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INFLUENCE OF PESTICIDES ON NITROGEN FIXING BACTERIA

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Abstract- Pesticide use is effective for the protection of plants from pest, but the extensive use of pesticide over the past four decades has resulted in the disturbance of natural biological system. Some pesticides used in agriculture can be harmful to nitrogen fixing bacteria. The objective of the present study was to study the effect of pesticides on nitrogen fixing bacteria. The study was actually carried out for observing the effect of pesticides Hilcyperil and Nuvan on desired nitrogen fixing bacteria, Rhizobium spp. and Azotobacter spp. which are very essential for the growth of plant as well as for more yields. Nuvan pesticide greatly inhibited the growth of both the nitrogen fixing bacteria as compared to that of Hilcyperil. It is concluded from this study that the pesticides have differential effect on the growth of nitrogen fixing bacteria, and their action vary at different sites. Indication has been observed that the pesticides which are under field condition possibly due to its high toxic nature reduced the population of these bacteria under field condition.

Keywords: Pesticides, Nitrogen Fixing Bacteria

I. INTRODUCTION

Pesticides are widely used against a range of pests infesting agricultural crops. Globally, about $3X10^9$kg of pesticides is applied annually with a purchase price of nearly $40 billion each year [1]. The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment [2]. With the growing use of pesticides in contemporary agriculture, the issue of the impact of these chemicals on the composition of soil microorganisms and the processes they direct has received more attention [3] [4]. The applied pesticides may harm the indigenous microorganisms, disturb soil ecosystem, and thus, may affect human health by entering in the food chain.

Soil contains many types of microorganisms such as bacteria, actinomycetes, fungi, and algae, which are important because they affect the physical, chemical, and biological properties of soil. Amongst the soil bacteria *Rhizobium* and *Azotobacter* have a beneficial effect on the growth of plants. It can live either in the soil or within the root nodules of host legumes [5]. For terrestrial plants, the phyllosphere represents the interface between the above-ground parts of plants and the air. In the last few decades, numerous soil microorganisms have been found to have a positive effect on plant development. Besides the well-known symbiotic nitrogen fixing bacteria (*Rhizobium*), free nitrogen fixers (*Azotobacter*) can also stimulate plant growth or reduce the damage caused by soil-borne plant pathogens [6] and has been used as a potential nitrogenous fertilizer to increase crop yield [7] [8]. Besides this, pesticides are used to control specific fungi, herbs, insects and other pests in crops [9].

Pesticide use is effective for the protection of plants from pest [10], but the extensive use of pesticide over the past four decades has resulted in the disturbance of natural biological system [11]. Pesticides in soil undergo a variety of degradative, transport, and adsorption/desorption processes depending on the chemical nature of the pesticide [12] and soil properties [13]. Pesticides interact with soil organisms and their metabolic activities [14] and may alter the physiological and biochemical behavior of soil microbes.

Biological nitrogen fixation (BNF) is an efficient and natural source of nitrogen, and the total BNF has been estimated twice (175 million tones) as compared to the total nitrogen fixation by nonbiological processes. Rhizobial symbioses with over 100 agriculturally important legumes contribute nearly half the annual quantity of BNF entering soil ecosystem [15]. Pesticide may influence the nodulation and BNF in legumes either by affecting virulence of attacking nodular bacteria, the root fibers of the plants in which the infection occurs or both.

Some pesticides used in agriculture can be harmful to nitrogen fixing bacteria, not only to inhibit the nitrogen fixation process in bacteria but also to reduce the bacterium's respiration rate and hence preclude its positive effects [16] [17]. Nitrogenase is the enzyme used by organisms to fix atmospheric nitrogen gas (N2). Application of pesticides affects the efficiency and activity of nitrogenase enzyme. In the previous study a decrease in total nitrogenase activity (measured from pots sown with *Pisum sativum* plants) with the application of herbicides was reported [18]. There is clear evidence that non-target soil bacteria are influenced by pesticides but the impacts are wide ranging-some are stimulatory, others highly inhibitory. Effects may be direct or indirect and are dependent upon several interacting factors that relate to the mode of application and the soil environment. A number of factors, for example, chemical nature of pesticides, concentration used, microbial community structure, type of soil, and soil conditions can contribute to divergent research findings [19]. Thus previous studies attributed such differences to the dual behavior of pesticides, both harmful and beneficial for soil microorganisms. The objective of the present study was to study the effect of pesticides on nitrogen fixing bacteria.

II. MATERIALS AND METHODS

Collection of Sample: The Root nodule and Phyllosphere soil sample of the four plants were collected from Dr. Panjabrao Deshmukh College of Agriculture, Nagpur. The samples were collected from following plants such as Maize *(Zea mays)*, Cotton *(Gossypium hirsutum)*, Lady finger *(Abelmoschus Esculantus)* and Brinjal *(Solanum melongena).*

Pesticides Tested: The pesticides Hilcyperil and Nuvan were obtained from Krushi Biyane Khat, Cotton Market, and Nagpur.

Isolation and Identification of N_2 Fixing Bacteria:

***Rhizobium* Isolation:** Total of eight samples of root nodules from Soyabean plant and Peas plant were collected. Healthy root nodules were washed with tap water thrice before streaking on agar plate [20]. The nodules were sterilized externally using 95% alcohol for 1-4 minute, followed by washing with calcium hypochlorite solution (10g/150ml distilled water) and crushing in a drop of sterile water. A loopful ground material was transferred to 5 ml of sterile water, of which 0.1 ml sample was spread on to the surface of Yeast Extract Mannitol Agar (YEMA). Plates were then incubated at 28^0C for 48 hours. Well isolated typical single colonies were restreaked on freshly prepared YEMA plates in order to obtain pure cultures.

***Azotobacter* Isolation:** Total eight phyllosphere soil samples were collected. Each soil sample (1 gm) was mixed with sterile distilled water (9 ml) in a test tube. One ml was taken and inoculated on Ashby's Glucose agar plate. The plates were incubated at 28^0C for 48 hours. After incubation the isolated colonies were subculture on nutrient agar slants. The organisms were further identified on the basis of morphological, cultural and biochemical characteristics [21] and results were compared with Bergeys's manual of determinative bacteriology, 9th edition.

Effect of Pesticides by Agar Well Diffusion Method: Nutrient agar plate was taken, on which a lawn of the bacteria was prepared with the help of sterile cotton bud. Two wells having 6 mm diameter were prepared with the help of sterile cork borer in which 10 µl of each pesticide were added in two different wells. The plates were incubated at 37^0C for 24 hrs. After incubation the zone of inhibition was observed around each well of pesticide [22].

III. RESULTS AND DISCUSSION

A total of two samples of root nodules of soyabeans and peas plants were collected. Out of which all the samples given the presence of *Rhizobium* spp. On the other hand, eight samples from phylloshere given eight *Azotobacter* spp. The influence of pesticides was carried out by agar well diffusion method.

Table 1: Effect of Pesticides on Nitrogen Fixing Bacteria

Sample	**Pesticide**	
	Hilcyperil	**Nuvan**
Root Nodules		
Soyabeans	18mm	22mm
Peas	14mm	10mm
Phyllosphere		
Maize	20mm	24mm
Cotton	16mm	26mm
Lady Finger	19mm	24mm
Brinjal	20mm	26mm

It was found that all the nitrogen fixing bacteria were inhibited by the pesticides tested. Out of the two pesticides Nuvan highly inhibited the isolated bacteria than Hicyperil. From the phyllosphere soil of four plant, it was found that Brinjal phyllosphere Bacteria has been greatly inhibited by Hilcyperil (20 mm), and Nuvan (24 mm) followed by maize phyllosphere soil organism got inhibited by Hilcyperil (20 mm) and Nuvan (24 mm). Cotton phyllosphere bacteria were inhibited by Hilcyperil (16mm) and Nuvan (26 mm). lady finger phyllosphere soil Bacteria were inhibited by Hilcyperil (19 mm) and Nuvan (24 mm) (Table 1).

On the other hand, root nodules investigation for the presence of *Rhizobium* spp. showed that soybean root nodule bacteria were inhibited by Hilcyperil (20 mm) and Nuvan (22 mm) followed by Peas root nodule bacteria Hilcyperil (14 mm) and Nuvan (10 mm) (Fig. 1). The result of present project showed that *Azotobacter* spp. was isolated from phyllosphere soil the result were similar with the previous findings in which the *Azotobacter* spp. was inhibited by two different pesticide used in the study [23]. Thus pesticide had a negative effect on the growth of nitrogen fixing bacteria from phyllosphere soil. It was reported that there was variable effect of pesticide on growth of nitrogen fixing bacteria from the root nodule of soyabean and pea plant *rhizobium* [24]. Both the organisms *Rhizobium* and *Azotobacter* were tested against pesticide and it was found that pesticides inhibited the growth of both the nitrogen fixing organisms.

The results of the present study revealed that pesticides had negative effect on nitrogen fixing bacteria. It was reported in some studies that above cited effect of cypermethrin and monocrotophos on bacteria was observed while fenvalerate had very low effect on soil microbes [25]. The present results contrast with some previous work in which cypermrehtirn had no adverse effect on soil microbes [26]. In case of endosulfan, significant reduction in soil microbes occurred at high concentration (1000 ppm) and this is supported in some studies [27] [28] which confirmed the same result for endosulfan. However, carbofuran significantly stimulated the populations of bacteria as well as N2 fixing bacteria in the agricultural soil while other tested insecticides reduced proportions of *Micrococcus* and *Rhizopus* in the soil [29] [30].

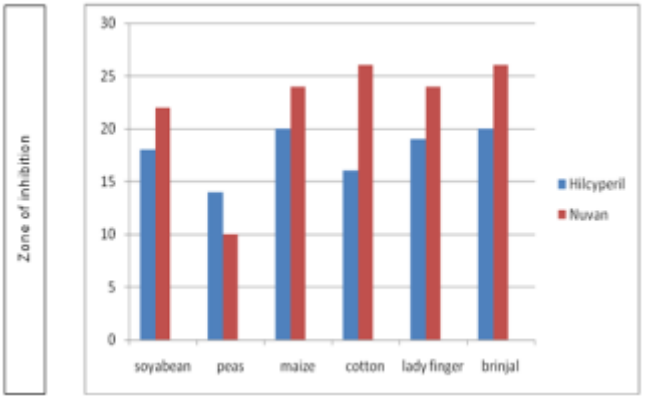

Fig. 1: Effect of Pesticides on Nitrogen Fixing Bacteria

It was reported that bacterial growth inhibition was due to agrochemical, and they contain similar active ingredient that reduced the number of nitrogen fixing bacteria [31-34]. Similar trend was reported in which fungicide treatment

decreased the number of viable N2-fixing bacteria [35]. Dimethoate decreased the growth of *Rhizobium* population [36]. The fixation of nitrogen was parallel to the population of both nitrogen fixing bacteria in soil treated with pesticides. It was found that organic phosphorous insecticides profenofos and chloropyrifos reduced the number of aerobic nitrogen fixers and significantly decreased nitrogen fixation [37] [38]. It is concluded from this study that the pesticides have differential effect on the growth of nitrogen fixing bacteria, and their action vary at different sites. Indication has been observed that the pesticides which are under field condition possibly due to its high toxic nature reduced the population of these bacteria under field condition.

IV. CONCLUSION

The project was actually carried out for observing the effect of pesticides Hilcyperil and Nuvan on desired nitrogen fixing bacteria, *Rhizobium* spp. and *Azotobacter* spp. which are very essential for the growth of plant as well as for more yields. Nuvan pesticide greatly inhibited the growth of both the nitrogen fixing bacteria as compared to that of Hilcyperil. Generally pesticides are used for the prevention of the crop from harmful organism. Therefore use of pesticides is good for crop but results of the present project concluded that pesticides are also harmful to nitrogen fixing bacteria which are essential for better yield of plant.

It is concluded from this study that the pesticides have differential effect on the growth of nitrogen fixing bacteria, and their action vary at different sites. Indication has been observed that the pesticides which are under field condition possibly due to its high toxic nature reduced the population of these bacteria under field condition.

REFERENCES

[1]. Pan-UK (2003) Current pesticide spectrum, global use and major concerns. (online) http://www.pan-uk.org/brie.ng/sida_fil/chap1.htm (14 February 2008).
[2]. Carriger J F, Rand G M, Gardinali P R, Perry, W B, Tompkins M S, Fernandez A M (2006) Pesticides of potential ecological concern in sediment from South Florida Canals: An ecological risk prioritization for aquatic arthropods. Soil Sed. Contam. 15: 21–45.
[3]. Pimentel D (1995) Amounts of pesticides reaching target pests: Environmental impacts and ethics. J. Agric. Environ. Ethics 8: 17–29.
[4]. Andrea M M, Peres T B, Luchini L C, Pettinelli A (2000) Impact of longterm pesticide application on some soil biological parameters. J. Environ. Sci. Health B 35: 297–307.
[5]. Baxter J, Cummings S P (2008) The degradation of the herbicide bromoxynil and its impact on bacterial diversity in a top soil. J. Appl. Microbiol. 104: 1605–1616.
[6]. Shahzad F, Shafee M, Abbas F, Babar S, Tariq MM and Ahmad Z (2012) Isolation and biochemical characterization of *Rhizobium meliloti* from root nodules of Alfalfa (Medico sativa). The Journal of Animal & Plant Sciences 22(2): 522-524.
[7]. Kloepper JW, Lifshitz R, Zablotowicz RM (1989). Free-living bacteria inoculum for enhancing crop productivity. Trends Biotechnol. 7: 39-44.
[8]. Steinberga V, Apsite A, Bicevskis J, Strikauska S, Viesturs U (1996) The effect of Azotobacterin on the crop yield and biologicalactivity of the soil. In: Wojtovich A, Stepkovska J, Szlagowska A editors. Proceedings of 2nd European Nitrogen Fixation Conference, pp. 191, Poznan.
[9]. MrkovaakiN KL, Åaåiã N, Mezei Sneÿana (2001) Primenamikro biološkog preparata u proizvodnji šeãernerepe. Zbornik instituta zaratar stvoipo vrtarstvo, 35: 67-73.
[10]. Johnsen K, Jacobsen CS, Torsovik V, Sorensen J (2001) Pesticides effect on bacterial diversity in agriculture soil-review. Biol. Fertil. Soil, Springer-Verlag 33:443-453.
[11]. Bolognesi C (2003) Genotoxicity of pesticides: a review of human bionitoring studies. Mutat. Res. 543: 251-272.
[12]. Ayansina AD, Oso BA (2006) Effect of two commonly used herbicides on soil microflora of two different concentrations. Afr. J. Biochem. 5(2):129-132.
[13]. Laabs V, Wehrhan A, Pinto A, Dores E, Amelung W (2007) Pesticide fate in tropical wetlands of Brazil: An aquatic microcosm study under semi-field conditions. Chemosphere 67: 975–989.
[14]. Weber J B, Wilkerson G G, Reinhardt C F (2004) Calculating pesticide sorption coefficients (K sub(d)) using selected soil properties. Chemosphere 55: 157–166.
[15]. Singh B K, Walker A (2006). Microbial degradation of organophosphorus compounds. FEMS Microbiol. Rev. 30: 428–471.
[16]. Tate R L (1995) Soil Microbiology (Symbiotic Nitrogen Fixation). pp. 307–333. John Wiley & Sons, New York.
[17]. San-Tos A, Flores M (1995) Effects of glyphosate on nitrogen fixation of free-living heterotrophic bacteria. *Lett Appl Microbiol*. 20: 349–352.
[18]. Singh G, Wright D (1999) Effect of herbicides on nodulation, symbiotic nitrogen fixation, growth and yield of pea (*Pisum sativum*). J. Agric. Sci. 133: 21–30.
[19]. Digrak M, Ozcelik S (1998). Effect of some pesticides on soil microorganisms. Bull. Environ.Contam. Toxicol., 60 (1): 916-922.
[20]. Ben-Gweirif S F, EI-Moshtty F I, Agouri S R (2005) Effect of some Pesticides on different isolates of *Rhizobium leguminosarum* in Libya. Proc. Symp, New Trends in Science 20-24.
[21]. Collee J G, Marr W (1996) Tests for identification of bacteria and laboratory control of antimicrobial therapy, Chapter 7 and 8. In: Mackie & McCartney Practical Medical Microbiology, Collee JG, Fraser AG, Marmion BP, Simmons editors 14th ed. Churchill Livingstone: New York; pp. 131-151.
[22]. Bonev (2008) Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J. Antimic. Chemother. 61(6):1295-1301.
[23]. Khudhur A M, Askar K A (2013) Effect of some pesticides on growth, nitrogen fixation and *nif*genes in *Azotobacter chroococcum* and *Azotobacter vinelandii* isolated from soil. Journal of Toxicology and Environmental Health Sciences 5(9): 166-171.
[24]. Sudbakar P, Chattopadhyay GN, Gangwar SK, Ghosh JK, Saratchandra B (2000) Effect of common pesticides on nitrogen fixing bacteria of mullberry (*Monusalbal*). Indian J. Agric. Res. 34(4): 211-216.
[25]. Rangaswamy V, Venkateswarlu K (1992) Degradation of selected insecticides, monochrotophos, quinalphos, cypermethrin and fenvalerate, by bacteria isolated from soil. Bull. Env. Contam. Toxic. 49 (6): 797-804.
[26]. Binner R, Berendes K H, Felgentreu D, Friesland H, Glitschka M (1999) Cypermethrin in bark and coniferous forest soil after pesticide treatment of single specimen of barked round wood in forests: persistence, distribution of diastereomers and effects on soil microorganisms. Nachrichtenblatt-des-Deutschen-Pflanzenschutzdienstes 51 (9): 227-237.

[27]. Digrak M, F Kazanici (2001) Effect of some organophosphorus insecticides on soil microorganisms. Turk. J. Biol. 25: 51-58.
[28]. Nasim G, Ilyas N, Shabbir A (2005) Study of effect of organic pesticides: Endosulfan and Bifenthrin on growth of some soil fungi. Mycopath 3(1 & 2): 27-31.
[29]. Dordevic S, Sestovic M, Raicevic V, Dordevic A (1998) Fluctuation of the abundance of microorganisms in the carbofuran treated soil. Pest 13 (4): 281 -288.
[30]. Das AC, Mukherjee D (1998) Insecticidal effects on soil microorganisms and their biochemical processes related to soil fertility. World J. Microbio. 14 (6): 903-906.
[31]. Gallori E, Casalone E, Coella CM, Daly S, Polsimelli M (1991) 1,8 Naphthalic anhydride antidote enhance the toxic effect of Captan and Thiram fungicides on *Azosprillium brasilense*. Res. Microbiol. 142: 1005-1007.
[32]. Revellin C, Leterme P, Catroux G (1993) Effect of some fungicide seed treatments on the survival of *Bradyrhizobium japonicum* and on the nodulation and yield of soya bean (*Glycine max* (L) *Merric*.) Soil Fertil. 16: 211-214.
[33]. Taiwo LB, Oso BA (1997) The influence of some pesticides on soil microbial flora in relation to change in nutrient level, rock phosphate solublization and P-release under laboratory conditions. Agric. Ecosystem, Environ. 65: 59-68.
[34]. Dunfield KE, Siciliano S, Germida JJ (2000) The fungicide Thiram and Captan effects the phenotypic characteristics of *R. leguminosarium* strain clas determine by FAME and Biology analysis. Biol. Fertil. Soil 31: 303-309.
[35]. Martensson AM (1992) Effect of agrochemical and heavy metals on fast-growing Rhizobia and their symbiosis with small seeded. Soil Biol. Biochem. 24 (5): 435-445.
[36]. Castro S, Vinocur M, Permingiani M, Halle C, Taurian T, Fabra A (1997) Interaction of the fungicide Dimethoate and *Rhizobium* sp. in pure culture and under field conditions. Biol. Fertil. Soil 25:147-151.
[37]. Martinez TMV, Salmeron V, GonzalezLopez J (1992) Effects of an organophosphorus insecticide, profenofos on agriculturalsoilmicroflora. Chemosphere 24(1):71-80.
[38]. Pozo C, Martinez TMV, Salmeron V, Rodelas B, Goles L, Opez J (1995) Effect of chloropyrifos on soil microbialactivity. Environ. Toxicol. Chem. 14(2): 187-192.