Applied Soil Ecology 120 (2017) 169–178

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Changes in the metabolic activities of two agricultural soils as influenced by the pesticides and insecticides combination

M. Madakka[a,*], N. Jayaraju[b], V. Rangaswamy[c]

[a] Department of Biotechnology and Bioinformatics, Yogi Vemana University, Kadapa. A.P, India
[b] Department of Geology, Yogi Vemana University, Kadapa, A.P,India
[c] Department of Mirobiology, Sri Krishnadevaraya University, Anantapur, A.P, India

ARTICLE INFO

Keywords:
Insecticides combination
Protease
Nitrogen mineralization
Sulphur oxidation
Invertase
Groundnut soil

ABSTRACT

Application of pesticides (thiram, difenoconazole, deltamethrin and profenofos) and insecticide combinations (profenofos + cypermethrin and deltamethrin + endosulfan) at concentrations ranging from 0.0 to 10.0 kg ha^{-1} were tested for their non-target effects towards the activities of protease, invertase, sulphur oxidation, and nitrogen mineralization in two agricultural soils of groundnut under laboratory conditions. In this study, protease activity was measured in terms of tyrosine equivalents formed from casein, and the invertase activity was measured in terms of glucose formed from sucrose. We found pronounced mineralization of peptone-nitrogen and sulphur oxidation in the soil samples treated with 2.5–5.0 kg ha^{-1} of the insecticide combinations and individual pesticides. Soil samples treated with 2.5 kg ha^{-1} –5.0 kg ha^{-1} of the insecticide combinations and individual pesticides have shown increased invertase and protease activity at 20th day and then decreased with increasing period of incubation. From the obtained results it was concluded that the influence of these insecticide combinations and individuals on enzyme activity, nitrogen mineralization, and sulphur oxidations were dose dependent.

1. Introduction

In order to meet the demands of a growing population, the use of pesticides has become indispensable in modern agriculture, and that use ultimately influences soil fertility and soil microbiota and their metabolic activities (Cerny et al., 2008; Chu et al., 2008; Munees et al., 2012). In addition, the effect of various combinations of pesticides may deviate from the behaviour of an individual pesticide due to the occurrence of synergistic, antagonistic or additive interactions between or among different pesticides (Schuster and Schroder, 1990). Therefore, in order to determine the ecological significance of pesticides, it has become necessary to study the effects of pesticide combinations applied at recommended levels (Reddy et al., 2011; Volodymyr et al., 2016). Concerns about the side effects of pesticides on soil fertility (Mousumi et al., 2014) led to the development of soil microbial testing programmes (Maleeka Begum and Rajesh, 2015). The testing programme includes measurement of soil enzyme activities which act as biological catalysts for various important biochemical reactions that maintain soil fertility (Ayansina and Oso, 2006). Generally, soil fertility is a reflection of the microbial status of soil, and the pesticides which settle in soil may interrelate with non-target microbiota and mediate significant biochemical transformations linked with nutrient recycling (Lakshmi et al., 2015; Maleeka Begum and Rajesh, 2015). Any disorder in the soil micro organisms may affect the primary function of the soil which is related to a continually cycled pool of nutrients for plant growth (Munees et al., 2012). Recycling of nutrients in soil organic matter involves the participation of soil microorganisms (Lakshmi et al., 2015) and pesticide application may adversely influence the nitrogen cycle, viz, ammonification, nitrification, denitrification and nitrogen fixation, which in turn may have serious effects on crop production (Pranita et al., 2015). Therefore, greater attention has been paid to study the influence of pesticides on a biological transformation of soils (Gundi et al., 2005; Jacobsen and Hjelmsø, 2014). In view of plant nutrition, sulphur stands next to nitrogen and phosphorus which enters the soil primarily in the form of plant residues, chemical fertilisers etc. In general, the sulphur transformation is microbially mediated by chemolithotrophs and has become increasingly apparent, since sulphate is the plant available source of sulphur (Wen et al., 2001; Srinivasulu et al., 2015). Despite the economic importance of sulphur, knowledge about pesticidal influence towards these microbial activities is scanty and therefore, the present investigation reports the interaction effects of pesticides viz., profenofos, deltamethrin, difenoconazole, thiram and

* Corresponding author.
E-mail addresses: madakka@gmail.com (M. Madakka), nadimikeri@gmail.com (N. Jayaraju), rangamanjula@yahoo.com (V. Rangaswamy).

http://dx.doi.org/10.1016/j.apsoil.2017.07.037
Received 2 May 2017; Received in revised form 26 July 2017; Accepted 27 July 2017

insecticide combinations involving cypermethrin + profenofos and deltamethrin + endosulfan on nitrogen mineralization, sulphur oxidation, protease and invertase activities in two agricultural soils.

2. Materials and methods

2.1. Soils

Two agricultural samples of black clay (13°57′45.35″ N and 77°50′13.15″ E) soil and red sandy loam (13°57′34.54″ N and 77°49′56.12″ E) soils were collected at a depth of 12 cm random sampling strategy of Anantapur district, a semi–arid zone in Andhra Pradesh, India. Later they were air–dried and sieved through a 2 mm mesh for further assessment.

2.2. Pesticides

Two fungicides, thiram (75% WP) (carbamate), difenoconazole (25% EC) (triazole), and two insecticides, deltamethrin (2.8% EC) (synthetic pyrethroid) and profenofos (50% EC) (organophosphate) and insecticide combinations like profenofos (organophosphate) + cypermethrin (25% EC) (synthetic pyrethroid) and deltamethrin (synthetic pyrethroid) + endosulfan (35% EC) (organochlorine) were used in the present study. Commercial formulations dissolved in distilled water of these tested pesticides were used for determining the microbial activities like soil enzymes, nitrogen mineralization, sulphur oxidation.

2.3. Soil incubation

The soil ecosystem stimulating non-flooded conditions consisting of ten gram of soil samples were added in test tubes (25 × 150 mm) and moistened to a water potential of 0.090 MPa, in order to maintain at 60% water holding capacity (Rangaswamy and Venkateswarlu, 2000).

2.4. Protease activity

Two and the five-gram portion of each soil, in duplicates, were treated with the selected pesticides at 1, 2.5, 5.0, 7.5 and 10 kg ha^{-1} concentration. Soil samples without pesticide treatment served as control. Soil samples in test tubes with and without pesticide treatment were incubated at room temperature in the lab (28 ± 4 °C). After 10 days of incubation, the soil extract was prepared in distilled water for an assay of proteases (Rangaswamy and Venkateswarlu, 1996).

2.5. Assay of soil protease

Soil samples including controls were incubated with 10 ml of 0.1 M Tris (2-amino-2 (hydroxy-methyl) propane-1:3-diol) (pH-7.5) containing sodium caseinate (2%w/v) for 24 h at 30 °C. An aqueous solution of trichloro acetic acid (4 ml, 17.5% w/w) was then added and the mixer was centrifuged. The supernatant liquid, in suitable aliquots, was treated with 3 ml of 1.4 M Na_2CO_3 and 1 ml of Folin-Ciocalteau reagent (33.3% v/v) with rapid swirling. The blue colour, thus formed after 30 min, was read at 700 nm in a spectronic −20 D Spectrophotometer (Milton and Roy). Tyrosine equivalents in soil extracts were estimated by referring to a calibration curve prepared with a known concentration of tyrosine.

2.6. Assay of soil invertase

The method employed for assay of invertase was developed by Cole (1997) and followed by Tu et al., 1981).The soil samples were transferred to 100 ml Erlenmeyer flasks and were treated with 1 ml of toluene to arrest the enzyme activity. After 15 min, 6 ml of 18 mM sucrose was added to the soil samples and incubated for 24 and 48 h respectively. The testing samples were then passed through Whatman No.1 filter paper, and the filtrate was assayed for the amount of glucose by Nelson Somogyi method in spectronic 20 D spectrophotometer. In another experiment, the rate of enzyme activity invertase was determined at 10, 20, 30, 40 days of soil incubation and further with the respective suitable substrate.

2.7. Nitrogen transformation in soil

The influence of selected insecticides at varying concentration on nitrogen mineralization was studied in two agricultural soils. Initially, 0.05 ml aliquots from stock solutions of the insecticides were applied with 0.1 ml pipette to the surface of a 10-g portion of the black and red soils contained in (25 × 200 mm) test tubes. Soil samples receiving only 0.05 ml acetone served as control. After the solvent had evaporated completely at room temperature, all the treatment including control was supplemented with 1000 ppm of nitrogen from AR grade peptone (organic N) for ammonification and 200 ppm nitrogen from $(NH_4)_2SO_4$ (inorganic N) for nitrification. The soil samples were then homogenised to distribute pesticide and nitrogen source. These soil samples were maintained at 60% water holding capacity throughout the incubation period. After 7 days of incubation, at room temperature (28 ± 4 °C) triplicates of each treatment were withdrawn and extracted with 2 M KCl for analyzing ammonia by Nesslerization method (Jackson, 1971) and with distilled water for estimation of nitrite by the diazotization method (Barnes and Folkard, 1951) and nitrate by the brucine method (Ranney and Barlett, 1972).

2.8. Estimation of ammonia

The ammonia ($NH4^+$ − N) formed in peptone amended soil samples with and without insecticide application was analysed by Nesslerization (Jackson, 1971). To suitable aliquots of the soil extract, 0.5 ml of the Nessler's reagent was added, and the volume was made up to 7 ml. The yellow colour that developed was read at 495 nm in a spectronic −20 D spectrophotometer (Milton Roy).

2.9. Estimation of nitrite

Nitrite was estimated by diazotization following the method of Barnes and Folkard (1951). Suitable aliquots from the filtrate of the soil extract were pipetted into test tubes and 1 ml of 1% sulphanilamide in 1 N HCl was added and shaken thoroughly. To the coloured diazo compound so formed was added 1 ml of 0.12%, N-(1-naphthyl)-ethylene diaminedihydrochloride in distilled water. The colour was allowed to develop for 25 min, and later the volume was made up to 25 ml with distilled water. The absorbance of the pink coloured solution was read at 520 nm in a Spectronic −20 D Spectrophotometer. The amount of nitrite was calculated by referring to a calibration curve prepared with the standard solution of known nitrite concentrations.

2.10. Estimation of nitrate

The nitrate (NO_3^- − N) produced due to the activity of soil microorganisms was determined by the method of Ranney and Bartlett (1972). Three drops of brucine reagent (2 g brucine dissolved in 50 ml methanol) were added to suitable aliquots of the soil extract followed by 2 ml of concentrated sulphuric acid. The solution was mixed by vortexing and placed in the dark for 30 min to ensure full-colour development, after which the volume was made up to 10 ml with distilled water, and the yellow colour was read at 410 nm in a spectronic − 20 D spectrophotometer. Further, the experiment was repeated with only 2.5 kg ha^{-1} of the selected insecticides and incubated for 1, 2, 3 and 4 weeks in order to determine the rate of ammonification. The impact of different concentrations of the insecticides after two weeks of the incubation period and the rate of nitrification at 2, 4, 6 and 8 weeks was determined following the same procedure as with ammonification.