Growth response study of fipronil degrading bacteria and abundance of microorganisms from cardamom plantation soils, Idukki district, Kerala

Keerthi. A.T and V. Salom Gnana Thanga

Department of Environmental Sciences, University of Kerala, Thiruvananthapuram, Kerala, India

Abstract- Insecticides are biologically active compounds and an unintended consequence of its application may lead to significant changes in microbial populations and activities influencing microbial and ecological balance affecting soil fertility. In the present work, three bacterial isolates capable of degrading fipronil were obtained by enrichment from soil samples collected from the cardamom plantations of Idukki district, Kerala. According to the observations we found that the isolates were capable of growing in the presence of fipronil in two different growth media. Most of the microbes were grown maximum in rhizosphere and had the capacity to grow in the presence of fipronil. This study suggested that the fipronil insecticide causes transient impact on microbial populations at a recommended field application rate.

Index Terms- fipronil, growth, tolerance, microbial density, sterilized and unsterilized soil

I. INTRODUCTION

In concerned with National Pesticide Information, fipronil is a broad spectrum insecticide. The International Union of Pure and Applied Chemistry (IUPAC) name for fipronil is (±) -5 amino-1-(2,6 dichloro- α, α, α- trifluoro - p- tolyl) - 4- trifluoromethyl sulfinyl pyrazole-3- carbonitrile. Hadjmohammadi et al (2006) observed that fipronil is a new soil and foliar broad spectrum insecticide discovered and developed by Rhone Poulene between 1985 and 1987 and placed on the market in 1993. It is active against a wide range of soil and foliar insects. It acts on gamma amino butyric acid (GABA) receptors, the principal nerve transmitter of insects preventing inhibition of GABA. USEPA (1996) revealed that fipronil has a half life of 122-128 days in oxygenated sandy loam soil and residues remain mainly in the upper 12 inches of soil.

Microbial density includes bacteria, fungi and actinomycetes. Microorganisms represent one of the largest reservoirs for essential nutrients. These microbes through their enzyme activities have a primary role in the degradation of plant and animal residues in the environment, which contribute the cycling of nutrients. Microorganisms being an intimate contact with the soil microbial environment make ideal monitors of soil pollution (Defo et al. 2011). Microbial population may be affected by many factors, including environmental changes and pollution with xenobiotic chemicals. In 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.

The sustainable agriculture involves optimizing agricultural resources and at the same time maintaining the quality of the environment and sustaining natural resources. The soil microbial community composition is of great importance, because they play a crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth (Chauhan et al., 2006; Tripathi et al., 2006; Pandey et al., 2007). A healthy population of soil microorganisms can stabilize the ecological system in soil (Chauhan et al., 2006) due to their ability to regenerate nutrients to support plant growth. Any change in their population and activity may affect nutrient cycling as well as availability of nutrients, which indirectly affect productivity and other soil functions (Wang et al., 2008).

At present, little is known about the effects of pesticides on soil micro flora and those recorded are restricted to fewer pesticides and also involve changes in single microbial activities or populations, which are regarded as having no importance in terms of overall activity. The impact of pesticides on microbial populations and on microbial activities such as respiration, soil enzymes and other soil biochemical processes has been studied by several workers. However, in India, the knowledge in this field is limited. Most of the studies were undertaken following a single application of pesticide for a short period, which does not represent a real situation in the field. Due to the extensive use of pesticides in agriculture and plantation crops these accumulate in the environment and exert adverse toxicological constraints and physiological stress on life forms. Since the pesticides are applied directly these may adversely influence the growth and activity of beneficial indigenous microorganisms. The majority of biochemical transformation in soil results from microbial activity and any compound that alters the number or activity of microorganisms could affect soil biochemical processes and ultimately influence soil fertility and plant growth.

II. MATERIALS AND METHODS

Collection of soil samples

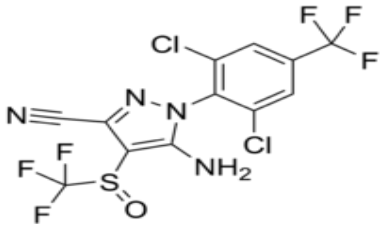
Surface soil (15 cm) depth was collected from rhizosphere (Rh) and non-rhizosphere (nRh) region of cardamom plantations using a hand auger. Two samples each were collected from 6 different stations (Anakkara, Vandanmedu, Puliyanmala, Pampadumpara, Nedumkandam and Udumbanchola) of varying altitudes. Each 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 of Fipronil of 98% and 97% purity was obtained from Sigma Aldrich chemicals Pvt Ltd, Mumbai.

Figure 1 : Molecular structure of Fipronil



Selection of bacterial strains

Three strains having fipronil degradation potential were selected by soil microcosm studies using High Performance Liquid Chromatography (Verma et al, 2014). These three strains were used for growth response studies in different growth media. Nutrient broth and Mineral salt medium was used for growth studies.

Growth tolerance studies of isolated bacterial strains in different growth media

In Nutrient broth (Table 1) take 25mL of broth to each correspondingly labelled test tubes and add 1mL pure cultures to it and assessed over a period of one week. Every day we take the optical density at620nm. In Mineral Salt Medium (Table 2), take the optical density at 660nm after Lowry's protein estimation of strains on the media assessed for one week.

Table 1 : Composition of Nutrient Broth

Peptic digest of animal tissue	5.0g
Beef extract	4.0g
Distilled Water	1000mL
pH (at 25^0c)	6.8±0.2

Table2:Composition of Mineral Salt Medium

Glucose	1.0g
Dipotassium hydrogen phosphate	0.8g
Potassium dihydrogen phosphate	0.2g
Magnesium sulphate	0.1g
Calcium chloride	0.1g
Ferric chloride	0.01g
Ammonium nitrate	0.5g
Distilled water	1000mL
pH	7.0

Microbial density of fipronil treated soils

Weighed 100g of three set soil samples in correspondingly labelled 250mL conical flasks and add 0.3% fipronil insecticide allowed to one week incubation at room temperature. According to this analysis the unsterilized soil without pesticide fipronil taken as control and other two for unsterilized soil with 0.3% fipronil and sterilized soil with 0.3% fipronil. The moisture content was maintained at regular intervals. To determine the microbial density, soil samples were subjected to the dilution plate method using different growth Media like Nutrient Agar for bacteria, Potato Dextrose Agar for fungi and Starch Casein Agar for Actinomycetes in different dilutions (Dubey et al 2004; Cappuccino et al 2004). The abundance and distribution of different groups of soil microorganisms were enumerated and expressed in terms of colony forming units per gram soil (cfu/g).

III. Results and Discussions

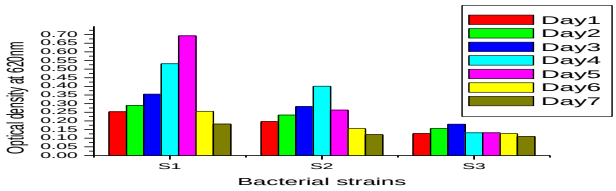
Growth tolerance of isolated strains in Nutrient Broth medium

The screened and selected 3 bacterial strains were incubated 7 days in the nutrient broth medium. Every day we take the optical density at 620nm.Comparitively strain S_1 showed the maximum capacity to grow on nutrient broth. According to the results the strains had a significant increase in growth up to 4^{th} day of incubation and then become reduced till 7^{th} day. According to Table 3and Figure 2 strain S_1 showed maximum growth (0.693) pattern in the nutrient broth medium.

Table 3: Growth tolerance of isolated bacterial strains in Nutrient Broth medium

Strains	Day1	Day2	Day3	Day4	Day5	Day6	Day7
S_1	0.253	0.290	0.355	0.531	0.693	0.256	0.182
S_2	0.196	0.234	0.283	0.401	0.263	0.156	0.120
S_3	0.126	0.156	0.181	0.131	0.131	0.126	0.110

Figure 2 : Growth tolerance of isolated strains in Nutrient Broth



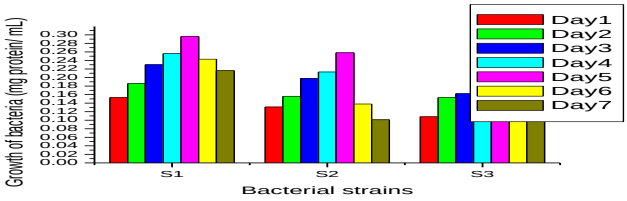
Growth tolerance of isolated strains on Mineral Salt Medium

According to this study similar to that of growth in nutrient broth S_1 showed maximum growth pattern in mineral medium and all strains had a significant increase in growth up to the 5th day of incubation. From 6th to 7th day the growth will become significantly reduced. Table 4 and Figure 3 showed that S_1 had the maximum capacity (0.296 mg protein/ml) to grow on mineral medium

Table 4 : Growth tolerance studies of isolated bacterial strains in Mineral salt medium (mg protein/ ml)

Strains	Day1	Day2	Day3	Day4	Day5	Day6	Day7
S1	0.153	0.186	0.230	0.256	0.296	0.243	0.216
S2	0.131	0.156	0.198	0.213	0.258	0.138	0.101
S3	0.108	0.153	0.162	0.168	0.132	0.128	0.118

Figure 3 : Growth tolerance of isolated strains in Mineral salt medium



Microbial density of fipronil treated sterilized and unsterilized soils

Bacterial population

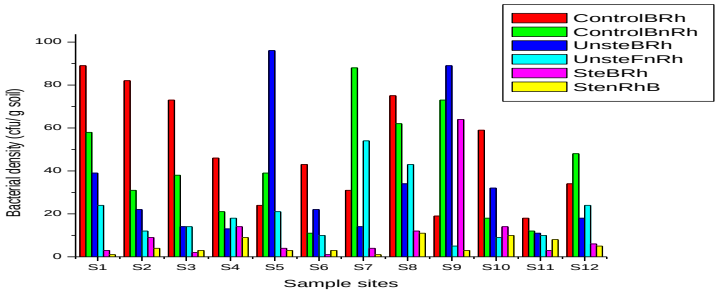
In rhizospheric region the bacterial diversity was ranged from 18x10^6 to 89x10^6 in control soil, 11 x10^6 to 96 x10^6 in unsterilized soil with 0.3% fipronil and ranged from 1x10^6 to 64x10^6 in sterilized soil with 0.3% fipronil treated soil. In non rhizospheric region the bacterial population was extended up to 88x10^6 in control, 5x10^6 to 34x10^6 in unsterilized soil and 1x10^6 to 11x10^6. Bacteria are considered to be very significant in soil fertility. The fluctuation in bacterial population may be attributed to nutritional and environmental changes, chemical pollution, etc. The insecticide treatment has an adverse effect on bacterial populations. From figure 4 observed that population was maximum in the control soil both in rhizosphere and nonrhizosphere

Table 5 : Bacterial Population of fipronil treated soil

Bacterial density (×10^6) cfu/g dry soil						
Samples	Control		Unsterilized soil+fipronil		Sterilized soil+fipronil	
	Rh	nRh	Rh	NRh	Rh	NRh
S_1	89±0.57	58±0.57	39±0.57	24±0.57	3±0.57	1±0.57
S_2	82±0.57	31± 0.57	22±0.57	12±0.57	9±0.57	4±0.57
S_3	73±0.57	38±0.57	14±0.57	14±0.57	2±0.57	3±0.57
S_4	46±0.57	21±0.57	13±0.57	18±0.57	14±0.57	9±0.57
S_5	24±0.57	39±0.57	96±0.57	21±0.57	4±0.33	3±0.57
S_6	43±0.57	11±0.57	22±0.57	10±0.57	1±0.57	3±0.57
S_7	31±0.57	88±0.57	14±0.57	34±0.57	4±0.57	1±0.57
S_8	75±0.57	62±0.57	34±0.57	43±0.57	12±0.57	11±0.57
S_9	19±0.57	73±0.57	89±0.57	5±0.57	64±0.57	3±0.57
S_{10}	59±0.57	18±0.57	32±0.33	9±0.57	14±0.57	10±0.57

S_{11}	18±0.57	12±0.57	11±0.33	10±0.57	3±0.57	8±0.57
S_{12}	34±0.57	48±0.57	18±0.57	24±0.57	6±0.57	5±0.57

Figure 4 : Bacterial population in fipronil treated sterilized and unsterilized soil



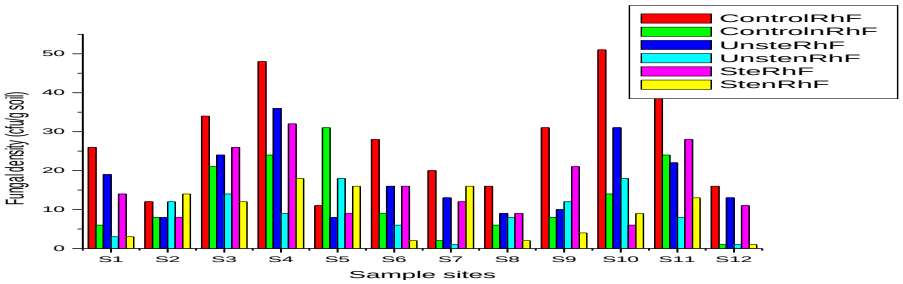
Fungal population

In rhizospheric region the fungal population was ranged from 11x10^3 to 51 x10^{3}in controls and 9 x10^3 to 36 x10^3 in unsterilized soil with fipronil. In the case of sterilized soil with 0.3% of fipronil the fungal population was ranged from 6 x10^3 to 32 x10^3. In nonrhizospheric region the fungal population was ranged from 1 x10^{3}to 31 x10^3 in control, 3 x10^3 to 18 x10^3 in unsterilized soil treated with 0.3% fipronil and in sterilized soil with fipronil it was ranged from 1 x10^3 to 18 x10^3. Fungi are decay organisms in the soil and have an important role in providing essential elements to higher plants. Most fungi are opportunistic. They grow and conduct their activities when environmental conditions are favorable. The acidic pH was generally more favorable for fungi.

Table 6 : Fungal population of fipronil treated sterilized and unsterilized soil

Fungal density (×10^3) cfu/g dry soil						
Samples	Control		Unsterilized soil+fipronil		Sterilized soil+fipronil	
	Rh	nRh	Rh	NRh	Rh	NRh
S_1	26±0.57	6±0.57	19±0.57	3±0.57	14±0.57	3±0.57
S_2	12±0.57	8±0.57	8±0.57	12±0.57	8±0.57	14±0.57
S_3	34±0.57	21±0.57	24±0.57	14±0.57	26±0.57	12±0.57
S_4	48±0.57	24±0.57	36±0.57	9±0.57	32±0.57	18±0.57
S_5	11±0.57	31±0.57	8±0.33	18±0.57	9±0.57	16±0.57
S_6	28±0.57	9±0.57	16±0.57	6±0.57	16±0.57	2±0.57
S_7	20±0.33	2±0.57	13±0.57	1±0.57	12±0.57	16±0.57
S_8	16±0.57	6±0.57	9±0.57	8±0.57	9±0.33	2±0.57
S_9	31±0.57	8±0.57	10±0.33	12±0.57	21±0.57	4±0.57
S_{10}	51±0.57	14±0.57	31±0.33	18±0.57	6±0.57	9±0.57
S_{11}	43±0.88	24±0.57	22±0.57	8±0.57	28±0.57	13±0.57
S_{12}	16±0.57	1±0.57	13±0.57	1±0.57	11±0.57	1±0.57

Figure 5 : Fungal population in fipronil treated sterilized and unsterilized soil



Actinomycetal population

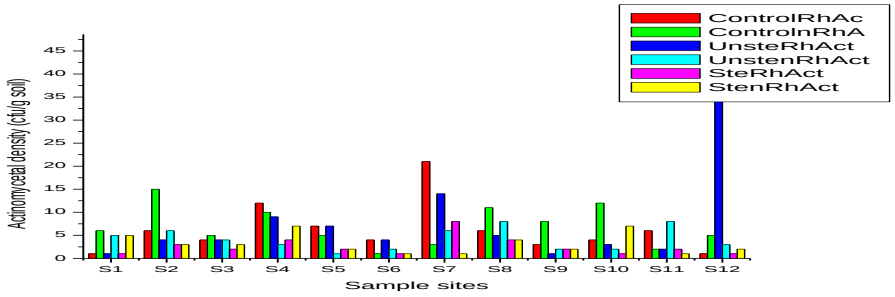
Actinomycetes compose 10% to 33% of the bacterial population and are more abundant in surface soils with high pH. In control the actinomycetal population was ranged from 1x10^4 to 21 x10^4 and in unsterilized soil with 0.3% fipronil it was ranged from 1 x10^4 to 14 x10^4. In the case of sterilized soil the density was extended from 1 x10^4 to 8 x10^4 in rhizospheric region. Actinomycetal density in unsterilized soil with pesticide

treatment was more than that of sterilized soil. In nonrhizospheric region actinomycetes were ranged from 1 $x10^4$ to15 $x10^4$ in control and very least population observed in unsterilized and sterilized soil treated with 0.3% fipronil ie $1x10^4$ to 8 $x10^4$ and 1 $x10^4$ to 7 $x10^4$ respectively. There was no distinct variation in both of two treatments according to the density in control.

Table 7 : Actinomycetal population of fipronil treated sterilized and unsterilized soil

Actinomycetal density ($\times10^3$) cfu/g dry soil						
Samples	Control		Unsterilized soil+fipronil		Sterilized soil+fipronil	
	Rh	nRh	Rh	nRh	Rh	nRh
S_1	1±0.57	6±0.57	1±0.57	5±0.57	1±0.57	5±0.57
S_2	6±0.57	15±0.57	4±0.57	6±0.57	3±0.33	3±0.57
S_3	4±0.57	5±0.57	4±0.57	4±0.57	2±0.57	3±0.57
S_4	12±0.57	10±0.57	9±0.57	3±0.57	4±0.33	7±0.57
S_5	7±0.57	5±0.57	7±0.33	1±0.57	2±0.57	2±0.57
S_6	4±0.57	1±0.57	4±0.57	2±0.57	1±0.33	1±0.57
S_7	21±0.57	3±0.57	14±0.57	6±0.57	8±0.57	1±0.57
S_8	6±0.57	11±0.57	5±0.57	8±0.57	4±0.57	4±0.57
S_9	3±0.57	8±0.57	1±0.33	2±0.57	2±0.57	2±0.57
S_{10}	4±0.57	12±0.57	3±0.33	2±0.57	1±0.57	7±0.57
S_{11}	6±0.33	2±0.57	2±0.57	8±0.57	2±0.57	1±0.57
S_{12}	3±0.57	1±0.57	4±0.57	2±0.57	3±0.57	4±0.57

Figure 6 : Actinomycetal population in fipronil treated sterilized and unsterilized soil



IV. Conclusion

According to the study, we can conclude that the isolated and selected fipronil degraded three strains had growth response on two different media. In nutrient broth , the strains have significant growth up to 4th day and in mineral salt media the strains had growth tolerance up to the 5th day of incubation. Both in rhizosphere and nonrhizosphere the microbial density was showed maximum in control than the sterilized and unsterilized soils amended with 0.3% fipronil. According to our observations unsterilized soil supplemented with 0.3% fipronil had a maximum microbial density than the sterilized soils. 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. The gradual increase in microbial counts may be attributed to their ability to temporarily mineralize and use fipronil as an energy source. Our plantation soil was the richest source of naturally occurring fipronil degrading bacterial strains. In future we can isolate and identify the fipronil degrading strains and use it as a bioremediator in fipronil contaminated soils.

Acknowledgment

We express our sincere gratitude to UGC, New Delhi for granting financial support in concern with a Major Research Project.

References

[1] United States Environmental Protection Agency.," Fipronil Pesticide Facts Sheet". EPA -737-F-96-005. U.S. Environmental Protection Agency. Washington.D.C, 1996.

[2] G.Zhu, H. Wu, J. Guo, M. E. Faithrest, " Microbial degradation of Fipronil in clay loam soil" Water, Air and Soil pollution, 153, 2004, 35-44.

[3] J.G. Cappuccino, Natalic Sherman, "Microbiology A Laboratory Manual" Pearson education, Pvt Ltd, 13, 2004.

[4] R.C. Dubey, D. K. Maheswari, "Practical Microbiology", S chand and company limited. Ramnagar. Newdelhi, 2004, 185-204.

[5] M.R. Haajmohammadi, S. M. Nikou, K. Kamal, "Determination of Fipronil Residue in Soil and Water in the Rice Fields in North of Iran by RP-HPLC Method, Acta Chim. Slov, 53, 2006, 517–520.

[6] A.K. Chauhan, A. Das, H. Kharkwal, A.C. Kharkwal, A. Varma, "Impact of microorganisms on environment and health", in A.K. Chauhan and A. Varma ed., Microbes: Health and environment, 2006.

[7] S.K. Tripathi, A. Sumida, K. Ono, H. Shibata, S. Uemura, Y. Kodama, T. Hara," Leaf litter fall and decomposition of different above-and below ground parts of birch (Betula ermanii) tree and dwarf bamboo (Sasa

kurilensis) shrub in a young secondary forest of Northern Japan, Biol. Fertil. Soils, 43, 2006, 237-246.

[8] R.R. Pandey, G. Sharma, S. K. Tripathi, A. K. Singh, "Litter fall, litter decomposition and nutrient dynamics in subtropical natural forest and managed plantations in northeastern India", For. Ecol. Manag. 240, 2007, 96-106.

[9] Q.K. Wang, S.L. Wang, Y.X. Liu, " Responses to N and P fertilization in a young Eucalyptus dunnii plantation; microbial properties, enzyme activities, and dissolved organic carbon", Appl. Soil Ecol. 40, 2008, 484-490.

[10] M.A. Defo, T. Njine, M. Nola, F. A. Beboua, " Microcosum study of the long term effect of endosulfan on enzyme and microbial activities on two agricultural soils of Yaounde-Cameroon", African Journal of Agricultural Research Vol. 6(9), 2011, 2039-2050.

[11] A. Kalia, S. K. Gosal, " Effect of pesticide application on soil microorganisms", Archieves of agronomy and soil science, 57, 2011, 569 – 596.

[12] A. Verma, A. Srivastava, S. S. Chauhan, P. C. Srivastav, " Effect of Sunlight and Ultraviolet Light on Dissipation of Fipronil Insecticide in Two Soils and Effect of pH on its Persistence in aqueous medium", Air, Soil and Water Research, 2014, 69-73 doi.10.4137/ASWR.S14847.

AUTHORS

First Author – Keerthi.A.T, MSc. MPhil, Research Scholar, Department of Environmental Sciences, University of Kerala, Thiruvananthapuram, Kerala

Second Author – Dr.V.Salom Gnana Thanga, Associate Professor, Department of Environmental Sciences, University of Kerala, Thiruvananthapuram, Kerala