IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Impact of selected pesticides alone and in combination on Amylase and Dehydrogenase activity in Ground nut (*Arachis hypogaea*. L) soils.

D.Meghana[1] , Bharathi. C[2] and V.Rangaswamy[3]

[1]Academic assistant, [2]Academic assistant, [3]Retired Professor

Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India.

ABSTRACT: An investigation is done in the laboratory level to study the impact of selected pesticides on the enzyme activities of two different soils collected from groundnut cultivated fields. During the experiment, there is clear evidence regarding the pesticide effect on the enzyme activities. When the concentration of pesticides is maintained at 2.5 kg ha^{-1} after 10 days of incubation enzyme activities were profoundly increased and gradually decreased along with the increase in the concentration of the pesticides to 7.5 kg $ha^{-1}$to 10.0 kg ha^{-1}. In the present study the results were clearly demonstrating that increase concentration will be more threat to the soil enzyme activities.

Key Words: Pesticides, Ground nut, Amylase, Dehydrogenase, Soil enzymes

Introduction:

India is large country mainly dependent on the quantity and quality of agriculture production. In India among the oil seed crops, groundnut (*Arachis hypogaea*. L) is one of the important, major cash crops grown throughout the year and India is a world leader in ground nut farming with 8 million hectares of cultivated area. Ground nut or peanut (*Arachis hypogaea*. L) is the species in the legume or "bean" family. Ground nuts are known by many other local names such as earthnuts, peanuts, goober peas, monkey nuts, pygmy nuts and pig nuts. They have rich nutty flavour, sweet taste, crunchy texture, and over and above relatively longer shelf life. In Anantapuramu, a semi arid region of Andhra Pradesh, agriculture state of Andhra Pradesh, ground nut is the predominant crop grown. Nearly one third of total of 2.17 X 10^6 ha ground nut cultivation is covered in Anantapuramu in Andhra Pradesh (Anonymous, 2015). Since several decades, xenobioitic substances have been widely used in agriculture as a part of pest control strategies with the growing use of pesticides. The issue of an impact of these chemicals on the composition of soil microorganisms and the process they direct have received more attention (Andrea *et al.*, 2000 and Baxter and Cummings, 2008) and their background levels in the environment have increased greatly. Pesticides also influences soil bio chemical processes driven by microbial enzymatic reactions. The microbial mineralization of organic compounds and associated biotransformations such as nutrient dynamics and their bio availability are also adversely affected by the pesticides (Demanou *et al.*, 2004; Kinney *et al.*, 2005; Mahia *et al.*, 2008 and Niewiadomska, 2004).

Soil enzymes help soil organisms in their efforts to satisfy nutrients. The use of these enzymes in the soil is very useful and each and every enzyme plays a important role in the biological reactions like dehydrogenase enzyme is present intracellularly in all living microbial cells and it is linked with microbial respiratory processes (Bolton *et al.*, 1985), it is an indicator of overall microbial activity in soils. Dehydrogenase enzyme is responsible for the oxidative activities which were done by the microorganisms. Amylase is the enzyme which plays an important role in catalyzing the hydrolysis and solubilisation of starch. These enzymes are usually extracellular and inducible.

In the Present research work, there is a clear explanation about the increase and decrease in the pesticide concentration will leads to the protection of soil microbial activities for long time and subjects for the proper farming.

Materials and Methods:

Soils

Two soil samples namely black clay soil and red sandy soils were collected from groundnut cultivating fields of Vidapankal village, Uravakonda mandalam, Ananthapuramu district, which is a semi-arid zone of Andhra Pradesh, India. Both soil samples were collected from the field to a depth of 12 cm by using a trowel. Soil samples were air dried and sieved through 2mm sieve prior to analysis.

Pesticides

Insecticides novaluron and indoxacarb, fungicides thiophanate methyl and propineb were selected for the determination of their influence on microbial activities and the microbial population. Insecticides and fungicides were used by dissolving them in the sterile distilled water for the analysis of enzymatic activities and population studies.

Amylase activity

Five gram portions of each soil samples, in triplicates, in test tubes (25 x 150 mm) were treated with the selected pesticides individually and in combination to provide the final concentration of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}. All the test tubes including the controls were incubated at room temperature in the laboratory (28 ± 4ºC). After particular time of incubation the triplicate soil samples were withdrawn for the assay of amylase enzyme

Assay of amylase enzyme

Amylase enzyme assay was developed by Cole (1977) and followed by Tu (1981a and b). Soil samples were taken in the Erlenmeyer flasks and were treated with 1 ml of toluene to arrest the enzyme activity. After 15 minutes, 6 ml of 0.2 M of acetate phosphate buffer (5.5 pH) containing 2 % starch was added to each of the testing samples and closed with cotton plugs. After 24 and 72 hrs of incubation the testing samples were made up to a volume of 50 ml with sterile distilled water and passed through Whatman No.1 filter paper. The filtrate was assayed for amount of glucose by Nelson's method (1944) in a U.V Visible Spectrophotometer (Thermo Scientific) Evolution 201.

Dehydrogenase activity

Five gram portions of each soil samples, in triplicates, in test tubes (25 x 150 mm) were treated with the selected pesticides individually and in combination to provide the final concentration of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}. All the test tubes including the controls were incubated at room temperature in the laboratory (28 ± 4ºC). After particular time of incubation the triplicate soil samples were withdrawn for the assay of dehydrogenase enzyme

Assay of Dehydrogenase

Dehydrogenase enzyme was assayed by the method of Casida *et al*., (1964) and Rangaswamy *et al*., (1994) and Srinivasulu and Rangaswamy (2013). Five grams of soil samples were treated with 0.1 g $CaCO_3$ and 1 ml of 0.18 M aqueous triphenyl tetrazolium chloride, and incubated for 24 hours at 37ºC. Then the reaction mixture was treated with methanol for extraction of triphenyl formazan and assayed at 485 nm in a U.V Visible Spectrophotometer (Thermo Scientific) Evolution 201. Dehydrogenase activity was measured at 7, 14, 21, 28 and 35 days of incubation.

Results and Discussion

Amylase activity

Both black and red soil samples were treated with different concentrations of pesticides alone and in combination (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) of novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl, were incubated for 10 days to determine the influence of the pesticides, on the soil enzyme amylase, by exposing it to starch for 24 and 72 hours at 28 ± 4ºC (Fig 1 and 2).

Amylase activity was enhanced by the application of pesticides novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl, in 1.0, 2.5 and 5.0 kg ha^{-1} of graded levels in both soil samples. The applied pesticides novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl, at 10, 25 and 50 ppm levels, showed increase in amylase enzyme activity as 82-135, 5-47, 12-88, 5-56, 5-41% and 52-96, 2-14, 2-68, 2-52, 2-40% over control, were observed following 24 and 72 hours of incubation respectively in black soil after 10-days (Table 1). The increasing levels in the amylase activity in red soil were 4-41, 2-19, 4-90, 2-67, 5-81% and 5-12, 1-5, 3-78, 1-68, 1-64% over control, were observed following 24 and 72 hours of incubation respectively after 10 days of incubation. (Table 2). Therefore it is clear that the activity of amylase was comparatively higher in black soil than in the red soil. The amylase activity was more stimulatory in treated soils than the control soil samples, which were incubated for 72 hours with starch, than 24 hours of incubation (Table 1 and 2). The significant stimulation of amylase activity, in terms of glucose released from starch was noticed from both soil samples.

Ross, 1965 and Tu, 1982 assayed amylase enzyme based on the hydrolysis of soluble starch and subsequent analysis of the reducing sugar content. The amylase activities increased during the germination in both control and cumin treated soils (Prasad and Mathur, 1983). There were only isolated reports on interaction effects between two chemical compounds in axenic culture studies with algae, cyanobacteria and fungi (Megharaj *et al*., 1989; Straton, 1984 and Stratton and Corke, 1982a and b)

Glucose accumulation was increased in all the treated soils as well as untreated soils, incubated for 20 days, whereas it progressively decreased with further incubation (Fig.1 and 2). Furthermore, this increase was more striking when the substrate was exposed to the soil samples for 72 hrs. The soil samples treated with and without pesticides were further incubated for 10, 20, 30, and 40 days, were exposed to starch (2% w/w) for 24 and 72 hours at 28ºC. In the present study, it is very clear that the stimulation on amylase activity by the applied pesticides is observed.

The starch hydrolysing enzyme amylase (Ross, 1976), is known to be constituted by α-amylase and β-amylase is synthesized mainly by plants (Pazur, 1965 and Thoma *et al*., 1971). The enzyme is widely distributed in plants and soils so it plays a significant role in the breakdown of starch. Research evidence suggests that several other enzymes are involved in the hydrolysis of starch, but of major importance are α-amylase which converts starch like substrates to glucose and β-amylase which converts oligosaccharides like substances to maltose (Thoma *et al*., 1971).

Studies have, however indicated that roles and activities of amylases may be influenced by different factors like cultural practices, type of vegetation, environmental soil types (Ross, 1968; Rose and Roberts, 1970; Pancholy and Rice, 1973 and Rose, 1975a). For example plants may influence the amylase enzyme activities of soil by directly supplying enzymes from their residues or excreted compounds, or indirectly providing substrates for the synthetic activities of microorganisms (Rose, 1975a), greater understanding the role of amylase influenced by and other chemical, biological, physical and agronomic factors.

Amylase catalyses the hydrolytic depolymerisation of polysaccharides in soil (Tu and Miles, 1976). Amylases are widely distributed among soil with a wide range of activities (Ladd and Butler, 1972) and properties (Ladd, 1972; Mayouden *et al*., 1975). Amylase activity was correlated significantly with fungal and bacterial numbers and moisture content and the pH of the litter (Joshi *et al*., 1993). Assays are based on the hydrolysis of soluble starch and subsequent analysis of the reducing sugar content (Ross, 1965 and Tu, 1982).

Dehydrogenase activity

Dehydrogenase enzyme is responsible for the oxidative activities which were done by the microorganisms. Pesticides influence on dehydrogenase enzyme activity is studied to know their effects. The activity of dehydrogenase is increased in all pesticide treated soils up to 2.5 kg ha^{-1} than the control after 21 days of incubation (Fig. 3 and 4). Novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl enhanced the dehydrogenase activity significantly at 21-day incubated soil samples, whereas the enzyme activity gradually decreased with increase in period of incubation up to 35 days. Sukul *et al*., (2006) stated that individual application of metalaxyl initially increased the dehydrogenase activity in fungicide treated soils and then gradually decreased after 30 days. Whereas quinolphos inhibited 30% of dehydrogenase activity in soils after 15 days of incubation.

Novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl at concentrations ranging from 1.0 and 2.5 kg ha^{-1} gradually increased dehydrogenase activity and reached a maximum at the concentration of 2.5 kg ha^{-1} in both black and red soils (Tables 3 and 4). Whereas amendment of novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl above 2.5 kg ha^{-1} resulted in minimum dehydrogenase activity while higher concentrations (7.5 and 10.0 kg ha^{-1}) showed inhibitory effect indicating antagonistic interactions (Tables 3 and 4). Neweke *et al*., (2007) also reported that atrazine and northrin stimulated the dehydrogenase activity at lower concentrations (0.2%) and inhibited it at higher concentrations (0.55%) in rhizoplane microbial community. After 7 days of incubation, 10-52, 16-56, 2-59, 19-46, 14-42% and 19-59, 17-50, 2-45, 17-52, 14-48% increase in dehydrogenase activity was observed by the application of pesticides in black and red soils respectively, when compared to controls (Tables 3 and 4).

Srinivasulu and Rangaswamy, (2013) observed when combination of monocrotophos and chlorpyrifos with mancozeb and carbendazim respectively showed increase in dehydrogenase activity at 1.0 and 2.5 kg ha^{-1} of each pesticide in red and black soil, but in the red soil the same combination (chlorpyrifos + carbendazim) increases dehydrogenase activity up to 5.0 kg ha^{-1}.

Cycon *et al*., (2010) observed that dehydrogenase activity was most sensitive to mancozeb + dimethomorph even at 15 mg kg^{-1} (1.5 kg ha^{-1}) soil when compared to loamy sand and sandy loam soils. Naumann, (1970) reported that methyl parathion at 15 kg ha^{-1} stimulated dehydrogenase activity.

Dehydrogenase activity was significantly enhanced under the influence of pesticides after 21 days of incubation. Further increase in incubation period up to 35 days dehydrogenase activity is decreased (Fig 3 and 4). The results of the present study shows that the enzyme activity gradually decreased gradually by the application of pesticides above 2.5 or 5.0 kg ha^{-1} in both black and red soils (Tables 3 and 4).

Dehydrogenase enzyme activity occurs in all living microbial cells, and it is linked with microbial respiratory processes (Bolton *et al*., 1985). This intracellular enzyme is an indicator of overall microbial activity in soils. The impact of pesticides on dehydrogenase activity has been widely reported. The insecticides are either neutral toward this activity (Caceres *et al*., 2009) or they inhibit it (Beulke and Malkomes 2001; Kalam *et al*., 2004; Yao *et al*., 2006 and Jastrzebska, 2011). Only endosulfon seems to stimulate dehydrogenase activity when it goes 100 to 200 times than standard rate of application (Kalyani *et al*., 2010 and Defo *et al*., 2011). There was a progressive increase in the accumulation of farmazan with increasing period of incubation up to 21 days, which gradually decreased further. Hence the dehydrogenase activity was enhanced significantly more at 5.0 kg ha^{-1} of the two insecticides. Infact application of insecticides to soils led to an initial striking increase in dehydrogenase activity at 100 ppm, quinolphos at 25 ppm, carbofuran and quintazene at 100 ppm was known to inhibit dehydrogenase activity (Rangaswamy, 1989; Rangaswamy *et al*., 1994 and Gundi *et al*., 2005).

The dehydrogenase was severely inhibited at higher doses of fungicides (Monkiedje *et al*., 2002 and Bello *et al*., 2008). Gu *et al*., (2009) observed dehydrogenase activities always were higher in P1312777 soil than in liaojing-a soil during the whole growth stages especially during late growth stages. Significant increase in dehydrogenase activity was noticed with permethrin FMC33297, FMC45498, shell WL41706, shell WL4367 and shell WL43775 in 0.5 and 5µg g^{-1} after 3 weeks of incubation (Tu, 1980b). Methyl parathion at 15 kg ha^{-1} was reported to stimulate soil dehydrogenase activity (Naumann, 1970). Similarly additive, synergistic and antagonistic interactions were recorded towards dehydrogenase activity in soils by the combination of several pesticides (Dzantor and Felsot, 1991 and Malkomes 1982). Gowda, (1973) reported inhibition of dehydrogenase activity in peptone amended soil by benomyl at 100 to 10,000 µg g^{-1} soil. In general the dehydrogenase activity was relatively less in the soil maintained under non flooded conditions as reported by Chendrayan *et al*., (1980).

Likewise, herbicides, except butacholr (Min *et al*., 2002 and Xia *et al*., 2011), have a repressive effect on dehydrogenase activity, whatever conditions of applications including dose and soil pH (Beulke and Malkomes, 2001; Benicelli *et al*., 2009 and Sebiomo *et al*., 2012).

Conclusion

From the present investigation results, there is clear evidence that soil enzymes amylase and dehydrogenase were affected by the application of Novaluron Indoxacarb, Thiophanate methyl and Propineb alone and in combination Navaluron +Thiophanate methyl at increased concentrations. There is no threat to the soil enzyme activities when the application rate of insecticides alone and in combination are in recommended levels but higher in the rate of application may leads to the loss of soil activities along with the controlling of pests and insects.

Ackowledgements

We are grateful to the INSPIRE PROGRAMME, Department of Science and Technology, Newdelhi, India for the financial assistance and very thankful to the Department of Microbiology, Sri Krishnadevaraya University for providing all the necessary facilities in working for our research.

Table 1. Influence of selected pesticides alone and in combination on amylase* activity in black soil incubated for 24 and 72 hours after 10 days.

Pesticide Concentration (kg ha^{-1})	Novaluron		Indoxacarb		T.methyl		Propineb		Novaluron + T.methyl	
	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs
0.0	170e (100)	250e (100)	170e (100)	150f (100)	170f (100)	250f (100)	170e (100)	250f (100)	170f (100)	250f (100)
1.0	310c (182)	380c (152)	180d (105)	255b (102)	190c (112)	255b (102)	180b (105)	255b (102)	180c (105)	255b (102)
2.5	440a (235)	490a (196)	250a (147)	285a (114)	320a (188)	420a (168)	270a (156)	380a (152)	240a (141)	350a (140)
5.0	350b (205)	420b (168)	220b (129)	268b (107)	150c (88)	270c (108)	100d (59)	200b (80)	160b (94)	210b (84)
7.5	270d (159)	290d (116)	200c (117)	210d (84)	90e (52)	100e (40)	105c (36)	165c (97)	170c (100)	190c (76)
10.0	150f (88)	180f (72)	100f (59)	140f (82)	60f (35)	90f (36)	90e (53)	150c (60)	140d (56)	200c (80)

*µg glucose g-1 soil formed after 24 and 72 hours incubation with 2% starch.
Figures, in parentheses, indicate relative production percentages.
Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test. Values in the table are means of triplicates. T. methyl= Thiophanate methyl.

Table 2. Influence of selected pesticides alone and in combination on amylase* activity in red soil incubated for 24 and 72 hours after 10 days.

Pesticide Concentration (kg ha^{-1})	Novaluron		Indoxacarb		T.methyl		Propineb		Novaluron + T.methyl	
	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs	24 hrs	72 hrs
0.0	210e (100)	280d (100)	210d (100)	280d (100)	210c (100)	280e (100)	210c (100)	280c (100)	210c (100)	282d (100)
1.0	220b (104)	296b (105)	225c (102)	285c (101)	220c (104)	290d (103)	215b (102)	285b (101)	220b (105)	285c (101)
2.5	240a (141)	315a (112)	250a (119)	296a (105)	400a (190)	500a (178)	350a (167)	470a (168)	380a (181)	460a (164)
5.0	210c (100)	286c (102)	230b (109)	286b (102)	350b (167)	420b (150)	200c (95)	300c (107)	200c (95)	310b (111)
7.5	120d (57)	146e (52)	150e(71)	198e (70)	150d(71)	360c (128)	150d (71)	205e (73)	170d (81)	203e (73)
10.0	90f (43)	128f (45)	120f (57)	158f (56)	110e (52)	220f (78)	140e (67)	180f (64)	130e (62)	160f (57)

*µg glucose g^{-1} soil formed after 24 and 72 hours incubation with 2% starch.
Figures, in parentheses, indicate relative production percentages.
Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test. Values in the table are means of triplicates. T. methyl= Thiophanate methyl.

Table 3. Influence of selected pesticides alone and in combination on dehydrogenase*activity in black soil after 7 days.

Pesticide Concentration (kg ha^{-1})	Novaluron	Indoxacarb	T.methyl	Propineb	Novaluron + T.methyl
0.0	240e(100)	240d(100)	240d(100)	240c(100)	240c(100)
1.0	265c(110)	278c(116)	245c(102)	285b(119)	275b(114)
2.5	366a(152)	374a(156)	382a(159)	350a(146)	340a(142)
5.0	315b(131)	305b(127)	280b(116)	225d(94)	205d(85)
7.5	225d(94)	230e(96)	200e(83)	211e(88)	200e(83)
10.0	190f(79)	200f(83)	170f(71)	160f(67)	150f(66)

*µg of formazan g^{-1} soil formed after 24 hours incubation with triphenyl tetrazolium chloride (TTC). Figures, in parentheses, indicate relative production percentages. Means, in each row, obtained for each sampling, followed by the same letter are not
significantly different (P≤0.05) from each other according to DMR test. Values in the table are means of triplicates.
T.methyl = Thiophanate methyl

Table 4. Influence of selected pesticides alone and in combination on dehydrogenase* activity in red soil after 7 days.

Pesticide Concentration (kg ha^{-1})	Novaluron	Indoxacarb	T.methyl	Propineb	Novaluron + T.methyl
0.0	210c(100)	210d(100)	210d(100)	210d(100)	210d(100)
1.0	250b(119)	245b(117)	215c(102)	245c(117)	240c(114)
2.5	335a(159)	315a(150)	305a(145)	320a(152)	310a(148)
5.0	210c(100)	220c(105)	247b(118)	278b(132)	250b(119)
7.5	170d(81)	200e(95)	142e(67)	200e(95)	210d(100)
10.0	120c(57)	155f(74)	56f(26)	160f(76)	120c(57)

*µg of formazan g^{-1} soil formed after 24 hours incubation with triphenyl tetrazolium chloride (TTC). Figures, in parentheses, indicate relative production percentages.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test. Values in the table are means of triplicates.

T.methyl = Thiophanate methyl

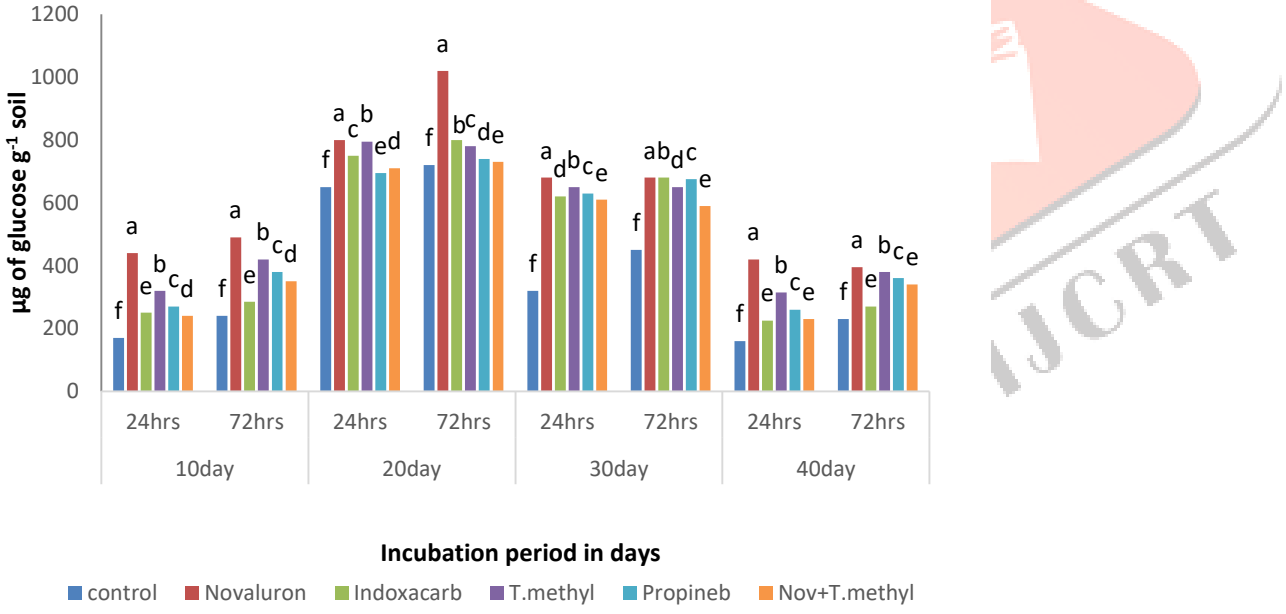

Fig 1. Influence of novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl at 2.5 kg ha^{-1} on amylase* activity in black soil

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test. *Values plotted in figure are means of triplicates.

Nov = Novaluron, T.methyl = Thiophanate methyl

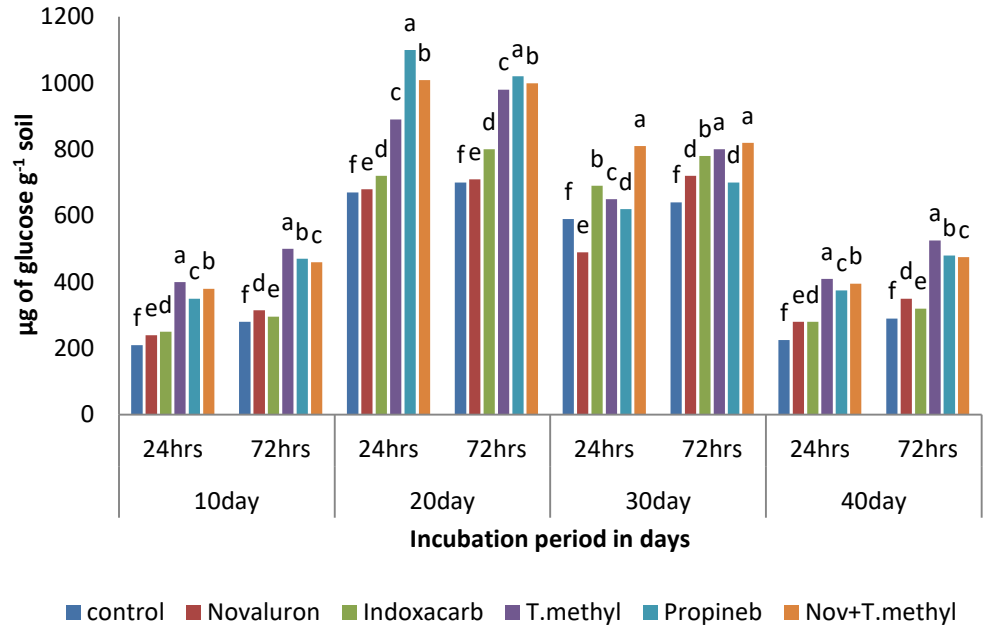

Fig 2. Influence of novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl at 2.5 kg ha^{-1} on amylase* activity in red soil
Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test. *Values plotted in figure are means of triplicates.
Nov = Novaluron, T.methyl = Thiophanate methyl

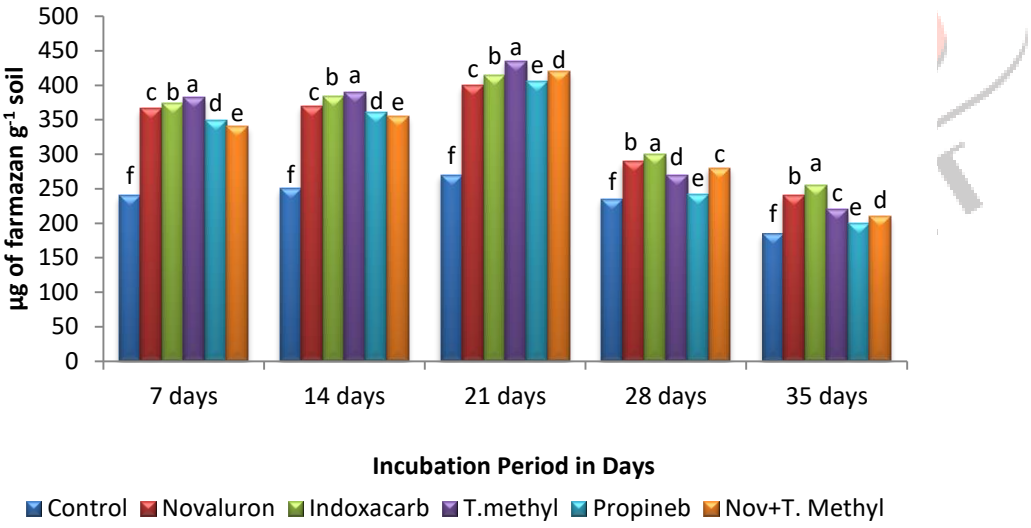

Fig 3. Influence of novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl at 2.5 kg ha^{-1} on dehydrogenase* activity in black soil.
Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test.*Values plotted in figure are means of triplicates.
Nov = Novaluron, T.methyl = Thiophanate methyl

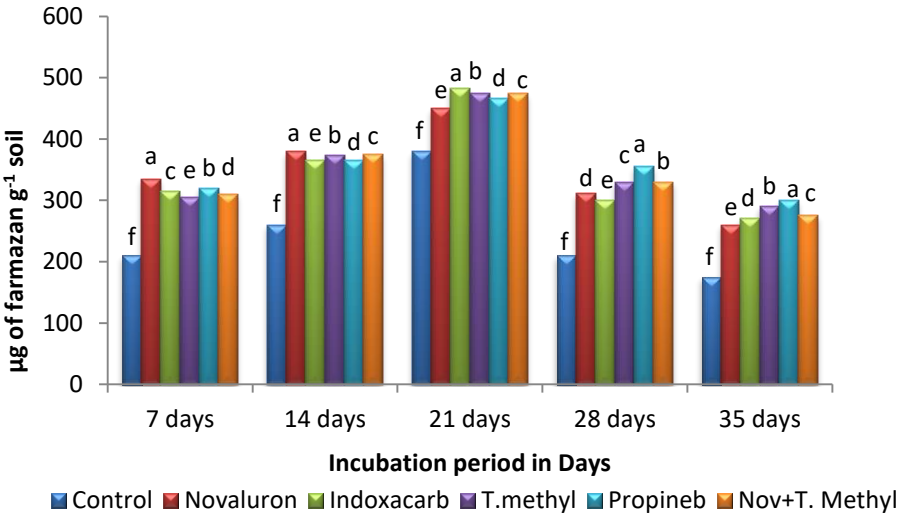

Fig 4. Influence of novaluron, indoxacarb, thiophanate methyl, propineb and novaluron + thiophanate methyl at 2.5 kg ha^{-1} on dehydrogenase* activity in red soil

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test. *Values plotted in figure are means of triplicates.

Nov = Novaluron, T.methyl = Thiophanate methyl

References

[1]. Anonymous, **2015**. Agriculture production plan for Anantapuramu district for Kharif season. Department of Agriculture, Anantapuramu, Andhra Pradesh, India.

[2]. Andrea, M. M., T. B. Peres, L. C. Luchini and A. Jr. Pettinelli. **2000**. Impact of long term pesticide application on some soil biological parameters. *J. Environ. Sci. Health B*, **35**: 297-307.

[3]. Baxter, T and P. Cummings. **2008**. The degradation of the herbicide bromoxynil and its impact on bacterial diversity in a top soil. *Journal of Applied Microbiology*, **104**: 1605-1616.

[4]. Demanou, J., A. Monkiedje, T. Njine, S. M. Foto, M. Nola, H. Serges, Z. Togouet. and N. Kemka. **2004**. Changes in soil chemical properties and microbial activities in response to the fungicide Ridomil gold plus copper. *Int. J. Environ. Res. Public Health*, **1**: 26-34.

[5]. Kinney, C. A., K. W. Mandernack. and A. R. Mosier. **2005**. Laboratory investigations into the effects of the pesticides mancozeb, chlorothalonil and prosulfuron on nitrous oxide and nitric oxide production in fertilized soil. *Soil Biol. Biochem*, **37**: 837-850.

[6]. Mahia, J., A. Cabaneiro, T. Carbellar. and M. Diaz-Ravina. **2008**. Microbial biomass and C mineralization in agricultural soils as affected by atrazine addition. *Biol. Fertil. Soils*. **45**: 99-105.

[7]. Niewiadomska, A. **2004**. Effect of carbendazim, imazetapir and thiram on nitrogenase activity, the number of microorganisms in soil and yield of red clover (*Trifolium pretense* L.). *Pol. J. Environ. Stud*, **13**: 403-410.

[8]. Bolton, H., L.F. Elliot, R.I. Papendick and D. F. Benzdick. **1985**. Soil microbial biomass and selected soil enzyme activities: Effect of fertilization and cropping practices. *Soil Biol. Biochem*, **17**: 297-302.

[9]. Cole, M. A. **1997**. Lead inhibition of enzyme synthesis in soil. *Applied Environmental Microbiology*, **33**: 262-268.

[10]. Tu, C. M. **1981a**. Effect of pesticides on activity of enzymes and microorganisms in a clay loam soil. *J. Env. Sci. Health*. **16**: 179-181.

[11]. Tu, C. M. **1981b**. Effect of some pesticides on enzyme activities in an organic soil. *Bull. Environ. Contam. Toxicol*. **27**: 109-114.

[12]. Casida, L. E., D. H. Klein, and T. Santoro. **1964**. Soil dehydrogenase activity. *Soil Sci*., **98**: 371-376.

[13]. Rangaswamy, V., B. R. Reddy. and K. Venkateswarlu. **1994**. Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin fenvalerate. *Agriculture Ecosystem Environment*, **47**: 319-326.

[14]. Srinivasulu, M. and V. Rangaswamy. **2013**. Influence of insecticides alone and in combination with fungicides on enzyme activities in soils. *Int. J. Environ. Sci Technol*, **10(2)**: 340-341

[15]. Ross, D. H. **1965**. A seasonal study of oxygen uptake of some pasture soils and activities of enzymes hydrolysing sucrose and starch. *J. Soil. Sci*, **16**: 73-85.

[16]. Tu, C. M. **1982**. Influence of pesticides on activities of amylase, invertase and level of microbial respiration in sandy soil. *Chemosphere*, **17**: 159-163.

[17]. Prasad, B. N. and S. N. Mathur. **1983**. Effect of metasystox and cumin-1 on seed germination, reducing sugar content and amylase activity in Vigna mungo (L.) Hepper. *Ind. J. Plant. Physiol*, **24(2)**: 209-213.

[18]. Megharai, M., K. Venkateswarulu. and A. S. Rao. **1989**. Interaction effects of insecticides combinations towards the growth of S*cenedesmus bijugatus* and *Synechococcus elongates*. *Plant Soil*. **114**: 159-13.

[19]. Straton, G. W. **1984**. Effects of herbicides atrazine and its degradation products alone and in combination on phototrophic microorganisms. *Arch. Environ*. *Contam.Toxicol*, **13**: 35-42.

[20]. Stratton, G. W. and C. T. Corke. **1982a**. Toxicity of the insecticide permithrin and some degradation products towards algae and cyanobacteria. *Environ. Pollut*. *A*, **29**: 71-80.

[21]. Ross, D. J. **1976**. Invertase and amylase activities in ryegrass and white clover plants and their relationships with activities in soils under pasture. *Soil Biol Biochem*, **8**:351–356.

[22]. Pazur, J. H. **1965**. Enzymes in the synthesis and hydrolysis of starch. In: Whistler R, Paschall EF (eds) Starch: chemistry and technology, vol 1, Fundamental aspects. Academic, New York, pp 133–175.

[23]. Thoma, J. A., J. E. Spradlin. and S. Dygert. **1971**. Plant and animal amylases. In: Boyer PD (ed) The enzymes, 5th edn. Academic, New York, pp 115–189

[24]. Ross, D. J. and H.S. Roberts. **1968**. A study of activities of enzymes hydrolysing sucrose and starch and of oxygen uptake in a sequence of soils under tussock grassland. *J. Soil Sci*, **19**: 186-196.

[25]. Ross, D. J. and H. S. Roberts. **1970**. Enzyme activnies and oxygen uptakes of soils under pasture in temperature and rainfall sequences. *J. Soil Sci*. **21**: 368-381.

[26]. Pancholy, S.K., and E. L. Rice. **1973**. Soil enzymes in relation to old field succession: amylase, cellulase, invertase, dehydrogenase, and urease. *Soil Sci. Soc. Am. Proc*, **37**: 47–50.

[27]. Rose, D. J. **1975a**. Studies on a climosequence of soils in tussock grasslands-5. Invertase and amylase activities of topsoils and their relationships with other properties. *NZ J. Sci*. **18**: 511-518.

[28]. Ladd, J. N. and J. H. A. Butler. **1972**. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol*. *Biochem*, **4**: 19-30.

[29]. Ladd, J. N. **1972**. Properties of proteolytic enzymes extracted from soil. *Soil. Biol*. *Biochem*, **4**: 227-237.

[29]. Mayaudon, J., L. Batistic. and J. M. Sarkar. **1975**. Properties des activities proteolitiques extradites des soils frais. *Soil Biol. Biochem*. **7**: 281-286.

[30]. Joshi, R., G. D. Sharma. and R. R. Mishra. **1993**. Microbial enzyme activities to litter decomposition near highway in a subtropical forest of north east India. *Soil. Biol*. *Biochem*, **25(12)**: 1763-1770.

[31]. Ross, D. H. **1965**. A seasonal study of oxygen uptake of some pasture soils and activities of enzymes hydrolysing sucrose and starch. *J. Soil. Sci*, **16**: 73-85.

[32]. Tu, C. M. **1982**. Influence of pesticides on activities of amylase, invertase and level of microbial respiration in sandy soil. *Chemosphere*, **17**: 159-163.

[33]. Sukul, P. **2006**. Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues. *Soil Biol Biochem*, **38**:320–326.

[34]. Srinivasulu, M. and V. Rangaswamy. **2013**. Influence of insecticides alone and in combination with fungicides on enzyme activities in soils. *Int. J. Environ. Sci Technol*, **10(2)**: 340-341.

[35]. Cycon, M., Z. Seget-Piotrowska, K. Ozdroz. **2010**. Response of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *International biodeterioration*, **64**: 316-323.

[36]. Naumann, K. **1970**. Zur Dynmik der Bodenmikroflora nach Anwwendung von pflanzenschutzmitteln.IV. Methyl auf die atmug und die Dehydrogenase – Aktivitat der Bodens. *Zentialbl. Bakteriool. Parasitenkd. Infektionsker. Hyg Abt II*, **125**: 119-133.

[37]. Bolton, H., L.F. Elliot, R.I. Papendick and D. F. Benzdick. **1985**. Soil microbial biomass and selected soil enzyme activities: Effect of fertilization and cropping practices. *Soil Biol. Biochem*, **17**: 297-302.

[38]. Caceres, T. P., W. X. He, M. Megharaj. and R. Naidu. **2009**. Effect of insecticide fenamiphos on soil microbial activities in Australian and education soils. *Journal of Environmental Science and Health Part B*, **44**: 13-17.

[39]. Beulke, S., H. P. Malkomes. **2001**. Effects of the herbicides metazachlor and dinoterb on the soil microflora and the degradation and sorption of metazachlor under different environmental conditions. *Biol. Fert. Soils*, **33**: 467-471.

[40]. Kalam, A., J. Tah. and A. K. Mukherjee. **2004**. Pesticides effects on microbial population and soil enzyme activities during vermicomposting of agricultural waste. *J. Environ. Biol*, **25**: 201-208.

[41]. Yao, X. H., H. Min, Z. H. Lu. and H. P. Yuan. **2006**. Influence of acetamiprid on soil enzymatic activities and respiration. *Eur J Soil Biol*, **42**:120–126.

[42]. Jastrzebska, E. **2011**. The effect of chlorpyrifos and teflubenzuron on the enzymatic activity of soil. *Pol. J. Environ. Stud*, **20**: 903-910.

[43]. Kalyani, S.S., J. Sharma, P. Dureja, S. Singh. and Lata. **2010**. Influence of endosulfon on microbial biomass and soil enzymatic activities of a tropical Alfisol. *Bull Environ Contam Toxicol*, **84**: 351-356.

[44]. Defo, M. A., T. Njine, M. Nola. and F. S. Beboua. **2011**. Microcosm study of the long term effect of endosulfon on enzyme and microbial activities on two agricultural soils of Yaounde-Cameroon. *Afr. J. Agric. Res*, **6**: 2039-2050.

[45]. Rangaswamy, V., P. B. B. N. Charyulu. and K. Venkateswarulu. **1989.** Effect of monocrotophos and quinolphos on soil population and nitrogen fixing activity of *Azospirillum* sps. *Biomed. Environ*, 225-230.

[46]. Rangaswamy, V., B. R. Reddy. and K. Venkateswarlu. **1994**. Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin fenvalerate. *Agriculture Ecosystem Environment*, **47**: 319-326.

[47]. Gundi, V. A. K. B., G. Narasimha. and B. R. Reddy. **2005**. Interaction effects of soil insecticides on microbial populations and dehydrogenase activity in a black clay soil. *J. Env. Sci. Heal*. **40**: 269-281.

[48]. Monkiedje, A., M. O. Ilori. and M. Spiteller. **2002**. Soil quality changes resulting from the application fungicides mefenoxam and metalaxyl to a sandy loam soil. *Soil. Biol. Biochem*. **34**: 1939-1948.1605-1616.

[49]. Bello, D., C. Traser-Cepeda, M.C. Lerios, and F. Gil-Stores. **2008**. Evaluation of various tests for the diagnostic of soil contamination by 2, 4, 5 – trichlorophenol (2, 4, 5 – TCP). *Environ Pollut*, **156**: 611-617.

[50]. Gu, Y., P. Wang. and C. H. Kong. **2009**. Urease, invertase, dehydrogenase and polyphenoloxidase activities in paddy soil influenced by allelopathic rice variety. *Euro. J. Soil. Biol*, **45(5-6)**: 436-441.

[51]. Tu, C. M. **1980b**. Influence of pesticides and some of the oxidised analogues on microbial population, nitrification and respiration activities in soil. *Bull. Environ. Contam. Toxicol*. **24**: 13-19.

[52]. Naumann, K. **1970**. Zur Dynmik der Bodenmikroflora nach Anwwendung von pflanzenschutzmitteln.IV. Methyl auf die atmug und die Dehydrogenase – Aktivitat der Bodens. *Zentialbl. Bakteriool. Parasitenkd. Infektionsker. Hyg Abt II*, **125**: 119-133.

[53]. Dzantor, E. K. and A. S. Felsot. **1991**. Microbial responses to large concentrations of herbicides in soil. *Environ. Tox. Chem*, **10**: 649-655.

[54]. Gowda, T. K. S. **1973**. Studies on the effect of bentate on microflora and other biological properties of soils of Mysore state. M.Sc thesis, University of Agricultural science, Bangalore, India, 160pp.

[55]. Chendrayan, K., T. Adhya, and N. Sethunathan. **1980**. Dehydrogenase and invertase activities of flooded soils. *Soil Biology and Biochemistry*, **12(3)**: 271-273.

[56]. Min, H., Y. F. Ye, Z. Y. Chen, W. X. Wu. and Y. F. Du. **2002.** Effects of butachlor on microbial enzyme activities in paddy soil. *J. Environ. Sci. China*. **14**: 413-417.

[57]. Xia. X. M., M. Zhao, H. Y. Wang, H. Ma. **2011**. Influence of butachlor on soil enzymes and Microbial growth. *J Food Agric Environ*, **9**:753–756.

[58]. Beulke, S., H. P. Malkomes. **2001**. Effects of the herbicides metazachlor and dinoterb on the soil microflora and the degradation and sorption of metazachlor under different environmental conditions. *Biol. Fert. Soils*, **33**: 467-471.

[59]. Bennicelli, R. P., A. Szafranek-Nakonieczna, A. Wolinska, Z. Stepniewska. and M. Bogudzinska. **2009**. Influence of pesticide (glyphosate) on dehydrogenase activity, pH, Eh and gases production in soil (laboratory conditions). *International Agrophysics*, **23**:117–122.

[60]. Sebiomo, A., V. W. Ogundero. and S. A. Bankole. **2012**. Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *Afr J Biotechnol*, **10**:770–778.