Protection of Tomato Seed Germination from the Inhibitory Effect of 2,4,5-Trichlorophenoxyacetic Acid by Inoculation of Soil with *Burkholderia cepacia* AC1100

K. P. Gangadhara and A. A. M. Kunhi*

Department of Food Microbiology, Central Food Technological Research Institute, Mysore-570 013, India

The effect of 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) on the germination and seedling vigor of different crop seeds was tested. Seeds of rice, maize, sorghum, finger millet, and horse gram were comparatively more tolerant to the chemical with no marked effect up to a concentration of 200 mg 2,4,5-T L^{-1} as tested by the filter paper method. Tomato and brinjal (egg plant) were highly susceptible. Even at 5 and 10 mg 2,4,5-T L^{-1}, marked reduction in the germination and seedling vigor of tomato and egg plant, respectively, was observed. At 20 and 30 mg L^{-1}, the germination of tomato and egg plant seeds, respectively, were completely inhibited on filter paper, whereas the inhibitory concentrations in soil was 40 mg 2,4,5-T kg^{-1} soil. Several abnormalities were observed in the chemically affected seedlings. Protease activity of the seeds germinating in the presence of the chemical was drastically reduced. Bioremediation of the chemically contaminated soil with *Burkholderia cepacia* AC1100, by inoculation of the soil 7 days before sowing the seeds, completely protected the seeds, resulting in normal germination and an improved seedling vigor.

Keywords: *2,4,5-Trichlorophenoxy acetic acid; seed germination; inhibition; seedling vigor; Burkholderia cepacia AC1100; soil bioremediation*

INTRODUCTION

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) is a weedicide which has been widely used for controlling various broadleaf weeds and as a growth regulator. The extensive use of this compound has resulted in toxicological and environmental pollution problems leading to the restriction of its use in various countries (Kilbane et al., 1982). Nevertheless, due to their high recalcitrance, its residues still persist in soils, particularly in the vicinity of waste dump sites. There is, however, only limited information on its deleterious effects on crop plants, particularly on seed germination. There are reports on the harmful effects of a number of other herbicides and insecticides. Cereals planted the year after the application of simazine (2-chloro-4,6-bis-(ethylamino)-s-triazine) and atrazine (2-chloro-4-(ethylamino)-6-isopropylamino-s-triazine) were reported to be severely injured (Splittsoesser and Derscheid, 1962). Seedlings of peas and soybean have been shown to be susceptible to even low levels of the herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) (Krueger et al., 1991). 2,4-Dichlorophenoxyacetic acid (2,4-D), a commonly used herbicide, was shown to cause dormancy in seeds of agricultural crops and malformation of root tips of seedlings (Moreland, 1967). Laboratory evaluation by Sund and Nomura (1963) of various herbicides revealed that Sudan grass, radish, and cucumber seeds were sensitive to 2,4-D and 2,4,5-T.

There seems to be no other report on the effect of 2,4-5-T on germination of crop seeds. Hence, we took up studies on this aspect taking representative seeds of cereals, legumes, and vegetables, and the data are presented in this paper.

Having found that 2,4,5-T causes inhibition of seeds germination, it was necessary to find out a way to protect the seeds from this inhibition. Kilbane et al. (1982) have reported the isolation of a bacterium, *Pseudomonas cepacia* AC1100 (now called *Burkholderia cepacia* AC1100) which can degrade up to 3 g L^{-1} of 2,4,5-T in shake flasks. The ability of this bacterium to degrade 2,4,5-T in soil was reported by Chatterjee et al. (1982). We tested the effectiveness of this bacterial strain in protecting the seeds from inhibition by 2,4,5-T by inoculating it into the soil. The results of these experiments are described in this paper, along with the data on the germination inhibition studies.

MATERIALS AND METHODS

Chemicals. 2,4,5-T (99% pure) was procured from British Drug House Ltd., England; 2,3,5-triphenyl tetrazolium chloride (TTC) was obtained from Hopkins & Williams Pvt Ltd., England. All other chemicals used in the culture medium and reagents were of analytical reagent grade and were procured from standard companies.

Seeds and Soil. Seeds of different representative crops such as rice (*Oryzae sativa* L. var PKB), sorghum (*Sorghum vulgare* L. var. CSH-3), maize (*Zea mays* L. var. Indaf-9), horse gram (*Dolichos biflorus* L. var local), tomato (*Lycopersicum esculentum* Moench var Araka Vikas), and brinjal or egg plant (*Solanum melongena* L. var. Iranagere) were procured either from Seed Testing Laboratory, Bangalore, or from local farmers.

A red loamy type soil used in this study was collected from Central Food Technological Research Institute Campus, Mysore. The soil had a good water holding capacity and had 1.0−1.5% organic matter. The soil was sieved (2.0 mm) to remove debris and pebbles.

* To whom correspondence should be addressed. Telephone: 0091-821-517539. Fax: 0091-821-517233. E-mail: aamkunhi@yahoo.com.

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Seed Germination Tests. Germination of seeds was tested on moist filter paper (ISTA, 1985) as well as in soil as follows: In the filter paper method, Whatman No.1 paper (9.0 cm disks) were kept in 10.0 cm diam Petri plates and moistened with aqueous solution of the required amount of 2,4,5-T (prepared by neutralizing to pH 7.0 by NaOH). In each Petri plate, 25 seeds were placed in and incubated at ambient temperature (20−27 °C) in a germinator under 12−12 h cycles of light and darkness. For each concentration of the chemical, 8 replicates of 25 seeds were taken. Controls were maintained on filter paper moistened with distilled water. Germination and seedling vigor were evaluated after 7 days by counting the seedlings and measuring the root and shoot lengths. The seedling vigor index was calculated as (mean root length + mean shoot length) × (percentage germination).

Germination of seeds in soil was tested in plastic cups (6.3 cm diam) filled with 80 g of soil (20% moisture). Different concentrations of 2,4,5-T were added to each cup as neutralized aqueous solution (1.0 mL) and were mixed thoroughly to obtain uniform distribution. The sides of the cups were pricked by a needle to facilitate aeration. To the control cups, 1 mL of distilled water was added in the place of the chemical solution. Ten seeds were sown in each cup at a depth of 0.5 cm. Eight replicates were taken for each variable. The cups were incubated in a germinator at ambient temperature (20−27 °C). Sterile distilled water (5 mL) was added to each cup, every alternate day, to maintain moisture. After 7 days, the germination percentage and the seedling vigor were evaluated.

Test of Viability of Tomato Seeds Exposed to 2,4,5-T. Living cells can take up 2,3,5-triphenyl tetrazolium chloride (TTC) and convert it into triphenyl formazan, whereas dead cells are incapable of this. Seed viability was tested using this principle. Ten tomato seeds were soaked for 24 h in aqueous solution of 2,4,5-T of different concentrations. The soaked seeds were cut along the margin to expose the embryo and placed in 0.1% aqueous solution of TTC for 24 h at 37 °C in darkness. The seeds were then removed, washed with distilled water, and soaked in 10 mL of 95% ethanol until all the color was extracted. The optical density of the extracted red color of triphenyl formazan was determined at 480 nm using a Shimadzu UV-160A (Japan) spectrophotometer.

Enzyme Assays. Protease and α-amylase activities in germinating tomato seeds, both exposed and unexposed to 2,4,5-T, were assayed. Triplicate samples of 50 seeds/seedlings were collected every 24 h for 7 days and were ground with acid-washed sand for 15 min in a cooled mortar maintained on an ice-bath. The extract was prepared in 0.2 M acetate buffer (pH 5.2), and the debris was removed by centrifugation at 10 000 rpm (revolution min^{-1}) for 10 min at 4 °C. The supernatants were made up to 5.0 mL.

A standard method (Laskowsky, 1955) was followed for protease assay. To a solution of bovine serum albumin (BSA) (10 mg mL^{-1}), an equal volume of enzyme extract was added and incubated at 30 °C for 30 min. The enzyme activity was expressed as OD_{600} of the BSA hydrolysate obtained when reacted with Folin−Ciocalteau reagent.

α-Amylase activity was assayed by measuring the release of reducing sugar from gelatinized soluble starch (1.0% in 0.1M acetate buffer, pH 5.2) according to the method of Bernfeld (1955).

Preparation of Bacterial Inoculum. The 2,4,5-T-degrading *B. cepacia* AC1100 (Kilbane et al. 1982) was a donation from A. M. Chakrabarty. For the preparation of inoculum, the bacterium was grown in shake flasks (1000 mL flasks containing 200 mL of medium) on a rotary shaker (150 rpm) at 30 °C for 24 h. The medium contained (g L^{-1}) KH_2PO_4, 2.72; Na_2HPO_4, 3.52; $(NH_4)_2SO_4$, 0.50; $MgSO_4 \cdot 7H_2O$, 0.20; $Ca(NO_3)_2$, 0.1; yeast extract, 0.050; and 2,4,5-T, 0.50 (pH 7.0). The cells were harvested by centrifugation at 10 000 rpm for 10 min.

Inoculation of Soil and Determination of Bacterial Growth. A wet cell pellet was suspended in sterile physiological saline in such a way as to obtain a biomass concentration of about 175 mg in 3 mL. A 3 mL aliquot of this cell suspension was added to 80 g of soil (20% moisture and different concentrations of 2,4,5-T) taken in plastic cups and mixed thoroughly to ensure uniform distribution. This inoculum gave an average colony-forming unit (cfu) value of 7.6×10^8 g^{-1} of soil. In one set of these cups, tomato seeds were sown immediately as described above and incubated at ambient temperature in a germinator. In the other two sets, seeds were sown after 3 and 7 days of incubation of the inoculated cups and incubated further for 7 days. Seedlings were evaluated 7 days after sowing for germination and seedling vigor. Soil without 2,4,5-T was also inoculated with bacterium and seeds were sown, which served as control.

The survival and growth of the inoculated strain were determined by making viable counts by plating appropriately diluted soil suspensions on minimal agar medium containing 2,4,5-T (0.5 g L^{-1}). The plates were kept at 30 °C for 1−2 days, and then colonies were counted.

The inorganic chloride (Cl^-) released to the soil during the degradation of 2,4,5-T was estimated by a slightly modified method of Bergmann and Sanik (1957). Soil samples were collected at 24 h intervals and dried. Dry soil (2 g) was extracted in distilled water, and to 2 mL of appropriately diluted aqueous extract, 0.2 mL of 0.25N ferric ammonium sulfate and 0.2 mL of saturated mercuric thiocyanate solution in ethanol were added and mixed. The color was measured at 460 nm by a spectrophotometer (Shimadzu UV-160A, Japan), against a reagent blank. The Cl^- concentration was computed from a standard curve prepared with NaCl as a reference standard.

RESULTS

Effect of 2,4,5-T on Seed Germination. The effect of different concentrations of 2,4,5-T on the germination and seedling vigor of maize, rice, sorghum, finger millet, horse gram, egg plant, and tomato seeds as tested by the filter paper method are shown in Table 1. In the cereals and the legume, there was no marked reduction in the germination percentage and seedling vigor up to a concentration of 200 mg L^{-1}. At 400 mg L^{-1} of 2,4,5-T, the germination percentage was reduced by almost half of that of the control. At 600 mg L^{-1}, there were drastic reductions in the germination percentage, with the values for maize, rice, sorghum, finger millet, and horse gram being 10, 19, 6, 21 and 7%, respectively. The vigor indices were also reduced to 3.6, 9.6, 3.2, 8.2, and 1.9% of the values for control seedlings of these plants, respectively (Table 1). At higher concentrations of the compound, all of the seedlings showed abnormalities such as reduced root and shoot lengths, negative geotropism, inhibition of primary root growth, and nonemergence of primary leaves from the coleoptile. In rice, the seedling and the husk of the seed turned black with 600 mg L^{-1} 2,4,5-T. In maize, the secondary root system showed red pigmentation. Tomato and egg plant seeds were the most susceptible to 2,4,5-T (Table 1). As tested by the filter paper method, complete inhibition of germination of tomato seeds occurred at a concentration of 20 mg L^{-1} and 50% inhibition occurred at 10 mg L^{-1} of 2,4,5-T. Even at a low concentration of 5 mg L^{-1}, the seedling vigor was reduced by almost half, although the inhibition of germination was partial. Egg plant seeds were slightly more resistant than tomato seeds but were much more susceptible as compared to cereals and legumes. The susceptibility of both these seeds were less pronounced when tested in soil as compared to what was observed on filter paper (Tables 1 and 2). More than 50% of the tomato seeds germinated in the presence of 20 mg kg^{-1} soil of 2,4,5-T, whereas the same concentration completely inhibited the germination on filter paper. Similarly, partial germination of egg plant seeds occurred in soil even at 40 mg kg^{-1} soil of 2,4,5-T,

Table 1. Effect of 2,4,5-T on Germination and Seedling Vigor of Different Crop Seeds as Tested by Standard Blotter Method

2,4,5-T concn (mg L^{-1})	germ. %	MSL[a]	MRL[b]	VI[c]	Ab[d] (%)
		Maize			
0	98	4.22 ± 0.463	4.35 ± 0.473	839.86	0
50	97	4.20 ± 0.461	4.20 ± 0.481	814.80	0
100	97	4.10 ± 0.471	4.13 ± 0.498	798.31	0
200	96	4.10 ± 0.331	4.12 ± 0.379	789.12	0
400	62	3.80 ± 0.678	3.81 ± 0.712	471.82	14
600	10	1.12 ± 0.484	1.89 ± 0.493	030.10	16
		Rice			
0	99	4.60 ± 0.809	5.30 ± 0.904	980.10	0
50	98	4.40 ± 0.824	5.50 ± 0.912	970.20	0
100	98	4.30 ± 0.923	5.40 ± 0.942	950.60	0
200	97	4.26 ± 0.934	5.32 ± 0.973	929.26	0
400	51	3.56 ± 1.072	3.69 ± 1.392	369.65	19
600	19	2.18 ± 1.832	2.77 ± 1.934	094.05	10
		Sorghum			
0	98	4.60 ± 0.845	4.70 ± 0.814	911.40	0
50	97	4.50 ± 1.132	4.62 ± 0.983	884.64	0
100	96	4.40 ± 0.957	4.56 ± 0.964	860.16	0
200	94	4.35 ± 0.884	4.30 ± 0.878	813.10	0
400	51	3.98 ± 0.842	4.00 ± 0.863	406.98	12
600	6	1.90 ± 0.932	2.98 ± 0.939	029.28	6
		Finger Millet			
0	90	4.19 ± 0.894	5.00 ± 0.529	827.10	0
50	90	4.10 ± 0.638	4.13 ± 0.661	740.70	0
100	88	4.08 ± 0.642	4.10 ± 0.648	719.84	0
200	87	4.08 ± 0.631	4.09 ± 0.693	710.13	0
400	54	3.74 ± 0.702	3.77 ± 0.718	405.54	14
600	21	1.23 ± 0.513	1.99 ± 0.519	067.62	5
		Horse Gram			
0	83	4.80 ± 0.533	5.20 ± 0.543	830.00	0
50	80	4.72 ± 0.648	4.93 ± 0.734	772.00	0
100	79	4.70 ± 0.674	4.77 ± 0.845	748.13	0
200	77	4.68 ± 0.810	4.74 ± 0.910	725.34	4
400	53	3.84 ± 1.211	3.93 ± 1.218	411.81	8
600	7	1.13 ± 1.343	1.17 ± 1.391	016.10	7
		Tomato			
0	87	4.46 ± 0.440	5.20 ± 0.422	840.42	0
05	70	3.21 ± 0.312	3.80 ± 0.312	470.70	4
10	51	1.72 ± 0.378	1.80 ± 0.388	179.52	4
20	0	0	0		
		Egg Plant			
0	81	4.60 ± 0.874	4.72 ± 0.724	754.92	0
10	78	4.00 ± 0.855	4.12 ± 0.892	633.36	6
20	18	3.20 ± 0.779	3.74 ± 0.872	124.92	16
30	4	1.20 ± 0.973	1.40 ± 1.012	010.420	4
40	0	0	0		

[a] Mean shoot length with standard deviation. [b] Mean root length with standard deviation. [c] Vigor index determined as described in the text. [d] Percent abnormal seedlings observed on the basis of the total seeds sown.

whereas on filter paper, germination was completely inhibited at the same concentration. This may be due to the reduced bioavailability of the chemical in the soil as a result of its adsorption to soil particles. Abnormalities of seedlings, very similar to what were observed in the cases of the cereals and the legume, were also observed in both tomato and egg plant seedlings at sublethal concentrations of 2,4,5-T, both in soil and on filter paper.

Viability of Tomato Seeds Exposed to 2,4,5-T. The viability of tomato seeds, which were found to be the most susceptible to 2,4,5-T, was tested by the standard TTC test, after exposing the seeds to different concentrations of the chemical. The losses of viability with increasing concentration of 2,4,5-T are shown in Figure 1. More than 50% viability was lost, as indicated by the failure of the seeds to convert TTC to triphenyl formazan, even at 10 mg L^{-1} of 2,4,5-T.

Table 2. Effect of Different Concentrations of 2,4,5-T on the Germination and Seedling Vigor of Tomato and Brinjal (Egg Plant) Seeds as Tested in Soil

2,4,5-T (mg kg^{-1} soil)	germ. %	MSL[a]	MRL[b]	VI[c]	Ab[d]
		Tomato			
0	90	3.82 ± 0.847	4.20 ± 0.923	721.80	0
5	88	3.14 ± 0.948	3.80 ± 0.994	610.72	3
10	73	2.40 ± 0.940	2.54 ± 0.982	360.62	8
20	55	1.40 ± 1.414	1.46 ± 1.429	157.30	18
40	0	0	0	--	--
		Egg Plant			
0	81	4.23 ± 0.872	4.96 ± 0.894	744.39	0
5	73	3.92 ± 0.675	4.10 ± 0.705	585.46	0
10	61	3.63 ± 0.968	3.72 ± 0.970	448.35	4
20	46	3.23 ± 0.686	3.32 ± 0.694	301.30	12
40	8	1.31 ± 0.496	1.07 ± 0.493	019.04	8

[a–d] As in Table 1.

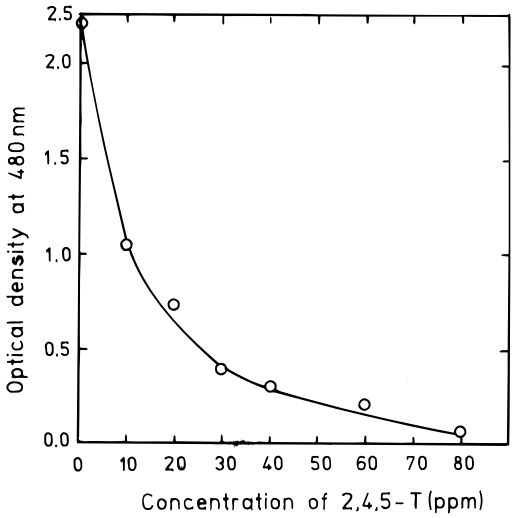

Figure 1. Effect of different concentrations of 2,4,5-T on the viability of tomato seeds as assessed by 2,3,5-triphenyl tetrazolium chloride test.

Effect of 2,4,5-T on the Enzyme Activities of Germinating Tomato Seeds. Enzymes such as protease and amylase normally are activated during germination of seeds to mobilize stored proteins and starches (Koller et al., 1962). Hence, it was thought that determination of these enzyme activities would provide some insight into the mechanism of inhibition of germination by 2,4,5-T. Amylase activity was not detected in tomato seeds germinating in either the presence or absence of 2,4,5-T. It may be because tomato seeds contain no or negligible amount of storage starch. On the other hand, the seeds germinating in the absence of the chemical showed fairly good protease activity (Figure 2). There was a steep increase in the activity up to 24 h after sowing the seeds, which continued to increase up to fourth day, but gradually. From day 4 onward, the activity started receding and reached minimum level on day 7. The seeds exposed to 2,4,5-T showed marked reduction in the protease activity, the reduction being more pronounced with higher concentrations (Figure 2).

Inoculation of Soil by *B. cepacia* AC1100 and Protection of Seed Germination. Each cup with 80 g of soil containing 0, 20, or 40 mg of 2,4,5-T kg^{-1} soil was inoculated with 175 mg of cells of *B. cepacia* AC1100, and tomato seeds were sown immediately, after 3 days or after 7 days of incubation. Normal germination did not occur in the cups containing 2,4,5-T (at both 20

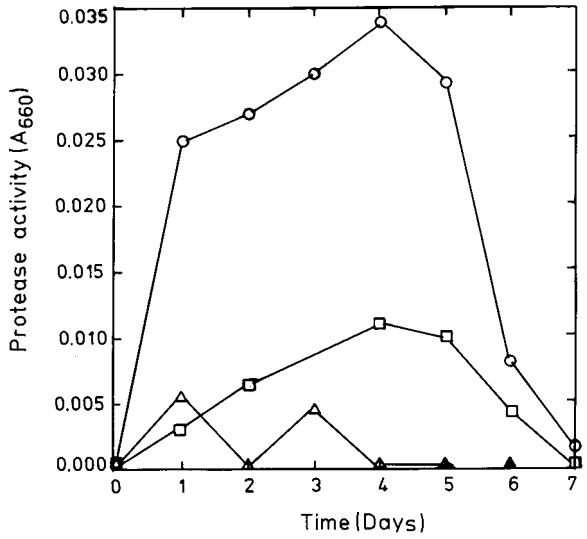

Figure 2. Effect of 2,4,5-T on the protease activity of the germinating seeds of tomato. Key: −○− control (without 2,4,5-T), −□− 20 mg L^{-1} of 2,4,5-T, and −△− 40 mg L^{-1} of 2,4,5-T.

Table 3. Effect of Inoculation of 2,4,5-T Treated Soil with *B. cepacia* AC1100 Cells on the Seed Germination and Seedling Vigor of Tomato†

2,4,5-T (mg kg^{-1} soil)	inoculum[d]	germ. %	MSL[a]	MRL[b]	VI[c]
0 (control)	−	90	4.30 ± 0.834	4.7 ± 0.821	810
0	+	90	4.40 ± 0.872	5.0 ± 0.734	846
20	−	50	2.10 ± 0.784	2.0 ± 0.812	205
20	+	90	4.32 ± 0.612	5.1 ± 0.512	848
40	−	0	0.0	0.0	0
40	+	90	4.5 ± 0.721	4.8 ± 0.610	837†

Seeds were sown 7 days after the inoculation of soil with bacterial cells. [a−c] As in Table 1. [d] + and − indicate inoculated and uninoculated soil.

and 40 mg L^{-1}) in which seeds were sown immediately or after 3 days of bacterial incubation, whereas in the cups with seeds sown after 7 days, the germination was normal. In the former two cases, all of the deleterious effects on the germination percentage and seedlings vigor, as observed in the uninoculated control cups containing 2,4,5-T, were observed (data not given as there was no noticeable difference). However, complete recovery of germination percentage and seedling vigor was observed in the cups inoculated with the bacterium (Table 3). Interestingly, an improved seedling vigor index was obtained in the bacteria-inoculated soils, irrespective of whether they contained 2,4,5-T.

An increase in cell population of *B. cepacia* AC1100 was observed in 2,4,5-T-treated soil (Figure 3). In soil containing both 20 and 40 mg of 2,4,5-T kg^{-1} soil, there was a steady increase in growth of the organism up to day 7, the growth being, from an initial cfu g^{-1} of soil of 7.5×10^8 to 6.4×10^9 and 7.8×10^9 in soil with 20 and 40 mg of 2,4,5-T kg^{-1}, respectively. In control soil, without 2,4,5-T, there was a marginal increase in cell density (from 8.4×10^8 to 2.2×10^9 cfu g^{-1} of soil) up to 3 days, beyond which there was no further growth. This growth would have occurred, probably, by utilizing the organic matter present in the soil.

The mineralization of 2,4,5-T by the bacterium was followed by estimating the Cl^- release into the soil. A gradual increase in the Cl^- concentration was observed in the 2,4,5-T-containing soil inoculated with the bacterium (Figure 4). Complete (100%) Cl^- release from 20 mg 2,4,5-T kg^{-1} soil occurred within 6 days of incubation

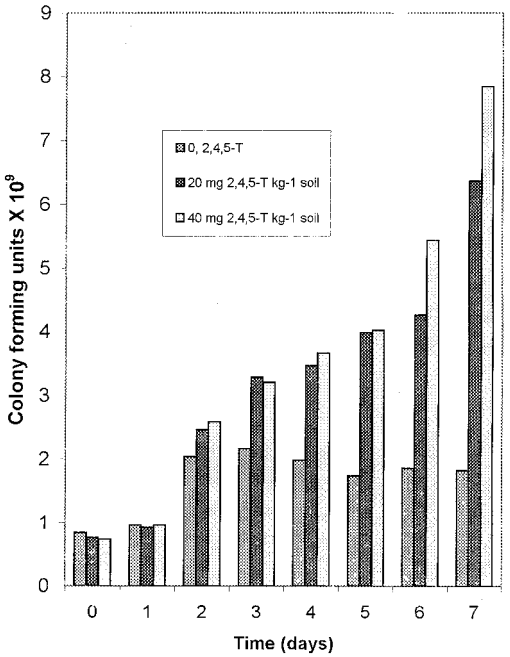

Figure 3. Growth of *B. cepacia* AC1100 in soil in the presence and absence of 2,4,5-T. Colony-forming units (cfu) were determined by dilution plating of soil suspensions on 2,4,5-T mineral-agar medium.

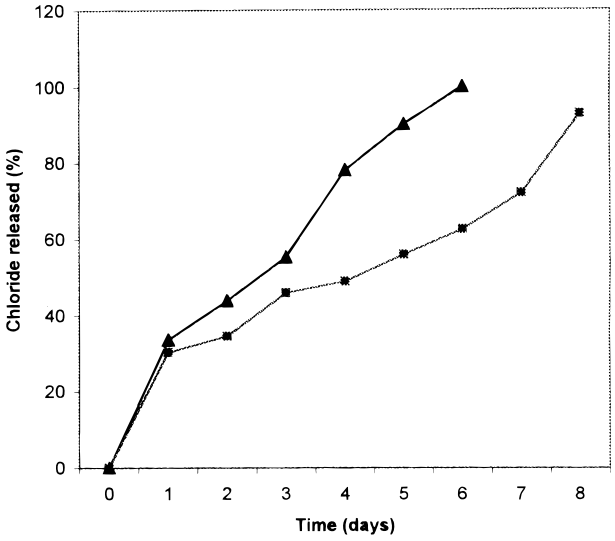

Figure 4. Release of inorganic chloride into the medium during the mineralization of 2,4,5-T by the inoculated *B. cepacia* AC1100. The Cl^- values are given as the percentage of the theoretical maximum of three chlorines released per 2,4,5-T molecule. The chlorine values obtained for control soil was deducted from the total Cl^- values obtained in the case of chemical-containing bacteria-treated soil to get the net Cl^- values and average of triplicate net values are plotted. Key: −▲− 20 mg and −■− 40 mg 2,4,5-T kg^{-1} soil.

after the inoculation of the bacterium, whereas in the cups with 40 mg kg^{-1} 2,4,5-T, even after 8 days, only about 93% Cl^- was released. By day 7, about 72% Cl^- release was detected.

DISCUSSION

2,4,5-T was found to adversely affect the germination and seedling vigor of a number of crop seeds (Tables 1 and 2). Considering the recalcitrance of the compound and its abundance in soil, particularly at waste dump sites, it is a serious matter. It is likely that leachates

from these sites may enter crop fields and cause havoc. Because important crop seeds such as tomato and other Solanaceae members are very sensitive to this compound, complete failure of germination can occur. Other crops are also susceptible, although to a lesser extent, and as a result, may end up with considerable reduction in crop productivity.

Complete inhibition of germination of tomato seeds on exposure to 400 mg L^{-1} of 3-chlorobenzoate (3-CBA)/4-chlorobenzoate (4-CBA) and marked reduction in seedling vigor at even lower concentrations of these compounds have been reported by Ajithkumar et al. (1998). It is also interesting to note that cereals and legumes were more resistant to 3-CBA and 4-CBA, too, whereas Solanaceae members such as tomato, egg plant, and tobacco were highly susceptible (Ajithkumar et al., 1998). Reduction in germination percentage and inhibition of seedling development (i.e., reduced root and shoot growth) of tall fescue (a grass) exposed to up to 30 mg 2,4,6-trinitrotoluene (TNT) L^{-1} or higher concentrations and 15 mg 4-amino-2,6-dinitrotoluene (4ADNT) L^{-1} was observed by Peterson et al. (1996). These compounds were shown to affect respiration, which may be a direct effect on mitochondrial respiration or an indirect result of inhibition of cell growth and division. Similar observation was made in the present study, too. Seed viability was drastically affected on exposure to 2,4,5-T as tested by treatment with TTC (Figure 1). A marked reduction in protease activity of the germinating seeds was also observed in the presence of 2,4,5-T (Figure 2). However, it would be difficult to assume whether the reduced activity was due to either the effect of the chemicals on the enzyme or inhibition of some other factors responsible for the induction and synthesis of the enzyme during the germination process.

Kilbane et al. (1982) have reported the isolation, for the first time, of a pure culture of the bacterium *P. cepacia* AC1100 (now *B. cepacia* AC1100) that can utilize 2,4,5-T as the sole source of carbon and energy. This strain can degrade more than 97% of 2,4,5-T present at 1000 mg L^{-1} within 6 days in shake flasks. The efficiency of this strain to degrade and grow in the presence of 2,4,5-T in soil was detected by Chatterjee et al. (1982). Up to 95% of 1000 mg 2,4,5-T kg^{-1} soil was degraded within 1 week when the soil containing 25% moisture was inoculated with *B.cepacia* AC1100 (2×10^7 cells g^{-1} soil) and incubated at 30 °C. The organism thrived well in soil utilizing the compound, and the cell population dwindled when the concentration of 2,4,5-T fell, indicating the dependence of the organism on the presence of the compound in the soil. On the basis of these findings, we tested the usefulness of this strain in protecting the germination of tomato seeds sown in 2,4,5-T-contaminated soil. As expected, the culture thrived well in the chemically treated soil with a marked increase in the cell population (Figure 3), which is indicative of the degradation of the compound in soil. Gradual release of Cl^- from 2,4,5-T into the medium was observed which was complete within 6 days in the case of 20 mg 2,4,5-T kg^{-1} soil, whereas only about 72% Cl^- was released from 40 mg kg^{-1} on day 7 and 93% on day 8. However, inoculation of the 2,4,5-T-containing soil with *B. cepacia* AC1100 effectively protected the tomato seeds, resulting in normal germination and seedling vigor, when sown 7 days after the inoculation with the bacterium. On the other hand, the seeds sown immediately or 3 days after inoculation failed to germinate normally, indicating that complete mineralization or degradation to a tolerable concentration of the compound is necessary before the seeds are sown. As per the Cl^- release data, 75% of 40 mg kg^{-1} and 100% of 20 mg kg^{-1} of 2,4,5-T was degraded by day 7, facilitating normal germination of the seeds. In a similar study, earlier, we have shown the degradation of 500 mg kg^{-1} of soil 3-CBA and 4-CBA within 2 days by inoculation of soil with *P. aeruginosa* strain 3mT with an initial titer of 2×10^7 cfu g^{-1} soil. Bioremediation of 3-CBA-contaminated sandy loam soil, amended with mineral salts medium by inoculation with *P. alcaligenes* C−O, has also been reported by Focht and Shelton (1987).

Protection of tomato seed germination from 3-CBA or 4-CBA by inoculating soil with the chlorobenzoate-degrading *P. aeruginosa* 3mT was demonstrated by Ajithkumar et al. (1998). Similarly, Krueger et al. (1991) have shown protection of soybean and pea seedling from the deleterious effects of the herbicide, dicamba by inoculating soils with dicamba-degrading bacteria. However, unlike in the present study, in both the above two cases, the germination of seeds occurred normally, irrespective of the time of sowing. This might be because of the fact that the compounds are getting completely degraded before the radicals emerge from the seed and get exposed.

A marginal increase in seedling vigor was observed in the bacterium-inoculated soil as compared to that of the uninoculated control (Table 3). A similar phenomenon was observed by Ajithkumar et al. (1998), too, in the soil inoculated with *P. aeruginosa* 3mT. However, it is difficult to explain the exact reason. It could be due to the production and secretion of some growth-promoting factor by the inoculated strain or could be due to the destruction of any growth-inhibiting substance present in the soil. It has been shown that bacterial inocula such as *P. flourescens, P. putida*, and *Azospirillum* can be used for improving soil fertility and suppressing plant diseases (Schroth and Hancock, 1982; Moores et al., 1984).

The inhibitory effect of 2,4,5-T on the germination of crop seeds, particularly those of tomato and egg plant, and its deleterious effect on seedling vigor have been established. It has also been shown that these effects can efficiently be eliminated by bioremediation of soil by inoculating with *B. cepacia* AC1100. However, the data presented here pertain to laboratory studies, and field trials have to be done to validate the findings and to ascertain the suitability of this bioremediation technique under natural conditions.

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