Effect of fipronil on the abundance of Phosphate Solubilizing Microorganisms (PSMs) in the cardamom plantation soils of Idukki district, Kerala, India

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Abstract - The aim of the present work was to investigate on the effect of fipronil insecticide on the density of phosphate solubilizing microorganisms. Phosphate solubilising microorganisms (PSMs) had a significant role in the conversion of inorganic phosphate into available forms. Fipronil is the insecticide has been found to be effective against the control of various insect pests. We selected soil samples of major cardamom plantations of Idukki District as the study material. According to this study, we can observe that the insecticide fipronil inhibits the growth of PSMs in soil. It leads to the Phosphorous nutrient deficiency in the soil available to plants and affect the crop yield.

Index terms - Phosphate Solubilizing Microorganisms, Fipronil, Abundance, Pikovskaya's medium

1. Introduction

India is an agriculture-based country. About 60-70% of its population depending on agriculture. A large portion of arable land already under cultivation is rapidly being depleted by industries and urban encroachments. On the other hand, the demand for agricultural crop is increasing day by day due to the rapidly increasing population. Hence there is a need for a huge increase in quality of agricultural produce as well as an improvement in its quality. The modern agriculture is readily associated with the use of different chemical inputs. Variant class of pesticides is used in managing different groups of pests to maximize crop production and meet the demand for higher supplies of food for the fast growing human population. The use of pesticides have become an integral and economically essential part of our modern agriculture. To meet these objectives, Agrochemicals like insecticides, fungicides and herbicides and also use of better quality seeds are being used on a large scale in agricultural lands. About 30% of agricultural produce is lost due to pests. Hence the use of pesticides have become indispensable in agriculture (Kavi et al, 2014). These pesticides affect the soil nutrients and fertility.

Phosphorous is one of the limiting factor of crop production of many kinds of soils in different geographical regions. In the form of chemical fertilizer a large amount of inorganic phosphate was added to the soil (Sanyal et al, 1991). Compared to mineral nutrients the concentration of soluble phosphorous in the soil is very low. The majority of the phosphorous fixed in the soil is rapidly into the forms that poorly available to the plant roots. It is a plant micronutrients and plays an important role in the plant metabolism and crop yields. The uptake of phosphorous by the plant is only a small fraction of added phosphate fertilizer (Vassilev et al, 2003). The remaining phosphorous are later converted into insoluble forms of phosphates and lost in the soil due to adsorption, precipitation or conversion to organic phosphates.(Holford ICR et al, 1997). Phosphorous fertilizers are required to maintain crop production due to widespread phosphorous deficiency.

Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which, through their hydroxyl and carboxyl groups, chelate the cation bound to the phosphate, the latter being converted into soluble forms. Phosphate solubilization takes place through various microbial processes or mechanisms, including organic acid production and proton extrusion (Nahas et al, 1996). PSMs dissolve the soil P through the production of low molecular-weight organic acids, mainly gluconic and ketogluconic acids (Duebel et al, 2000). Phosphorous solubilizing bacteria have a significant role in phosphorous nutrition through the release of inorganic and organic soil P pools by solubilization and mineralization. The highest portion of the PSM is concentrated on the rhizosphere and they are metabolically active from other sources. Phosphate solubilizing microorganisms can increase crop production up to 70%. Crop production increases due to the solubilization of fixed soil P and applied phosphates by phosphate solubilizing bacteria (Khan et al, 2009). Microbial population may be affected by many factors, including environmental changes and pollution with xenobiotic chemicals. In the short term experiments pesticides may stimulate, inhibit or have no effect on microbial numbers. According to Kalia and Gosal (2011) the application or extensive use of pesticides has led to a rapid decline in the quality of organic matter in soil it also affects the diversity of microbial flora and fauna. Fipronil is an insecticide widely used in the cardamom plantation soils against

nematodes.

Soil microorganisms play an important role in mineralizing the organic phosphate available to plants. These microorganisms were isolated from different soils of India (Vikram et al, 2007). Several varieties of PSMs have been isolated from the rhizosphere soils of crops. Majority of the PSMs are bacteria and some of them are fungi can solubilize phosphates. These bacteria and fungi have the potential to be used as biofertilizers. They have a significant role in improving the nutrient value of soil and crop yield (Kundu et al, 2009). Microorganisms with phosphate solubilizing potential increases the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixations. Enhancing microbial activity through P solubilizing inoculants may contribute considerably to plant P uptake. PSB were very effective against increase as well as the growth and yield of crops. So exploitation of phosphate solubilizing bacteria through biofertilization has enormous potential for making use of ever increasing fixed P in the soil and natural reserves of phosphate rocks. Phosphate Solubilizing Microorganisms were isolated from each sample by serial dilution and spread plate method. One gram of soil sample was dispersed in 9mL of autoclaved distilled water and was thoroughly shaken.1mL of the above solution was again transferred to 9mL sterile distilled water to form 10^{-2} dilution. Similarly 10^{-3},10^{-4},10^{-5},10^{-6} and 10^{-7} serials were made for each sample.0.1mL of each dilution was spread on Pikovskaya's agar medium (PVK) and incubated at 27-30^{0}c for 7 days. The density was calculated in cfu/g soil.

2. Materials and Methods

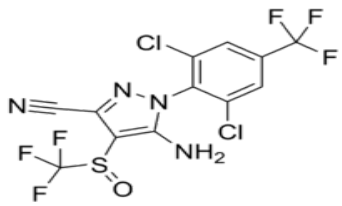
Collection of soil samples

Surface soil (15 cm) depth was collected from rhizosphere (Rh) and non-rhizosphere (nRh) region of cardamom plantations using a hand auger. Two samples each were collected from 6 different stations (Anakkara, Vandanmedu, Puliyanmala, Pampadumpara, Nedumkandam and Udumbanchola) of varying altitudes. Each sample was placed in a sterile plastic bag, sealed and placed on ice during transportation to the laboratory. All samples were passed through a 2.0 mm sieve and stored at 4°C for further analyses.

Fipronil- Insecticide

Fipronil insecticide 5-amino-1-[2,6,-dichloro-4(trifluoromethyl)phenyl]-4-[(1R,S) (trifluoromethyl)sulfinyl]- 1H-pyrazole-3-carbonitrile, was discovered in 1987 and first registered as a pesticide in the United States in 1996. Fipronil is a member of class phenyl pyrazoles and was introduced by Rhone-Poulenc Agrochemical company to control a broad-spectrum of crop pests (Zhu et al. 2004). Technical grade in Fipronil was obtained from Sigma Aldrich chemicals Pvt Ltd, Mumbai.

Figure 1 : Molecular structure of Fipronil



Abundance of Phosphate Solubilizing Microorganisms

Phosphate Solubilizing Microorganisms were isolated from each sample by serial dilution and spread plate method. One gram of soil sample was dispersed in 9mL of autoclaved distilled water and was thoroughly shaken.1mL of the above solution was again transferred to 9mL sterile

Table 1 : Composition of Pikovskaya's medium (PVK)

Composition	Gram/mL
Glucose	10
Yeast extract	0.5
(NH4)2SO4	0.5
MagnesiumSulphate (MgSO47H2O)	0.1
Calcium Phosphate(Ca3(PO4)2)	5
NaCl	0.2
KCl	0.2
MnSO4.2H2O	0.002
FeSO47H2O	0.002
Agar	1.5

Effect of fipronil on Phosphate Solubilizing Microorganisms (PSMs)

Weigh 250g of soil in correspondingly labeled 250mL Erlenmeyer flasks and supplemented with various concentrations of fipronil having 1000ppm,1500ppm and 2000ppm. And are allowed to incubate for one month at room temperature. The moisture content was maintained at regular intervals. After one month PSM was isolated from each sample by serial dilution and spread plate method. One gram of soil sample was dispersed in 9mL of autoclaved distilled water and was thoroughly shaken.1mL of the above solution was again transferred to 9mL sterile distilled water to form 10^{-2} dilution. Similarly 10^{-3},10^{-4},10^{-5},10^{-6} and 10^{-7} serials were made for each sample. 0.1mL of each dilution was spread on Pikovskaya's agar medium (PVK) and incubated at 27-30^{0c} for 7 days.

3. Results and Discussion

Density of Phosphate Solubilizing Microorganisms

Plants acquire phosphorous from the soil solution as phosphate anion. It is the least mobile element in the plant. Phosphorous solubilising bacteria play role in phosphorous nutrition by enhancing its availability to plants through release from inorganic and

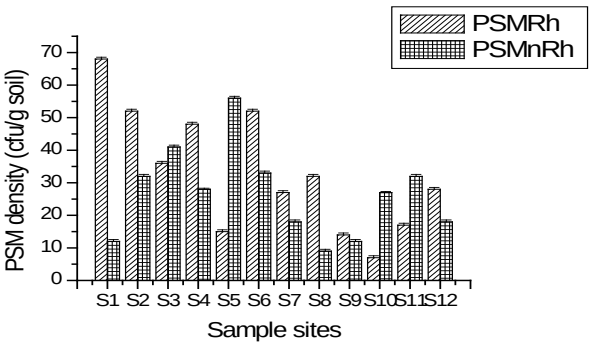
organic soil P pools by solubilisation and mineralization. Principal mechanism in organic acids and mineralization of organic P by acid phosphatase. The use of phosphorous solubilizing bacteria as inoculants increases P uptake. These bacteria also increase prospects of using phosphatic rocks in crop production. Table 2 and figure 2 showed that high

Table 2 : Density of Phosphate Solubilizing Microorganisms (PSMs) in untreated soil

Samples	PSM ($\times 10^3$) cfu/g dry soil	
	Rh	nRh
S_1	68±0.57	12 ±0.57
S_2	52 ±0.57	32 ±0.57
S_3	36 ±0.57	41 ±0.57
S_4	48 ±0.57	28 ±0.33
S_5	15±0.57	56 ±0.57
S_6	52 ±0.57	33 ±0.57
S_7	27 ±0.57	18 ±0.57
S_8	32 ±0.57	9 ±0.57
S_9	14 ±0.57	12 ±0.57
S_{10}	7 ±0.57	27 ±0.33
S_{11}	17 ±0.57	32 ±0.57
S_{12}	28 ±0.57	18 ±0.57

proportion of PSM were concentrated on the rhizosphere region and they were metabolically more active than from other sources. In rhizosphere (Rh) region, it was ranged from $7x10^3$ to $68x10^3$ cfu/g dry soil and in non rhizosphere (nRh) region it was from $9x10^3$ to $56x10^3$ cfu/g dry soil.

Figure 2 : PSMs density in untreated soil



Effect of fipronil on Phosphate Solubilizing Microorganisms (PSMs)

Pesticides applied to the soil are generally considered to have deleterious effects on soil microbial populations. The fluctuation in microbial population may be attributed to nutritional and environmental changes. Table 3 shows that the PSM population in the rhizosphere with concentration of 1000 ppm ranged from 6 cfu/g to 41cfu/g soil, in 1500 0 to 16 cfu/g soil in 1500 ppm and 0 to 6 cfu/g soil in 2000 ppm.

From the results (Fig 3 & 4) we observed that the plantation soil treated with various concentrations of fipronil inhibits the growth of Phosphate solubilizing microorganisms. Which leads to Phosphorous nutrient deficiency in soil and affects the plant growth and crop yields. PSM population was considerably lower in higher concentration 2000 ppm of fipronil than lower concentration 1000 ppm. In every concentrations PSM density was rich in rhizosphere region than the nonrhizosphere region. According to figure 3 PSMs density was seen more in rhizosphere but it was less in concerned with PSMs density of untreated soil.

Figure 3 : PSMs density on rhizospheric soil (Rh) supplemented with fipronil

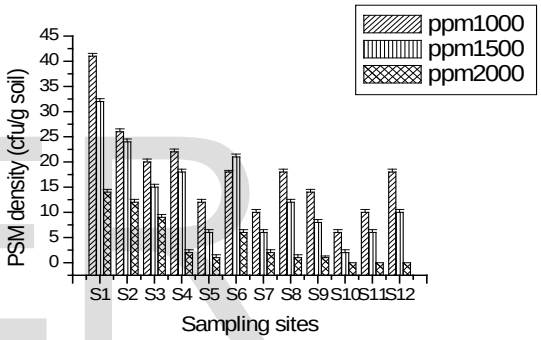


Figure 4 : PSM density on nonrhizospheric soil (nRh) supplemented with fipronil

ppm it was ranged from 2 cfu/g to 32 cfu/g soil and in 2000 ppm 0 to 14 cfu/g soil. In nonrhizospheric region the PSM population ranged from 2 to 18 cfu/g soil in 1000 ppm

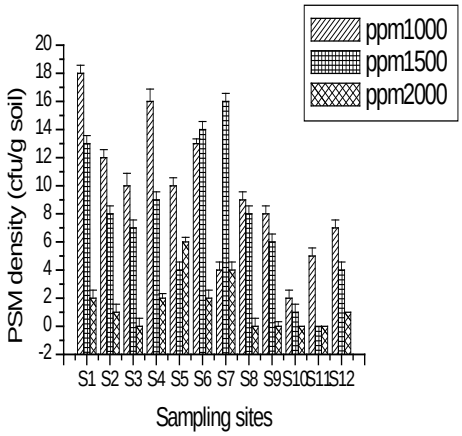


Table 3 : Abundance of PSMs on treated soil samples

Abundance of Phosphate solubilizing microorganisms ($\times 10^3$) cfu/g dry soil)						
Samples	1000ppm		1500ppm		2000ppm	
	Rh	nRh	Rh	NRh	Rh	nRh
S_1	41±0.57	18±0.57	32±0.57	13±0.57	14±0.57	2±0.57
S_2	26±0.58	12±0.57	24±0.57	8±0.57	12±0.57	1±0.57
S_3	20±0.57	10±0.88	15±0.57	7±0.57	9±0.57	0±0.00
S_4	22±0.57	16±0.88	18±0.57	9±0.57	2±0.57	2±0.33
S_5	12±0.57	10±0.57	6±0.57	4±0.57	1±0.57	6±0.33
S_6	18±0.33	13±0.57	21±0.57	16±0.57	6±0.57	2±0.57
S_7	10±0.57	4±0.57	6±0.57	10±0.57	2±0.57	4±0.57
S_8	18±0.57	9±0.57	12±0.57	8±0.57	1±0.57	0±0.00
S_9	14±0.57	8±0.57	8±0.57	6±0.57	1±0.33	0±0.00
S_{10}	6±0.57	2±0.57	2±0.57	1±0.57	0±0.00	0±0.00
S_{11}	10±0.57	5±0.57	6±0.57	0±0.00	0±0.00	0±0.00
S_{12}	18±0.57	7±0.57	10±0.57	4±0.57	0±0.00	1±0.57

4. Conclusion

Phosphorous is essential for growth and productivity of plants. It plays an important role in plants in many physiological activities such as cell division, photosynthesis and development of good root system and utilization of Carbohydrates. Application of insecticide seemed to affect the PSMs count in treated soil than the plantation soil. PSMs population will progressively inhibit by the used insecticide fipronil. In plantation soil and insecticide treated soil, PSM density was seen mostly in rhizosphere region. Application of biofertilizers and phosphate solubilizing microorganisms to the soil for getting better crop yield and protecting soil fertility.

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References

- Sanyal S.K, De Datta S.K, "Chemistry of phosphorus transformations in soil", Adv Soil Sci 16: 1-20, 1991.
- Nahas E. "Factors determining rock phosphate solubilization by microorganisms isolated from soil", World J Microb Biotechnol 12: 18-23, 1996.
- Holford ICR, "Soil phosphorus: its measurement and its uptake by plants", Aust J Soil Res 35, 227-239, 1997.
- Duebel A, Gransee A, Merbach W. "Transformation of organic rhizodeposits by rhizoplane bacteria and its influence on the availability of tertiary calcium phosphate", J Plant Nutr Soil Sci 163: 387-392, 2000.
- Vassilev N, Vassileva M, "Biotechnological solubilization of rock phosphate on media containing agroindustrial wastes", Appl Microbiol Biotechnol 61, 435-440,2003.
- Zhu G, Wu H, Guo J, Faithrest M.E, "Microbial degradation of Fipronil in clay loam soil" Water, Air and Soil pollution, 153. 35-44, 2004.
- Vikram, Alagawadi A.R, Hamzehzarghani H, "Factors related to the occurrence of phosphate solubilizing bacteri and their isolation in vertisols", Int J Agri Res 2: 571-580, 2007.
- Kundu B.S, Nehra, K, Yadav R, "Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana " Ind J Microbiol 49: 120-127, 2009.
- Ahmad Ali Khan , Ghulam Jilani , Mohammad Saleem Akhtar , Syed Muhammad Saqlan Naqvi , Mohammad Rasheed, " Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production ", J. AGRIC. BIOL. SCI. 1(1), 48-58, 2009.
- Kalia. A, Gosal S.K, "Effect of pesticide application on soil microorganisms", Archieves of agronomy and soil science, 57. 569 – 596, 2011.
- Kavi K.S, Saranraj, P "Toxic effects of pesticide pollution and its biological control by microorganisms: A review", Agricultural Journal of Hygiene, 3(1). 1 – 10, 2011.