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Pendimethalin Degradation in Soil and Its Interaction with Soil Microorganisms

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Abstract

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Pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2, 6-dinitrobenzenamine] is a herbicide used worldwide to control most annual grasses and common weeds in cereals, fruit, and vegetables. Its degradation in Haplic Chernozem under controlled greenhouse conditions was studied in this paper. The effect of recommended and doubled pendimethalin doses, as well as the effect of the biopreparate EM-EKO ProBio Plus on pendimethalin degradation in soil and on soil microorganisms was investigated. Pendimethalin half-life ranged from 24.4 to 34.4 days and the double dose did not increase the pendimethalin half-life. Thirty-eight days after pendimethalin application there was no statistical difference between the pendimethalin concentration in soil when applied at the recommended and doubled dose. No effect of pendimethalin on the amount or the activity of soil microorganisms was observed. The effect of EM-EKO ProBio Plus was apparent only on the first sampling of double-dose pendimethalin, however, this bio-preparate had no significant effect on the half-life of pendimethalin, as observed at the end of the experiment.

Keywords: biodegradation; half-life; persistence; pesticides

Pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2, 6-dinitrobenzenamine] is a synthetic selective herbicide belonging to the group of dinitroaniline substances. According to the Central Institute for Supervising and Testing in Agriculture, the consumption of pendimethalin in the Czech Republic was 113 702.88 kg in 2014, placing it among the most widely used herbicides. Pendimethalin is absorbed by plant roots and leaves and inhibits mitosis and cell division. Pendimethalin is a herbicide used worldwide to control the spectrum of monocotyledonous and dicotyledonous weeds in crops of corn (*Zea mays* L.), sunflower (*Helianthus annuus* L.), soybean (*Glycine max* L. Merr.), peas (*Pisum sativum* L.), winter cereals, vegetables, pome fruit and stone fruit orchards, and vineyards. Pendimethalin can be applied as preplant-incorporated, preemergence, or postemergence with or without incorporation, because it has relatively low volatility. Due to its low water solubility (0.33 mg/l) and high Soil Organic Carbon-Water Partitioning Coefficient (Koc = 17 581), pendimethalin has low potential for leaching. Pendimethalin GUS (groundwater ubiquity score) is extremely low (0.59). Despite this, pendimethalin can be leached from the root zone and enter the aquatic environment in average concentrations exceeding the EU limit value for groundwater (0.1 µg/l) (Kjær *et al.* 2011).

The half-life of pendimethalin can differ according to soil and climatic conditions, from 4 days (Sevage & Jordan 1980) to 563 days (Walker & Bond 1977). Other factors such as cultivation practices, mode of

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application, repeated application (Piutti *et al.* 2002), and herbicide incorporation into the soil after its application (Talbert & Press 1997), or application of Fenton's reagent (Miller *et al.* 1996) can affect the pendimethalin half-life. The effect of soil moisture and soil temperature was described by Zimdahl and Clark (1984). They described increased pendimethalin degradation with the increase of both soil moisture and temperature, and they suggested that soil type may have less influence than temperature and soil moisture. Half-lives in sandy loam and clay loam were equal, but those in the clay and clay loam were different. However, the difference in the rate of degradation between soils was not great (42, 45, and 54 days).

After its application to soil, pendimethalin may dissipate through volatilization, 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) volatized if it was applied on the soil surface (Oliver 1979). When incorporated 5 cm into the ground in concentrations equivalent to 1.2 mg/kg, the loss was only 1% (Strandberg & Scott-Fordsmand 2004).

After pendimethalin reaches the soil, it is the subject of many transportation and transformation processes. Residual pendimethalin that has not been leached from the soil or volatilized may be degraded physically or chemically, or can be also metabolized. For example, photochemical decomposition may be responsible for up to 10% dissipation (Dureja & Walia 1989).

Application of pendimethalin also affects soil microorganisms. The initial reduction of soil microorganisms after pendimethalin application into soil and stimulation of soil microorganisms 50 and 75 days after pendimethalin application was reported by Nayak *et al.* (1994). The effect of pendimethalin on soil microorganisms and their activity was also studied by Miller *et al.*(1996), Shetty and Magu (1998), and Chikoye *et al.* (2014).

The aim of this study was to determine the half-life of pendimethalin in Haplic Chrenozem, to evaluate the effect of recommended and double doses, as well as the effect of the biopreparate EM-EKO ProBio Plus on pendimethalin degradation in soil and its interaction with soil microorganisms.

MATERIAL AND METHODS

Experiment set up. A Haplic Chernozem from the experimental field of the Czech University of Life Sciences Prague in Prague 6-Suchdol was used in this experiment. Soil samples were collected from topsoil (0–20 cm) in February 2014 and the principal soil properties were determined (sand 25%, silt 56%, clay 19%, pH_{H2O} 7.76, pH_{KCl} 7.33, oxidizable carbon (C_{OX}) 1.47%, cation exchange capacity (CEC) 23.5 mmol(+)/100 g, hydrolytic acidity 0.99 mmol(+)/100 g). Half of the soil was inoculated by the biopreparate EM-EKO ProBio Plus (EM-EKO, s.r.o., Prague, Czech Republic), which is a complex of microbial strains, containing lactic acid bacteria, yeast, fungi, Gram-positive actinomycetes, and photosynthetic bacteria. Ten ml of the biopreparate EM-EKO ProBio Plus was used for each pot with the volume of 650 cm^3 (tweight of wet soil in each pot was approximately 850 g). The inoculation was performed by spraying under continuous homogenization of the soil. The soil was transferred into pots immediately after inoculation. The second half of the soil was directly filled into the pots without any treatment. The next day, pendimethalin was applied by spraying on the soil surface (of both inoculated and uninoculated treatments) in two doses using the herbicide Stomp 330 (BASF, Ludwigshafen, Germany). The recommended dose corresponding to 5.3 l/ha and the double-dose corresponding to 10.6 l of Stomp 330/ha were used. Two treatments (inoculated and uninoculated soil) without pendimethalin were used as controls to evaluate the effect of pendimethalin and biopreparate EM-EKO ProBio Plus on soil microorganisms. In total, the experiment contained six treatments (each treatment in three replicates); the scheme is shown in Table 1. Soil samples for determining the pendimethalin concentration in soil were taken 5, 15, 38, and 84 days after the herbicide application. The soil samples for microbiological analyses were taken at the beginning (one day after pendimethalin spraying) and at the end of the experiment.

Analysis of soil microorganisms. Total numbers of fungi and mesophilic bacteria were determined using

Table 1. Scheme of the experiment

Treatments	Pendimethalin dose (kg/ha)	Inoculation
C	0	no
CI	0	yes
10	1.75	no
1I	1.75	yes
20	3.5	no
2I	3.5	yes

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the plate method on Potato Dextrose Agar (ME096; HiMedia, Mumbai, India) and Thornthon Agar (KNO_3 0.5 g, KH_2PO_4 1 g, $MgSO_4$ 0.2 g, $CaCl_2$ 0.1 g, NaCl 0.1 g, $FeCl_3$ 0.05 g, asparagine 0.5 g, mannitol 1 g, agar 10 g, distilled water 1000 ml) respectively. The dilution rate for bacterial spores determination was heated for 10 min at 80°C and the Most Probable Number (MPN) method with Nutrient Broth No. 2 (CM67; Oxoid, Ltd., Basingstoke, UK) was used to calculate the total number of bacterial spores. The dehydrogenase activity and soil respiration were determined in accordance with the methodology of Öhlinger (1995).

Pendimethalin determination in soil samples. Soil samples for determining pendimethalin concentration were frozen, freeze-dried, ground, and sieved using a 2 mm mesh sieve. Subsequently, the samples were homogenized and 10 g of soil were used for the extraction by 10 ml of methanol. The soil suspension was shaken for 20 h, and then centrifuged in a refrigerated centrifuge at 13 000 rpm for 20 min. The supernatant was filtered through a glass injection filter (0.7 µm) into vials. The concentration of pendimethalin in the soil extract was determined using a HPLC instrument from Dionex (Sunnyvale, USA). The instrument was assembled using a P680 HPLC Pump. The mobile phase was prepared by mixing 63% of acetonitrile, 37% of redistilled water, and 1 ml HCOOH per 1 l of the mixture. The flow rate of this mobile phase was maintained at the level of 1 ml/min. 10 µl of sample were injected via the Automated Sample Injector ASI-100 (Dionex, Sunnyvale, USA). The separation took place in a Kinetex 2.6 µm, C18, 100A Column (50 × 4.6 mm) placed in the Thermostatted Column Compartment TCC-100 (Dionex) set to 25°C. To prolong the lifetime of this column, the ChromSep Guard Column SS 10 × 3MM (Varian, Lake Forest, USA) was used. The detection of pendimethalin was performed on line in the UV region (240 nm) by means of PDA-100 Photodiode Array Detector (Dionex). The signal from the detector was processed and stored using chromatographic software Chromeleon, Ver. 6.70 (Dionex). The pendimethalin detection limit was 0.0015 mg/l.

Statistical analyses. Statgraphics Centurion Ver. 17 was employed for statistical evaluation of the results. The multifactor ANOVA was used to evaluate the effect of both the double-dose pendimethalin and the biopreparate EM-EKO ProBio Plus on pendimethalin concentration in soil as well as to evaluate the dehydrogenase activity and soil respiration between the treatments at the beginning and at the end of the experiment. The total number of bacteria, fungi, spores, and actinomycetes in soil samples at the beginning and at the end of the experiment was compared using the *t*-test.

RESULTS AND DISCUSSION

Soil microorganisms. The total number of bacteria, fungi, spores, and actinomycetes in soil samples at the beginning of the experiment is given in Figure 1. The effect of inoculation was noticeable on the total number of bacteria. In both samples treated with pendimethalin, a significantly higher bacteria content was found in inoculated treatments. For the lower pendimethalin dose, the *P*-value was 0.0095. For the higher pendimethalin dose, the *P* was 0.03. In the control samples no difference was determined between the inoculated and uninoculated treatments ($P = 0.749$). The inoculation affected actinomycetes only in the case of lower pendimethalin concentration ($P = 0.046$). In the case of fungi ($P = 0.532$) and spores ($P = 0.720$) no effect of inoculation was found between inoculated and uninoculated treatments.

At the end of the experiment there was no difference in the concentration of bacteria, fungi, spores, and actinomycetes between the inoculated and uninoculated treatments (data not shown). The number of microorganisms (bacteria, fungi, and spores) ranged in a similar interval as at the beginning of the experiment with the exemption of actinomycetes, which showed a significant increase at the end of the experiment ($P = 0.0001$) (Figure 2). The increase of actinomycetes at the end of the experiment could be connected with their ability to better utilize the complex substrates.

Nayak *et al*. (1994) also investigated the effect of pendimethalin on the soil populations of bacteria, fungi, and actinomycetes, in a sandy loam soil in Bhubaneshwar, India. They found that pendimethalin (0.5 kg/ha) significantly reduced bacteria (61%) after 25 days but not after 50 and 75 days, when a slight stimulation was noted as compared with the control. Fungi were significantly reduced (by 19%) after 25 days and stimulated after 50 and 75 days as compared with the control. Actinomycetes were substantially reduced (by 21%) after 25 days and stimulated after 50 and 75 days.

The effect of herbicide on soil microorganisms was also studied by Chikoye *et al*. (2014). They observed a reduction of N_2 fixation and vesicular arbuscular

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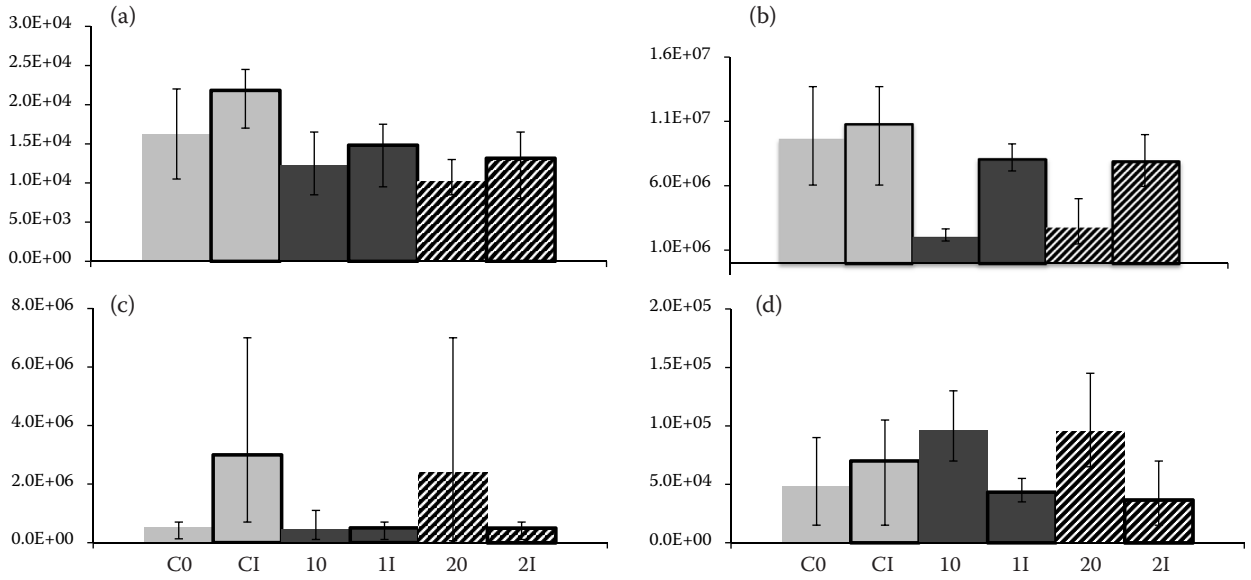

Figure 1. Total number of fungi (a), bacteria (b), spores (c), and actinomycetes (d) at the beginning of the experiment CO – no pendimethalin, uninoculated soil; CI – no pendimethalin, inoculated soil; 10 – low dose of pendimethalin, uninoculated soil; 1I – low dose of pendimethalin, inoculated soil; 20 – high dose of pendimethalin, uninoculated soil; 2I – high dose of pendimethalin, inoculated soil

mycorrhizal (VAM) fungi colonization due to pendimethalin and imazaquin application. The degradation of pendimethalin by *Bacillus* species was also studied by Megadi *et al.* (2010). They found that *B. circulans* degraded the herbicide pendimethalin by nitroreduction to yield 6-aminopendimethalin. Pendimethalin was also degraded by oxidative *N*-dealkylation to yield 3,4-dimethyl-2,6-dinitroaniline and pentane.

Dehydrogenases are enzymes that are responsible for oxidation of organic compounds. Increased dehydrogenase activity caused by pesticides has been reported by Jenkinson and Powlson (1976), Zelles *et al.* (1985), and Tu (1995). The same results were observed in the present study. No differences were found among all studied treatments at the beginning (P = 0.059) and at the end (P = 0.116) of the experiment. However, a significant reduction (approximately five times) of dehydrogenase activity was found at the end of the experiment ($P < 0.0001$) in comparison to its onset.

Soil respiration, as indicated by oxygen consumption and CO_2 evolution, is considered an indicator of microbial activity. The rate of soil respiration depends on the physiological condition of the organisms, as

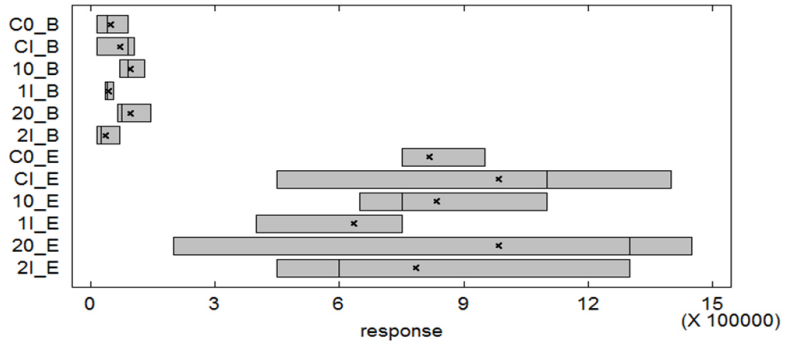

Figure 2. Concentration of actinomycetes in all treatments at the beginning (_B) and at the end (_E) of the experiment CO – no pendimethalin, uninoculated soil; CI – no pendimethalin, inoculated soil; 10 – low dose of pendimethalin, uninoculated soil; 1I – low dose of pendimethalin, inoculated soil; 20 – high dose of pendimethalin, uninoculated soil; 2I – high dose of pendimethalin, inoculated soil

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well as the edaphic conditions such as temperature and soil moisture. In our study, the ammonium and glucose (NG) potential respiration showed no statistical difference between the studied treatments at the beginning of the experiment ($P < 0.397$) and at its end ($P < 0.207$). However, a significant increase of respiration was found at the end of the experiment in all studied treatments ($P < 0.0001$). This reaction is a signal that mineralization activity of microorganisms was not affected by the experimental conditions.

The basal respiration showed no statistical difference between the studied treatments at the beginning of the experiment ($P < 0.473$) and at its end ($P < 0.756$). However, a significant increase ($P < 0.0001$) of basal respiration was found at the end of the experiment in all studied treatments in comparison to the values at the beginning of the experiment. These results correspond with the results of Popelářová *et al.* (2008) highlighting that correlations among the tested activities (potential and basal respiration, ammonification, nitrification) and counts of bacteria were mostly insignificant (except the nitrification). It might indicate that some other factors (moisture, temperature, pH, aeration, composition of nutrient sources) could be of higher importance for the measured activities than the microorganism counts. This result does not correspond to the results of Miller *et al.* (1996). They found a decrease of potential heterotrophic microbial activity using ^{14}C-labelled glucose. Glucose mineralization decreased with increasing pendimethalin concentration to a minimum of 13% at 100 mg/kg. Furthermore, Shetty and Magu (1998) observed a decrease of CO_2 evolution and dehydrogenase activity after pendimethalin application.

Pendimethalin dissipation from soil. The average pendimethalin concentration in soil during the experiment is given in Figure 3. Pendimethalin concentrations during the experiment in all treatments were compared. The ANOVA showed that both the time and the treatments have a significant effect on pendimethalin concentration ($P < 0.001$ for both time and treatments). However, the effect of treatment was significant only between the higher and lower pendimethalin concentration. Therefore, the concentration of pendimethalin in each sampling term was studied by ANOVA in detail. Five days after pendimethalin application, a statistical difference ($P = 0.0007$) was determined between both pendimethalin doses and between the inoculated and uninoculated treatments of higher pendimethalin doses. The inoculated soils showed lower pendimethalin concentration than uninoculated soil. In the case of lower pendimethalin dose, no difference between the inoculated and uninoculated soil was found.

Fifteen days after pendimethalin application a statistical difference ($P = 0.0004$) was found between only the higher and lower pendimethalin dose. No effect of inoculation was observed in this sampling term.

Thirty eight days after pendimethalin application there was no significant difference ($P = 0.705$) between any treatments. The same results were also observed 84 days after pendimethalin application ($P = 0.056$). Similar results were presented by Zimdahl and Clark (1984). They found no effect of pendimethalin dose (1.2 and 2.4 kg/ha) on its half-life calculated 45 days after pendimethalin application. Similar results were also presented by Tsiropoulos and Miliadis (1998) and Kewat *et al.* (2001). Opposing results were presented by Lin *et al.* (2007). They estimated the pendimethalin half-life in soil at 14 and 21 days using the dose of 1.19 and 2.38 kg a.i. per ha, respectively.

The dissipation of pendimethalin with time was calculated using the first order equation (Hurle & Walker 1980):

$$C = C_0 e^{-kt}$$

where:
C – concentration at time t (mg/g)
C_0 – initial concentration (mg/g)
e – Euler's number
k – rate constant
t – time (days)

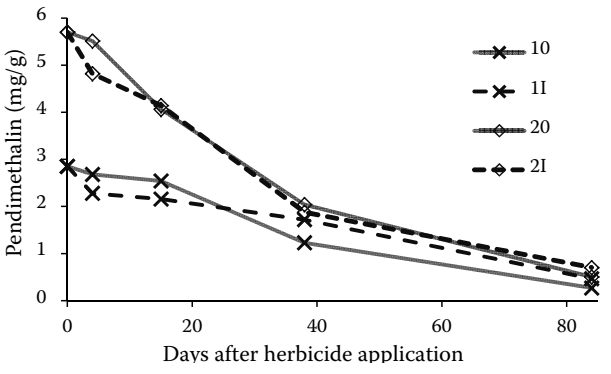

Figure 3. Average pendimethalin concentration in soil during the experiment
10 – low dose of pendimethalin, uninoculated soil; 1I – low dose of pendimethalin, inoculated soil; 20 – high dose of pendimethalin, uninoculated soil; 2I – high dose of pendimethalin, inoculated soil

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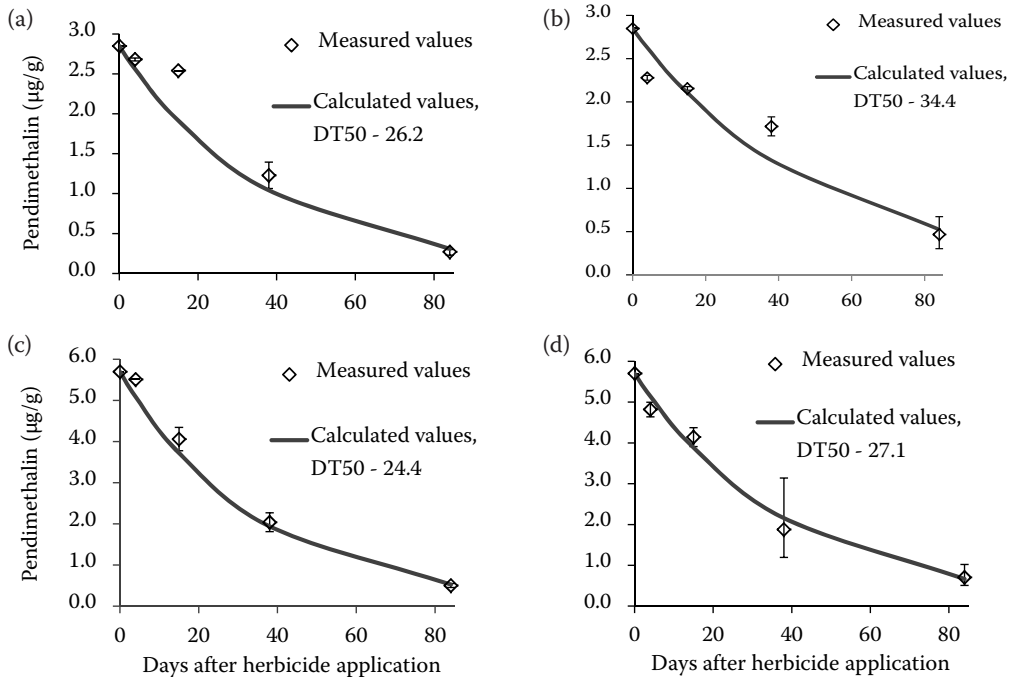

Figure 4. Measured and calculated values of pendimethalin concentration in soil: 5.3 l/ha of Stomp 300, uninoculated treatment (a), 5.3 l/ha of Stomp 300, inoculated treatment (b), 10.6 l/ha of Stomp 300, uninoculated treatment (c), 10.6 l/ha of Stomp 300, inoculated treatment (d)

The pendimethalin half-life was then calculated using the equation of Hurle and Walker (1980):

$$t_{\frac{1}{2}} = \frac{0.6932}{k}$$

The measured values, with bars indicating the maximum and minimum values, as well as the calculated values are shown in Figure 4. The calculated half-life of pendimethalin was 26.2 days for uninoculated soil with low pendimethalin dose (R^2 = 0.94); 34.4 days for inoculated soil with low pendimethalin dose (R^2 = 0.92); 24.4 days for uninoculated soil with high pendimethalin dose (R^2 = 0.99) and 27.1 days for inoculated soil with high pendimethalin dose (R^2 = 0.99). The observed half-life is similar to the pendimethalin half-life reported by Kewat *et al.* (2001), who observed half-lives of 24 and 36 days in sandy loam soils in New Delhi. However, the pendimethalin half-life can differ on the basis of soil and climatic conditions from 4 days (Sevage & Jordan 1980) to 563 days (Walker & Bond 1977).

Interestingly, in this study the half-life of pendimethalin at high doses was shorter than the half-life of low doses of pendimethalin. It can probably be explained by the microorganisms' adaptability to the higher pendimethalin doses. It is also evident from Figure 3 that the low pendimethalin dose degraded slowlier at the beginning of the experiment (during the first 15 days of the experiment) than the doubled pendimethalin dose. The half-life of pendimethalin in uninoculated soil was slightly shorter than the pendimethalin half-life in inoculated soil.

CONCLUSIONS

- The pendimethalin half-life ranged from 24.4 to 34.4 days.
- The doubled dose of pendimethalin has a lower half-life than recommended doses.
- The effect of inoculation was significant just 5 days after pendimethalin application.
- The number of microorganisms (bacteria, fungi, and spores) at the end of the experiment ranged in a similar interval as at its onset.
- The number of actinomycetes increased significantly at the end of the experiment, however, no difference was found between the treatments at the beginning as well as at the end of the experiment.
- The dehydrogenase activity and the potential and basal respiration showed no difference between the studied treatments at the beginning as well as at the end of the experiment. However, a signifi-

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cant reduction of dehydrogenase activity and a significant increase of potential and basal respiration were observed at the end of the experiment.

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