Effect of quinalpos on enzyme activity in two ground(Arachishypogaea L.)soils.

P.Ramanamma and V.Rangaswamy.

Abstract: Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes. Soil dehydrogenase enzymes are one of the main components of soil biogeochemical cycles. Introduction of pesticides into soil may have lasting effects on soil microbial activities and thus affect soil health. The effect of selected pesticides i.e. quinalphos on selected soil enzymes dehydrogenases were determined in two different soil samples (red sandy loam and black clay soils) of groundnut (*Arachis hypogaea L.)* cultivated fields of Anantapuramu District of Andhra Pradesh, India. A laboratory experiment was conducted to determine the effect of selected pesticides, quinalphos at different concentrations ranging from 1.0 to 10 kg ha^{-1} on the activity of dehydrogenase in two groundnut *(Arachis hypogaea L.)* soils. The activity of dehydrogenase was significantly more at quinalphos levels of 5.0 kg ha^{-1} in black and red soils respectively. But at higher concentrations of 7.5 and 10.0 kg ha^{-1} respectively, quinalphos were toxic to dehydrogenase activity. The activity of dehydrogenase was drastically decreased with increasing period of incubation up to 28 and 35 days.

Key words: Quinalphos, dehydrogenase, groundnut (Arachis hypogaea L.) soils.

1.INTRODUCTION

India is an agro based country. Large population is dependent on agriculture. Pesticides are necessary to protect crops and losses that may account for about 45 % of total food production worldwide (Tomlin, 1997). The use of various pesticides has become a matter of concern in modern agricultural practice in the context of environment in general and ecotoxicology in particular (Perucci *et al*., 2000). Pesticides in the soil affect the non target and beneficial microorganisms (Singh Prasad, 1991 and Bhuyan *et al*., 1992) and their activities which are essential for maintaining soil fertility. India occupies the top position in the world with regard to the production of groundnut which is 5-6 million acres and 4-5 million tons respectively. Modern agriculture depends upon a wide variety of synthetically produced chemicals including insecticides, fungicides, herbicides and other pesticides (Zhang WJ, Jiang FB, Ou JF *et a.,l* 2011*).* India is one of the largest producers of oil seeds in the world and occupies an important position in the Indian agricultural economy. Soil is a dynamic living system containing many free enzymes, immobilised extra cellular enzymes and some enzymes within microbial cells (Vineela Deborah *et al*, 2014). Groundnut is called as the 'King' of oilseeds. In spite of the maximum potential of soil enzymes in maintaining, soil biodynamic, only limited studies were available (Nannipieri and Landa, 2000 and Ramesh *et al*., 2003) on influence of soil enzymes with organo chemicals.

Research scholar, Department of Microbiology, SriKrishnadevaraya University, Anantapuramu–515001, Andhra Pradesh, India.+918790683357,rama.ramamsc@gmail.com

Professor,Department of Microbiology,SriKrishnadevaraya University, Anantapuramu–515001, Andhra Pradesh.India,+91 8333832874

pesticides in the soil environment depends not only on the properties of the pesticides but also on the properties of the soil and the prevailing climatic conditions (Khan ,1980).Within the state, Anantapuramu District, a semiarid region occupies a predominant place in groundnut cultivation (Anonymous, 2003). Soil microbial populations play an endorsed role in biochemical transformations by producing enzymes. These enzymes may serve as useful indicators for changes in the biology and chemistry of the soil due to management and environment factors (Dick, 1997, Yun Long *et al.,* 2006 and Pandey, Singh, 2006). Currently, soils are becoming more and more polluted by pesticide molecules because of their wide use in agriculture practices. Pesticides may be toxic to some important bacterial groups; other Microorganisms are able to use some pesticides as energy and nutrient sources (Johnsen *et al*., 2001; Mokiedje and Spiteller, 2002). The present day agriculture involves abundant cultivation of the crop because of its vital role in edible oil seeds production (Kori *et al*., 2002).This condition led to inherent risk of yield loss due to drought, diseases and pests (Galgunde and Kurundkar et al., 2002). Reduction in number of microorganisms in different soil types and at various depths was investigated and insecticides and / or their residues inhibited the growth of microorganisms (Digrak Ozcelik 1998 Tawfic *et al*., 1998 and Hashem *et al*., 1999). These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh *et al.,* 1991). They are important in catalyzing several vital reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling, hence playing an important role in agriculture (Dick *et al*., 1994 and Dick 1997). All soils contain a group of enzymes that determine soil metabolic processes (McLaren 1975) which, in turn, depend on its physical, chemical, microbiological, and

biochemical properties. While different pesticides may be toxic to some important bacterial groups (e.g., nitrogen-fixing and nitrifying bacteria), other microorganisms are able to use some pesticides as energy and nutrient sources (Johnsen *et al*., 2001 and Monkiedje *et al*., 2002). As microorganisms are scavengers in soil and possess physiological variability, they degrade a great variety of chemical substances including the insecticides to derive energy and other nutrients for their metabolism (Bhuyan *et al., 1993)*. Repeated and extensive application of the pesticide ultimately reaches the soil, which in turn may interact with soil organisms and their metabolic activities (Sharma and Roomiro 2002).Therefore the aim of the present research work is to evaluate the effect of selected organo phosphorous pesticide i.e.quinalphos on selected agricultural soil enzymes dehydroginase was determined in two different soil samples(black clay &red sandyloan) of ground nut cultivated fields of Anantapuramu Distric of Andhra Pradesh, India.

2.MATERIALS AND METHODS

2.1 Soils used in the present study

Agricultural soil samples such as samples of black clay soil and red sandy loam soil, collected from groundnut cultivated fields of Anantapuramu District of Andhra Pradesh, India in a semi arid zone from the depth of 12 cm and mixed thoroughly to prepare a homogenate composite sample, air dried at room temperature samples were cleaned by removing plant material and other debris and passed through a 2 mm sieve and stored at 4°c prior to analysis.

2.2.Analysis of Physico- chemical characteristics of soil samples:

Mineral matter of soil samples such as sand, silt and clay contents were analyzed with the use of different sizes of sieves by following the method of Alexander (1961). Water holding capacity of the soil samples were determined by adding distilled water up to the saturation point and then 60 % water holding capacity of the soil samples was calculated by Johnson and Ulrich (1960). pH of soil samples was determined by mixing soil and water in the ratio of 1:1.25 using systronics digital pH meter with calomel glass electrode. Organic carbon content in soil samples was estimated by Walkey-Black method and the organic matter was calculated by multiplying the values with 1.72 (Jackson 1971). Electrical conductivity of soil samples was measured by a conductivity bridge. Total nitrogen content in soil samples was determined by the method (Jackson 1971). The inorganic ammonium nitrogen content in the soil samples after extraction of 1M KCL by Nesslerization method (Jackson 1971) and contents of nitrite - nitrogen (Barnes and Folkard 1951), and the contents of nitrate-nitrogen by Brucine method (Ranney and Bartlett, 1972) after extraction with distilled water were determined respectively. Physico-chemical characters of the two soil samples are listed in table 1.

2.3Table1 Physico-chemical properties of the soils

Properties	Black clay soil	Red sandy soil
Sand (%)	66.4	52.4
Silt (%)	23.6	26.9
Clay (%)	9.3	19.4
pH[a]	7.9	6.8
Water holding capacity (ml g-1 soil)	0.45	0.32
Organic matter (%) b	1.44	0.74
Total nitrogen (%) [c]	0.089	0.048
NH^{+}_{4} - N (μg g^{-1} soil) [d]	8.57	7.02
No^{-}_{2}-N(μg g^{-1} soil) [e]	0.45	0.66
No^{-}_{3}-N (μg g^{-1} soil) [f]	0.92	0.74

[a] 1:1.25=soil: water slurry

[b] Walkley- Black method (johnson and Ulrich 1960)

[c] Micro-Kjeldahl method (Johnson and Ulrich 1960)

[d] Nesslerization method (Johnson and ulrich 1960)

[c] Diazotization method (Ranney and Bartlett 1972)

[f] Brucine method (Barnes and Folkard 1951)

2.4.INSECTICIDES USED IN THE PRESENT STUDY

To determine the effect of selected insecticide on soil enzyme activities quinalphos (o, o-dimethyl-o-quinaxaline-2-yl phosporothioate) (17.8 mg/L soluble) were obtained from Syngenta India Limited.Theused commercial grade insecticides were dissolved in water.

3.SOIL INCUBATION STUDIES

3.1Dehydrogenase activity (E.C. 1.1.1.1)

To study the effect of quinalphos on dehydrogenase, 5 g of dried black clay soil and red sandy loam soil were taken separately in test tubes (12 × 125 mm) containing different concentrations of insecticides 10, 25, 50, 75, and 100 μg g^{-1} soils which are equal to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1} of field

application rates. In order to maintain 60% water holding capacity (WHC), about 2 ml of deionized water was added to test tubes containing black clay soil and 1 ml into tubes containing red soil. Untreated soil samples served as controls. All the treatments, including controls were incubated in the dark at 28 ± 4°C for 1, 2, 3, 4, and 5 weeks. During the incubation period certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the enzyme assay.

3.2 Assay of dehydrogenase

The method employed for the assay of dehydrogenase was developed by (Casida *et al.*, 1964). This method is based on the reduction of 2,3,5-triphenyltetrazoliumchloride (TTC) to triphenyl formazan (TPF). Each soil sample was treated with 0.1 g of $CaCO_3$ and 1 ml of 0.18 mM aqueous solutions of TTC and incubated for 24 hours at 30°C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a spectrophotometer.

4.STATISTICAL ANALYSIS

The concentration of dehydrogenase was calculated on the basis of soil weight (oven dried). Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test (DMRT) (Jaffer *et al.*, 2010). All statistical analysis was performed at $p \leq 0.05$ using SYSTAT Statistical software package.

5.RESULTS AND DISCUSSION

5.1Dehydrogenase activity:

To determine the effect of pesticide on dehydrogenase activity the soil samples treated with different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) of the pesticides for 7 days and the treated samples were exposed to TTC, which is water soluble and its redox potential is about -0.08 mV and function as electron acceptor for several dehydrogenases (Thalman 1968). Nearly all microorganisms reduce TTC in to TPF. Hence, the results in the present study clearly indicated that the activity of dehydrogenase in black soil was comparatively higher than in red soil. In genaral black soil is having high organic matter than in red soil (Srinivasulu et al., 2011) and also black soil is having high WHC.The maximum dehydrogenase activity was significantly enhanced at 5.0 kg ha^{-1} (stimulatory).The stimulatory concentration of the above insecticide (5.0 kg ha^{-1}) induces the highest enzymatic activity after 2, 3, 4, 5 weeks of incubation in both soils. A further increase in the stimulatory concentration of insecticides decreased the rate of dehydrogenase activity after 3 weeks of incubation and then decline phase was started from 3 to 5 weeks of incubation. In contrast, (Cycon *et al.*,2010) reported that dehydrogenase activity decrease in sandy loam soils in combination of mancozeb and dimethomorph at higher concentrations. Similar observations were made by (Monkiedje *et al.*,2002) with mefenoxam and metalaxyi and as well as azoxystrobin, tebuconazole and chlorothalonil by (Bending *et al.*, 2007). In the same manner dehydrogenase activity was decreased to 39.3% in unamended polluted soils (with MCPA) (Tejda *et al.*, 2010). Rasool and reshi (2010) reported a significant increase in dehydrogenase activity was stimulated by fenamiphos (Caeeres *et al.*, 2009) in Australian and Ecuadorean solis. Akmal and Xu (2008) noticed a significant decrease in dehydrogenase (50%) enzyme activity with different concentrations (0, 200, 400,600, 800 and 1000 mg kg^{-1}) of pb-contaminated soil.

Table .2 Activity of dehydrogenase* under the impact of different concentrations of selected in secticides in soils(both black & red) for 24 hr after 7 days.

centration of insecticides (kg h^{-1})	Black soil	Redsoil
0.0	1649±5.664	1020±2.335
1.0	2150±4.338	1549±11.535
2.5	222±4.224	2030±11.535
5.0	2850±5.773	2520±4.533
7.5	1830±11.447	1671±10.773
10.0	1420±5.770	1375±10.355

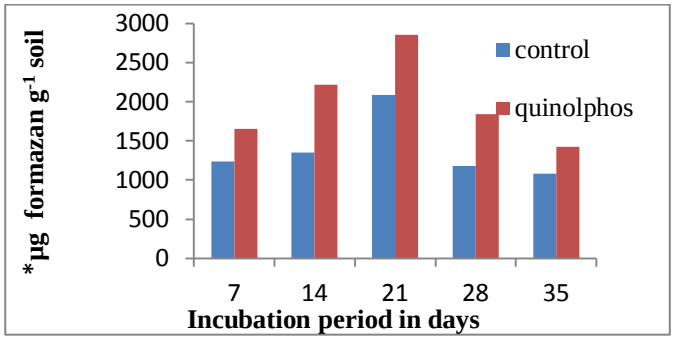

Fig.1 Influence of selected insecticides at 5.0 kg ha^{-1} on dehydrogenase activity in black soil after 24 hr *ug formazan g^{-1} soil formed after 24 hr incubation with triphenyl terazolium chloride (TTC), Means in each time period, followed by the same letter are significantly different ($p \leq 0.05$) from each other acording to DMR test.

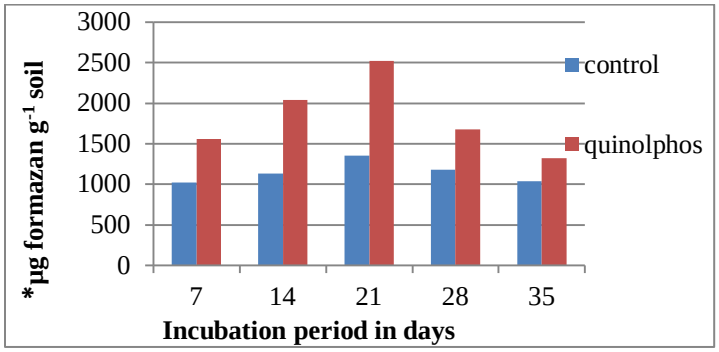

Fig.2 Influence of selected insecticides at 5.0 kg ha^{-1} on dehydrogenase activity in red soil after 24 hr *ug formazan g^{-1} soil formed after 24 hr incubation with triphenyl terazolium chloride (TTC), Means in each time period, followed by the same letter are significantly different ($p \leq 0.05$) from each other acordimg to DMR test.

The data presented in the Table 2 reveals that significant inhibition of dehydrogenase activity occured higher concentrations (10 Kg ha^{-1}) of quinalphos in both soils Groundnut is the most important oilseed crop in India. Especially in drought prone district of Anantapuramu, the farmers are mainly depends on groundnut cultivation. Due to lack of irrigation facilities and poor alternative cropping pattern in rain fed areas likeAnantapuramuand in other Rayalaseema districts the farmers have been cultivating groundnut crop from the last several decades. But of nine oil seed crops grown in India, the area under groundnut accounts for about 45 percent of the total cropped area and 55 percent of the total oilseeds area. India is the major groundnut producing country in the world. It stands third place in exporting of groundnut and earned an amount of Rs. 52,579 lakhs during 2005-2006. The effect of pesticides generally decreased with the increase of incubation period. Increase in the concentration of pesticides decreased the rate of enzyme activities.Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles (Ladd, J.N., *et al*., 1985). The soil dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in soil. Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to accepters. Enzyme activity in soil results from the accumulated enzymes and from enzymatic activity of proliferating microorganisms (Kiss *et al*., 1975). Inhibition of dehydrogenase activity by quinalphos was also reported by (Menon *et al*., 2005). In the other hand some studies showed dehydrogenase activity increasing after pesticides application (Fragoeiro and Magan, 2008; Singh and Kumar, 2008). Higher activities of dehydrogenases have been reported at low doses of pesticides, and lower activities of the enzyme at higher doses of pesticides (Baruah and Mishra 1986).

6.Conclusion:

The results of the present study thus, clearly indicated that the insecticide, quinalphos were profoundly enhanced the activity of dehydrogenase at 5.0. kg ha^{-1} Based on the above results, it is concluded that the biological activities were not affected, by the insecticides applied at recommended levels in agricultural systems to control insect pests.

7.Acknowledgment

The author (PR) are grateful to the University Grants Commission UGC-BSR, New Delhi, India, for financial assistance and at the same time we are very much thankful to the Department of Microbiology, Sri Krishna Devaraya University for providing all the necessary facilities in fulfilling our research.

8.REFERENCES:

1. Anonymous (2003). Agriculture production plan for Anantapuramu district for Kharif season. Depatrment of Agriculture, Ananatapuramu, Andhra Pradesh, India, opp.1-10.
2. Barnes, H., and Folkard, A.R. 1951. The determination of nitrites. Analyst, 76(907): 599 603.
3. Baruah M, Mishra RR (1986). Effect of herbicides butachlor, 2,4-d and oxyfluorfen on enzyme activities and CO2 evolution in submerged paddy field soil. Plant Soil 96: 287–291.
4. Bending GD, Rodriguez-croz SD (2007). Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere 69,82-88.
5. Bhuyan,S., Sreedharan, B., Adhya, T.K. and Sethunathan, N., (1993), Enhanced biodegradation of □-hexachlorocyclohexane [□- HCH) in HCH (commercial) acclimatized flooded soil: factors affecting its development and persistence, Pesticide Science, 38, pp 49-55
6. Caceres TP, He w-x, Megharaj M, Naidu R (2009). Effect of insecticide fenamiphos on soil microbial activities in Australian and Education soils. Journal opf Environmental sscience and Health part B 44,13-17.Casida LE jr., Klein DA, santoro T (1964). SOIL dehydrogenase activity. SOLI SCIENCE 98,371-376.

7. Cycon M, piotrowska- seget Z,K ozdroj (2010). Response of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. International biodeterioration 64 316-323.

8. Dick, R.P. 1997. Soil enzyme activities as integrative indicators of soil health, In Biological indicators of soil health. (Ed. J.W. Doran et al.,) SSSA Spec. Pub. 49. Madison, WI. 247- 271.

9. Dıgrak, M.and S. Ozcelik, 1998. Effect of some pesticides on soil microorganisms. Bull. Environ. Contam. Toxicol. 60 (1):916-922.

10. Fragoeiro, S. and N. Magan, 2008. Impact of trametes versicolor and phanerochaete chrysosporium on differential breakdown of pesticide mixtures in soil microcosms at two water potentials and associated respiration and enzyme activity. Int. Biodeter. Biodegr., 62: 376-383.

11. Galgunde, LP and Kurundkar, BP (2002).Occurrence of groundnut diseases in Rabi crop j Maharasha, Agric.Sci. 115:1517.

12. Jaffer Mohiddin G, Srinivasulu M, Madakka M, Rangaswamy V(2010). Influence of insecticides on the activity of amylase and celluase in groundnut (Arachis hypogea L.) Soil.Ecology, Environemt and conservation 16(3) 383-388.

13. Jackson ML (1971) Soil chemical Analysis, prentice Hall,New Delhi, India, pp 535-538.

14. Johnson, C.M., Ulrich, A. 1960. Determination of moisture in plant tissues. Calif. Agricult. Bull 766:112-115.

15. Nannipieri, P. and L. Landa. 2000. Soil enzymes. In: Handbook of Soil Sci. (Ed. H.E. Sumner) CRC Press, New York, C 129 - C 137.766:112- 115.

16. Khan SU (1980), pesticides in soil Environment, Elsevier, Amsterdam Kumar M,Philip L (2006). Adsorption and desorption characteristics of hydrophobic pesticide endosulfan in four Indian soils. Chemosphere, 62:1064-1077.

17. Kiss, S., M. Dragan-Bularda and D. Radulescu, 1975. Biological significance of enzymes in soil. Adv. Agron., 27: 25-91.

18. Kori, RN, Patil, SL, Salakinakop, SR, Hunshal, CS and Nagagoud, BT (2002). Economics of integrated pest management in irrigated groundnut. J.oil.seeds.Res. 017(1);61-65.

19. Ladd, J.N., 1985. Soil enzymes. In: Soil Organic Matter and Biological Activity. Nijhoff, Dordrecht.

20. McLaren AD (1975) Soil as a system of humus and clay immobilised enzymes. Chem Scripta 8:97–99.

21. Monkiedje A, IIori MO, spiteller M (2002) soil quality changes resulting from the application of the fungicides mefenoxam and metalaxy to sandy loam soil. Soil biology and biochemistry 34, 1939-1948.

22. Menon, P., M. Goal and R. Parsed, 2005. Effects of chlorpyrifos and quinalphos on dehydrogenase activities and reduction of Fe3+ in the soils of two semi-arid fields of tropical India. Agr. Ecosyst. Environ. 108: 73-83.

23. Perucci, S. Dumontet, S. A. Bufo, A. Mazzatura, and C. Cauca., (2000), Effects of organic amendment and herbicide treatment on soil microbial biomass, Biology and Fertility of Soils, 32, pp 17-23.

24. Ranney, T.A., Bartlett, R.J. 1972. Rapid field determination of nitrate in natural waters. Common. Soil Sci.Plant Anal., 3: 183- 186.

25. Rangaswamy V, Reddy BR, Venkateswaralu K (1994). Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermerthrin fenvalerate. Agriculture, Ecosystems and Environment 47,319-326.

26. Rasool N, Reshi ZA (2010). Effect of the fungicide Mancozeb at different application rates on enzyme activity in a silt loam soil of the Kashmir Himalaya, India. Tropical Ecology 51(2),199-205.

27. Sinsabaugh RL, Antibus RK, Linkins AE (1991) an enzymic approach to the analysis of microbial activity during plant litter decomposition. Agric Ecosyst Environ 34:4354.

28. Singh and Prasad (1991). Effect of phorate and gamma BHC on mineralization of nitrogen in soil, Journal of Indian soil sciences society, 39, pp 183–185.

29. Sharma HC, Romero Ortiz (2002). Host plant resistance to insects: An eco-friendly apporach for pest management and environment conservation. J.Environ.Biol.23 (2):111-135.

30. Srinivasulu M, Jaffer Mohiddin G, Subramanyam K, Rangaswamy V. Effect of insecticides alone and in combination with fungicides in nitrification and phosphatase activity in two groundnut (Arachis hypogaea L.) soils. Environ Geochem Health, 2011.

31. Tejada M, Garcia-Martinez AM, Gomez I, parrado J(2010). Application of MCPA herbicide on soils amended with biostimulants: Short-time effects on soil biological properties.Chemosphere 80, 1088-1094.

32. Tomlin CDS (1997).The Pesticide Manual, 11th edn.London: British Crop Protection Council pp.128-141.

33. United states Department of Agriculture (USDA) (2011). Foreign Agricultural Service (FAS)

34. Vineela Deborah B, Jaffer Mohiddin G, and Jaya Madhuri R, (2013). Interaction effects of selected pesticides on soil enzymes. Toxicology International, Vol-20/ Issue -3.

35. Zhang WJ, Jiang FB, Ou JF. Global pesticides consumption and pollution: With China as a focus. Proc Int Acad Ecol Environ Sci. 2011; 1:125–44.

.