

Full Length Research Paper

Bioremediation of pendimethalin-contaminated soil

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One strain of microorganisms was isolated from soil previously treated with pendimethalin using enrichment technique and identified using 16S rDNA as *Pseudomonas putida* (E15). The effect of pH and temperature on the growth ability of the tested strain was investigated. The results show that the optimum pH and temperature for the growth of pendimethalin dissipating strain were 7 and 30°C, respectively. *P. putida* was used to dissipate pendimethalin from mineral liquid medium with half-life of 5.46 days. Pendimethalin half-life was 51.9 days in untreated mineral liquid medium. *P. putida* and compost were also evaluated for detoxification of pendimethalin in clay soil. *P. putida* and compost were effective in pendimethalin dissipation in soil with half-life of 4.67 and 5 days, respectively. Pendimethalin half-life was 62.43 days in untreated soil. Pendimethalin treatment affected analysis of the microbial population growing in *P. putida* or compost treating soil leachates showed an overall increase in the population of microorganisms. There is no toxicity of pendimethalin detected in soil on cucumber plants after treatment with *P. putida* or compost. Pendimethalin significantly decreased germination and increased cucumber seedlings mortality rate. *P. putida* and compost treatments increased the growth parameters. Moreover, no significant difference was observed in the most growth parameters between *P. putida* and compost treatments. Abnormal development of xylem tissue was observed in pendimethalin contaminated soil as a result of phytotoxicity. The results suggest that bioremediation by *P. putida* and compost was considered to be effective method for detoxification of pendimethalin in soil.

Key words: Pendimethalin, soil, biodegradation, phytotoxicity, seedling mortality.

INTRODUCTION

Pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine) has the empirical formula C₁₃H₁₉N₃O₄, a selective pre-emergent herbicide a dinitroaniline group, is used extensively for weed control in cotton, rice, soybean and tobacco (Smith et al., 1995). Pendimethalin acts by inhibiting the steps in plant cell division responsible for chromosome separation and cell wall formation. It is used before crop emergence or planting (Appleby and Valverde, 1988). The inhibition of root and shoot growth results in stunting of aerial plant Portions (Parka and Soper, 1977).

Studies in terrestrial ecosystems showed that 10–20% of the herbicide vaporizes within the first week or two week after application (Strandberg and Scott-Fordsmand, 2004). The observation of phytotoxicity to crops and weeds 200 days after application confirms that the dissipation time of pendimethalin is high enough to harm plants far beyond the period during which it is intended to be active (Strandberg and Scott-Fordsmand, 2004). The US Environmental Protection Agency (EPA) has classified pendimethalin as a persistent bioaccumulative toxic (PBT).

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Pendimethalin is also highly toxic to fish and aquatic invertebrates. However, its use may adversely affect endangered species of terrestrial and semi aquatic plants and invertebrates including mollusks, fishes and birds. Care should be taken to minimize excessive pendimethalin applications to the soil in order to minimize possible injury to sensitive rotation crops. It is important to develop methodologies to prevent pesticide contamination from point sources. Microorganisms can use a variety of xenobiotic compounds including pesticides for their growth, mineralize and detoxify them (Belal et al., 2008). Bioremediation is an accepted technology for accelerating the rate of cleanup of contaminated water and soil. Soil microorganisms that are repeatedly exposed to pesticides may develop new capabilities to degrade such chemicals (Vidali, 2001). There are some reports on the degradation of pendimethalin by microorganisms comprising *Azotobacter chroococcum*, *A. vinelandii* and *Bacillus circulans* (Saha et al., 1991; Singh and Kulashrestha, 1991; Kole et al., 1994; Megadi et al., 2010). The success of bioremediation depends not only on the high degradation ability but also on the stability of active microorganisms under varied conditions, such as changes in pH and temperature (Pattanasupong et al., 2004). Therefore, it is necessary to investigate the effects of various environmental factors on the growth ability of the tested microorganisms (Pattanasupong et al., 2004). On the other hand, it was found that various materials were used as soil amendments, nutrients, to increase and enhance the degradation potential of xenobiotics such as Yard manure compost (Guo et al., 1991; Cole et al., 1995; Gan et al., 1996; Zheng and Cooper, 1996; Vogel, 1996; Grigg et al., 1997; Leoni et al., 1997), biogas slurry and compost (Kadian et al., 2008; Belal et al., 2008). However, after remediation toxicity assessments are needed.

Firstly, is providing valuable and complementary information to compound analysis. Secondly, the major advantage of toxicity tests is the direct assessment of the potential hazard to the environmental system by both original pollutants and its metabolites (Tiainen et al., 2002). Therefore, this study attempted to isolate and identify efficient bacterial strain for bioremediation of pendimethalin in aquatic system and to evaluate this strain and compost in remediation of pendimethalin contaminated soil. In addition to confirm the complete detoxification of pendimethalin by measuring the toxicity of the treated soil in the presence of bacterial strain or compost against sensitive target such as cucumber plants.

MATERIALS AND METHODS

Chemicals

Pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine) standard was obtained from Ehrenstorfer (Germany). All other chemicals were of analytical grade.

Microbial degradation of the pendimethalin

Media

Minimal medium as mineral salt liquid (MSL) and Luria Bertani (LB) a complete medium were used through this study as described by Sambrook et al. (1989).

Isolation by enrichment culture

Enrichment cultures of microorganisms capable of dissipating pendimethalin were established from soil previously treated with pendimethalin. Samples of soils were collected from Kafr El-Sheikh and Elbeheira, Governorates, Egypt. Ten grams soil were suspended in 90 ml sterilized mineral salt medium in 500-ml bottle containing (100 µg/ml) of pendimethalin as a sole source of carbon, then incubated at 30°C and 150 r/min for 28 days. Thereafter, 10 ml of the cultures were transferred into fresh 90 ml MSL medium containing the same concentration of pendimethalin. This procedure was repeated four times. Series dilutions were prepared after the final time from enrichment culture in a glass tube containing 9 ml MSL medium up to 1:10⁶ and then 100 µl of their were spread on plates of MSL medium + pendimethalin (100 µg/ml) using glass spreader. The plates were sealed in polyethylene bags, then incubated at 30°C for 7 days and monitored for appearance of colonies. Single colony growing on each plate was isolated by picking the colony using sterile inoculating needle and was further purified by the standard spatial streaking on complex agar media (Luria Bertani LB).

The isolated colonies were then tested for their ability to grow in MSL medium containing (100 µg/ml) of pendimethalin. The number of cells of each strain was determined by plating appropriate dilutions of liquid medium onto mineral salt agar medium containing pendimethalin. Bacterial populations were estimated by counting the number of colonies on plates.

Identification

The efficient selected pendimethalin degrading bacterial isolate was identified depending on the morphological and physiological characteristics as described by John (1984) and 16S rDNA as follow: DNA extraction was carried out following the CTAB method according to the method of Azadeh and Meon (2009). Oligonucleotide primer for 16S rDNA gene was 16S-1f (5'-GCTAGTTGGTGGGGTAA-3', 17 mer) and 16S-2r (5'-GCCATCTCAGTTCGGATTG-3'; 18 mer) were designed on the basis of the sequence of *E. coli* 16S gene (corresponding to positions 247 to 263 and 1291 to 1309; *E. coli* numbering system). Amplification reaction for *P. putida* (E15) was performed according to the method of Wilems and Collins (1993).

The PCR products was purified using a commercial kit (QIA Quick PCR purification kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instruction. After purification, the PCR products were sent for sequencing services at Sigma Co. Germany. The 16S gene sequences were aligned using BioEdit software versions 7.0.8 (<http://www.mbio.ncsu.edu/bioedit>) and searched for sequence similarity to other sequences which are available in the NCBI database at <http://www.ncbi.nih.gov> using Basic Local Alignment Search Tool (BLAST) algorithm. Multiple sequence alignments were performed on the selected closely related sequence accessions available using bioedit software (<http://bioedit.edu/>). Phylogenetic analysis was done based on the nucleotides sequences of 16S gene using mega4 or tree view software provided by the Biology Workbench Program (<http://workbench.sdsc.edu/>). Number of Data base JYZVMFRR015, K12FNS3P01S.

Effect of pH and temperature on the growth of the tested strain

To determine the effect of temperature and pH on the growth of tested strain, a 30 ml MSL medium supplemented with 100 µg/ml of pendimethalin was used as a sole source of carbon for bacterial strain. MSL medium was inoculated by 1 ml from bacterial cell suspension at 10^7 cfu/ml. To determine the optimum pH, experiments were carried out at pH 6, 6.5, 7, 7.5 and 8. Cultures were incubated on a rotary shaker at 30°C and 150 r/min for 7 days. To determine the effect of temperature, MSL medium with pH of 7 was incubated at 20, 25, 30, 35 and 40°C under 150 r/min. Cells number of the bacterial strain were determined by plating appropriate dilutions of liquid medium onto mineral salt agar medium containing pendimethalin.

Dissipation of pendimethalin by *P. putida* (E15) in mineral liquid medium

P. putida (E15) was cultured onto MSA medium + pendimethalin for 7 days and then the growing colonies were washed with 3 ml sterilized MSL medium. The bacterial cell suspension (10^7 cfu/ml) was then used to inoculate 100 ml MSL medium containing (100 µg/ml) of pendimethalin. The cultures were incubated at 30°C and 150 r/min for 0, 7, 14, 21 and 28 days. The percentage of dissipation and the half-life of pendimethalin were determined as described afterward. Control flasks of equal volume of liquid mineral medium and pendimethalin without any microbial population were run in parallel at all intervals to assess a biotic loss.

Bioremediation of pendimethalin contaminated soil and phytotoxicity test

The phytotoxicity bioassay of pendimethalin was performed in the contaminated soil after 28 days treatment with *P. putida* (E15) and compost. Cucumber plants (*Cucumis sativus* L., cultivar Hisham) were used as the test organism. The phytotoxicity was determined as deformation in morphological and histological cucumber plants comparing to the treatments with *P. putida* (E15) and compost. All treatments were compared with the control treatment (untreated soil).

Clay soil with no previous history of pendimethalin concentration was collected from top 12–15 cm randomly following standard procedure and sieved through 2 mm size sieve (Gupta, 2000). The experiments were conducted in 1000 g capacity pots (polyethylene pots, 20 cm inner diameter and 30 cm in depth), each having 1000 g dried clay soil. Soil was contaminated with pendimethalin (100 µg/gm soil) at 2% moisture level in their respective treatment pot before one week from cucumber sowing.

P. putida (E15) was cultured onto MSA + pendimethalin for 7 days and then the growing colonies were washed with 3 ml sterilized MSL liquid medium. One hundred milliliter from cell suspension (10^7 cfu/ml for bacterial strains) was then used to inoculate 1 kg clay containing (100 µg/gm) from pendimethalin before one week from cucumber sowing, mixed well and kept under incubation for 28 days at temperature $30 \pm 2^\circ\text{C}$. Compost was used as soil amendments. The calculated quantity that is 100 g of compost was applied before sowing of trial in respective treatment pot, mixed well and kept under incubation for 28 days at $30 \pm 2^\circ\text{C}$ (Belal et al., 2008) under greenhouse conditions. Five cucumber seeds of (*Cucumis sativus* L., cultivar Hisham) were sown in each pot after one week from soil contaminated with pendimethalin.

The residue half-life (RL_{50}) for pendimethalin residues was calculated using the equation of Moye et al. (1987). Control pots of equal weight of soil and pesticide without any microbial population or compost were run in parallel at all intervals to assess a biotic

losses as well as measuring of the botanical parameters on cucumber plants as follow:

Growth characters and chlorophyll pigment contents

Germination percentage was determined on the 15th day from sowing. Percentages of seedling mortality were calculated as percentages of total number of germinated seeds. For seedlings characters, samples were taken at 15 days from sowing to estimate seedling hypocotyl length (cm), fresh and dry weights of cucumber seedlings (dried in an oven at 70°C for 72 h) g/plant. Chlorophyll a, b and total were determined in cotyledonary leaf and the first true leaf using spectrophotometer method as described by Moran and Porath (1980).

Histological parameters

The seedling hypocotyl specimens were taken from the middle region. The leaf specimens including the midrib were taken from the first true leaf. Specimens were taken on the 10th day of sowing. Specimens were fixed in formalin alcohol acetic acid mixture (FAA, 1: 18: 1; v/v), washed and dehydrated in alcohol series. The dehydrated specimens were infiltrated and embedded in paraffin wax (52–54°C m. p.). The embedded specimens were sectioned using a rotary microtome (Leica RM 2125) at a thickness of 8 – 10 µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranin and azur II (Gutmann, 1995), cleared in xylol and mounted in canada balsam (Ruzin, 1999). Ten reading from 3 slides were examined with microscope (Lieca DM LS) with digital camera (Lieca DC 300), and then photographed. The histological feature of the hypocotyl was thickness of hypocotyl, vascular and cortex tissues as well as number of vessels/bundle. Moreover, the histological features of the first true leaf were thickness of lamina, midrib region, midrib vascular bundle, mesophyll (palisade and spongy tissues) and vascular tissues (xylem and phloem) in addition to the No. of vessels/midrib vascular bundle. The histological manifestation was calculated using Lieca IM 1000 image manager software. Lieca software was calibrated using 1 cm stage micrometer scaled at 100 µm increment (Leitz Wetzler, Germany 604364) at 4 and 10 X magnifications.

Analytical procedure

Extraction and determination of pendimethalin residues was carried out by the described method of Jażwa et al. (2009) at Central Agric. Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Pendimethalin residue in soil was monitored weekly after application date. At each sampling time four soil samples were taken from randomly selected pots of cucumber plants. At the end of that test, pendimethalin residues were determined. Soil samples were air-dried, ground and stored at room temperature prior to analysis but no more than three days. Subsamples (20 g) were extracted by shaking for one hour with 100 ml of dichloromethane-acetone mixture (9:1 v:v) on a rotary shaker. The extract obtained, was decanted by a layer of anhydrous sodium sulphate and the soil was rinsed two times with 10 ml of dichloromethane (Ambrus et al., 1981; Luke et al., 1975, 1981; Sadlo, 1998). The extract was cleaned using florisil (Valverde-Garcia et al., 1991). The analysis of the extract was performed using a Hewlett Packard 5890A gas chromatograph, equipped with a nitrogen – phosphorus detector (GC-NPD). The column used in this study was an HP fused – silica capillary column coated with cross-linked methyl silicone (length 25 m, ID 0.31, film thickness 0.52 µm). Nitrogen was used as both the carrier and make-up gas at a flow rate of 30 ml/min. Hydrogen was used at a flow rate of 3.5 ml/min. and air at 120 ml/min. The oven temperature was programmed as follows: initial temperature 150°C (1 min.), rate of 10°C/min. and final tem-

Table 1. Growth ability of the isolated bacterial strains in MSL supplemented with pendimethalin .

Bacterial strain	Growth ability (Log CFU/ml)	
	MSL (control)	MSL + pendimethalin
E15	0	8.6
E16	0	6.3
E17	0	4.6
E18	0	4.5
E19	0	4
E20	0	4
B1	0	3.3
B2	0	3
Act1	0	2.3
Act2	0	2

perature 250°C. Recovery studies were carried out regularly by spiking analytical samples with stock solution of pendimethalin standard.

Statistical analysis

Data were subjected to statistical analysis of variance according to the method of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Isolation of the pendimethalin-dissipation isolates

The pre-treated soil samples with pendimethalin were used to isolate the pendimethalin-dissipating microorganisms in the present study. By using enrichment techniques, a total of 10 morphologically different microorganisms capable of dissipating pendimethalin were isolated from the soil (Table 1). A preliminary classification based on the morphology of the isolates revealed that, the pendimethalin- dissipating microorganisms belongs to the group of bacteria. Six out of 10 bacterial isolates were Gram-negative, motile, rods and oxidase positive. Two out of 10 were Gram positive, motile, spore forming rod shaped bacterium as well as 2 out of 10 were Gram positive and filamentous shaped bacterium. Results in (Table 1) show that, one strain (E15) gave the highest growth on MSL medium supplemented with pendimethalin as the sole source of carbon comparing with the other strains. This indicates that this strain may show a high potential for pendimethalin degradation. The obtained results were compared with the growth of the strains in MSL medium only (no pendimethalin enriched). The bacterial isolates were also routinely streaked onto plates of LB for bacterial isolate.

This bacterial strain (E15) was identified according to the morphological, physiological as well as using analysis of 16S rDNA (Figure 1). This bacterial strain (E15) was Gram-negative, motile, rods and oxidase positive. According to the 16S rDNA analysis, the phylogenetic tree of

the isolated bacteria (E15) and related bacterial species based on the 16S rDNA sequence is provided in Figure 1. It can be clearly seen that the isolated bacteria was included in the genus *Pseudomonas* and closely related to the species *P. putida*. It showed the highest sequence similarities with *P. putida* F1 (98%).

Our results are in agreement with previous finding reported by Kopytko et al. (2002), Karpouzias et al. (2005), Belal et al. (2008), Derbalah and Belal (2008) and Megadi et al. (2010). It was found that enrichment culture technique led to the isolation of two bacterial strains, which were able to degrade different pesticides rapidly in liquid cultures. The application of pendimethalin promotes the evolution of microorganisms that are capable of degrading this xenobiotic compound in the soil (Chaudhry and Ali, 1988). Chaudhry and Ali (1988) reported that, actinomycetes have considerable potential for the bio-transformation and biodegradation of pesticides. Members of this group were Gram-positive bacteria and have been found to degrade pesticides with widely different chemical structures including organochlorines, s-triazines, triazinones, carbamates, organophosphates, organophosphonates, acetanilides, sulfonylureas and herbicide metolachlor (Krause et al., 1985; De Schrijver and De Mot, 1999).

Effect of environmental factors (pH and temperature) on the growth of *P. putida* (E15)

Normally, the pH and temperature influence the growth of microorganisms and hence, these factors will influence also the degradation process of the pesticides. Karpouzias and Walker (2000) reported the degradation of ethoprophos by *P. putida* strains epl and II affected by pH and temperature. Hong et al. (2007) found that various factors including pH and temperature affected degradation of fenitrothion-contaminated soil using *Burkholderia* sp. FDS-1. Belal et al. (2008) and Derbalah and Belal (2008) found also that the pH and temperature affected cadusafos, carbofuran and cymoxanil degrading microorganisms

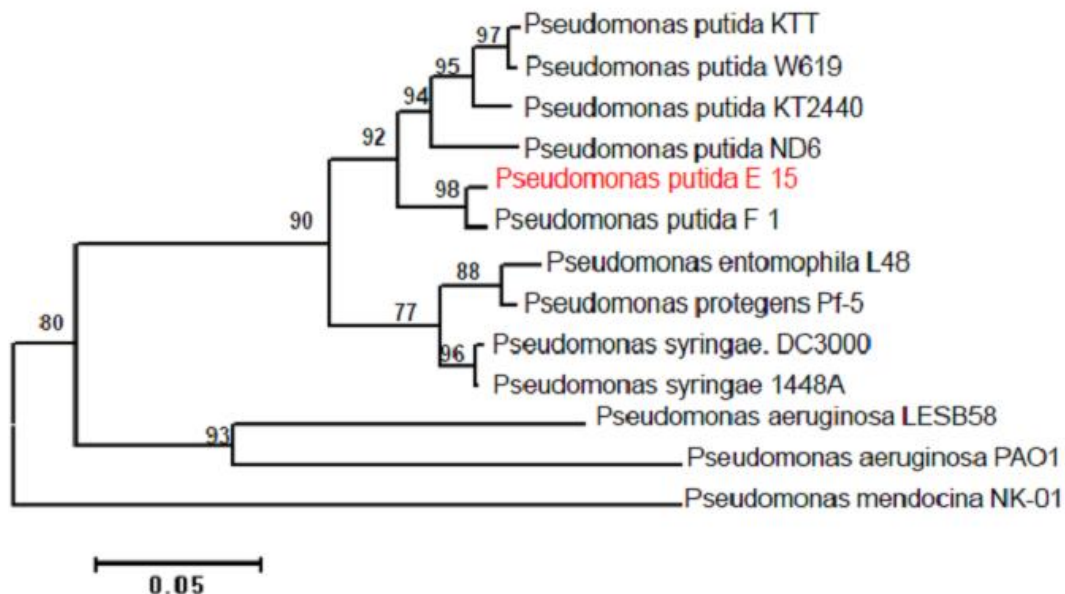


Figure 1. Dendrogram illustrating the genomic relationship among seventy isolates belonging to genus *Pseudomonas* revealed by UPGMA cluster analysis.

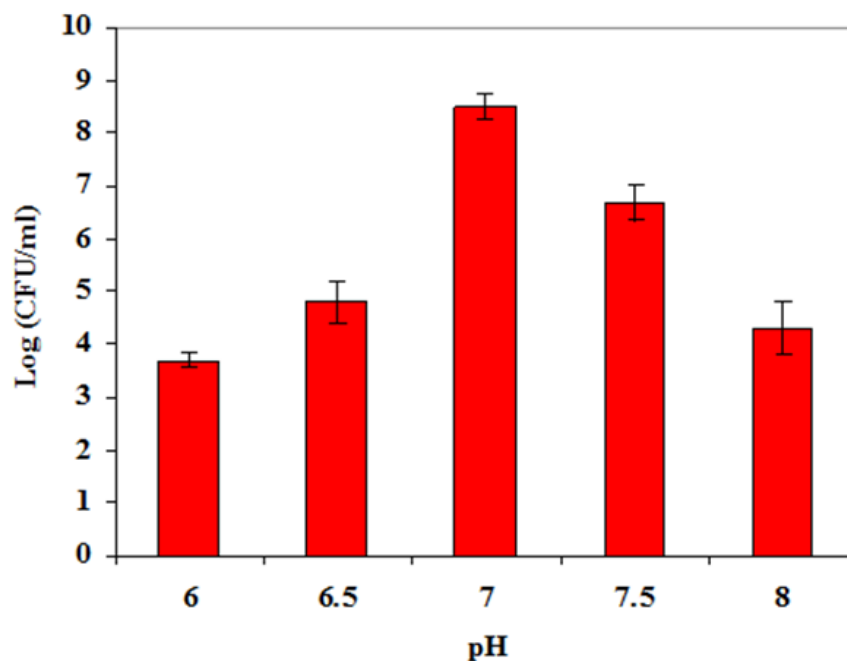


Figure 2. Effect of pH on growth ability of *P. putida* (E15).

comprising fungi and bacteria.

The influence of pH on biomass yield by the tested isolates is shown in Figure 2. Generally, the optimum pH was 7 for either bacterial or fungal isolates. Since, the maximum growth for *P. putida* (E15) was recorded at pH 7 (Figure 2). It is known that the most of bacterial isolates prefer the neutral pH. However, the tested bacterial strain in this study can grow at range of pH from 6 to 8.

Ross and Marco (1978) reported previously that metaxyl acid (N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine) resulting from the hydrolysis of the methyl ester group of pendimethalin was determined as the major metabolite in field soils at potato harvest and this may allow the degrading effect of metaxyl.

The effect of different temperatures on the growth of *P. putida* (E15) is shown in Figure 3. The temperature of

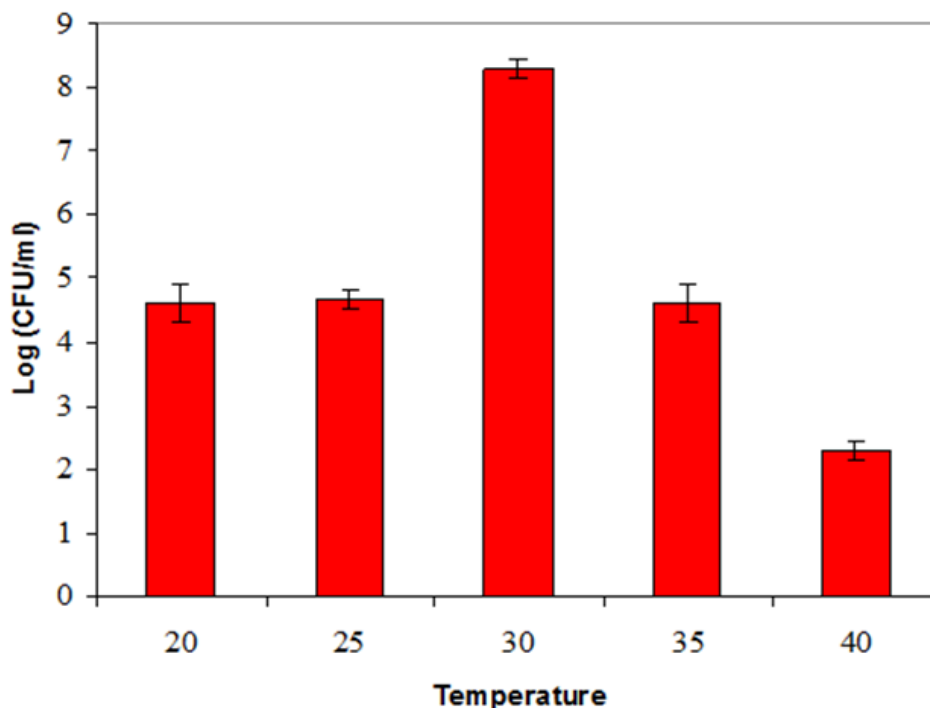


Figure 3. Effect of temperature on growth ability of *P. putida* (E15).

30°C appears to be the optimum degree for growth of *P. putida* (E15). Moreover, the tested microbial isolates exhibited growth at 40°C of *P. putida* (E15) was used for further studies under the optimum growth conditions to evaluate their degradation potential for pendimethalin at different incubation times (0, 7, 14, 21, 28 days).

Dissipation of pendimethalin by *P. putida* (E15) in mineral liquid medium

The ability of *P. putida* (E15) to dissipate pendimethalin is illustrated in Figure 4. The results indicate that *P. putida* (E15) was the most efficient strain in pendimethalin dissipation with a half-life of 5.46 days. One hundred percent of pendimethalin initial concentration was dissipated within 4 weeks by *P. putida* (E15). Pendimethalin half-life was 62.43 days in untreated liquid medium as control treatment.

The growth response of pendimethalin *P. putida* (E15) increased gradually with the pendimethalin dissipation rate increasing as shown in Figure 4.

This is suggesting that different microbial types, which may be using different enzymes, have different degradation preferences. On the other hand, pendimethalin dissipation percentage reached to 17% at the end of incubation time in the control or non-inoculated samples. This implies that the quote of pendimethalin decay due to temperature effect and volatilization (Strandberg and Scott-Fordsmand, 2004). Many authors reported earlier that *Pseudomonas* has considerable potential for the bio-

transformation and biodegradation of pesticides. Members of this group are Gram-negative bacteria and have been found to degrade pesticides with widely different chemical structures (Spain and Nishino, 1987; Kyria et al., 1997). The degradation of some pesticides may be attributed to the secretion of enzymes from either tested bacterial or fungal strains which are capable of degrading of pesticides (Bollag and Liu, 1990).

With regard to biological metabolization, in vitro degradation of pendimethalin has been demonstrated by numerous authors. For instance, Kole et al. (1994) observed that 45 and 55% metabolism of pendimethalin caused by *Azotobacter chroococcum* after 10 and 20 days of incubation, respectively. *Azotobacter vinelandii* was isolated from a pendimethalin-treated barley rhizosphere. *A. vinelandii* utilized pendimethalin as the sole source of carbon to fix N₂ (Saha et al., 1991; Singh and Kulashrestha, 1991). Pendimethalin was degraded by oxidative N-dealkylation to yield 3, 4-dimethyl-2,6-dinitroaniline and pentane. However, 6-aminopenimethalin and 3, 4-dimethyl-2,6-dinitroaniline were not further metabolized because they neither supported growth of organism nor stimulated oxygen uptake. But the pentane, released by oxidative N-dealkylation of pendimethalin, was utilized as the sole source of carbon and energy for the growth of the organism. The acetylation, aryl methyl oxidation and cyclization products of pendimethalin, as reported in *Azotobacter chroococcum* (Holding and Collee, 1971; Kole et al., 1994). Megadi et al. (2010) reported that pendimethalin degradation with *Bacillus circulans* was by

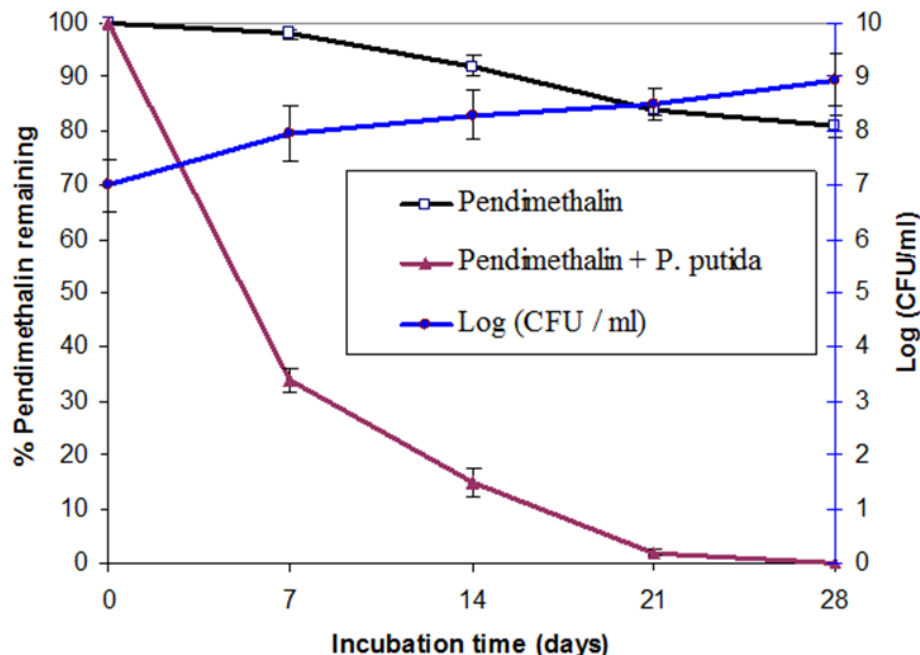


Figure 4. Dissipation of pendimethalin by *P. putida* (E15) in mineral liquid medium.

nitroreduction and oxidative N-dealkylation via secretion of pendimethalin nitroreductase and pendimethalin N-dealkylase. The microbial degradation of dinitroaniline herbicides, pendimethalin and trifluralin has been reported to occur most often by oxidative N-dealkylation and nitroreduction. The nitro group reduction and oxidative N-dealkylation destroys the herbicidal activity of pendimethalin, leading to its detoxification (Zayed et al., 1983; Singh and Kulashrestha, 1991; Kole et al., 1994; Megadi et al., 2010).

Bioremediation of pendimethalin contaminated soil and phytotoxicity test

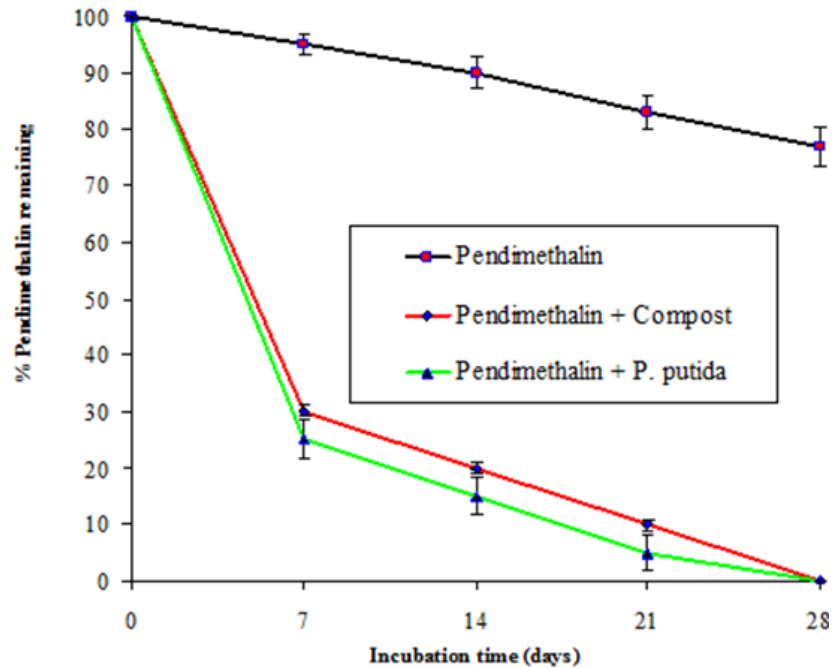
Results in Figure 5A show the dissipation rate of pendimethalin by *P. putida* (E15) and compost in clay soil. Pendimethalin dissipation rate by *P. putida* (E15) was similar with compost. Pendimethalin half-lives were 4.67 and 5 days for *P. putida* (E15) and compost in clay soil, respectively. Pendimethalin half-life was 51.9 days in untreated clay soil. The loss of pendimethalin in untreated clay soil was 23% and this may be due to evaporation, dirt or leaching. The trend of dissipation rate of pendimethalin by bacterial strain and compost was similar in the tested clay soil. The obtained results showed that the bacterial strain and compost play an outstanding role in degradation of pendimethalin in clay soil. The results exhibited that increasing in loss of pendimethalin after initial phase (7 days), and thereafter dissipation of the pendimethalin increased gradually till the end of the incubation time 28 days and this may be

due to accumulation of biodegradation products. Data in Table 2 show characterization of clay soil and compost.

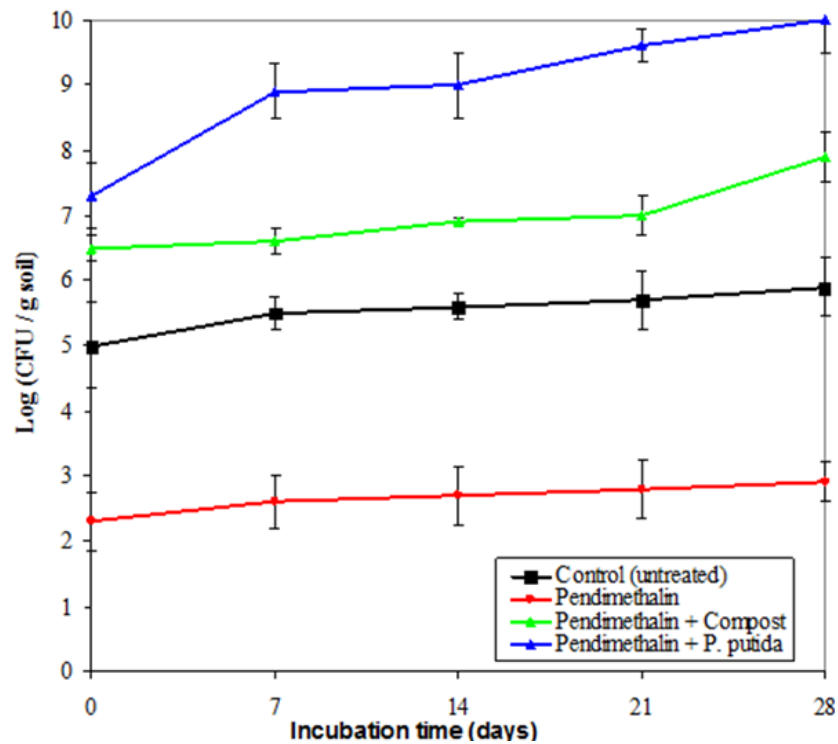
The obtained results in Figure 5B showed that, pendimethalin treatments affected the analysis of the microbial population growing in *P. putida* (E15) or compost treated soil leachates showed an overall increase in the population of microorganisms. Pendimethalin treatment decreased the population of the microorganisms compared with the other treatments. The population of the microorganisms was reduced after one weeks and a slight stimulation was noted in the next weeks in pendimethalin treatment as compared with the unweeded control.

Biodegradation of pesticides in soil was reported with microorganisms and compost (Cole et al., 1995; Belal et al., 2008). Previous studies by Karpouzas and Walker (2000) have reported the isolation of two ethoprophos-degrading *P. putida* strains, which were also able to degrade cadusafos but in a less efficient way compared to the *Flavobacterium* and *Sphingomonas* strains. *Flavobacterium* strains have been reported to be responsible for the degradation of carbofuran (Chaudhry and Ali, 1988).

More potent strains that degraded pendimethalin rapidly were obtained from a soil samples which pendimethalin had been applied or exposed for a number of years or the time an enrichment technique. This indicates that repeated applications or exposure of soil or mature compost to xenobiotic compounds for a long period of time can result in the evolution of microorganisms capability of degrading these compounds rapidly and more extensively.



A



B

Figure 5. A, Disipation of pendimethalin contaminated soil by *P. putida* (E15) and compost; B, Population of microorganisms in pendimethalin contaminated soil.

Although addition of these bioprocessed materials has been an integral part of sustainable agriculture practices and offers a good nutrient source for microbes (Laine and

Jorgensen, 1996) and enhancers of microbial activity include moisture, inorganic nutrients, and oxygen. There are many well-established bioremediation technologies

Table 2. Physicochemical characteristics of clay soil and compost.

Parameter	Compost	Clay soil
pH	7.2	7.2
Electrical conductivity	1.8	2.2
organic matter%	17.5	1.43
Nitrogen (ppm)	311.4	130.2
Potassium (ppm)	9.8	0.042
Phosphates (ppm)	126.3	12.14
Cadmium (ppm)	0.3	1.72
Nickel (ppm)	0.83	3.04
Lead (ppm)	1.3	9.5
Manganese (ppm)	5.2	7.98
Copper (ppm)	3.5	4.44
Iron (ppm)	10.1	13.26
Seed germination test %	93	92
Total count of microorganisms	6×10^6 cfu/g	1×10^5 cfu/g
Phytopathogenic agents (fungi, bacteria and nematodes)	Negative	Negative
Total coli form counts	Negative	Negative
Total Salmonella counts	Negative	Negative

applied commercially at contaminated sites. One of such technology is the use of compost material and biogas slurry. Compost is rich sources of microorganisms, which can degrade contaminants to innocuous compounds such as carbon dioxide and water.

Earlier studies have also reported that bioprocessed materials such as compost and biogas slurry were used to degrade atrazine herbicide in contaminated soil using various bioprocessed materials (Cole et al., 1995; Kadian et al., 2008). Due to their high organic matter content, all bioprocessed materials accelerated cadusafos and carbofuran breakdown. Earlier studies have also reported high microbial biomass in soil that received the organic carbon amendment (Drenovsky and Richards, 2005), who found that addition of compost provided a rich source of microorganisms. Production of compost with a high nutritional content, that could be further used as inocula for the treatment of hazardous waste. Kulshrestha and Singh (1992) observed that 11–14% of pendimethalin degradation could be attributed to microbial transformation in sandy soil after 91 day. Oliver (1979) reported that, after application to soil, pendimethalin may dissipate through evaporation, drift, leaching, and runoff. A laboratory experiment simulating winter conditions showed that as much as 10% of the applied pendimethalin (0.6 mg/kg applied) evaporated if it was applied on the soil surface. Nayak et al. (1994) investigated the effect of pendimethalin on populations of bacteria, fungi, and actinomycetes in sesame soil (sandy loam, pH 5.8, available N, P, and K 21, 23.7 and 53.75 kg/ha, respectively) at Bhubaneswar, India. The dilution plate method was used to enumerate populations of bacteria, fungi, and actinomycetes from soil samples. It was found that pendimethalin (0.5 kg/ha) significantly reduced bacteria

(61%) after 25 days but not after 50 and 75 days, at which time a slight stimulation was noted as compared with the unweeded control. Fungi were significantly reduced by 19% after 25 days and stimulated after 50 and 75 days as compared with unweeded control. Actinomycetes were substantially reduced by 21% after 25 days and stimulated after 50 and 75 days. Sidhu et al. (1985) and Barua et al. (1991) studied the effect of pendimethalin on populations of fungi, bacteria, and actinomycetes. A significant decrease was observed on the first few days after the application, but after a period of 6 weeks, recovery to the level of the control was reached or almost reached. Bacteria were almost unaffected after 42 days, while actinomycetes were the most one.

Phytotoxicity assessment

The effect of the remaining toxicity of pendimethalin in clay soil on germination, growth and anatomical characters of cucumber plants was estimated after treatment with compost and *P. putida* (E15).

The results in Table 3 show the influence of the remaining toxicity of pendimethalin in clay soil on germination and seedling mortality percentage of cucumber seedling after treatment with compost and *P.*

putida (E15). Pendimethalin caused the highest value in reduction germination and increasing seedling mortality percentage of cucumber seedling compared with the control treatment. These parameters were improved with compost and *P. putida* (E15) treatment compared with pendimethalin treatment. Compost treatment was more effective in the reduction of seedling mortality compared with *P. putida* (E15) treatment. The efficacy of compost and *P. putida* (E15) was similar in increasing germination

Table 3. Effect of the remaining toxicity of pendimethalin in clay soil on cucumber seed germination and seedling mortality percentage after treatment with compost and *P. putida* (E15).

Treatment	% Germination	% Seedling mortality
Pendimethalin	80	25
Pendimethalin + Compost	90	11
Pendimethalin + <i>P. putida</i> (E15)	87	18
Control	92	0
LSD (0.05)	3.52	4.6

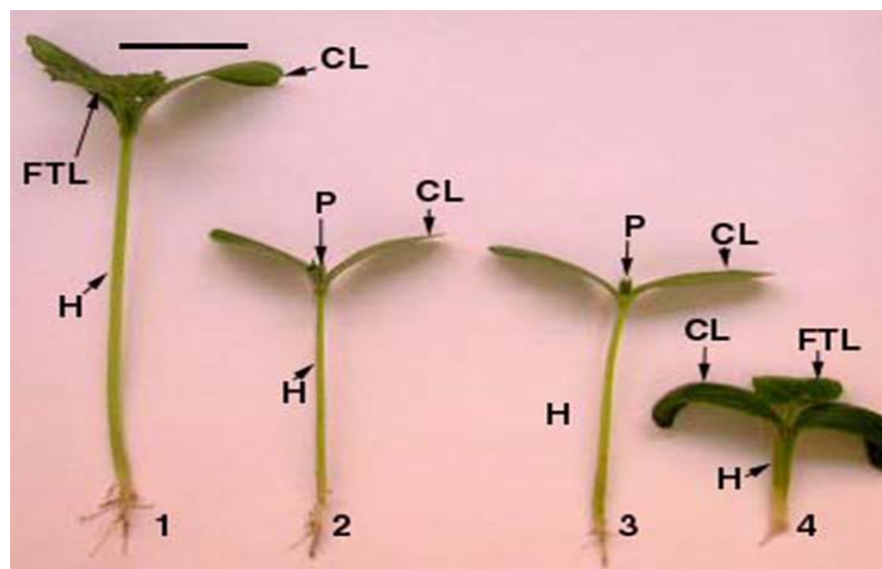


Figure 6. Cucumber seedling 15 days after sowing, 1- Control (untreated), 2- Pendimethalin + Compost, 3- Pendimethalin + *P. putida* (E15), 4- Pendimethalin; Hypocotyl (H), Cotyledonary leaf (CL), Plumule (P), First true leaf (FTL), Bar = 3cm.

percentage of cucumber seedlings compared with pendimethalin treatment. All treatments were compared with unweeded treatment (control) after 15 days from sowing.

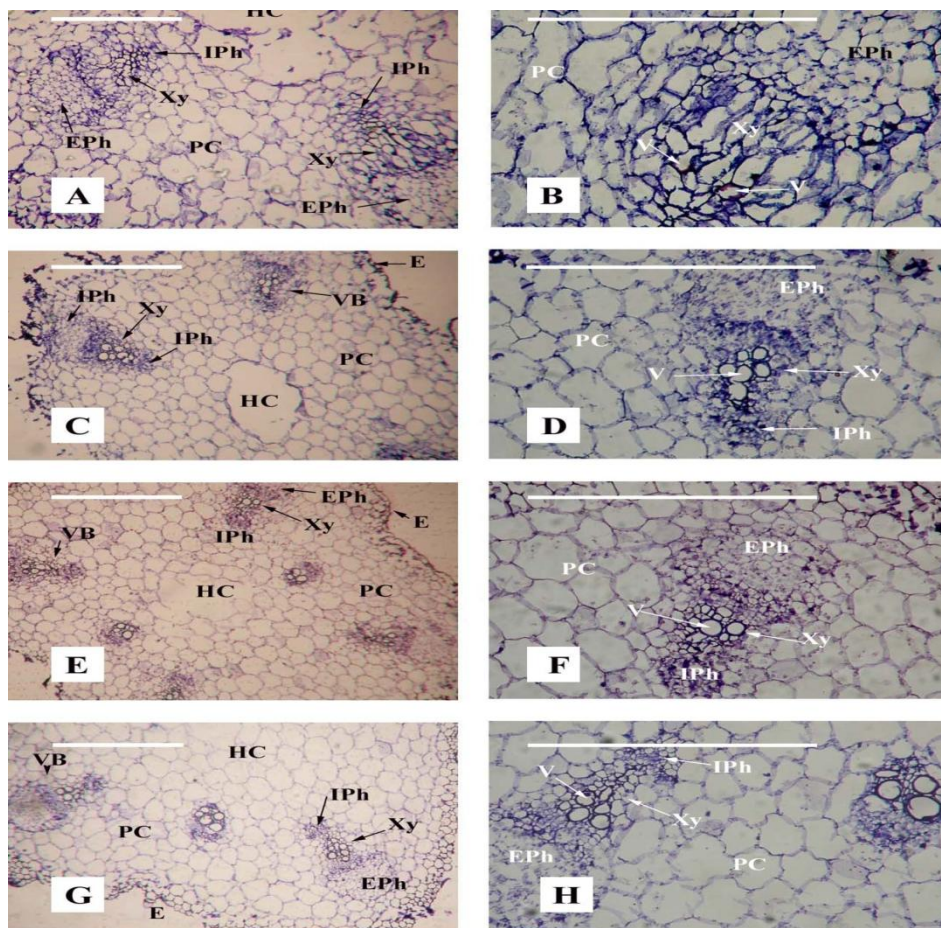
Data presented in Table 4 and Figure 6 illustrate the effect of the remaining toxicity of pendimethalin in clay soil on hypocotyl length and fresh and dry weight of cucumber seedlings after treatment with compost and *P. putida* (E15). The plant parameters (hypocotyl length, fresh and dry weight) of cucumber seedlings were reduced in the case of treatment with pendimethalin compared with other treatments. These parameters increased with compost and followed by *P. putida* (E15). The control (without pendimethalin) treatment recorded the highest value for the measured plant parameters compared with the other treatments. Pendimethalin treatment reduced the measured botanical parameters more than the other treatments and this is due to pendimethalin residues in soil which were 95, 30 and 25% with pendimethalin, compost and *P. putida* (E15) treatments, respectively. These plant parameters were improved gradually when pendimethalin residues disappeared.

Application of pendimethalin significantly increased chlorophyll pigment (chlorophyll a, chlorophyll b and total contents of chlorophyll) in cotyledonary and the first true leaf of cucumber seedling compared with the other treatments. Chlorophyll pigment contents in cotyledonary leaf were higher than in the first true leaf (Table 5). It is interesting to note that, the increase in chlorophyll pigment contents is accompanied with the increase in mesophyll tissue thickness (Table 5 and Figure 7).

The hypocotyls internal structure of cucumber is similar to stems of dicotyledon plants. The hypocotyl structure of cucumber plants as seen in transverse sections consists of the epidermis, ground tissue and vascular system (Figure 7). The regions between the bundles are parenchymatous. The vascular bicollateral bundles arranged in complete cylinder (Siphonostele: eustele). Two types of bicollateral vascular bundles are present, that is, large and small bundles. Data presented in Table 6 and Figure 7 revealed that, effect of the remaining toxicity of pendimethalin in clay soil on some anatomical parameters of cucumber seedling hypocotyl after treatment with compost and *P. putida* (E15).

Table 4. Effect of the remaining toxicity of pendimethalin in clay soil on hypocotyl length, fresh and dry weight of cucumber seedlings after treatment with compost and *P. putida* (E15).

Treatment	Seedling hypocotyl length (cm)	Fresh weight (g/seedling)	Dry weight (g/seedling)
Pendimethalin	3.1	0.521	0.057
Pendimethalin + Compost	6.2	0.986	0.113
Pendimethalin + <i>P. putida</i> (E15)	6	0.882	0.104
Control	9.4	1.886	0.284
LSD (0.05) =	1.23	0.102	0.005

**Figure 7.** Cross sections through cucumber hypocotyle, A-B: Pendimethalin, C-D: Pendimethalin + *P. putida* (E15), E-F: Pendimethalin + Compost, G-H: Control (untreated), Hypocotyls cavity (HC), Xylem (Xy), External phloem (Eph), Internal phloem (IPh), C = Cortex (Co), Vessel (V), Bar = 500 µm.

Application of pendimethalin increased seedling hypocotyl cross section, cortex and conductive vascular tissues (xylem and phloem) thickness compared with other treatments.

On the other hand, the lowest number of vessels per bundle was reduced by pendimethalin treated soil in comparison with the other treatments. Application of compost and *P. putida* (E15) decreased these anatomical parameters compared to the control. Abnormal development

of xylem tissue was noticed by pendimethalin treated soil treatment as a result of phytotoxicity.

The leaf lamina internal structure of cucumber plants is similar to other dicotyledons plants. It consists of upper and lower epidermis and mesophyll tissue, which differentiate into palisade and spongy parenchyma. Epidermis, one layer of completely arranged parenchymatous cells, which are flattened parallel to the leaf surface. The palisade parenchyma cells are elongated and completely

Table 5. Effect of the remaining toxicity of pendimethalin in clay soil on chlorophyll pigment (a, b and total chlorophyll) of cotyledonary and the first true leaves of cucumber seedlings after treatment with compost and *P. putida* (E15).

Treatment	Chlorophyll content in cotyledonary leaf			Chlorophyll content in the first true leaf		
	chlorophyll a	chlorophyll b	Total chlorophyll	Chlorophyll a	chlorophyll b	Total chlorophyll
Pendimethalin	8.21	3.80	11.09	7.61	3.39	11.02
Pendimethalin + Compost	6.76	2.97	9.73	4.43	1.90	6.33
Pendimethalin + <i>P. putida</i> (E15)	5.5	2.48	8.02	4.35	2.18	6.52
Control	6.76	3.32	10.08	5.15	2.30	7.85
LSD (0.05) =	1.18	0.43	1.001	0.70	0.273	0.176

Table 6. Effect of the remaining toxicity of pendimethalin in clay soil on some anatomical parameters of cucumber seedling hypocotyl after it treatment with compost and *P. putida* (E15).

Treatment	Seedling hypocotyl cross section thick (mm)	Cortex thick (μm)	Xylem thick (μm)	Number of Vessels/bundle	External phloem thick (μm)
Pendimethalin	4.3	1720	464.8	3	398.4
Pendimethalin + Compost	1.96	640	166	7	182.6
Pendimethalin + <i>P. putida</i> (E15)	1.78	800	128	6	166
Control	2.12	880	186	8	199.2

arranged. The spongy parenchymatous cells are loosely arranged with numerous large intercellular spaces. Data presented in Table 7 and Figure 8 indicated that, anatomical parameters of the first true leaf of cucumber show similar trend as those of seedling hypocotyl. Lamina, palisade, spongy, conductive vascular tissues as well as midrib thickness were induced by pendimethalin soil treatment in compared with other treatments. Number of vessels per bundle was 3 for pendimethalin treatment and 13, 12, 14 for compost, *P. putida* (E15) and control (untreated with pendimethalin), respectively. It is interesting to indicate that, the internal growth parameters were concomitant with the growth parameters. No available literature was found concerning the anatomical differences, which may be useful for understanding the effect mechanisms of pendimethalin on cucumber plants.

Herbicides play an important role in the production of vegetables but their residues may cause numerous environmental problems. First of all, they may contaminate surface and groundwater through leaching and run-off. They may also remain on the soil surface and potentially affect quality and yield of the next crop cultivated on the same field. Finally, stable herbicides may be taken up by a plant forming unwanted residues. With regard to plants, pendimethalin shows differential toxicity to various species, and there is a formulation-dependent toxicity to non target plant species.

The efficacy of pendimethalin action against target organisms has been the objective of a large number of

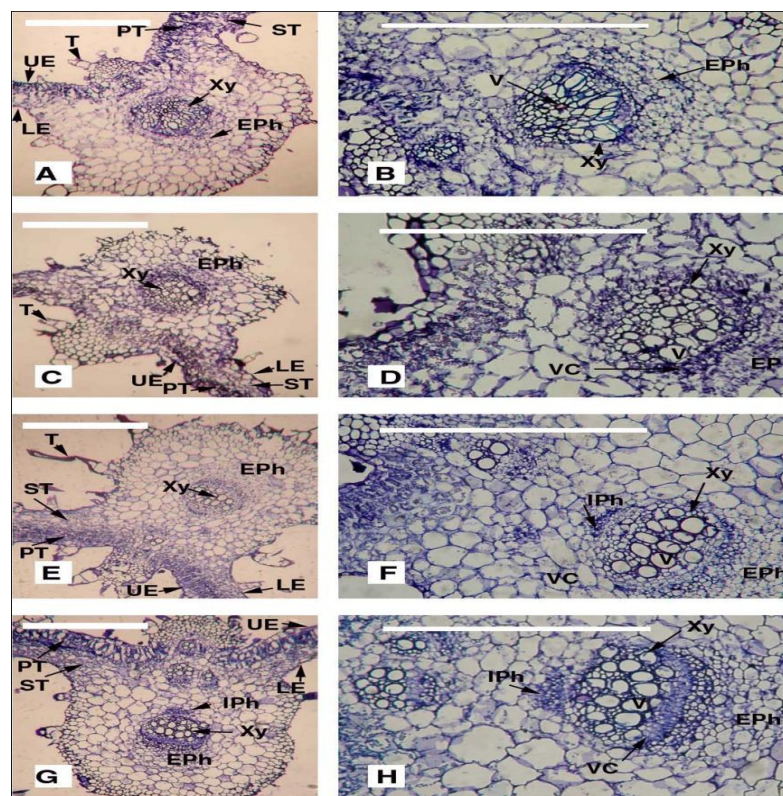
investigations, including, for example, control of *Trianthema portulacastrum* and effect on yield of fodder oats (Brar and Walia, 1995), control of the population of broad-leaved weeds, grass, sedges, and total weeds at definite intervals after application (Gowda and Devi, 1984), control of common vetch, *Vicia sativa*, and black medic, *Medicago lupulina* (Norcini et al., 1997).

Pendimethalin is similar to other broad-spectrum herbicides in that it is phytotoxic to crop species to some extent. In the development of herbicides, screening experiments are conducted to ascertain extent of phytotoxicity to crops. The phytotoxicity of pendimethalin to crop species has been the focus in numerous experiments, for example, rice grain, and straw yield (Devi and Gowda, 1985), root suppression of pampas grass (Green et al., 1997), effect of repeated application on cotton yield and quality, cotton fiber quality and yield (Keeling et al., 1996).

There is indication that dinitroaniline herbicides (include Pendimethalin) inhibit photosynthesis, oxidative phosphorylation, protein, nucleic acid and lipid synthesis (Moreland et al., 1972). Cotyledonary leaf and hypocotyls and first true leaf of seedling treated with pendimethalin seem to be dark green colors, swelling and brittleness. Pendimethalin treatment caused reduction in primary root length and number of lateral roots. This may be due to the fact that the radicle is the first organ to come directly in contact with pendimethalin in the soil. Compost and *P. putida* (E15) disappeared the dark green colors and

Table 7. Effect of the remaining toxicity of pendimethalin in clay soil on some anatomical parameters of the first true leaf of cucumber plants after treatment with compost and *P. putida* (E15).

Treatment	Lamina thick (μm)	Palisade tissue thick (μm)	Spongy tissue thick (μm)	Midrib thick (μm)	Midrib vascular bundle thick (μm)	Xylem thick (μm)	Number of Vessels/bundle	Phloem thick (μm)
Pendimethalin	215.8	66.4	116.2	1095	448.2	265.6	3	166
Pendimethalin + Compost	99.6	16.6	66.4	999	365.2	215.8	13	116
Pendimethalin + <i>P. putida</i> (E15)	167	49.8	99.6	1328	400.6	196.2	12	166
Control	189	66.4	100	1329	431.8	200.1	14	149

**Figure 8.** Cross sections through the first true leaf of cucumber plants, A-B: Pendimethalin, C-D: Pendimethalin + *P. putida* (E15), E-F: Pendimethalin + Compost, G-H: Control (untreated), Hypocotyls cavity (HC), Xylem (Xy), External phloem (EPh), Internal phloem (IPh), Vascular cambium (VC), Vessel (V), Upper epidermis (UE), Lower epidermis (LE), Trichome (T), Palisade tissue (PT), Spongy tissue (ST), Bar= 500 μm .

improved the lateral roots. Smith (2006) recorded, that pendimethalin markedly inhibited the growth of both seedling weeds and crops. It was found that, dinitroaniline herbicides kill seedling weeds by inhibiting the development of lateral roots in susceptible plants, stunting the above-ground parts, with the development of a dark green color, swelling and brittleness of the stem or seedling hypocotyl (Parka and Soper, 1977). Severe crop phytotoxicity and damage symptoms reported in literature range from reduced or inhibited germination, reduced root length, protein and nucleic acid contents of root tips, injured flowers, to complete crop failure and residual persistence of herbicides in crop and soil (Henderson and Webber, 1993; Sinah et al., 1996). Pendimethalin caused seedling mortality (Aluka, 1997), but does not prevent seedling emergence (Akobundu, 1987; Smith, 2004; Smith, 2006). In the present study, applications of pendimethalin reduced seedling emergence lower than the other treatments. The higher seedling phytotoxicity could be attributed to pendimethalin concentration in the soil. Thus, a carryover of herbicide residues from one crop season to the following one may occur even through the development of modern herbicides has been directed toward a short half-live in the environment (Fayez and Kristen, 1996).

Conclusion

In the present study, dissipation of pendimethalin-contaminated soil was studied by the addition of pure culture from *P. putida* (E15) and compost in 28 days. *P. putida* (E15) and compost showed high ability in Pendimethalin dissipation. There is no toxicity of pendimethalin detected in clay soil after treatment with *P. putida* or compost on cucumber plants, therefore these residues do not affect the following economical crops. It was observed that clay soil without any amendment (that is control) showed least dissipation of pendimethalin. Pendimethalin significantly decreased germination rate and increased cucumber seedling mortality rate. The results suggest that bioremediation by *P. putida* (E15) strain and compost were considered to be the effective method for detoxification of pendimethalin in soil system.

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