International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 7 No. 4 Aug. 2014, pp. 1534-1543

http://www.ijias.issr-journals.org/

The effect of Paraquat and Fipronil on the soil and rhizosphere microflora of tea (*Camellia sinensis* (L) O. kuntze)

Yumnam Devashree[1], B.K. Dutta[1], S.B. Paul[2], and Sudip Choudhury[2]

[1]Microbial & Agricultural Ecology and Biodiversity Conservation Laboratory,
Department of Ecology and Environmental Science, Assam University, Silchar, 788011, Assam, India

[2]Department of Chemistry, Assam University, Silchar, 788011, Assam, India

ABSTRACT: The effect of two pesticides Paraquat and Fipronil on the soil and rhizosphere microflora of tea (i.e. fungi, actinomycetes and bacteria) was studied. The experiment was conducted over a period of 65 days and the observations were made at different interval of days (5, 20, 35, 50 and 65 days) respectively for both the rhizosphere and non rhizosphere soil. Paraquat and Fipronil had a transient negative effect on the rhizosphere and non rhizosphere soil microorganisms, however the negative effect was observed at the initial stage only. The dehydrogenase and phosphatase activity in the said soil also showed an inhibitory response at the initial stage but was able to recover with time.

KEYWORDS: Actinomycetes, Bacteria, Dehydrogenase activity, Fungi, Pesticide, Phosphatase Activity, Rhizosphere.

1 INTRODUCTION

The constant and widespread use of pesticides has resulted in the pollution of the environment. These chemicals not only affect the target organisms but also the microbial communities of the soil [3]. Maximum microbial population is found in the plant roots or rhizosphere [15]. However, the rhizosphere communities differ significantly from the bulk soil communities. Observations on the microbes isolated from the soil and rhizosphere reveal that the rhizosphere bacteria are physiologically more active than the soil bacteria [15]. Apart from quantifying the microbial population as an indicator of the effect of pesticides in soil, the effect of pesticide application on the soil enzyme study is also a good indicator of the degree of damage caused to the ecosystem.

Two of the most commonly used pesticides in tea agroecosystem of Barak Valley is Paraquat and Fipronil. Paraquat is one of the most widely used herbicide in the world to control broad leaf weeds and grasses [5]. It does not harm mature bark and is thus widely used for weed control in tea and coffee plantations [14]. Fipronil is an insecticide that belongs to the phenylpyrazole chemical family [13]. It is mainly used to control termites in tea agroecosystem. Termites are a very destructive pest of the tea plantations of Barak Valley, South Assam.

Enzymes have been considered as markers of soil environment purity [9]. Therefore in the present investigation, the effect of Paraquat and Fipronil on the total fungal, actinomycetes and bacterial population in the soil and tea rhizosphere and also on the dehydrogenase and phosphatase activity of the tea soil have been studied.

2 MATERIALS AND METHOD

Soil and tea saplings were obtained from the agricultural field (i.e, tea agro ecosystem) nursery of Rosekandy Tea Estate. The soil was sieved prior to the treatment with Paraquat and Fipronil. Soil @ 5 kg per earthen pots was taken and aqueous solution of Paraquat and Fipronil was applied at 10 ppm, 100 ppm and 1000 ppm concentration. Healthy seedlings of tea were planted in each pot. Soil and rhizosphere soil samples for analysis were collected at 5, 20, 35, 50 and 65 days interval

Corresponding Author: Yumnam Devashree

after the application of the said pesticides. Three pots were taken for each treatment and control. To obtain the rhizosphere samples, the complete root system of the tea plant was dug out and put in the polythene bag where it was tapped gently to remove loosely attached soil. The root with attached rhizospheric soil was transferred to a 500 ml conical flask and mixed with distilled water for 10 minutes. Serial dilution was prepared from this suspension. Corresponding non rhizosphere soil samples were also collected. Serial dilution was prepared from this sample for determining the rhizosphere and non rhizosphere fungal, bacterial and actinomycetes population. The total number of microorganisms was determined using the dilution plate method [11].

The number of colony forming units (CFU) in a gram of sample was calculated by the formula given by Angle et. al [17]. Nutrient Agar medium was used for determining bacterial population while Rose Bengal Agar and Starch Casein Agar were used for fungal and actinomycetes population, respectively.

The dehydrogenase and phosphatase activity of soil microbes was assayed by the method as described by Casida *et.al*. [7] and Tabatabai *et.al*. [10] respectively.

3 Results And Discussion

3.1 Effect of Paraquat and Fipronil on the tea rhizosphere and soil microflora

The fungal population of the rhizosphere was significantly inhibited on the 5^{th}, 25^{th} and 35^{th} day after the application of Paraquat (Fig .1). With time, the population recovered from the initial inhibitory effect. The non rhizosphere fungal population was also inhibited by the treatment with paraquat as compared to control soil (Fig.1).

The rhizosphere and non rhizosphere actinomycetes population were able to overcome the initial deleterious effects of Paraquat (Fig.2). The bacterial population of the rhizosphere was significantly affected at the initial stages of the observation (Fig.3). However the inhibition was overcome and the population remained constant until the last observation on the 65^{th} day after the application of Paraquat. However, the population of bacteria in the non rhizosphere soil was stimulated by Paraquat (Fig.3).

Fipronil also had a significant inhibitory effect during the initial observation on the 5^{th} and 25^{th} day after the application on the rhizosphere and non rhizosphere fungal population (Fig. 4). However, they were able to overcome the adverse effect with time and maintained a constant population.

The actinomycetes population also showed varied response to Fipronil. Both the non rhizosphere and rhizosphere population were observed to overcome the initial adverse effect of the pesticide treatment (Fig.5). Fipronil was seen to be most effective which adversely affected the bacterial population of both rhizosphere and non rhizosphere soil (Fig 6). The effect was significant throughout the course of the study.

3.2 Assessment on the rhizospheric effect (R/S Ratio)

A considerable amount of change was observed in the rhizosphere of the plants grown in the Paraquat and Fipronil treated pots (Table 1&2). Compared to control, the treated soil showed a marked stimulatory effect on the fungal population. In case of actinomycetes also, treatment with both the pesticides had stimulatory effect. However, inhibitory effect was observed during the subsequent observations. The R/S ratio for bacteria shows that Paraquat and Fipronil also had an inhibitory effect on the bacterial population compared to control (Table 1&2).

3.3 Effect of Paraquat and Fipronil on the dehydrogenase and phosphatase activity of tea soil

The results of the dehydrogenase and phosphatase activity of tea soil as influenced by Paraquat and Fipronil are presented in Fig. 7, 8, 9 & 10 respectively. Dehydrogenase activity in soil treated with Fipronil was recorded to be lower compared to control, while the Fipronil treated soil showed inhibition of dehydrogenase activity at the initial stage. However, the activity increased after 35 days of treatment (Fig. 7). The highest dehydrogenase activity was observed on the 65^{th} day after the application of Fipronil (665 µg TPF g^{-1} dry soil 24 h^{-1}).

Paraquat also showed a similar effect on the dehydrogenase activity in the experimental soil (Fig. 8). The effect was inhibitory at the initial stage and the activity increased with time, till the last observation i.e after 65 days of treatment.

The phosphatase activity was observed to be lower compared to control soil (Fig. 9) in the soil treated with Fipronil. It showed an inhibitory effect up to 25 days following the pesticide application but the activity increased gradually and maintained a constant value until the last observation made on the 65th day.

In the soil treated with Paraquat, inhibition of the microbial population was recorded on the 5th and 25th day after application of the pesticide. However the inhibition was found to have reduced during the subsequent observations (Fig.10). The control soil showed a gradual increase in phosphatase activity with time.

Similar consistent result was also seen in which the soil was treated with three pesticides to observe their effect on the total viable bacterial population [2]. The pesticides were found not to have any inhibitory effect on the total microbial population. An experiment was conducted to observe the effect of four insecticides HCH, Phorate, Carbofuran and Fenvalerate on the growth and development of bacteria, actinomycetes and fungi in the treated soil. The applied pesticides were found to have increased the population of the microorganisms in soil significantly, which showed that the microorganisms are not so sensitive to the said insecticides and they utilized the released nutrients and increased in numbers [1]. It was indicated that greater utilization of insecticidal residues as well as their degraded products by the soil microorganisms to derive energy and other nutrient elements for their cellular metabolism and survival [18].

A study on the effect of a fungicide Chlorothalonil on the population of soil microorganisms showed that after initial population variations, soil bacteria, fungi and actinomycetes adapted gradually to Chlorothalonil and the negative effect became transient in the subsequent observations [8]. Similar findings were also reported in which tests were conducted to determine the effect of four nematicides on the microbial activities in loamy sands. It was observed that the bacterial and fungal population initially decreased with the nematicide treatments, but recovered rapidly to the level similar to those in control [4].

Stimulation in phosphatase activity under the influence of Paraquat, Trifluralin, Glyphosate and Atrazine was reported [6]. The effect of the pesticide Brominal and Secleron on the soil was studied [16]. They reported that acid and alkaline phosphatase activity has been accelerated by the treatment.

The effect of fungicide Mancozeb on the enzyme activities in a silty loam soil of Kashmir was studied [12]. It was observed that the phosphatase activity increased by 41 % after fourteen days of incubation. The dehydrogenase activity is measured to evaluate the microbial activity. The activity was observed to have been stimulated in response to different concentrations of the said fungicide treatment.

4 CONCLUSION

The result of the present study suggests that the pesticides Paraquat and Fipronil had a transient negative effect on the rhizosphere and non rhizosphere microflora. The dehydrogenase and phosphatase activity are indicative of the physiologically active microorganisms, which recovered with time from the initial inhibitory effect of the above mentioned pesticide treatments. Therefore, they seem to be fairly safe to be used in the tea agroecosystem at large.

Table no.1: Rhizosphere effect of the fungal, bacterial and actinomycetes population in the tea soil rhizosphere treated with Paraquat.

Days	Rhizosphere effect in the tea soil treated with Paraquat					
	Rhizosphere microorganisms in thousands per gram of dry soil			Rhizosphere effect *(R.S/C.S)		
	Fungi	Actinomycetes	Bacteria	Fungi	Actinomycetes	Bacteria
5	5	106.6	10.6	1.66	1.18	0.41
20	4.3	128	118.6	0.44	1.60	2.06
35	13	266.6	240	2.16	0.86	2.27
50	17.3	273.3	245.3	1.73	0.85	2.16
65	27.6	278.6	336	1.62	0.84	2.11

**Rhizosphere soil/Control soil.*

Table no.2: Rhizospheric effect of the fungal, bacterial and actinomycetes population in the soil rhizosphere treated with Fipronil.

Days	Rhizosphere effect in the tea soil treated with Fipronil					
	Rhizosphere microorganisms in thousands per gram of dry soil			Rhizosphere effect *(R.S/C.S)		
	Fungi	Actinomycetes	Bacteria	Fungi	Actinomycetes	Bacteria
5	5	294.6	49.3	0.48	1.57	0.68
20	5.3	27.6	27.6	1.47	1.93	1.22
35	15.3	129.3	23.3	2.42	0.95	1.07
50	17	161.6	40.3	1.88	1.10	1.02
65	17.3	164	41.6	1.73	1.09	1.02

**Rhizosphere soil/Control soil.*

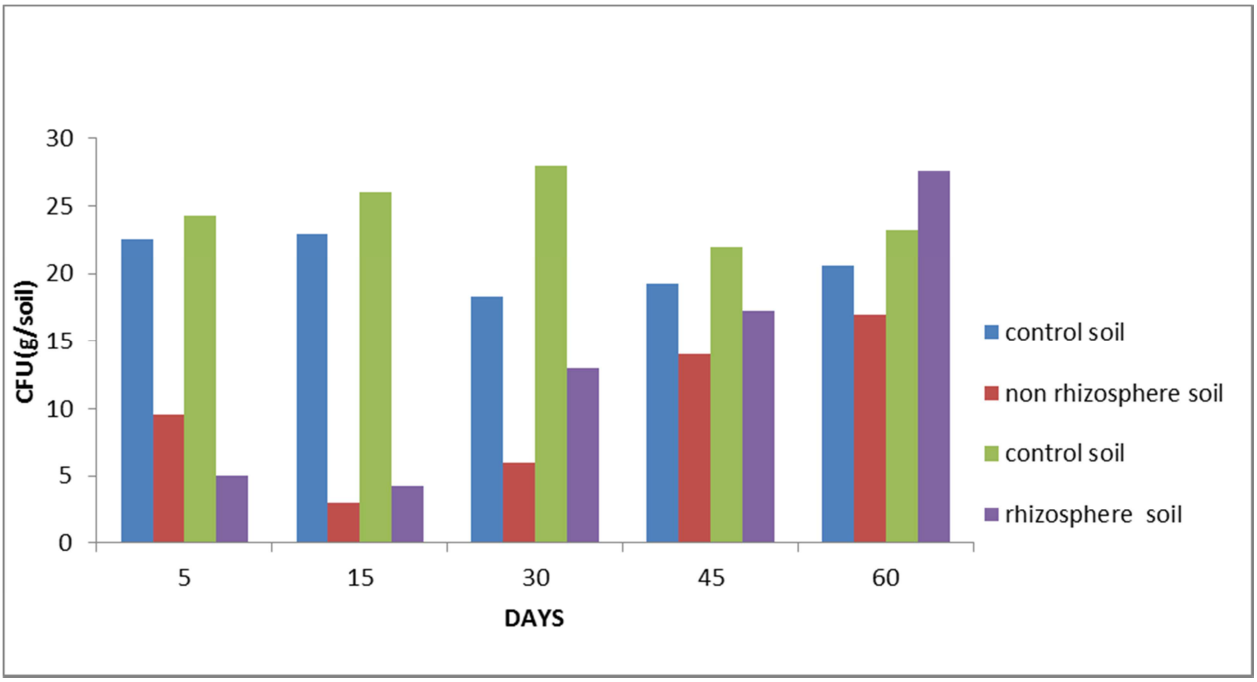

Fig.1: The effect of Paraquat on the non rhizosphere and rhizosphere fungal population.

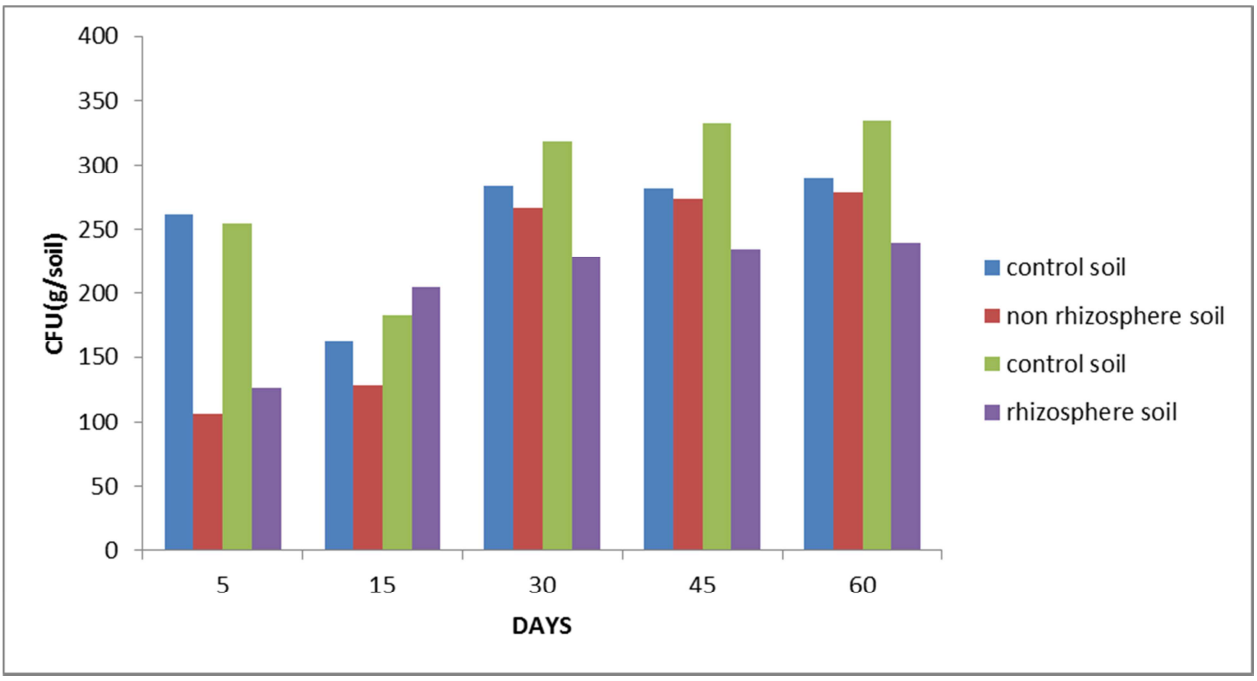

Fig.2: Effect of Paraquat on the non rhizosphere and rhizosphere actinomycetes population.

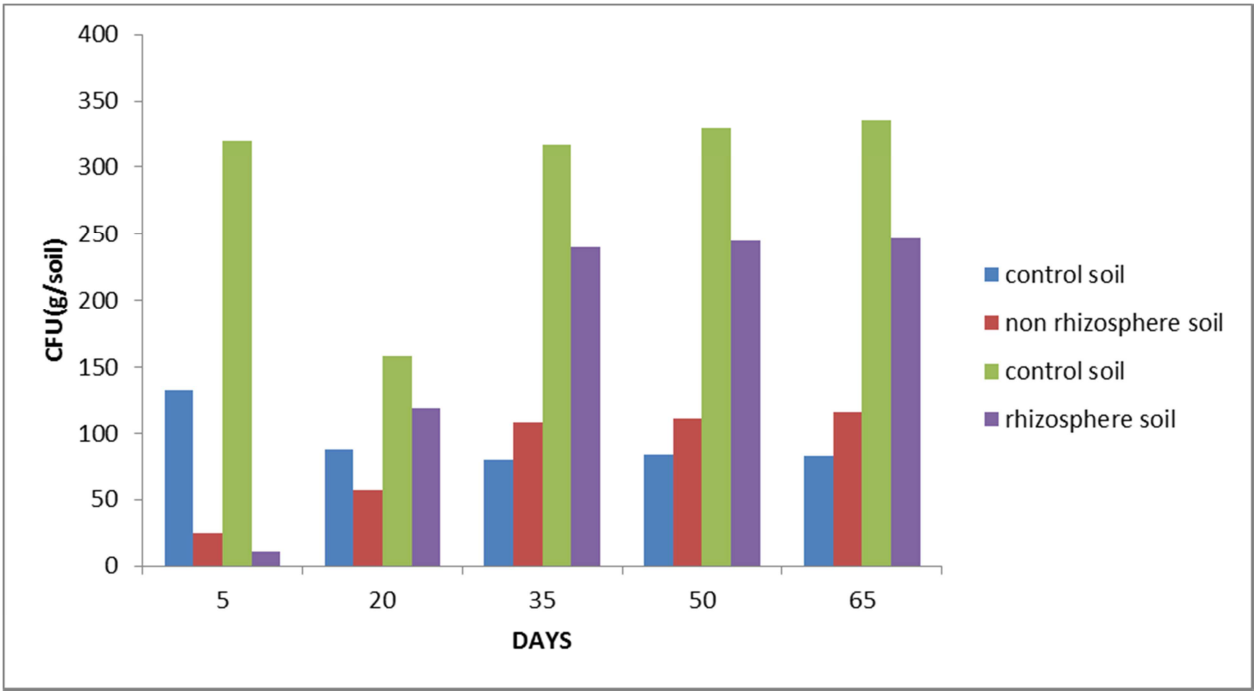

Fig.3: Effect of Paraquat on the non rhizosphere and rhizosphere bacterial population.

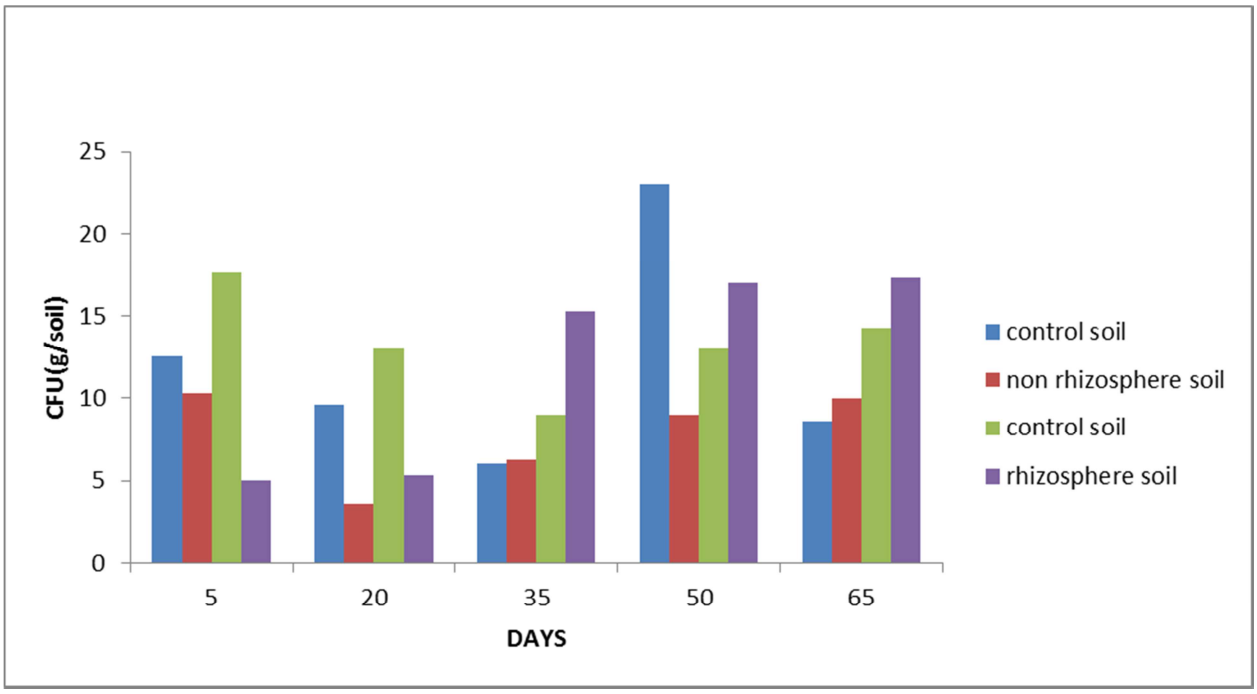

Fig. 4: Effect of Fipronil on non rhizosphere and rhizosphere fungal population.

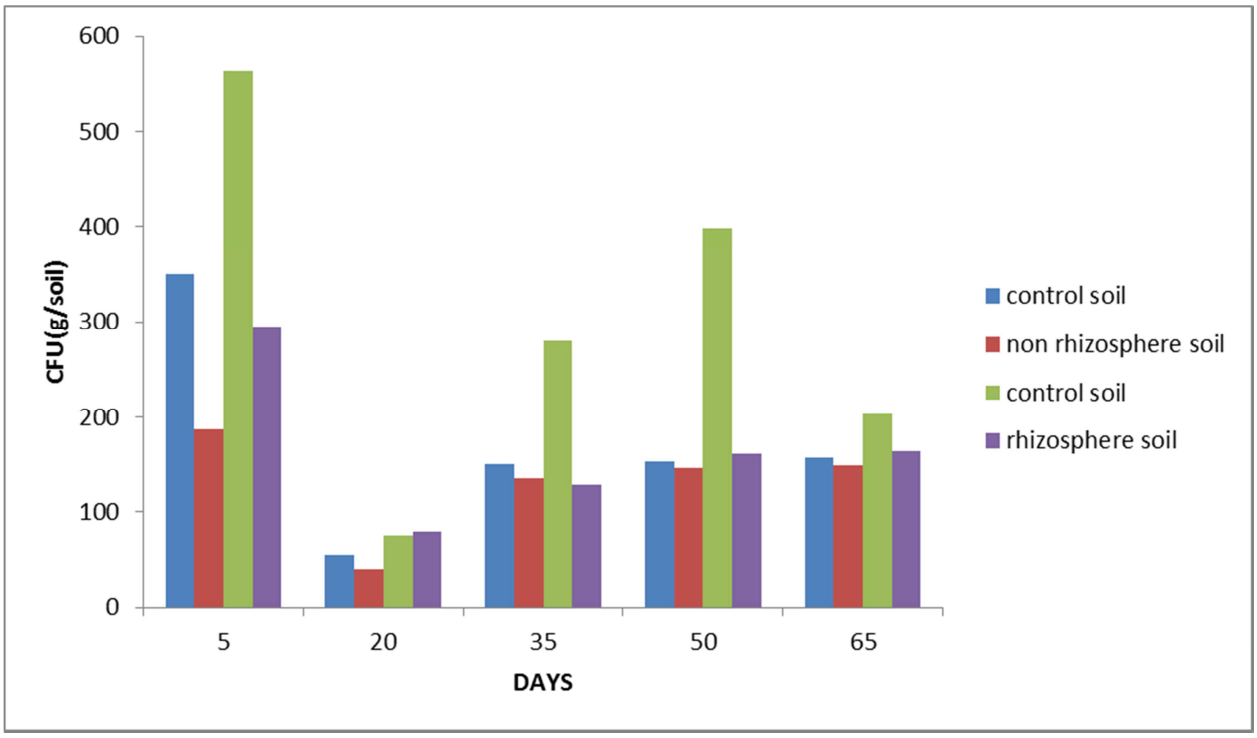

Fig. 5:Effect of Fipronil on non rhizosphere and rhizosphere actinomycetes population.

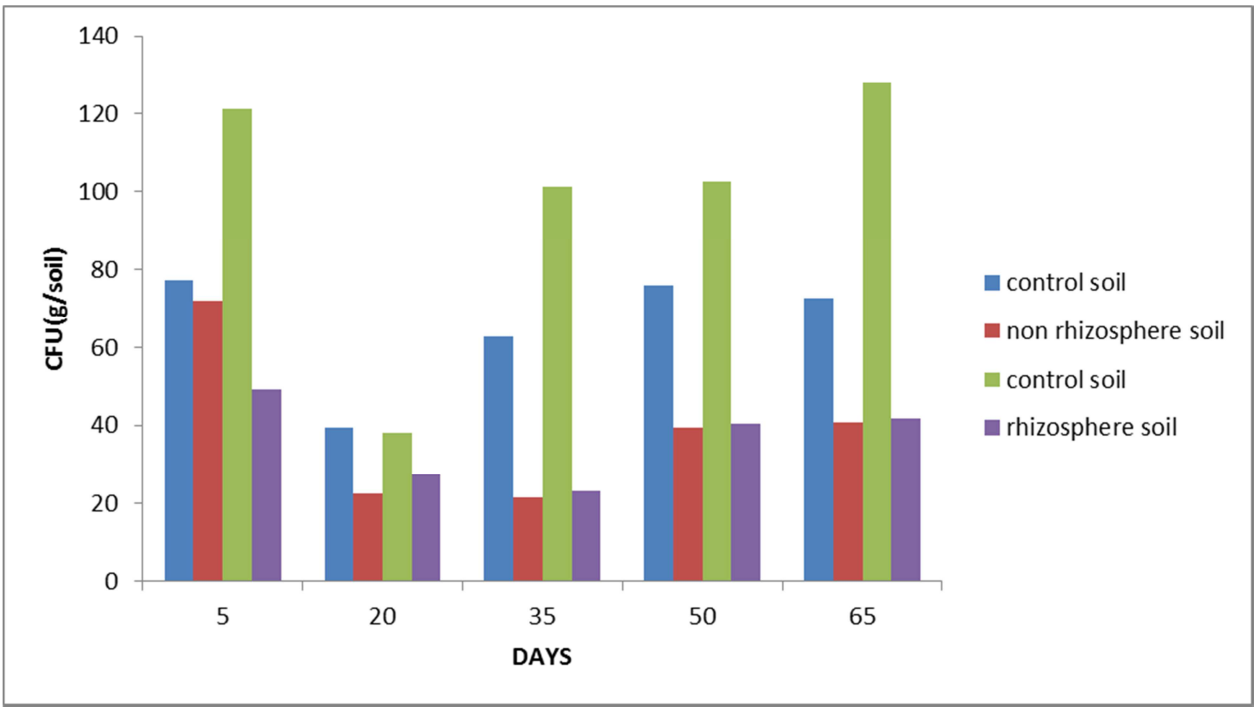

Fig. 6: Effect of Fipronil on non rhizosphere and rhizosphere bacterial population.

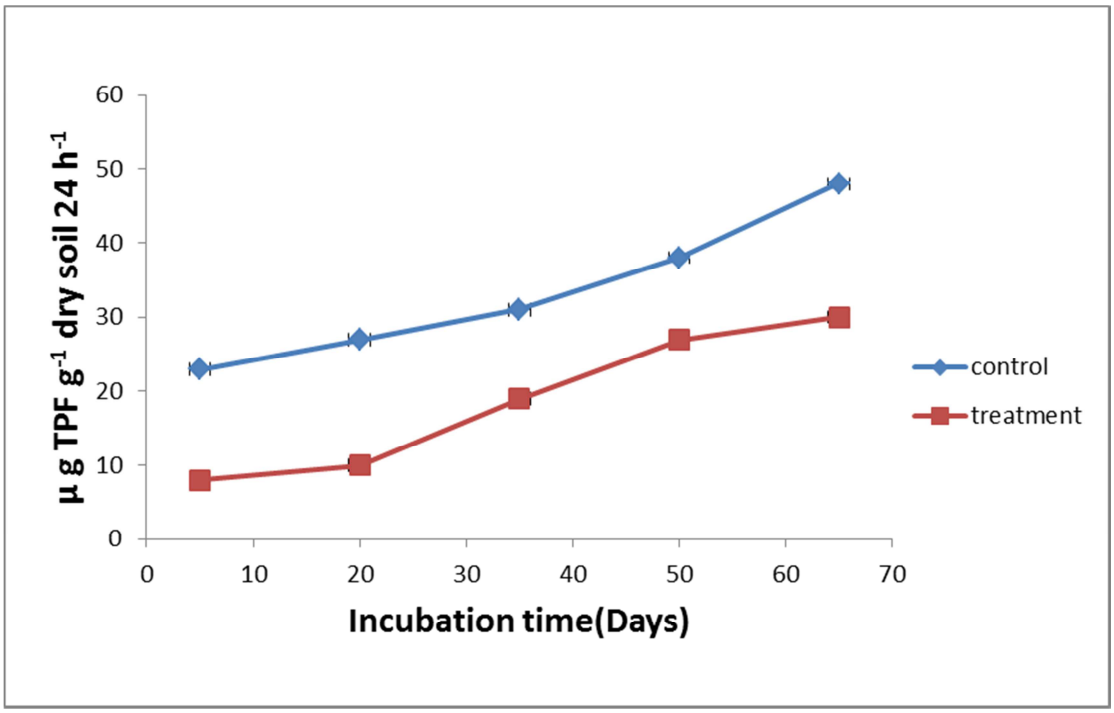

Fig.7: Effect of Fipronil on the dehydrogenase activity of soil.

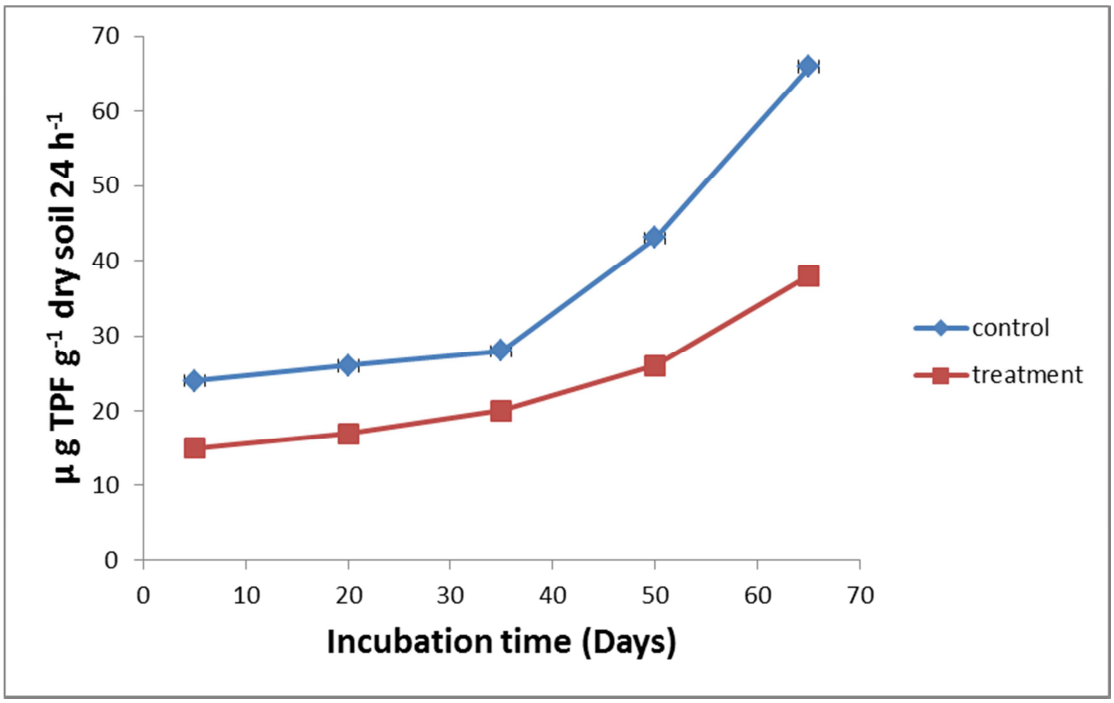

Fig.8: Effect of Paraquat on the dehydrogenase activity of soil.

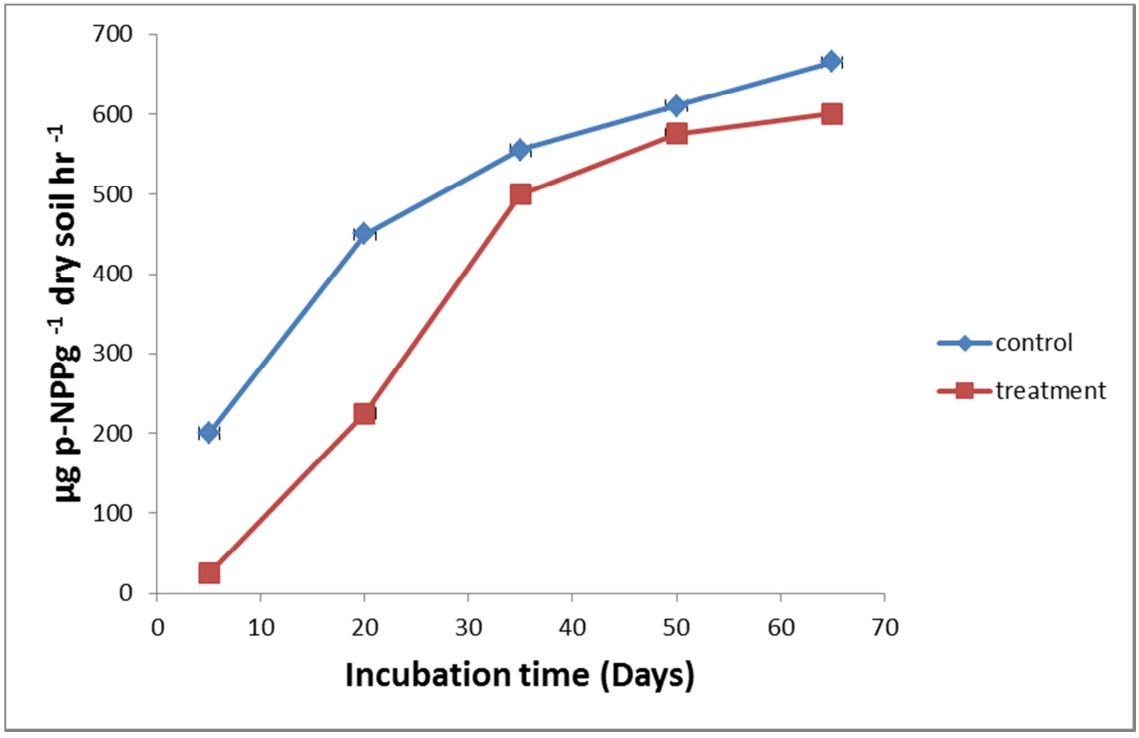

Fig.9: Effect of Fipronil on the phosphatase activity of soil.

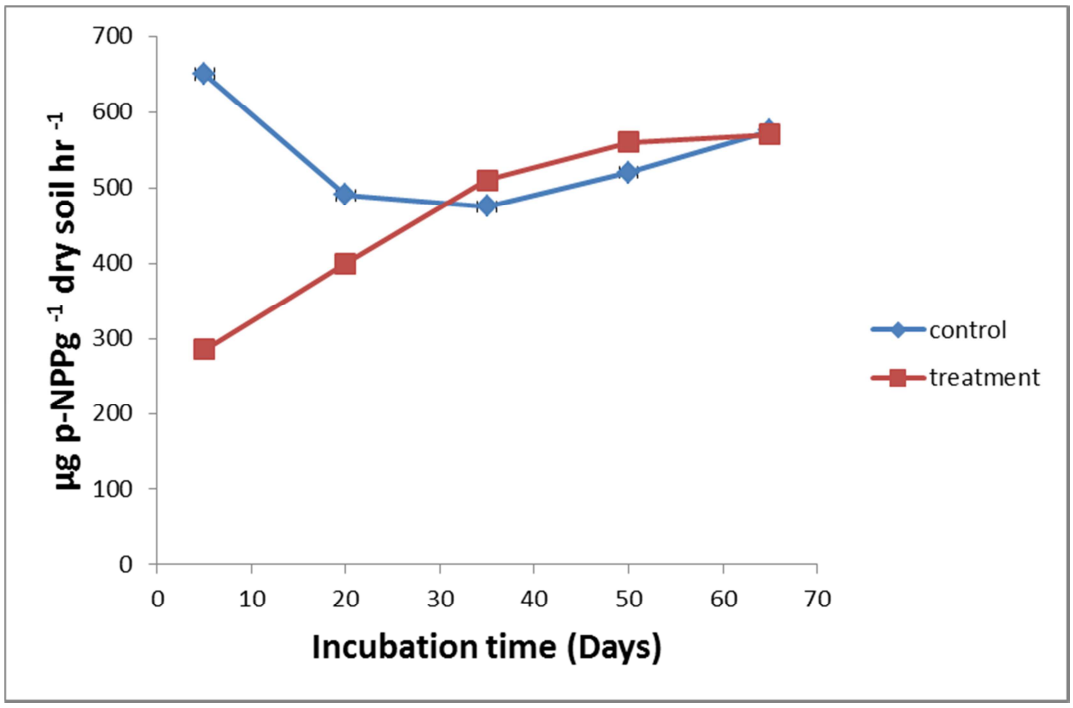

Fig.10: Effect of Paraquat on the phosphatase activity of soil.

REFERENCES

[1] A.C. Das and D. Mukherjee, "Soil Application of Insecticides Influences Microorganism and Plant Nutrients," Applied Soil Ecology, vol.14,pp.53-62,1999.

[2] Aurelia Onet, "Study of the effect of some pesticides on soil microorganisms," International Symposia Risk Factors for Enironment & Food Safety and Natural Resources and Sustainable Development, Faculty of Environment Protection. November 6-7, Ordea

[3] B.W Hutsch, "Methane oxidation in non flooded soils as affected by crop production."Invited paper. Eur. J. Agron, vol.14,pp. 237-260, 2001.

[4] C.M. Tu, "Effect of four nematicides on activities of microorganisms in soil," Applied Microbiology, vol. 23, no.2,pp. 398-401,1972.

[5] D. Riley and W. Wilkinson., "Biological unavailability of bound paraquat residues in soil. Bound and conjugated Pesticide Residues," ACS Symp. Series. Vol 29.pp.301-353, 1976.

[6] Hazel A. Davies and M.P. Greaves, "Effects of some herbicides on soil enzyme activities," Weed Research, vol 21, No.5, pp.205-209, 1981.

[7] L.E. Casida J.R, D.A. Klein and J. Santon, "Soil dehydrogenase activity," Soil Science, vol.98, pp.371-376,1964.

[8] L.Y.Yu, Min Shan, Hua Fang, Wang Xiao and Q.X. Chu, "Responses of Soil Microorganisms and Enzymes to Repeated Applications of Chlorothalonil, J. Agric. Food Chem, vol.54, pp.10070-10075,2006.

[9] M.A. Aon and A.C. Colaneri, "Temporal and spatial evolution of enzymatic activities and physico- chemical properties in an agricultural soil," Appl. Soil Ecol, pp.18:25,2001.

[10] M.A. Tabatabai and J.M.Bremner, "Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity," vol.1, Issue. 4, pp.301-307. 1969.

[11] M.I. Timonin. "The interaction of higher plants and soil microorganisms. I. Microbial populations of rhizosphere of seedlings of certain cultivated plants," Canad.J.Res, vol.18, no.7, pp. 307-317, 1940.

[12] Nazima Rasool and Zafar Reshi, "Effect of the fungicide Mancozeb at different application rates on enzyme activities in a silt loam soil of the Kashmir Himalaya, India," Tropical Ecology, vol.51, no. 2, pp.199-205,2010.

[13] Pesticide News No.48. June 2000. pp. 20. http://www.pan-uk.org/pestnews/Actives/fipronil.htm

[14] Pesticides News No. 32.June 1996. p20-22. http://www.pan-uk.org/pestnews/Actives/paraquat.htm

[15] R.L. Starkey, "Some influences of the development of higher plants upon the microorganisms in the soil,VI. Microscopic examination of the rhizosphere," Soil Sci., vol. 45, pp.207-249, 1938.

[16] S.A. Omar and M.A. Abdel – Sater, " Microbial populations and enzyme activities in soil treated with pesticides," Water, Air and Soil Pollution .vol. 127, pp. 49-63,2001.
[17] S. Angle , R.W. Weaver, P. Botztomley , D. Bezdicek, S. Smith, A. Tabatabai and A. Wollum, Methods of soil analysis, part 2- Microbiological and biochemical properties.- Soil Science society of America, Inc., pp.1121.1994.
[18] S. Bhuyan, B. Sreedharan, T.K. Adhya, N. Sethunathan, " Enhanced biodegradation of ϒ-hexachlorocyclone (ϒ-HCH) in HCH (commercial) acclimatized flooded soil and factor affecting its development and persistence," Pestic. Sci., vol. 38,pp.49-55,1993.