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Original Research Article

Effects of Synthetic Pyrethroids on Phosphate Solubilizing Activity of Microorganisms

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A B S T R A C T

Pesticides are used widely to control a variety of pests and often residues of these pesticides or their metabolites are left in soil. Present study was designed to determine the influence of Synthetic Pyrethroids on growth and Phosphate-Solubilization activity of certain soil microorganisms. Amongst the four insecticides studied, all of the four insecticides showed remarkable effect on the populations of P-solubilizing microorganisms. The effect of Deltamethrin on *Bacillus, Rhizobium* and *Pseudomonas* was more pronounced as compared to Alpha-Cyhalothrin, Fenvelerate and Cypermethrin. The application of Cypermethrin inhibited the activity of *Pseudomonas* and *Serratia*, significantly as compared to Fenvalerate, Alpha-Cyhalothrin and Deltamethrin. The inhibitory effect of Fenvelerate was more pronounced in case of *Pseudomonas, Azotobacter and Rhizobium* as compared to other three insecticides. Lastly alpha-Cyhalothrin did not show any remarkable inhibitory effect on Phospahte solubilizing activity as compared to remaining three insecticides.

Keywords

Synthetic pyrethroids; Cypermethrin; Fenvelerate; Deltamethrin; Alpha-Cyhalothrin; Phosphate Insecticides.

Introduction

Pesticides are the important agrochemicals used for prevention of crops from pests. Their use has been largely increased in last few decades. The application of pesticides starts from the pre sowing stage. Different treatments include soil application, seed treatment, foliar spray, etc. Repeated applications of pesticides contaminate the soil. Soil is the most important site of biological interactions. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including microflora of soil, and also the physicochemical properties of soil like pH, salinity, alkalinity leading to infertility of soil (Sarnaik *et al.*, 2006).

The important microflora, beneficial for the growth of plants includes nitrogen fixing bacteria and phosphate solubilizing bacteria, present in the rhizosphere of the plant. The excess application of these pesticides may adversely affect the function of these rhizospheric microorganisms. Since the fertility of the soil depends on the number and type of microorganisms present in the soil, studies on effect of pesticide applications on soil were carried out (Sarnaik *et al.,* 2006).

Phosphorus is one the most essential elements for unavailable to the plants after its application in the soil. However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of the genetic traits.

Phosphate solubilizing microorganisms refer to a group of soil microorganisms that as components of phosphorus cycle, can release it from insoluble sources by different mechanisms (Salehrastin, N., 1999). Phosphate solubilizing fungi and bacteria are known as effective organisms in this process (Reyes *et al.*, 1999). Among the soil bacteria communities, *Pseudomonas strata* S., *Bacillus sircalmous* and intrubacters could be referred to as the most important strains (Subbarao, W.S., 1988). In particular, *Pseudomonas florescens* is considered as an important member of rhizosphere organism community.

Phosphate solubilizing microorganisms include largely bacteria and fungi, which can grow in media containing tricalcium, Iron and Aluminium phosphate, hydroxyapetitie, rock phosphate and similar insoluble phosphate compounds as a sole phosphate source. Such microbes not only assimilate phosphorous but a large portion of soluble phosphate is released in quantities in excess of their own requirement (Gaur, A.C. 1990). Microbial P-Solubilization can, therefore, improve the affectivity of mineral P fertilization.

The fixed phosphorous in the soil can be recycled through the use of certain microbes which have the capacity to convert inorganic, unavailable 'P' form viz., hydroxyapatite and tricalcium phosphate and secondary orthophosphates to sole phosphate source. Phosphate solubilizing bacteria (PSM) include different types of microorganisms that convert insoluble phosphatic compounds into soluble forms (Prerna *et al*., 1997; Raju and Reddy, 1999).

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) with the resulting increase in the biomass of the insecticide-utilizers which may favourably influence nutrient transformations in soil (Das and Mukherjee., 1994).

On the other hand, there are some insecticides that exert adverse effect on the proliferation of microorganisms and their associated transformations in soil. The effect of three herbicides namely Agroxone 50SC and 2, 4-Damine on *Azotobacter binelandii*, *Rhizobium phaseoli* and *Bacillus subtilis* were studied. The results revealed that 2,4-Damine was the most toxic of the three herbicides studied and *Azotobacter* was found to most sensitive to the herbicides. The percentage survival decreased with increased concentration of herbicides and days for *Rhizobium* and *Azotobacter* while an initial reduction in population was followed by increased percentage survival of organisms for *Bacillus* (Adeleye *et al*.,2004) .

In microorganisms, pesticides have been shown to interfere with respiration, photosynthesis and the synthetic reactions as well as cell growth, division and molecular composition (Martinez Toledo *et al*., 1992). A study was conducted to investigate the effects of synthetic pyrethroids (Cypermethrin 25%EC, Fenvalerate 20%EC, Deltamethrin 98%TC (Technical material) and Alpha Cyhalothrin 2.5%EC) on the activity of phosphate solubilizing microorganisms.

Materials and Methods

Inoculation and growth conditions

The cultures of microbial strains were obtained from University of Rajasthan Jaipur, BISR Jaipur and IMTech Chandigarh. 10ml of nutrient broth medium was inoculated with bacteria from the nutrient agar slant in case of Bacteria *Bacillus subtilus, Pseudomonas florenscens and Serratia marcescens,* and incubated at 37ºC ± 1ºC for 24h while 10ml of Yeast extract Mannitol and Ashby medium were inoculated with *Rhizobium leguminosorum* and *Azotobacter chrococcum* and incubated at 30ºC ± 1ºC for 24h.

The Synthetic pyrethroids formulations which were used in the agricultural field were obtained from Durgapura Agricultural Institute, Jaipur. The bacterial cultures were enumerated with and without adding synthetic pyrethroid. 0.2ml aliquot of 24h culture grown in nutrient broth was inoculated into 10ml nutrient broth flask containing 1000ppm of each synthetic pyrethroid (Cypermethrin 25%EC, Fenvalerate 20%EC, Deltamethrin 98%TC (Technical material) and Alpha Cyhalothrin 2.5%EC) and incubated in incubator shaker. Control flasks of equal volume of nutrient broth medium containing culture but no synthetic pyrethroid were run in parallel.

Inoculation and growth Conditions

Erlenmeyer conical flasks (100ml) containing 10ml Pikovskaya's broth medium of composition (in g/l); Glucose 10, TriCalciumPhosphate 5.0, Ammonium sulphate 0.5, Sodium Chloride (NaCl) 0.2, Magnessium sulphate heptahydrate($MgSO_4$. $7H_2O$) 0.1, Potassium chloride (KCl) 0.2, Yeast extract 0.5, Mangnese sulphate ($MnSO_4$. H_2O) 0.002 and Ferrous sulphate heptahydrate (Fe SO_4. $7H_2O$) 0.002; pH adjusted to 7 were autoclaved. They were inoculated with 0.5ml of the inoculum and 0.1ml of Synthetic Pyrethroids from stock solution and incubated at 28^0 C in incubator shaker for 2, 4, 6 and 8days for TriCalcium Phosphate solubilization.

Phosphorous estimation

At periodic intervals the flasks were withdrawn and centrifuged at 10000g for 20min. In the supernatant soluble Phosphorous was estimated by chlorostannous reduced molybdophosphoric acid blue method.

Results and Discussion

The Phosphate Solubilizing activity of *Bacillus* was significantly reduced during the application of all the four synthetic pyrethroids. Alpha-Cyhalothrin exposure caused highly significant reduction in the enzymatic activity (Figure 1).

In case of *Pseudomonas* the phosphate-solubilzing activity was found to be significantly enhanced during the treatment of Alpha-Cyhalothrin. Other three insecticides: Cypermethirn, Fenvalerate and Deltamethrin also cause reduction in the activity of bacteria (Figure 2).

The phosphate-solubilzing activity of *Serratia* was found to be more pronounced during the treatment of Deltamethrin and was slightly enhanced after the treatment of Cypermethrin, Fenvalerate and Alpha-Cyhalothrin (Figure 3).

The inhibitory effect of Fenvalerate on phosphate-solubilzing activity of the Azotobacter was more severe than Cypermethrin, Deltamethirn and Alpha-Cyhalothrin (Figure 4).

The effect of Alpha-Cyhalothrin on phosphate-solubilzing activity of *Rhizobium* was more pronounced as compared to Cypermethrin, Fenvalerate and Deltamethrin (Figure 5).

The proliferation of phosphate-solubilizing microorganisms was augmented due to the application of insecticides in medium and the augmentation was highly significant when the medium was treated with Synthetic Pyrethroids. This clearly indicates that phosphate-solubilizing microorganisms preferentially utilized insecticides to derive energy and other nutrients resulting in greater increase in their populations in medium. The higher proliferation of phosphate-solubilizing microorganisms under synthetic pyrethroids on 8th day of sampling indicates that the phosphate solubilizers were able to use the insecticides and/or their degraded products for their growth and metabolism (Gaur, A.C., 1990; Bhuyan *et al*.,1993).

The solubilization of insoluble phosphates concomitantly increased with the proliferation of phosphate-solubilzing microorganisms in medium. The effect of four insecticides, viz. HCH, phorate, carbofuran and fenvalerate at their field application rates (7.5, 1.5, 1.0 and 0.35 kg a.i. ha^{-1}, respectively), on the growth and development of bacteria, actinomycetes and fungi as well as their role in the transformations and availability of some plant nutrients in laterite soil (*Typic Orchragualf*). All the insecticides in general, and HCH and phorate in particular, significantly increased the population of microorganisms in soil. The most predominant genera of microorganisms, such as *Bacillus*, *Micrococcus* and *Aspergillus* were not affected by most of the insecticides. However, some of the insecticides stimulated the growth and development of *Bacillus*, *Proteus*, *Corynebacterium*, *Streptomyces*, *Fusarium*, *Trichoderma* and *Rhizopus*. On the other hand, some insecticide exerted deleterious effect on the proportions of *Pseudomonas* (Debneth *et al*., 1994).

In some cases pesticide residues contributing to the contamination of soil may influence microbial population of the soil and in turn fertility of soil. The reports showed that the application of pesticides to soybean i.e. phorate, carbofuran, carbosulfan, thiomethoxam, imidacloprid, chlorpyriphos and monocrotophos results in the viable count of rhizobia and phosphate solubilizing bacteria which ranged between 10(7)-10(8) cfu/g soil which was comparable to the count of bacteria from untreated (control) soil. No significant change in the total viable count of any kind of bacteria due to application of pesticides has been found showing their ability to degrade these pesticides (Samaik *et al*., 2006).

Figure.1 Effect of Synthetic pyrethroids on Phosphate solubilizing activity of *Bacillus subtilis*

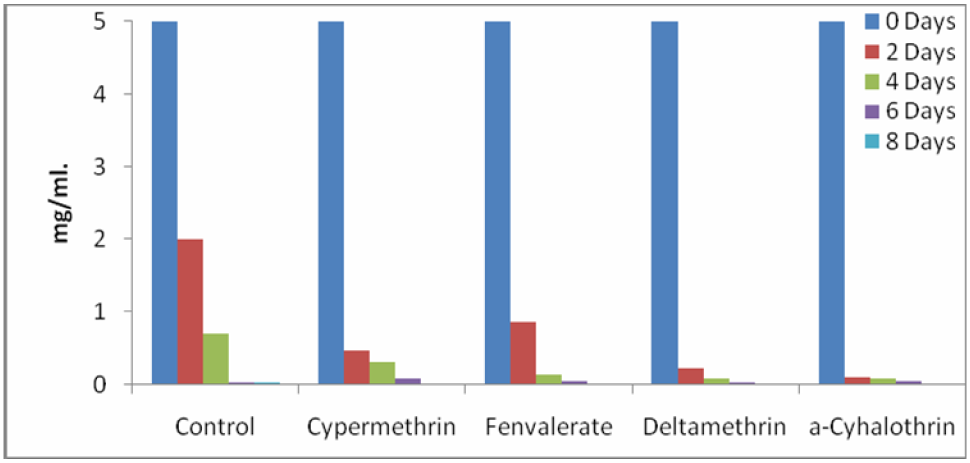


Figure.2 Effect of Synthetic pyrethroids on Phosphate solubilizing activity of *Pseudomonas fluorescens*

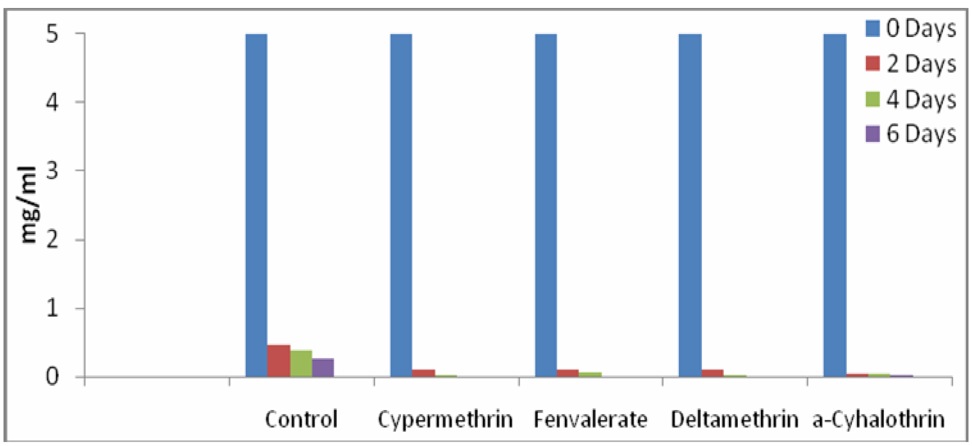


Figure.3 Effect of Synthetic pyrethroids on Phosphate solubilizing activity of *Serratia marscens*

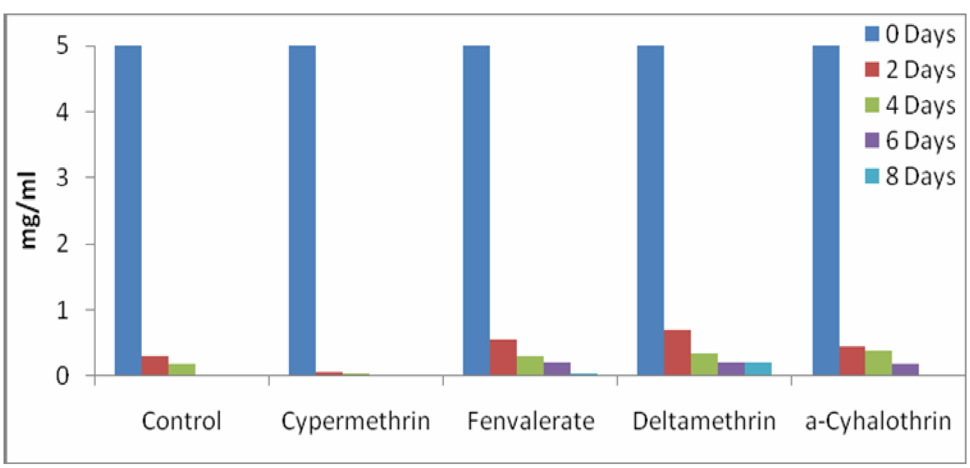


Figure.4 Effect of Synthetic pyrethroids on Phosphate solubilizing activity of *Azotobacter chrococcum*

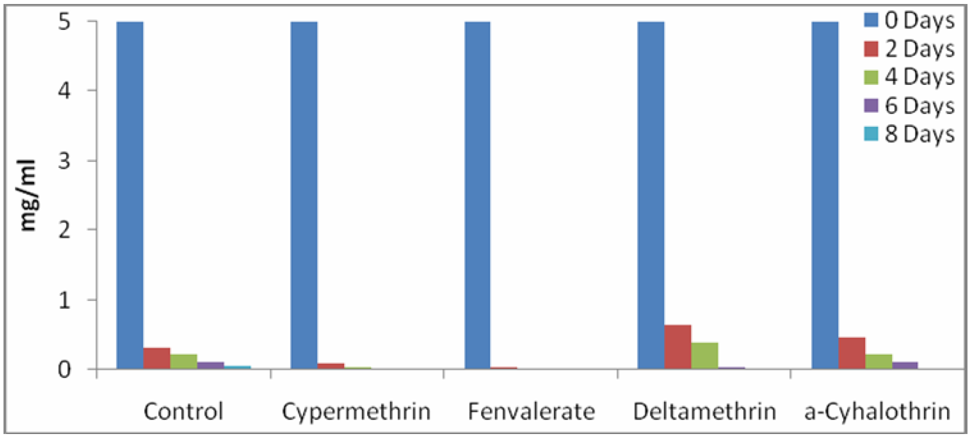
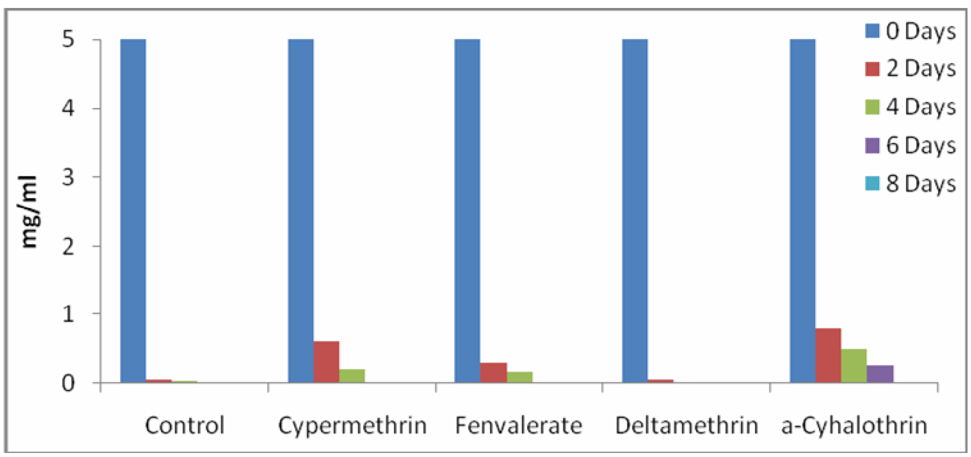


Figure.5 Effect of Synthetic pyrethroids on Phosphate solubilizing activity of *Rhizobium leguminosorum*



The results of the present investigation thus clearly indicate that application of different Synthetic Pyrethroids in general, at 1000ppm concentrations, brought about significant effects on growth and activities of beneficial microorganisms, which are responsible for the transformations of nutrient elements to make them more available in soil. Though the Synthetic Pyrethroids are toxic to nontarget organisms but they are showing positive effect on the microbial activity i.e. Phosphate solubilizing activity.

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