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Effect of Novaluron Pesticide on Phosphatase Activity in Black Clay and Red Sandy Ground Nut (*Arachis hypogaea* L.) Soils

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Abstract: ***Biocatalysts have a vital role in all the biological activities. They show maximum influence in all the soil ecological reactions. Agricultural soils were frequently subjected with pesticides to control the pests. To get a better yield of agriculture food, better agriculture soil should be used for cultivation. The study was designed to determine the effect of novaluron pesticide used now a day for the pest control, on phosphatase activity. Soil samples collected from groundnut cultivating fields at Anantapur District, Andhra Pradesh randomly from a depth of 12cms. Air dried and sieved soil samples were taken and a laboratory experiment was conducted to investigate the effects of pesticides at their recommended field rates and higher rates on phosphatase activity in both soil samples after 10, 20, 30 and 40 days exposure under controlled conditions. Lower stimulatory activity is noticed at 10 days interval of time and striking stimulatory activity is noticed at 2.5 kg ha⁻', persists for 20 days in both soils. The higher concentration of the novaluron (5.0 - 10.0 kg ha⁻') were toxic to the enzymatic activity. The results of the present study clearly indicate that the field application rates show no effect on enzyme activity for longer period of incubation (40 days). But higher concentration (10 kg ha⁻') of pesticide leads to the inhibition of the enzymatic activity.***

Keywords: Phosphatase, Novaluron, Pesticide, Groundnut soils

1. Introduction

Pesticides are indispensable to modern agriculture. Currently among the various groups of pesticides that are being used world over, organophosphates form a major and most widely used group accounting for more than 36 %of the total world market. The wide spread use of these pesticides over the years has resulted in problems caused by their interaction with the biological systems in the environment. India is primarily an agriculture based country with more than 60-70 % of its population dependent on agriculture. The increase in agriculture output and the phenomenal progress we have made is due to the application of improved technology, increased use of important agriculture inputs like fertilizers and pesticides. Crops worth rupees 12,000 crores are just eaten away by pests (Shroff, 2000). Therefore extensive use of pesticides is inevitable.

However intensive use of common pesticides can lead to the toxicity to soils, which may inhibit several biochemical reactions. Due to a higher degree of toxicity, some pesticides particularly those persistent in soils constitute a very important group of contaminants. When pesticides are applied to soils, they may interact with non-target microorganisms and exhibit chronic diverse effects on soil micro flora (Omar and Abdel stater, 2001; Moorman, 1989; Tu, 1995; Piomentol and Levitan 1986 and Sarafraz *et al*., 2009). The living dynamic nature of organisms is one of the important features of soill quality and often used as a bio indicator for soil health (Gainfreda *et al*., 2005; Sukul, 2006). Entry of insecticides in soil due to agriculture practices may disturb a delicate balance of micro flora, thereby effecting recycling of nutrients and soil fertility (Alexander, 1997). Increasing pesticides usage in agriculture adds to the rise in concern for the environmental contamination (Zhu *et al*., 2004). Pesticides reaching the soil may affect non targeting microorganisms, thereby disturbing pesticide degradation processes (Pal et al., 2006). The overall dissipation of pesticides from soil results in the interaction of biotic and abiotic mechanisms (Racke et al., 1997).

Phosphatase is a soil enzyme find widely in the soil environment which is responsible for hydrolytic cleavage of a variety of ester phosphate bonds of organophosphates and anhydrides of orthophosphoric acid (H_3PO_4) into inorganic phosphate (Rahmansyah *et al*., 2009). Phosphatase is concentrated in the surface layer and rhizosphere where most of the fresh and less humified organic matter is prevailing (Rojo *et al*., 1990 and Tarafdar *et al*., 2001). Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms and thus in the cycling of it within the soil (Schneider et al., 2001). The objective of the present study is to evaluate the effect of novaluron [1-[3- Chloro-4-(1, 1, 2- triflouro-2- triflouromethoxyethoxy) phenyl]-3-(2, 6- difluorobenzyol) urea], a relatively new benzylphenyl urea insect growth regulator which inhibits the chitin formation on larvae of various insects (*Lepidoptera sp, Coleoptera sp, Homoptera sp and Diptera sp*) (Tomlin, 1997). The effect of novaluron on microbial biomass, respiration and flourescein diacetate hydrolyzing activity in tropical soils were studied (Das et al., 2007). Influence of biotic and abiotic factors on the degradation of novaluron in tropical soils was studied (Das et al., 2008). However the effect of novaluron pesticide on enzyme activity is very meager therefore the present study have been taken up in order to evaluate the information of phosphatase activity in groundnut soils.

2. Materials and Methods

2.1 Soils used in the Present Study

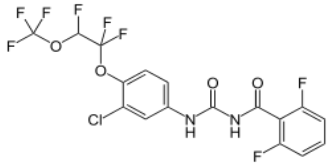
Groundnut growing fields in Anantapur district Andhra Pradesh were selected for the present study and soil was collected randomly near the rhizosphere zone using trowel at a depth of 0-12 cms and mixed thoroughly to prepare a homogenous composite sample, air dried at room temperature. Samples were cleaned to remove plant material and other debris and passed through 2 millimeter sieve and stored at 4ºC prior to analysis. Mineral matter of soil samples was done by following the method (Johnson and Ulrich., 1960). Soil pH was determined by using 1: 1.25 soils to water ratio in systronic digital pH meter. Organic matter in soil samples was estimated by walkley- black oxidation. Total nitrogen content in soil samples was determined by Micro-Kjeldhal method (Jackson., 1971). Electrical conductivity was measured by Conductivity Bridge and contents of nitrite – nitrogen (Barnes, Folkared., 1951). Contents of nitrite – nitrogen by Brucine method (Ranney, Bartlett., 1972). The important Physico-chemical properties of the two soils are presented in Table1.

Table 1: Physico chemical characteristics of soil samples

Properties	*Black clay soil*	*Red sandy soil*
1.sand	65.8	55.3
2. silt	25.2	27.2
3. clay	9.0	17.5
4. pH	7.2	6.2
5. Water holding capacity (mg $^{-1}$soil)	0.47	0.27
6. Electrical conductivity(m. mhos)	260	244
7. Organic matter	1.33	0.72
8. Total Nitrogen	0.082	0.046
9. NH_4(µg g^{-1}soil)	7.93	7.92
10. NO_2(µg g^{-1}soil)	0.54	0.43
11. NO_3(µg g^{-1}soil)	0.86	0.62

2.2 Chemical - Novaluron - pesticide

Novaluron [1-[3- Chloro-4-(1, 1, 2- triflouro-2-triflouromethoxyethoxy) phenyl]-3-(2, 6- difluorobenzyol) urea]. The molecular formula is $C_{17}H_9ClF_8N_2O_4$. It is a chemical with pesticide properties belonging to the class of insecticides called insect growth regulators. It is a benzoyl phenyl urea developed by Maktheshim Agan Industries Ltd. This pesticide inhibits chitin formation in the insects, targeting specifically larval insect stages that actively synthesize chitin.



Structure of novaluron

2.3 Phosphatase activity (E.C. 3.1.3.1.)

The activity of phosphatase under the influence of the insecticide novaluron, at different concentrations was determined in black and red sandy soils. Two gram portions of soil samples, transferred into testubes (12 x 125 mm), was treated with novaluron insecticide to provide final concentrations of 10, 25, 50, 75, and 100 µg g^{-1}soil (equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 Kg ha^{-1} field application rates). The soil samples without insecticide treatment were served as control. All the treatments, including controls were maintained at 60% water holding capacity (WHC) and the tubes were incubated in the laboratory at 28 ± 4ºC. After ten days of incubation, triplicate soil samples were withdrawn for the assay of phosphatase (Tabatabai and Bremner., 1969 and Srinivasulu *et al*., 2012). Similarly, the influence of insecticide at stimulatory concentrations (5.0 Kg ha^{-1}) on the rate of phosphatase activity in two different soils was also determined at 10, 20, 30 and 40 days of incubation.

2.4 Assay of Phosphatase

Each soil sample was treated with 6ml of 0.1 M Maleate buffer (pH 6.5) and 2 ml of 0.03 M *p*-nitro phenyl phosphate. After incubation for 30 minutes at 37ºC, the tubes were passed through Whatmann No.1 filter paper. To suitable aliquots of the extract, 1ml of 5 M $CaCl_2$ and 4 ml of 0.05 M NaOH was added, and the yellow color developed was read at 405nm in a Spectronic 20 D spectrophotometer (Milton Roy Company).

2.5 Statistical Analysis

The concentration of the phosphatase enzyme was calculated on a soil weight (over dried) basis. The insecticide treatments were contrasted with untreated controls and the significant level $P \leq 0.05$ between values of each sampling, and each insecticide was performed using SYSTAT statistical software package to find the results by Duncan's Multiple Range (DMR) test.

3. Results and Discussion

For the cultivation of groundnut crop in Anantapur district of Andhra Pradesh, India the black and red soils were predominantly used. The major constraint in groundnut crop is insect and fungi pests. For this reason, insecticides and fungicides were frequently used for crop protection. Continues and indiscriminate use of these pesticides cause major risk of soil health. Hence, these soils were selected to study the effect of insecticide on the phosphatase activity. In general, the organic matter content is high in black soil. Therefore the biological activity (phosphatase activity) was also pronounced more in black soil than in red soil under the influence of insecticide. Red soil indicates the presence of iron oxides, and black color in soil indicates that the soil has high organic matter content (Getenga and Weil, 2006)

3.1 Phosphatase enzyme activity

Phosphatases, a group of enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid. The mineralization of organic phosphorous by the activity of phosphatase in soils makes one of the essential elements, phosphorous in soil for plant growth. Hence, phosphatase activity was measured under the influence of novaluron at different concentrations (1.0, 2.5, 5.0, 7.5, 10.0 Kg ha^{-1}).

Phosphatase activity was increased in treated soils up to 5.0 Kg ha $^{-1}$ than the controls in 10 days incubated soil samples. The enhancement of enzyme activity continued up to 20 days and then gradually decreased after 30 and 40 days of incubation. Novaluron significantly enhanced in increasing of the phosphatase activity in 10 days incubated soil samples. Novaluron at concentrations ranging from 1.0 to 5.0 Kg ha $^{-1}$ gradually increased the phosphatase activity and reached maximum at the concentrations of 5.0 Kg ha $^{-1}$ in both soil samples. Beyond 5.0 Kg ha $^{-1}$ novaluron showed minimum phosphatase activity and also at 10.0 Kg ha $^{-1}$. At the end of the 10 days incubation, about 28-90% increase in phosphatase activity was observed in black soil and 12-95% increase was observed in red soil treated with novaluron when compared with controls. Rangaswamy and Venkateswarulu (1996) reported comparable stimulatory effects of two organophosphorous pesticides at 1.0-5.0 Kg ha $^{-1}$ on the phosphatase activity. Similarly stimulatory effect by chloropyriphos on phosphatase activity in the field conditions was noticed by Sikors *et a.,* (1990). On the contrary telfuthrin, DOWCO 429X and DPX 43,898 when applied even at 10 mg kg $^{-1}$, induced a reduction in phosphatase activity in an organic soil, but stimulation in sandy soil was reported by Tu (1990). But higher concentrations of pesticides at the levels 7.5-10.0 Kg ha $^{-1}$ show inhibitory effect on the phosphatase activity and represents antagonistic interaction. Rangaswamy and Venkateswarulu (1996) reported that though exact mechanisms for the proliferation/non-proliferation of biological activities are not known, lowering of phosphatase activity at higher concentrations of pesticides alone in soils may affect availability of phosphate for growth of plants. The results obtained from the experiment are represented in table 2 and table 3 and graphically shown in figure 1 and figure 2.

Table 2: Activity of Phosphatase under the impact of different concentrations of selected insecticide in black clay soil for 24hrs after 7 days

Concentration of Insecticide (Kg ha $^{-1}$)	*µg p- nitrophenol (pnp) g^{-}1soil*
0.0	1850±15.773
1.0	2560±5.774
2.5	2650±5.773
5.0	2215±8.660
7.5	1950±11.547
10.0	1450±5.773

Table 3: Activity of Phosphatase under the impact of different concentrations of selected insecticide in red sandy soil for 24hrs after 7 days

Concentration of Insecticide (Kg ha $^{-1}$)	*µg p- nitrophenol (pnp) g^{-}1soil*
0.0	1020±11.547
1.0	1460±11.547
2.5	1490±5.773
5.0	1440±23.094
7.5	1300±11.547
10.0	1220±11.547

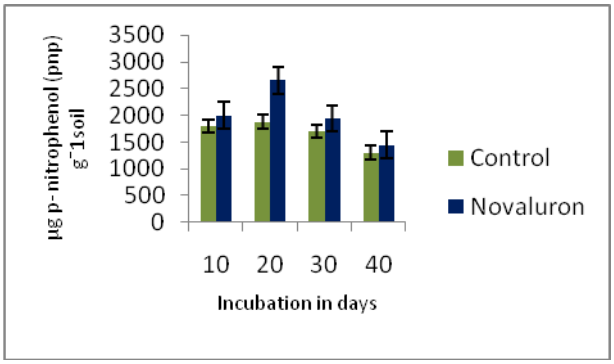


Figure 1: Effect of Novaluron on Phosphatase activity in Black clay soil

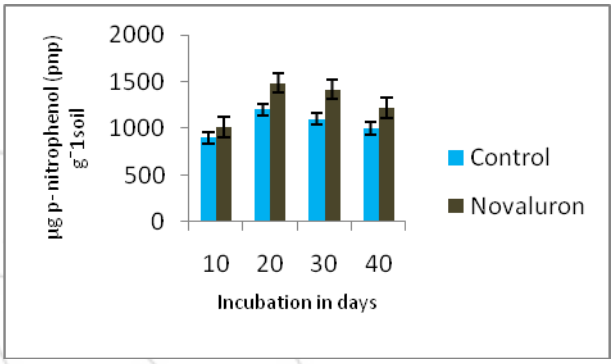


Figure 2: Effect of Novaluron on Phosphatase activity in Red sandy soil

4. Conclusion

The results of the present research work clearly indicated that the selected pesticide Novaluron alone at the levels ranging from 2.5 to 5.0 Kg ha^{-1}profoundly enhanced the phosphatase activity in both the soil samples of groundnut cultivated fields. Further research is needed to evaluate the influence of novaluron on other enzyme activities in groundnut soils.

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