amylase Activity of Green Gram Seeds



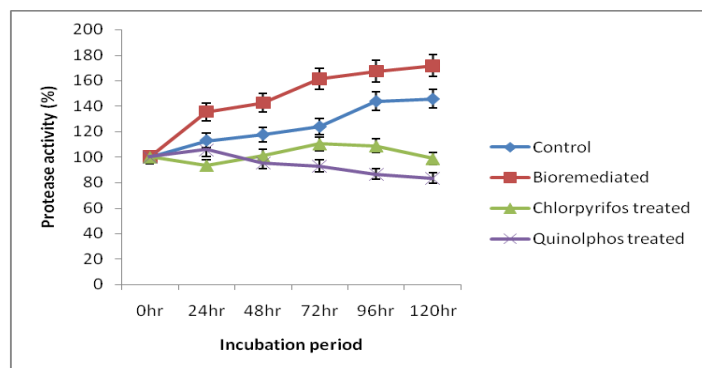
**Fig.1b** Effect of Chlorpyrifos and Quilophos on  $\alpha$ -amylase Activity of Radish Seeds



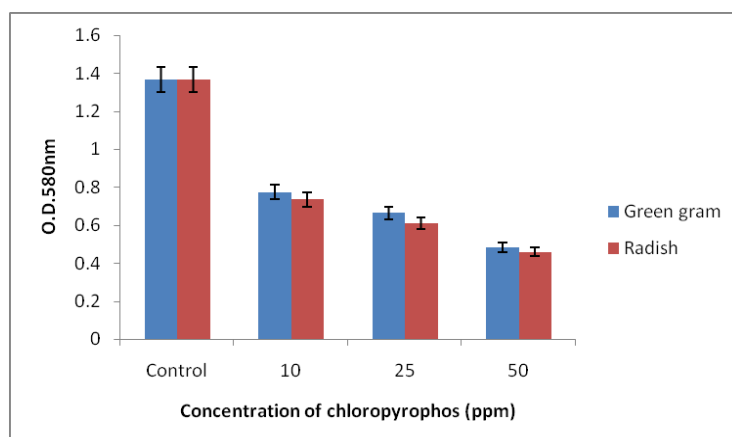
**Fig.2a** Effect of Chlorpyrifos and Quilophos on Protease Activity of Green Gram Seeds



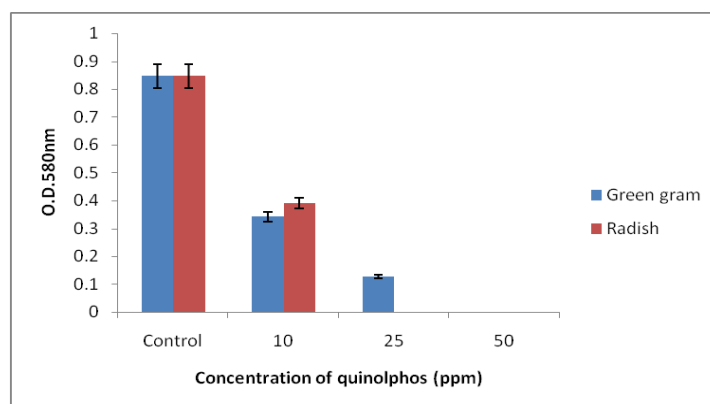
**Fig.2b** Effect of Chlorpyrifos and Quinolphos on Protease Activity of Radish Seeds



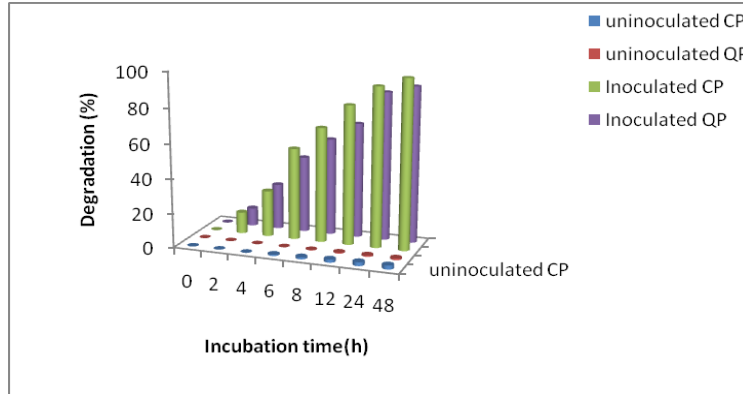
**Fig.3** Effect of Chlorpyrifos on the Viability of Radish and Green Gram Seeds as Tested by TTC



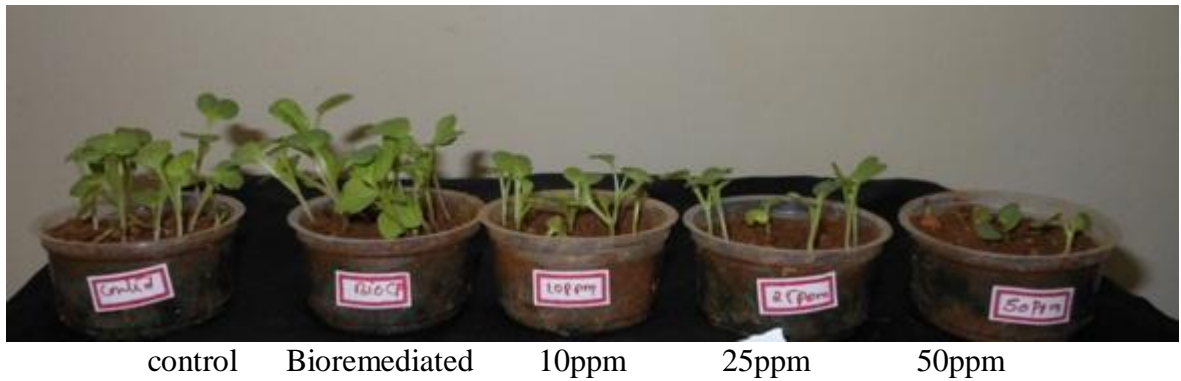
**Fig.4** Effect of Quinolphos on the Viability of Radish and Green Gram Seeds as Tested by TTC



**Fig.5** Biodegradation of Soil Spiked with Chlorpyrifos and Quinolphos



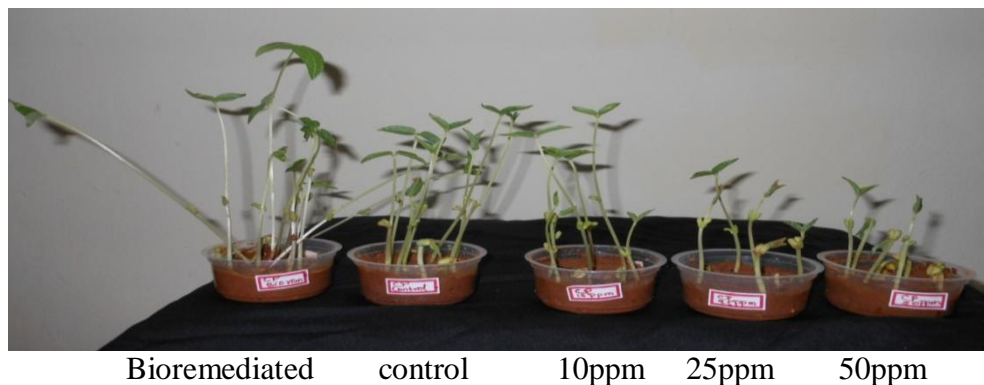
**Plate.1** Radish seeds grown in soil spiked with Chlorpyrifos. (1) Control without Chlorpyrifos (2) Bioremediated soil (3) 10 µg Chlorpyrifos per gram soil; (4) 25 µg Chlorpyrifos per gram soil; (5) 50 µg Chlorpyrifos per gram soil; showing the effect of Chlorpyrifos on the development of the roots and shoots in each case



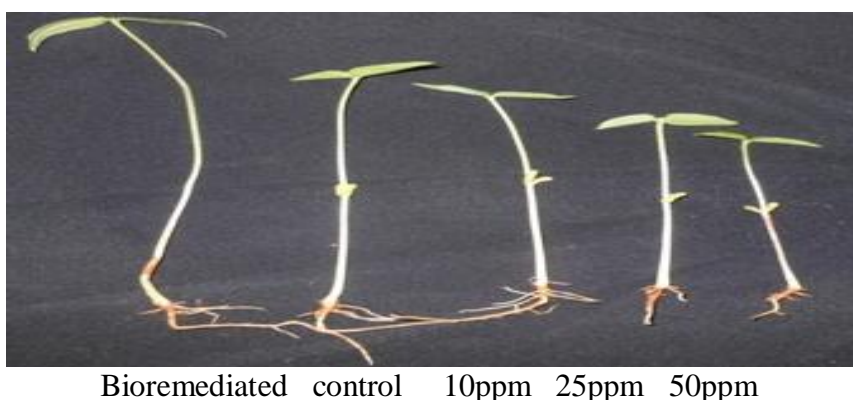
**Plate.2** Radish seeds grown in soil spiked with Chlorpyrifos. C=control; (1) Bioremediated soil (2) Control without Chlorpyrifos (3) 10 µg Chlorpyrifos per gram soil; (4) 25 µg Chlorpyrifos per gram soil; (5) 50 µg Chlorpyrifos per gram soil



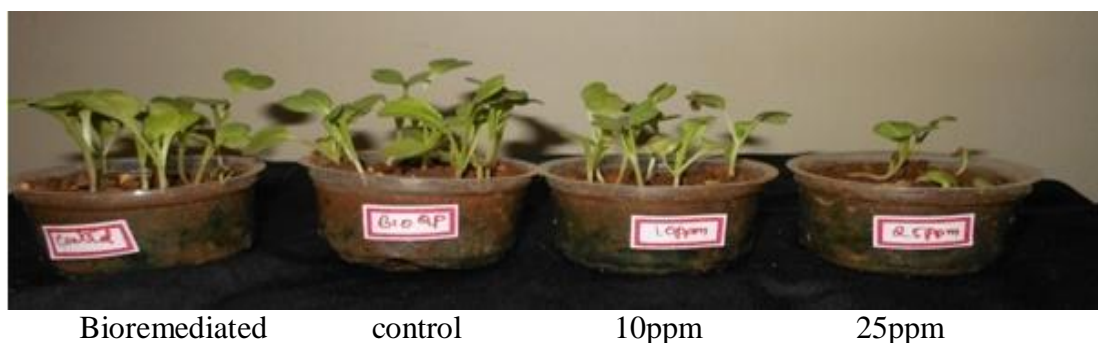
**Plate.3** Green gram seeds grown in soil spiked with Chlorpyrifos 1= Bioremediated 2=, Control (without Chlorpyrifos treatment), 3= 10µg Chlorpyrifos 4= 25 µg Chlorpyrifos per gram soil and 5= 50 µg Chlorpyrifos showing the effect of Chlorpyrifos on the development of the roots and shoots in each case



**Plate.4** Green gram seeds grown in soil spiked with C Chlorpyrifos . C=control; (1) Bioremediated soil (2) Control without Chlorpyrifos (3) 10 µg Chlorpyrifos per gram soil; (4) 25 µg Chlorpyrifos per gram soil; (5) 50 µg Chlorpyrifos per gram soil



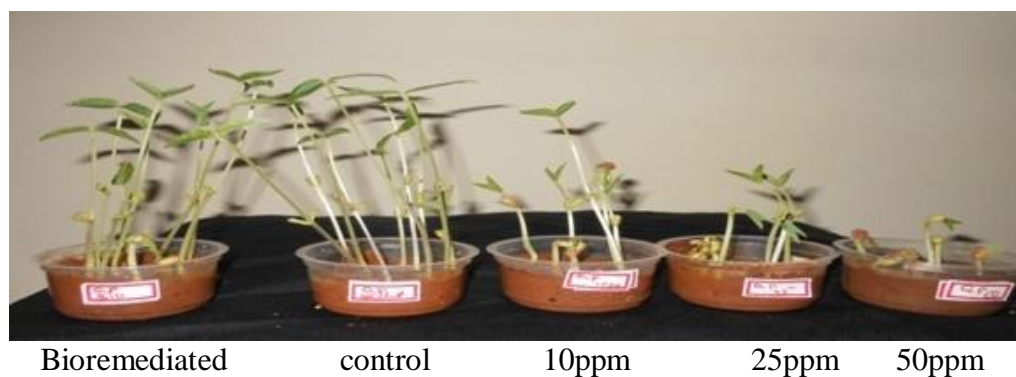
**Plate.5** Radish seeds grown in soil spiked with Quinolphos. (1) Bioremediated soil (2) Control without quinolphos (3) 10 µg quinolphos per gram soil; (4) 25 µg quinolphos per gram soil



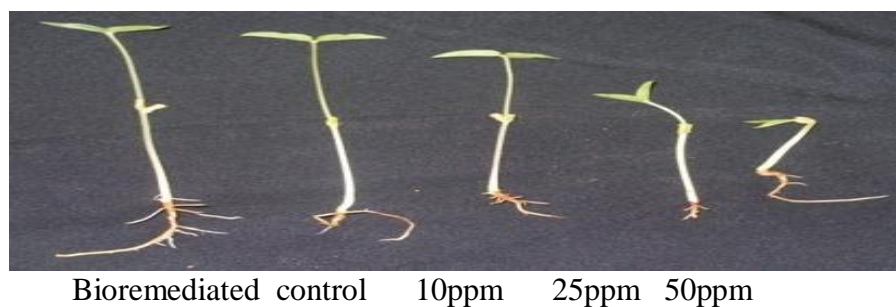
**Plate.6** 1= Bioremediated 2=Control (without quinolphos treatment),, 3= 10  $\mu\text{g}$  quinolphos 4= 25  $\mu\text{g}$  quinolphos per gram soil showing the effect of quinolphos on the development of the roots and shoots in each case



**Plate.7** Green gramh seeds grown in soil spiked with Quinolphos. (1) Bioremediated soil (2) Control without quinolphos (3) 10  $\mu\text{g}$  quinolphos per gram soil; (4) 25  $\mu\text{g}$  quinolphos per gram soil; (5) 50  $\mu\text{g}$  quinolphos per gram soil



**Plate.8** 1= Bioremediated 2=Control (without quinolphos treatment),, 3= 10  $\mu\text{g}$  quinolphos 4= 25  $\mu\text{g}$  quinolphos per gram soil 5= 50  $\mu\text{g}$  quinolphos per gram soil showing the effect of quinolphos on the development of the roots and shoots in each case



In soils, bioremediated with the quinolphos degrading microbial consortium, normal germination and seedling vigour of both radish and green gram seeds was observed (Table 3). The amylase and protease activities were either same or slightly higher than control seeds (Figs. 1 and 2). Perhaps quinolphos is degraded either completely or degraded to non-toxic metabolites quinolphos degrading microbial consortium.

Organophosphates such as Chlorpyrifos and quinolphos were found to adversely affect seed germination and seedling vigour of radish and green gram seeds. This is the first report on the effect of chlorpyrifos and quinolphos on seed germination. It is clear from our results that even low concentrations of Chlorpyrifos and quinolphos effected seed germination and seedling vigour of both radish and green gram seeds, Thus will considerably reduce the crop yield. A reduction in both  $\alpha$ -amylase and protease activities have been observed in the seeds sown in Chlorpyrifos and quinolphos treated soils. The reduction in the enzyme activity might be due to effect of these pesticides on enzyme activity or due to inhibition of induction of the enzyme or inhibition of some other biochemical process.

The TTC test, showed complete inhibition of seed viability after exposure to Chlorpyrifos and quinolphos even at very low concentrations. The deleterious effects were more pronounced in both in filter paper test and soil tests. The Chlorpyrifos and quinolphos treated soil, when bioremediated with the microbial consortium, showed complete elimination of the parent compound by 120 h of incubation, with slight increase in cell population.

The microbial consortium used in our study, is capable of degrading 10 and 25  $\mu\text{g}$

Chlorpyrifos and quinolphos completely within five days. Bioremediation of Chlorpyrifos and quinolphos from spiked soil by the microbial consortium appeared to protect the germinating seeds resulting in the normal germination and seedling vigour. In a similar study Kruegor et al. (1991), observed the elimination of soybean and pea seedling susceptibility to the herbicide dicamba (3,6-dichloro-2-methoxy benzoic acid), by inoculating the herbicide contaminated soil by dicamba degrading microorganisms. In a study by Ajith Kumar et al. (1998) the effect of 3-CBA and 4-CBA on germinating tomato seeds was nullified by treatment of contaminated soil with the strain *P. aeruginosa* 3 mT.

Bidlan et al (2004) studied the effect of Tech-HCH on the germination of few seeds. In all these cases, they observed that the germination of the seedlings was normal irrespective of the time of planting i.e. seedling planted either immediately or two days or five days after inoculation. The consortium is ideally suited for remediation of Chlorpyrifos and quinolphos contaminated soils having various physico-chemical properties such as different water holding capacities, pH, temperature and organic matter etc. The removal of toxicity by this consortium could be either elimination of the parent compound from the soil to less phytotoxic levels by the time of seed germination or complete elimination of the parent compound (Kruegor et al., 1991).

In conclusion, the toxic effects of Chlorpyrifos and quinolphos were efficiently eliminated by inoculating the Chlorpyrifos and quinolphos degrading microbial consortium under laboratory conditions. However, the data has to be made practical by translation of laboratory results to field trials and to ascertain the suitability of the bioremediation process.

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