Развитие и внедрение современных технологий и систем ведения сельского хозяйства, обеспечивающих экологическую безопасность окружающей среды

Материалы международной научно-практической конференции, посвященной 100-летию Пермского НИИСХ.

В 3 томах

Том 1
Агрохимия и земледелие
Часть 1
Пермь, 2013
THE EFFECT OF AZADIRACHTIN ON MICROBIAL BIOMASS AND BASAL RESPIRATION IN SOIL

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Annotation. The main objective of this study was to determine the effect of soil contamination by azadirachtin ($\text{C}_{30}\text{H}_{51}\text{O}_{17}$) on microbial biomass $C (C_{mic})$ and basal respiration (BR) under field conditions in Perm, Russia. The tests were conducted on loamy soil ($\text{pH}_{\text{H}_{2}O} 6.7$, EC $0.213$ dS$m^{-1}$, 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 $C_{mic}$ and BR 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 $C_{mic}$ and BR at different soil sampling times. The increased doses of azadirachtin applied resulted in the higher level of $C_{mic}$ and BR in soil. The soil $C_{mic}$ and BR showed the highest activity on the 21$^{th}$ day after 60 mL azadirachtin da$^{-1}$ application doses.

INTRODUCTION

Agrochemicals are continually becoming a more intricate part of modern society, and are used in varying amounts on crops to maintain yield and quality. Although complete and accurate analytical data concerning losses are lacking, the most comprehensive estimate was published by several research. To reduce damage to crops, many kinds of synthetic pesticides are
applied for insect, diseases and weed control. Their use, however, has resulted in the disruption of ecosystems because of the effects on non-target species, accumulation of pesticide residues in the environment and in food, and build-up of pesticide resistance in the target species (Agyarko et al. 2006). Although benefits associated with pesticide use are most frequently identified as a reduction in losses due to pests, other less apparent factors contribute to the dependence of modern agriculture on the use of pesticides. Generally in developing countries, people suffer from short-term exposure to synthetic chemicals (including that resulting in suicide) and chronic effects of long-term exposure (Kizilkaya, 2000). These negative effects of synthetic pesticides have led to the search for alternative means of pest control (Sarathchandra et al. 1996). An ideal pesticide should be toxic only to the target organism; biodegradable and undesirable residues should not affect non-target surfaces. One such alternative is use of natural plant products such as azadirachtin that have pesticide activity. Azadirachtin possesses insecticidal activity against many economically important insect pests (Schmutzer and Singh, 2002). Currently, there is an upswing in the use of azadirachtin as an insecticide owing to its plant origin (Akça et al. 2005). The belief that such natural insecticides are safer or less damaging to the ecosystem is also necessary to be further validated, as their effect on non-target organisms have been reported to be very close to threshold chronic toxicity (Scott and Kaushik, 1998). It is well known that indiscriminate methods of application and concentrations of insecticides are rampant, often allowing high loads of xenobiotics to reach the soil matrix.

Soil is a complex environment, where microorganisms play a crucial role in nutrient cycling and the stabilization of soil structure (Kizilkaya et al. 2004). They responsible for the global C, N, P and S cycles and mineralization of essential nutrients from soil organic pool (Kizilkaya, 2008). On the other hand, microbial activities are strongly dependent on nutritional and other chemical and physical conditions of the soil and respond rapidly to changes in soil properties. Microorganisms are considered sensitive indicators when monitoring changes in soil status affected by agricultural management, but the meaningful set of microbiological indicators still remains an object of debate (Truu et al. 2008).

The overall aim of the studies was to evaluate the effects of azadirachtin on microbial response variables in soil. Specific objectives were as follows: (i) to determine the effects of different azadirachtin application doses on microbial biomass carbon (C microbi), and basal respiration (BR), and (ii) to determine changes in the C microbi and BR were determined in soil samples taken in 7, 14 and 21 days after the field experiment.

**MATERIAL AND METHODS**

*Experimental field:* The field experiment was conducted at the Experimental Station of Perm State Agricultural Academy named after academian D.N. Pryanishnikov, Perm, Russia (57°56'00" N, 56°14'59" E) at an altitude of 127 m above mean sea level.

*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 physical-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; CaCO₃ content: 0.04%; total organic C: 0.99%; total N: 0.086%; available P (0.5M NaHCO₃, extractable P): 13.34 mg.kg⁻¹, and exchangeable K (1N NH₄OAc 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 ($C_{19}H_{23}O_{6}$): The azadirachtin (NeemAzal®-T/S) was imported by VIT, Turkey. This insecticide (10 g 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 homogenous 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°C until the analyses were carried out. The acclimatized soil samples were used for the enzyme analyses.

Microbiological assays
To assess the microbiological assays to the adjustments in the soil variables, with and without pesticides, Microbial biomass carbon and Basal respiration were estimated by following methods.

Microbial biomass carbon ($C_{mic}$) was determined by the substrate-induced respiration method of by Anderson and Domsch (1978). A moist sample equivalent to 10 g oven-dry soil was amended with a powder mixture containing 40 mg glucose. The $CO_2$ production rate was measured hourly using the method described by Anderson (1982). The pattern of respiratory response was recorded for 4 h. Microbial biomass carbon ($C_{mic}$) was calculated from the maximum initial respiratory response in terms of mg C g$^{-1}$ soil as 40.04 mg CO$_2$C g$^{-1}$ + 3.75. Data are expressed as mg C g$^{-1}$ dry soil.

Basal respiration (BR) at field capacity ($CO_2$ production at 22°C without addition of glucose) was measured, as reported by Anderson (1982) by alkali (Ba(OH)$_2$.8H$_2$O+BaCl$_2$) absorption of the CO$_2$ produced during the 24-h incubation period, followed by titration of the residual OH$^-$ with standardized hydrochloric acid, after adding three drops of phenolphthalein as an indicator. Data are expressed as μg CO$_2$.C g$^{-1}$ dry soil.

RESULTS AND DISCUSSION

Soil microbial biomass C ($C_{mic}$) and basal respiration (BR) varied significantly in response to azadirachtin application doses over time (Table 1). The $C_{mic}$ and BR in different doses of azadirachtin applied soils during the experiment are shown in figure 1.

| Table 1. Azadirachtin Impacts on $C_{mic}$ and BR in soil at different sampling times |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **Microbial biomass C**       | **Soil variables**              | **Azadirachtin application doses** |
| **Response variables**         | **sampling times**              | **15 mL da$^{-1}$**             | **30 mL da$^{-1}$**             | **60 mL da$^{-1}$**             |
|                                | **Control**                     | **(1,09)**                      | **(1,60)**                      | **(2,52)**                      |
| **7 days**                      |                                | **65,14**                       | **74,82**                       | **91,59**                       |
|                                |                                | (0,66)                          | (1,66)                          | (2,52)                          |
| **14 days**                     |                                | **72,42**                       | **82,44**                       | **94,60**                       |
|                                |                                | (1,41)                          | (1,32)                          | (2,35)                          |
| **21 days**                     |                                | **80,46**                       | **81,57**                       | **97,46**                       |
|                                |                                | (1,08)                          | (2,38)                          | (2,13)                          |
| **7 days**                      |                                | **47,71**                       | **17,95**                       | **19,90**                       |
|                                |                                | (0,29)                          | (0,75)                          | (0,46)                          |
| **14 days**                     |                                | **17,04**                       | **20,43**                       | **24,66**                       |
|                                |                                | (0,17)                          | (0,30)                          | (0,59)                          |
| **21 days**                     |                                | **18,64**                       | **26,87**                       | **31,83**                       |
|                                |                                | (0,24)                          | (0,64)                          | (0,83)                          |

Notes: Standard deviation are shown in parentheses.

$C_{mic}$ = Microbial biomass C (mg C g$^{-1}$ dry soil)
BR = Basal respiration (mg CO$_2$.C g$^{-1}$ dry soil).

Our research results also indicate that considerable alterations in $C_{mic}$ and BR tested were found for the different doses of azadirachtin.
at soil application. In general, high doses of azadirachtin application had high amounts of $C_{mic}$ and BR (Table 1). The increase of $C_{mic}$ in recommended application doses of azadirachtin (300 mL da$^{-1}$) had a higher than in other doses (Fig. 1a). However, the increase of BR in high application doses of azadirachtin (600 mL da$^{-1}$) had a higher than in low and recommended doses (Fig. 1b). We accept that $C_{mic}$ gives an idea of the potential microbial activity of a soil (Nannipieri et al. 1990). The incorporation of different azadirachtin application doses in the soil raised the $C_{mic}$ level significantly, which reflects the increased number of microorganisms. The general increase in $C_{mic}$ noted can be attributed to the incorporation of easily biodegradable organic materials. The greatest increase in $C_{mic}$ occurred in soil amended with high application doses of azadirachtin, probably due to this material being obtained by processes involving a high degree of microbial activity. In addition the high application doses of azadirachtin has a higher content substrate and energy source, which acts as energy source for the microorganisms, thus contributing to an increase in their activity and biomass. Anderson (1982) defined BR as a useful parameter in measuring a soil's biological activity. The addition of azadirachtin significantly raised the BR in soil, and was greater high application doses of azadirachtin.

![Figure 1. The changes of microbial response variables in different doses of azadirachtin applied soils](image)

**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 $C_{mic}$ and BR studied. In field conditions, the amounts of azadirachtin entering the soil depend on the dosage of azadirachtin. The study revealed that microbial response variables, $C_{mic}$ and BR can be used as a sensitive indicator including soil C sequestration in response to insecticides application practices. Moreover, a routine measurement of $C_{mic}$ and BR can be used as an early indicator of both soil microbiological quality and C sequestration. 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.

**ACKNOWLEDGEMENTS**

Financial supports from Ondokuz Mayas University, Samsun, Turkey (PYO ZRT.1901.12.004), and Perm State Agricultural Academy named after academician D.N. Pryanishnikov are gratefully acknowledged.

**REFERENCES**


