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Effect of soil contamination with azadirachtin on dehydrogenase and catalase activity of soil

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Abstract

Insecticides are used in modern agriculture in large quantities to control pests and increase crop yield. Their use, however, has resulted in the disruption of ecosystems because of the effects on non-target soil microorganisms, some environmental problems, and decreasing soil fertility. These negative effects of synthetic pesticides on the environment have led to the search for alternative means of pest control. One such alternative is use of natural plant products such as azadirachtin that have pesticidal activity.

The aim of this experiment was to study the effect of soil contamination by azadirachtin ($C_{35}H_{44}O_{16}$) on dehydrogenase (DHA) and catalase activity (CA) of soil under field conditions in Perm, Russia. The tests were conducted on loamy soil (pH_{H2O} 6.7, EC_{H2O} 0.213 dSm^{-1}, organic carbon 0.99%), to which the following quantities of azadirachtin were added: 0, 15, 30 and 60 mL da^{-1} of soil. Experimental design was randomized plot design with three replications. The DHA and CA analyses were performed 7, 14 and 21 days after the field experiment was established.

The results of field experiment showed that azadirachtin had a positive influence on the DHA and CA at different soil sampling times. The increased doses of azadirachtin applied resulted in the higher level of DHA and CA in soil. The soil DHA and CA showed the highest activity on the 21th day after 60 mL azadirachtin da^{-1} application doses.

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Introduction

One of the side effects of the application of pesticides is that these chemicals accumulate in soil. Pesticides are biologically highly active, therefore they will have an effect not only on the organisms subjected to their activity but also on a number of other organisms present in this environment, many of which are useful in nature. Such side effects are compounded by long degradation times of some pesticides in soil (Wyszkowska, 2002). Persistence of these xenobiotics in soil depends on several factors determining the rate of their disappearance from the environment, of which the chemical structure of the active substance of a preparation, its chemical properties, formation of bonds with other compounds and biodegradability are most significant (Chapman et al., 1981; Demoute, 1989). An important role is also played by environmental

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and agricultural factors, including temperature, pH, moisture, soil type, organic matter content, fertilisation and count and activity of soil microorganisms (Pedziwilk, 1995; Kızılkaya, 1997; Wyszkowska, 2002).

Degradation of pesticides is catalysed by enzymes excreted by microorganisms, producing in effect some intermediate metabolites, which may have a selective influence on soil microflora. Accurate determination of such modifications occurring during microbiological and biochemical processes is essential for sustaining and regenerating the fertility of soil (Wyszkowska, 2002). An assay of the enzymatic activity of soil, especially the activity of enzymes involved in the conversion of C, N and P, can be regarded as a good indicator of the effect of pesticides on soil metabolism, and also soil enzyme activities are very sensitive to both natural and anthropogenic disturbances, and show a quick response to the induced changes (Dick, 1997). Therefore, enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices.

The aim of the studies was to evaluate the effects of azadirachtin on dehydrogenase and catalase enzyme activity in soil. Specific objectives were as follows: (i) to determine the effects of different azadirachtin application doses on dehydrogenase (DHA) and catalase (CA) activity, and (ii) to determine changes in the DHA and CA were determined in soil samples taken in 7, 14 and 21 days after the field experiment.

Material and Methods

Experimental field and climate

The field experiment was conducted at the Experimental Station of Perm State Agricultural Academy, Perm, Russia (57°56′00″ N, 56°14′59″ E) at an altitude of 127 m above mean sea level (Figure 1). The data on climatic parameters such as precipitation and temperature are shown in Figure 2.

Figure 1. Location of the experimental field in Perm, Russia

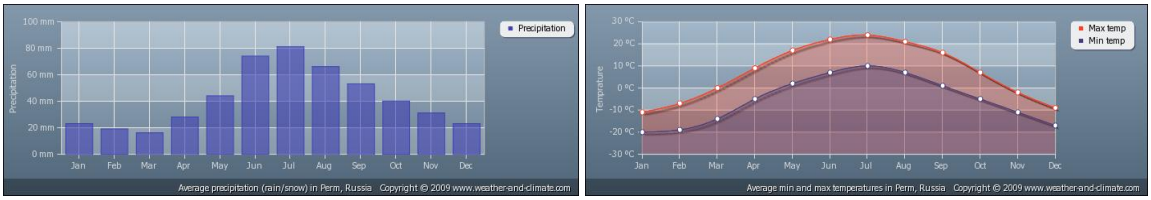


Figure 2. Climatic data in Perm, Russia

Soil

The soil of the experimental site is loam (31.4% sand, 45% silt, 23.6% clay). A composite surface soil sample from 0-20 cm depth was collected from the experimental site before initiating the experiment and was analyzed for physicochemical properties according to Rowell (1996) and Jones (2001). Soil samples were air dried at room temperature; sieved with <2 mm screen. The basic physico-chemical characteristics of the soil are as follows: pH (1:1, soil:water): 6.70, electrical conductivity (1:1, soil:water): 0.81 dSm^{-1}; $CaCO_3$ content: 0.04%; total organic carbon: 0.99%; total nitrogen: 0.086%; available phosphorus (0.5M $NaHCO_3$ extractable P): 13.34 $mg.kg^{-1}$, and exchangeable potassium (1N NH_4OAc extractable K): 1.382 $cmol(+).kg^{-1}$. The soils had no history of receiving any pesticide treatment six months prior to this study. Experimental soil was classified as "Albic Luvisol" according to the FAO (2006).

Azadirachtin ($C_{35}H_{44}O_{16}$)

The azadirachtin (NeemAzal®–T/S) was imported by VIT, Turkey. This insecticide (10g azadirachtin L^{-1}) was used as technical and added to soil.

Experimental design

This experiment was conducted to determine the effects of azadirachtin on soil enzyme activities under field conditions. Experimental design was a randomized plot design with three replications, and was established June 26, 2011. Each plot was an area of 1 x 1 m. The treatments were: (1) control: 0 mL azadirachtin da^{-1}, (2) low application doses: 15 mL azadirachtin da^{-1}, (3) recommended application doses: 30 mL azadirachtin da^{-1}, (4) high application doses: 60 mL azadirachtin da^{-1}, respectively. In order to homogenoeus azadirachtin application in soil, azadirachtin were applied in 2.5 L water per m^2. Changes in the dehydrogenase and catalase activities were determined in soil samples taken in 7, 14 and 21 days after the field experiment was conducted.

Soil sample preparation

Field moist soils were collected and brought to the laboratory in properly labeled and sealed polythene bags. The sieved soil samples (<2 mm) were homogenized and kept in polyethylene boxes, and also stored at 4 ^{0}C until the analyses were carried out. The acclimatized soil samples were used for the enzyme analyses.

Enzyme analyses

To assess the enzyme analyses to the adjustments in the soil variables, with and without pesticides, enzyme analyses were estimated by following methods.

Dehydrogenase activity (DHA) was determined according to Pepper et al (1995). To 6 g of sample 30 mg glucose, 1 ml of 3% TTC (2,3,5-triphenyltetrazoliumchlorid) solution and 2.5 ml pure water were added and the samples were incubated for 24 h at 37^{0}C. The formation of TPF (1,3,5 triphenylformazan) was determined spectrophotometrically at 485 nm and results were expressed as μg TPF g^{-1} dry soil.

Catalase activity (CA) was measured by the method of Beck (1971). Ten ml of phosphate buffer (pH, 7) and 5 ml of a 3% H_2O_2 substrate solution were added to 5 g of soil. The volume (ml) of O_2 released within 3 minutes at 20^{0}C was determined. Three replicates of each soil were tested and controls were tested in the same way, but with the addition of 2 ml of 6.5% (w/v) NaN_3. Results were expressed as ml O_2 g^{-1} dry soil.

Statistical analysis

All data were analyzed using SPSS 11.0 statistical software (SPSS Inc.). Analysis of variance (ANOVA) was carried out using one-factor randomized complete plot design; where significant *F*-values were obtained, differences between individual means were tested using the LSD (Least Significant Difference) test, with a significance level of $P<0.01$. All figures and tables presented include standard deviations of the data and *F*-values. The asterisks, * and ** indicate significance at $P<0.05$, and 0.001, respectively.

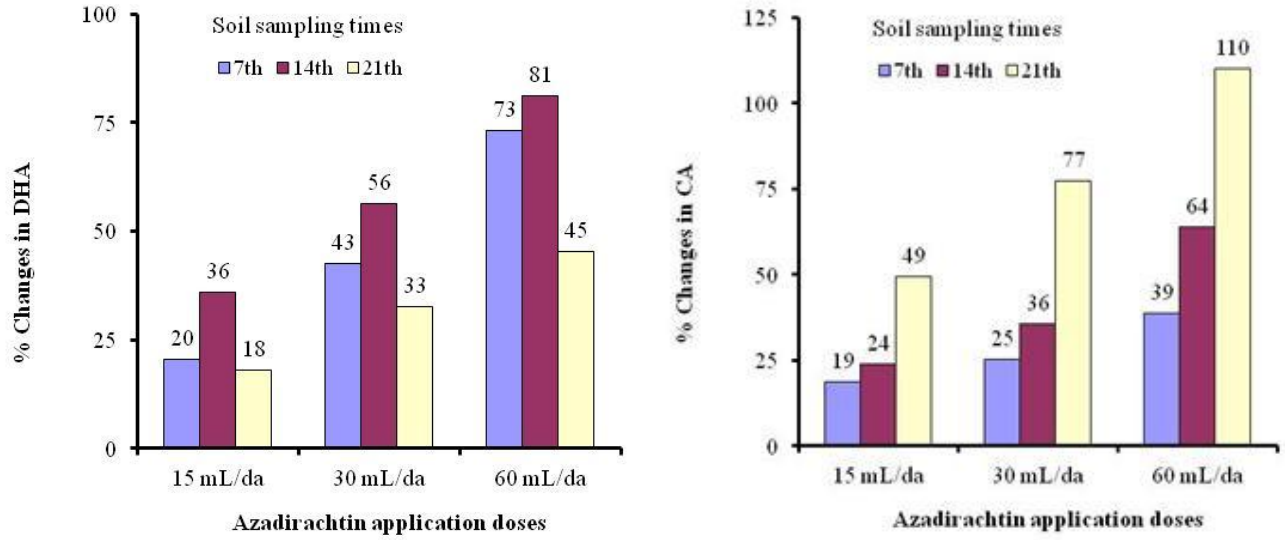
Results and Discussion

Dehydrogenase (DHA) and catalase (CA) activity varied significantly in response to azadirachtin application doses over time (Table 1). The DHA and CA in different doses of azadirachtin applied soils during the experiment are shown in figure 3.

Table 1. Azadirachtin impacts on dehydrogenase and catalase activity in soil at different sampling times

Enzyme activity	Soil sampling times	Azadirachtin application doses			
		Control	15 mL da^{-1}	30 mL da^{-1}	60 mL da^{-1}
DHA	7 days	101,01 (4,02)	121,59 (3,68)	144,08 (6,37)	174,95 (6,37)
	14 days	91,48 (4,57)	124,26 (2,88)	142,93 (7,50)	165,80 (5,72)
	21 days	99,10 (6,99)	117,01 (3,49)	131,50 (5,72)	144,08 (4,12)
CA	7 days	40,14 (2,04)	47,71 (2,06)	50,29 (3,28)	55,72 (2,88)
	14 days	40,50 (2,72)	50,15 (3,55)	54,94 (3,10)	66,38 (5,53)
	21 days	40,91 (2,40)	61,14 (1,60)	72,59 (2,07)	85,97 (2,28)

Notes: Standard deviation are shown in parentheses.
DHA = Dehydrogenase activity (μg TPF g^{-1} dry soil), CA = Catalase activity (ml O_2 g^{-1} dry soil)



(a) (b)

Figure 3. The changes of DHA and CA in different doses of azadirachtin applied soils
a) Dehydrogenase activity (DHA) b) Catalase activity (CA)

Considerable variations in DHA and CA were found for the different doses of azadirachtin at different sampling times. Statistically significant variations were found in DHA and CA at various azadirachtin application doses. The DHA and CA were also affected by incubation period. The analysis of variance of the results obtained in our experiment on the periodic sampling times with azadirachtin showed that all factors (azadirachtin doses and soil sampling times) significantly influenced DHA and CA (Table 2). After azadirachtin application a rapid and significant increase in DHA and CA were observed in soils. At the end of the sampling times, the DHA and CA measured in azadirachtin applied soils were statistically different from those measured in the control soils.

Biological oxidation of organic compounds is a dehydrogenation process (Tabatabai 1982); mediated by many different intracellular and specific dehydrogenases. Therefore, dehydrogenase activity (DHA) of soil is supposed to reflect microbial activity (Skujins 1976; Kumar and Tarafdar, 2003). The activity of dehydrogenase is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity (Skujins 1973), because, being exclusively intracellular, it is linked to viable cells (Quilchano and Maranon, 2002; Kızılkaya, 2008). At all sampling times, significant positive effects were observed for all azadirachtin application doses. Dehydrogenase activity was found to be max. stimulated by doses of 600 mL azadirachtin da^{-1}. The maximum increase value was 81% (600 mL azadirachtin da^{-1}, 21 days after application) (Figure 3a). The experimental data are consistent with results reported by other authors on the effect of different pesticides on this enzyme. Dinelli et al. (1998) and Accinelli et al. (2002) reported that sulfonylurea herbicides at a rate up to 20 mg kg-1 inhibited dehydrogenase activity. The results Radivojević et al. (2008) showed a decreased activity of dehydrogenase under all atrazine concentrations

(8.0, 40.0 and 80.0 mg kg^{-1}) from the 1st to the 30th day after atrazine application. The decrease ranged: 12.5-18.2% for 8.0 mg concentration, 4.8-24.8% for 40.0 mg, and 6.6-39.6% for 80.0 mg.

Table 2. Results of ANOVA

Variables	DHA		CA	
	F-value	$LSD_{\alpha=0.01}$	*F*-value	$LSD_{\alpha=0.01}$
AAD	243.440***	6.994	145.020***	3.996
SST	17.426***	6.057	98.740***	3.461
AAD x SST	6.419***	12.113	14.037***	6.922

AD = Azadirachtin application doses, Soil sampling times
ns not significant * $P<0.05$, ** $P<0.01$, *** $P<0.01$

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The CA is sensitive to both natural and O_2 level, and shows a quick response to induced changes. Also it may be affected by cast formation by earthworm in anaerobic condition. The CA is based on the rates of oxygen release from the added hydrogen peroxide, and may be related to the metabolic activity of aerobic organisms (Glinsky et al., 1986; Kızılkaya et al., 2004; Kızılkaya and Hepşen, 2007). The changes in CA as influenced by the application of azadirachtin are presented in Table 1 and Figure 3b. All application doses where an azadirachtin was applied were found to increase the CA significantly in comparison with control. The maximum increase value was 110% (600 mL azadirachtin da^{-1}, 21 days after application).

Conclusion

In this investigation, azadirachtin was applied at different concentrations on the soil for 21 days. Short-term changes or stimulation were observed in the activities of the enzymes studied. However, at the end of the experimental period, these activities were significantly stimulated. The present findings mean that the azadirachtin is only relative safe pesticides which could not cause environmental risk and would not cause an ecological problem from the microbial point of view.

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