Journal of Applied Biology and Biotechnology Vol. 3 (05), pp. 019-025, Sep-Oct, 2015
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2015.3504

Effect of cypermethrin, a pyrethroid insecticide on dynamics of soil microflora

Bijay Kumar Sethi[1,2*], Subhashree Pradhan[1], Narayan Behera[1], Santi Lata Sahoo[1]

1 Applied Microbiology Laboratory, Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar-751004, Odisha, India.
[2]Department of Biotechnology, MITS School of Biotechnology, 2 (P), Infocity, Patia, Chandaka Industrial Estate, Bhubaneswar-751024, Odisha, India.

ARTICLE INFO

Article history:
Received on: 19/06/2015
Revised on: 06/07/2015
Accepted on: 22/07/2015
Available online: 22/10/2015

Key words:
Aspergillus, cypermethrin, protease, soil.

ABSTRACT

Soil samples were collected from garden of P.G. Department of Botany, Utkal University, Odisha India. The physical analysis of soil revealed that texture of the soil was sandy loam with pH 5.7, temperature 34.6ºC, 5.7% water content and 67.3% water holding capacity, respectively. Utmost numbers of *Aspergillus* species with other fungi were isolated from garden soil (5×10^5 CFU/g). Total 19 fungal taxa were isolated from all the samples studied. The isolated species were belonging to 8 genera representing the genus *Aspergillus* as dominant one. Total twelve fungal species of six genera were recorded from air of the pesticide treated soil area. Soil treated with 600ppm cypermethrin exhibited ten fungal species. Out of which, *A. flavus* appeared in greater number as compared to other fungi. Similarly, *A. candidus* was contributed highest percentage followed by *A. flavus* in dilution plate technique. Total eight species of fungi belonging to three genera were found when soil was treated with cypermethrin at a concentration of 800 ppm. *A. candidus* and *A. flavus* were isolated in highest number at 1000ppm cypermethrin. But, in direct plate technique, *A. flavus* was found to be the dominant species followed by *A. terreus* and *A. niger* at 1000 and 1200ppm concentration of cypermethrin, respectively. Soil treated with cypermethrin at 1400ppm concentration exhibited that *A. awamori* was the dominant species isolated followed by *A. terreus* and *Mucor hiemalis* both in direct and dilution plate techniques. Among all the isolates screened for protease activity, *A. terreus* was potent enough for this production.

1. INTRODUCTION

Soil is the most vibrant living habitat which comprises a variety of flora and fauna and microorganism (actinomycetes, arthropods, bacteria, crustaceans, earthworms, fungi and nematodes). But in the contemporary era of agriculture and farming, the pesticides have been used extensively and occupied a pivotal place in cultivation of crops. Its uncontrolled use has generated countless hazards and dilemma including the environmental pollutions. The pesticides have cumulative effect on target and non-target microflora that may greatly hamper the biological processes like, decomposition, degradation, transformation, clay–humus-microbes' interaction, microbe-microbe interaction and plant-microbe interaction. It also acts on the resistance factors of microorganisms tending towards the vanishing from the soil. Frequent long term application of pesticides to the soil may accumulate the recalcitrant chemicals that may possess permanent detrimental effect on soil micro-biological and biochemical activities. Currently, microorganisms are exploited in many ways to get valuable products which include enzymes, secondary metabolites, therapeutic agents and industrial products. Such potential microorganisms are usually isolated from the soil sample. Among such microbes, filamentous fungi dominate our globe as sources of food, plant and animal pathogens and for biosynthesis of other worthy products.

Besides to this, they are the critical components of our natural and agricultural ecosystem. Due to the application of a variety of hazardous chemicals to the cultivated lands, the growth, survival, continuity and community of fungi is significantly affected. During 1930s, the organophosphorus pesticides in Germany have possessed high mammalian toxicity and also perturb the functional dynamics of many microbial communities. For example, due to the long-term application of atrazine, cellulase-producing-bacteria were permanently removed from an apple orchard kept free from direct vegetative cover [1].

** Corresponding Author*
Bijay Kumar Sethi,Applied Microbiology Laboratory, Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar-751004, Odisha, India. Email :reach4bijay@gmail.com

Repeated annual applications of paraquat up to 18 years was found to have escalating populations of aerobic & cellulolytic bacteria and actinomycetes, but diminished fungal populations [2]. Rai [3] reported that 15 years of annual application of 2,4-D decreased microbial biomass by 15-20%, while long-term applications of paraquat [2], trifluralin [4] and atrazine [5] were all reported to have a decline in microbial biomass. Fungicides of both systemic and non-systemic are reported to have an undesirable effect on the colonization of the roots of crop plants by arbuscular mycorrhizal (AM) fungi with reduced phosphorus uptake [6]. The fungicide mancozeb and the nematicide "aldicarb" when applied to soil individually did not affect the colonization of sugarcane roots by arbuscular mycorrhizal fungi, but reduced colonization when applied in combination [7]. Besides that, Other similar reports include a decrease in dehydrogenase and urease activity following long-term (15 years) application of 2,4-D (isoctyl ester formulation) [3], a decrease in dehydrogenase and arylsulfatase in South Australian soils following long-term applications of atrazine [5], and a decrease in phosphatase activity following long-term applications of glyphosate [8]. A number of pesticides used in Australian agriculture (endosulfan, paraquat, glyphosate and diuron) have a short-term harmful impact on soil respiration which may persevere for 6-8 weeks after application. The prospective for pesticides to amend the soil microbial community without necessarily affecting the soil biology has encouraged the addition of novel methods for swift diagnosis of microbial miscellany in pesticide approval protocols concerning the environmental insinuations of amended microbial diversity to soil productivity [9]. Therefore, appraisal of soil microbial community is regarded to be a constructive technique to evaluate the impact of agricultural practices on soil health [7, 10, 11].

Therefore, as the search for new microbial sources is a continual and essential exercise, the present study aimed at the isolation, identification and effect of various pesticides on dynamics of soil microflora.

2. MATERIALS AND METHODS

2.1Chemicals, media and reagents

All the chemicals, media and reagents used in this study were of Analytical grade (AR) and procured from Hi-Media, Merck and Sigma Chemicals. The pesticide, cypermethrin was purchased from a local agricultural based outlet of Bhubaneswar, Odisha, India.

2.2 Selection of the experimental field

An experimental field (5×5 m) was selected inside the garden of the P. G. Department of Botany, Utkal University, Odisha, India. All small plants and other litter deposited were removed completely. The field was left under natural condition for about fifteen days before the soil sampling. Surface soil was removed carefully and freed from decomposed plant residues.

Soil samples were collected in two different parts for soil and microbial population analysis. New polythene bags were used to collect soil samples for edaphic properties. Sample in bulk, about 1 Kg was brought to the laboratory, dried in shade under fan and gently crushed to get semi fine soil particles. Crushed soil was passed through a general mash and about 100g soil was sealed in a small polythene packet and stored under laboratory condition until use. Ten such packets were prepared and kept in laboratory for study of soil parameters.

Other part of the soil was collected aseptically for fungal population analysis. Ten such samples of the soil within a depth of 5cm were collected, brought to laboratory immediately and stored in refrigerator at 4 °C.

The samples thus collected were processed for fungal enumeration on the same day or on the next day positively to avoid the loss of viability of certain microbes that fail to stand condition away from nature.

2.3 Soil analysis

Physical parameters of the soil were studied such as, soil texture, pH, temperature, water content and water holding capacity (WHC).

2.4 Effect of pesticides on soil microflora

Cypermethrin was selected to evaluate its effect on soil microflora. Cypermethrin is generally available in 10% E.C. in the market with a recommended dose of 1000 ppm. Five different concentrations i.e. 600, 800, 1000, 1200 and 1400 ppm were applied to the experimental soil. Same size earthen pots were taken and 2 kg hand crushed garden soil was filled in each pot. The pesticide solutions were separately added and mixed in each pot with an amount so as to provide 60% WHC to the soil .Samples were drawn after 7days of treatment for microflora analysis.

2.5. Isolation of microorganisms

2.5.1 Dilution plate technique

The collected soil samples were processed for analysis by serial dilution followed by pour-plate technique as per Sethi *et al.* [12] method. The dilutions of 10^{-4}, 10^{-5} and 10^{-6} thus prepared were platted using sterilized potato dextrose agar (PDA) medium separately in three replicates taking 1ml from individual dilution tubes. The prepared plates were incubated upside down in an incubator at 28°C±2°C. Number of Colony Forming units developed after 3, 5 and 7days on PDA plates were counted.

Similarly, prepared PDA plates were exposed for a period of 15 min in the garden to ensure the number of microorganisms present in the air.

2.5.2 Soil plate technique

About 0.1g of soil sample was taken in a clean and sterilized perti plate. One to two drops of sterile water was added to moisten the soil followed by proper teasing to get fine particles. The plate with sample was shaken repeatedly for proper dispersion of soil particles. To it, 15-20 ml of sterilized, warm (~45°C) agar medium was added and mixed properly. Plates thus prepared were incubated at 28±2°C for seven days and examined for total count

of colonies and identification. Enumeration of fungal population was calculated from dilution plate technique and percentage frequency from direct soil plate technique.

2.6. Identification of the isolated fungi

All the isolated fungi were identified based on macro (colony morphology) and microscopic characteristics [13, 14].

2.7. Screening for proteolytic activity

Screening for protease activity was performed on prepoured Czapek Dox agar plates supplemented with 1% casein (w/v) by point inoculation with mycelium/spores of fungal isolates [15]. Petri plates were stained with Nessler's reagent and the hydrolyzing zones were measured and photographed.

2.8. Statistical analysis

All the experiments were performed in triplicates. The mean, standard deviation and standard error of mean were calculated and represented in the results.

3. RESULTS AND DISCUSSION

Soil is the house of all living forms and the laboratory within which alterations are carried out that facilitate the life to go on. Hence, Soil is also universally known as the elixir of many organisms. But, among different layers of soil, the upper surface of the layer within 10-12 inch depth is the most productive zone, densely populated with flora, fauna and microbes, which accomplish major transformations in soil. Nonetheless, garden soil was also reported to be a preferred source for isolation of microbes, presumably because of the various biological activities that create suitable conditions for life to exist [16].

Soil samples were collected mainly from garden of P.G. Department of Botany, Utkal University, Odisha, India. This area was selected because it is rich in organic pollutants due to various garden practices and dumping of plant materials.

3.1. Properties of soil

Before stepping into the different aspects of the study, the physical properties of soil were analyzed. The texture of the soil was sandy loam with a pH of 5.9, temperature 31.5ºC, 5.7% water content and 69.2% water holding capacity, respectively. The pH values clearly indicate that the soil is moderately acidic in nature. Acidic soils are usually deprived in nutrient content and consequently, organic carbon, organic matter, organic nitrogen and available phosphorus are least. Water holding capacity of sandy soil is constantly less as compared to other types of soil and lofty in pit soils where humus content is very high. Texturally, the present soil is sandy loam with low organic content and, therefore, the WHC is not high.

3.2. Soil mycoflora study

Maximum numbers of fungal species were isolated from the air followed by soil of garden of P.G. Department of Botany. Utmost numbers of *Aspergillus* species and other fungal species were obtained from the garden soil of P.G. Department of Botany (5×10^5 CFU/g). The frequency distribution of all examined samples for fungi and *Aspergillus* species count varied within the range ≤5% to 15%. In all soil samples, *Aspergillus* species was the most dominated species followed by *Aspergillus terreus*. Fleet and Mian [17] have also reported similar type of findings for the garden soil. All soil samples were enumerated using both dilution (pour) plate and direct plate methods. The mixed fungal plates thus obtained were pure cultured for further separation of the individual colony. Most of the fungi isolated belong to the sub division Deuteromycotina and two members were recognized under Zygomycotina. A total of 19 fungal taxa were isolated from all the samples studied (Table 1). All the species isolated were belonging to 8 genera and the genus *Aspergillus* was the dominated one. *Aspergillus* species were the dominant group having about 9 taxa. *Curvularia* and *Alternaria* have represented by 2 taxa each. *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus nigricans* and *Trichoderma* recorded here were represented by only one species each (Table 1).

Mucor hiemalis and *Rhizopus nigricans* were the two isolates recorded from the sub division Zygomycotina (Fig 1a). One unsporulated white sterile mycelium was also reported from the air sample (Fig. 1b). Surface of the soil was initially scraped by a sterilized spatula followed by horizontally running a sterilized test tube to collect about 10-20 gm of soil.

3.3. Aeromycoflora study

Total twelve fungal species belonging to six genera were recorded from air of the pesticide treated soil area. Genus *Aspergillus* was comprised of four species, *Curvularia* two species and others represented by single species. It is clear from the Fig. 1b that *Curvularia lunata* was the dominant species that contributed maximum to the total isolates and constituted 16.1% of the sum total colonies followed by *Curvularia pallascens* which contributed about 13.8% of the total isolates followed by *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus* sp., *Mucor hiemalis*, *Penicillium* sp., white sterile mycelia, *Rhizopus nigricans* and *Fusarium oxysporum*. Fungus, *Fusarium oxysporum* was contributed only by 2.09% of the total population (Fig. 1b). Air sampling is a most suitable technique to get spores settled on agar medium and grow to colonies in a period of 3-4 days. In spite of certain minor inadequacies, this is widely accepted by various workers. This technique of sampling is more appropriate to check the contamination of air spora [18]. The presence study on aeromycoflora was mainly conducted in the garden environment of Department of Botany, Utkal University. Of all the members *Aspergillus niger*, *A. terreus* and *A. flavus* were the dominant ones. *A. niger* is an aeroallergens causes aspergillosis, a serious bronchital infections. *Aspergillus flavus* is very well known for production of aflatoxin. This toxin is fatal to birds and hazardous to metabolic function. Chakraverty and Sinha [19] have also studied the incidence of *A. parasiticus* for a period five months in indoor and outdoor environment.

Table 1: Occurrence of fungal species in air, soil and pesticide treated soil.

Name of the Fungus	Samples							
	Air	Soil (SP)	Soil (DP)	Cypermethrin treated soil (conc. in ppm)				
				600	800	1000	1200	1400
Alternaria alternata	-	+	-	-	-	-	-	-
Alternaria tenius	+	-	-	-	-	-	-	-
Aspergillus awamori	-	+	+	-	-	-	-	+
Aspergillus candidus	-	-	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+	+	+
Aspergillus fumigatus	-	+	+	+	+	-	-	+
Aspergillus japonicus	-	-	-	-	-	-	+	+
Aspergillus niger	+	+	+	+	+	+	+	
Aspergillus tamari	-	+	+	+	+	+	+	+
Aspergillus terreus	+	+	+	+	+	+	+	+
Aspergillus species	+	+	+	+	+	+	-	-
Curvularia lunata	+	-	-	-	-	-	-	-
Curvularia pallusens	+	-	-	-	-	-	-	
Fusarium oxysporum	+	-	-	+	-	-	-	-
Mucor hiemalis	+	-	+	-	+	+	+	+
Penicillium sp.	-	+	-	-	-	-	-	-
Rhizopus nigricans	+	-	-	+	-	-	-	-
Trichoderma viridae	-	+	-	-	-	-	-	-
White sterile mycellium	+	-	-	-	+	-	-	-

+: present; -: absent; **SP**: Soil plate technique; **DP**: Dilution plate technique

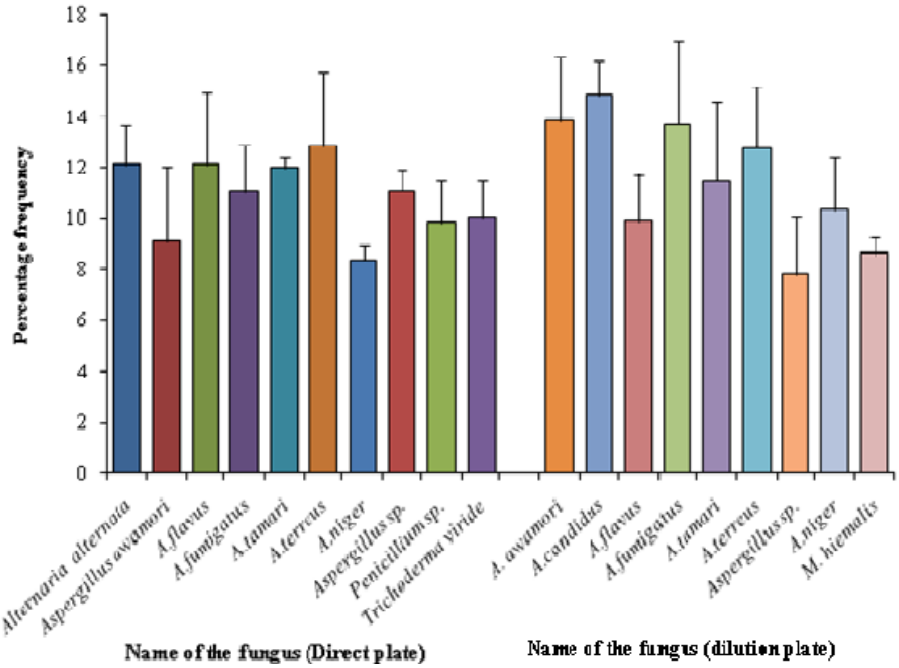


Fig. 1a: Percentage frequency of the isolated fungi recorded from soil (direct and dilution plate) (mean values ± SEM) of botanical garden, P.G. Department of Botany, Utkal University, Odisha, India.

3.4. Identification of isolated fungi

All these fungi were identified by the aid of laboratory experiences, references of certain monographic books [13, 14] and NCFT, New Delhi (Fig. 2a).

The colony colour and texture of *A. terreus* on PDA was as follows; colour: cinnamon to brown, RPC: brownish yellowish, appearance: powdery to dusty: form: irregular to rounded, margin: erose, elevation: raised and furrowed. Phase contrast microscopic study revealed that the conidiophores of *A. terreus* were found to be 100-250×4.5-6 µm, vesicle: hemispherical (10-15 µm long), merging into supporting conidiophores, biseriate sterigmata with primaries crowed, parallel (5-7 × 2-2.5 µm), secondaries closely packed (5.5-7×1.5-2 µm). Conidia were found to be short, smooth, colourless, globose to slight elliptical with 1.5-2.5µm in diameter. *Philides* were biseriate; vesicle: round with loosely radiate head. Hulle cells were solitary, round and produced directly on hyphae with colourless head (Fig. 2b, c, d).

3.5. Effect of cypermethrin on fungal community in soil

The fungal taxa isolated by direct plate method from the soil treated with cypermethrin (600ppm concentration) with their percentage contribution recorded by direct plate method are given in Fig.3a. It was shown from the figure that *Aspergillus flavus* appeared in greater numbers than rest of the fungi. In total ten

species of fungi belonging to four genera were noted. *Aspergillus* was the dominant genus comprised of seven species and others were found as single species. Among the taxa, *Mucor hiemalis* was represented with lesser frequencies as compared to the others. Similarly, *Aspergillus candidus* was contributed highest percentage followed by *Aspergillus flavus* in dilution plate technique. The third dominant one was *Aspergillus terreus*. *Mucor hiemalis* was found to have minimum contribution as compared to the total population isolated (Fig. 3a). Total eight species of fungi belonging to three genera were found when soil was treated with cypermethrin at a concentration of 800ppm. Genus *Aspergillus* was comprised of seven species, *Mucor hiemalis* was only one along with a single white sterile mycelium (WSM). Among the taxa, *A. fumigatus* was found with lesser frequencies as compared to other isolates (Fig. 3b). Results of the dilution plates treated with 800ppm cypermethrin are also given in (Fig. 3b). Data are presented in percentage contribution of each individual isolation. *A. flavus* was always contributed highest percentage followed by *A. candidus*. Among all, the third dominated species was found to be *A. terreus*. Figure 3b explains about the fungal taxa isolated from soil treated with cypermethrin (1000ppm) with their percentage frequencies and percentage contribution recorded by direct and dilution plate methods. *A. candidus* and *A. flavus* were appeared in greater number than other fungi. In total, only six species of fungi were belonging to the genus *Aspergillus*. Among all the genera, *M. hiemalis* was found in least number. In direct plate technique, *A. flavus* was found to be the dominant species as compared to other fungal isolates at 1000 ppm concentration of cypermethrin. Genus *Aspergillus* was comprised of six species followed by only one species of *M. hiemalis*. Except *A. flavus*, others fungi were found to be merely equal in number. Results of the dilution plate treated with cypermethrin 1000 ppm concentration are presented in percentage contribution of individual isolation (Fig. 3b). *A. candidus* and *A. flavus* were found in highest number followed by *A. niger* and *A. terreus*. Results of the dilution plate treated with cypermethrin (1200 ppm concentration) are represented in percentage contribution. In this finding, *A. flavus* was recorded in greater number followed by *A. terreus* and *A. niger* (Fig. 3c). Fungi isolated from soil treated with cypermethrin at 1400 ppm concentration with their percentage frequencies recorded by direct plate method are represented in Fig. 3d. *A. awamori* was found in highest number followed by *A. terreus* and *M. hiemalis*. Results of the dilution plate treated with cypermethrin 1400 ppm are shown in Fig. 3d. In this study, *A. awamori* was found in highest number followed by *A. flavus* and *A. tamari*. *Mucor hiemalis* was represented with least number.

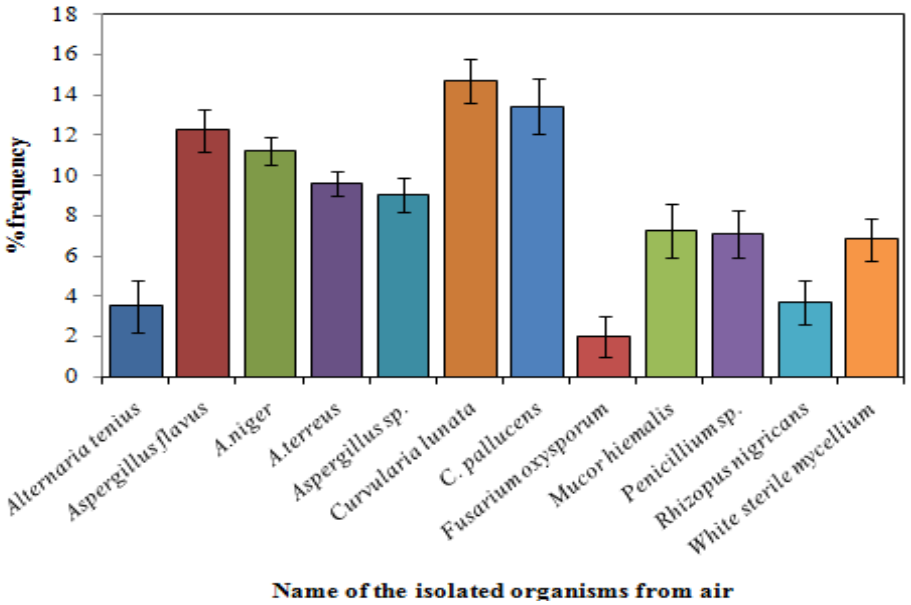


Fig. 1b: Percentage frequency of the isolated fungi recorded from air (mean values ± SEM) of botanical garden, P.G. Department of Botany, Utkal University, Odisha, India.

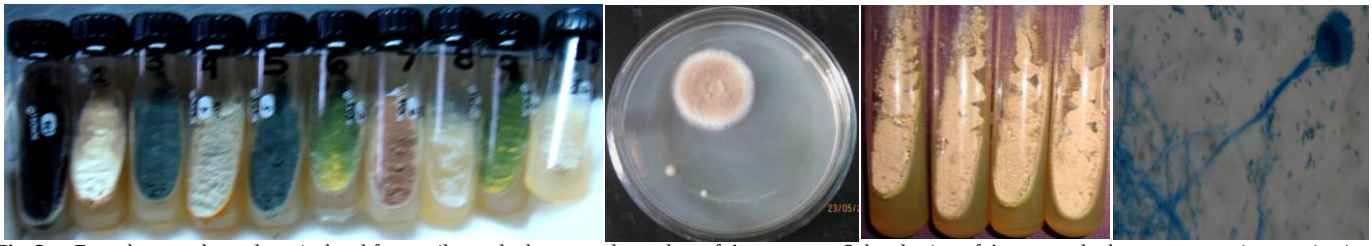
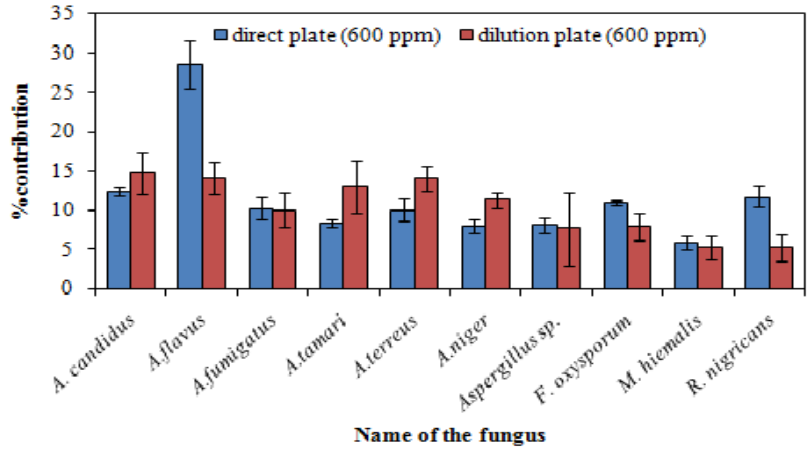
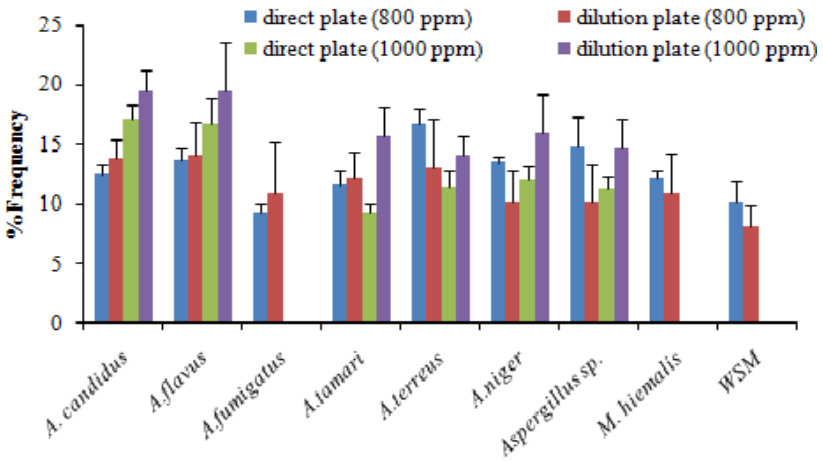


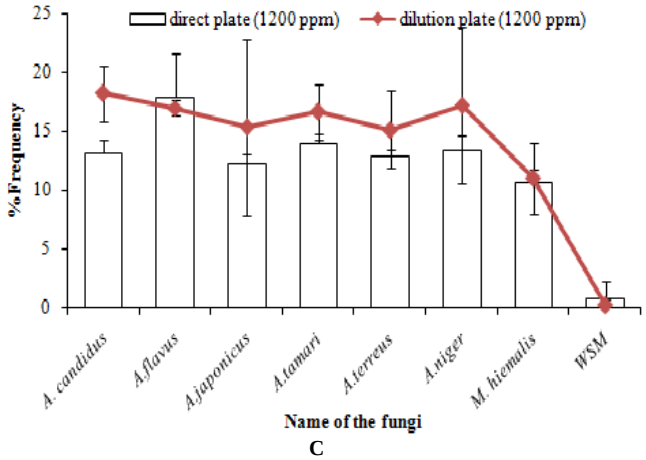
Fig. 2 a: Fungal pure culture slants isolated from soil sample; b: pure culture plate of *A. terreus*, c: Sub culturing of *A. terreus* d: phase contrast microscopic view of *A. terreus*



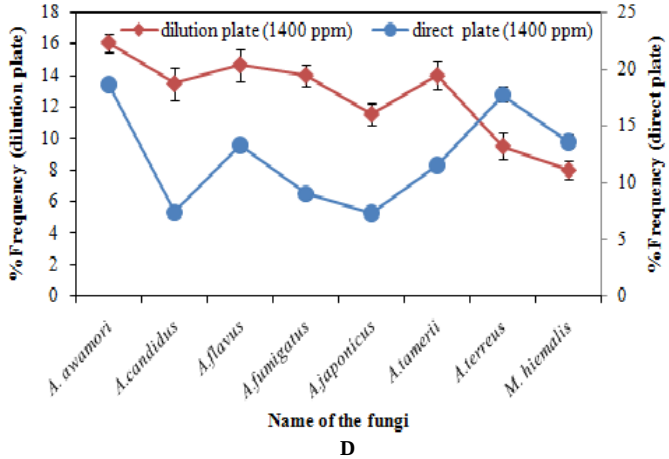
A



B



C



D

Fig. 3 (a-d): Fungi with their percentage contribution and percentage frequency recorded from cypermethrin treated soil (Direct and Dilution plate) (mean values ± SEM)

3.6 Protein hydrolyzing ability of isolated fungi

For the biosynthesis of industrially important enzymes in bulk, isolation, identification and characterization of novel strain is a never-ending process. Furthermore, proteases produced from microorganisms are either constitutive or induced in the presence of specific substrates and their biosynthesis is generally persuaded by alteration in fermentation conditions [20]. So, casein, a milk protein was supplemented with the medium and evaluated for protease production by the native isolates. All test strains showed best hydrolyzing zone formation potential at pH 8.5±0.2. Among

the ten test strains, performance of *A. terreus* was the best in casein hydrolyzing activity (Fig. 4). Strains like *M. hiemalis* and white sterile mycelium were comparatively least efficient (data not shown). Many species of genus *Aspergillus* have also been reported in literature for the biosynthesis of protease [21, 22, 23].

Fig. 4: screening plate of *A. terreus* NCFT 4269.10 displaying protease activity.

4. CONCLUSION

The innate microorganisms of soil mostly respond in three different ways to various chemicals applied. Most of them exhibit a suppressed growth with very low population, some are not greatly affected and few of them are greatly influenced exhibiting high population utilizing the chemicals. The attention may be focused on the sensitive microorganisms that negatively respond to the chemicals applied by greatly reducing the growth and diminish the pattern of transformation of organic and inorganic materials in soil. Though, remarkable developments have been achieved in understanding of the responsibility of soil dwelling organism and their biological action for upholding the soil health; more detailed study is obligatory in achieving feasible protocols for assessment of soil health.

5. ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial assistance provided by UGC-RGNF Scheme, New Delhi, India. One of the authors (BKS) also gratefully acknowledges the assistance of Dr. P. N. Chowdhry, Principal Mycologist, National Center of Fungal Taxonomy, New Delhi, India and Prof. Narayan Behera, Former Visiting Professor, P.G. Department of Botany, Utkal University, Bhubaneswar, India in identifying the isolated microorganisms.

6. REFERENCES

1. Voets J.P., Meerschman P., Verstraete W. Soil microbiological and biochemical effects of long-term atrazine applicationsSoil Biology and Biochemistry. 1974, 6, 149-152.
2. Duah-Yentumi S., Johnson D.B. Changes in soil microflora in response to repeated applications of some pesticides. Soil Biology and Biochemistry. 1986, 18, 629-635.
3. Rai, J.P.N. Effect of long-term 2,4-D application on soil microbial populations. Biology of Fertile Soils. 1992, 13, 427-431.
4. Dumontet S., Perucci P. The effect of acifluorfen and trifluralin on the size of microbial biomass in soil. The Science and Total Environment. 1992, 123-124.
5. Megharaj M. Kondinin Landcare Group Magazine, Farming Ahead, 2002, 121, 37-38.
6. Smith M.D., Hartnett D.C., Rice C.W. Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. Soil Biology and Biochemistry. 2000, 32, 935-946.
7. Pankhurst C.E., Doube B.M., Gupta V.V.S.R. Biological Indicators of Soil, edn. 1997.
8. Sannino F., Gianfreda L., Chemosphere. Pesticide influence on soil enzymatic activities. Chemosphere. 2001, 45, 417-425.
9. Johnsen K., Jacobsen C.S., Torsvik V. Pesticide effects on bacterial diversity in agricultural soils-A review. Biology of Fertile Soils. 2001, 33, 443-453.
10. Doran J.W., Parkin T.B. Defining and assessing soil quality. In: JW Doran, DC Coleman, DF Bezdicek and BA Stewart (ed.) Defining Soil Quality for a Sustainable Environment. Soil Science Society of America Special Publication, 1994, 35, pp 3-21.
11. Gregorich E.G., Carter M.R., Doran J.W., Pankhurst C.E., Dwyer L.M. Biological attributes of soil quality. In: EG Gregorich, and Carter MR. (ed,) Soil Quality for Crop Production and Ecosystem Health. Elsevier, 1997, pp. 81-113.
12. Sethi B.K., Nanda P.K., Sahoo S.L. Isolation, Identification And Conservation Of Potent Hydrolase Producer From Different Soils Of Odisha, India. International Journal of Pharma and Bio Sciences. 2013, 4(2)(B), 89-100.
13. Alexopoulos C.J., Mims C.W. Introductory Mycology. 3rd ed., John Wiley and Sons, New York, 1979.
14. Watanabe T. Pictorial Atlas of Soil and Seed Fungi. 2nd Ed., CRC Press, London, 2002.
15. Hankin L., Anagnostakis S.L. The use of solid media for detection of enzyme production by fungi. Mycologia. 1975, 67(3), 597-607.
16. Grant W.D., Mwatha W.E., Jones B.E. Alkaliphiles: ecology, diversity and applications. FEMS Microbiology Review. 1990, 75, 255-70.
17. Fleet G.H., Mian M.A. The occurrence and growth of yeasts in dairy products. Internet Journal of Food Microbiology. 1987, 4, 145-155.
18. Parkinson D., Gray T.R.G., William S.T. Methods for studying the Ecology of soil microorganisms. International Biological Programme Hand book No-19. Black well Scientific Publications, Oxford, 1971.
19. Chakraverty R., Sinha S. Grana. The Incidence of Aspergillus Parasiticus in the Indoor and Outdoor Environments of Calcutta, India. Grana. 1985, 24(2), 133-135.
20. Beg K.Q., Gupta R. Purification and characterization of an oxidation-stable, thiol-dependent serine alkaline protease from Bacillus mojavensis. Enzyme and Microbial Technology. 2003, 32, 294-304.
21. Ali U.F. Utilization of whey amended with some agro-industrial by-products for the improvement of protease production of Aspergillus terreus and its compability with commercial detergents. Research Journal of Agriculture and Biological Science. 2008, 4(6), 886-891.
22. Negi S., Banerjee R. Characterization of amylase and protease produced by*Aspergillus awamori* in a single bioreactor Food Research International. 2009, 42, 443-448.
23. Saravanakumar K., Thiyagarajan A., Kaviyarasan V. Optimization of medium constituents for the production of extra cellular alkaline Protease by As p e rg illus fis c h e ri using response surface experimental designJournal of Bioscience Research. 2010, 1(3), 118-129.

How to cite this article:

Bijay Kumar Sethi, Subhashree Pradhan, Narayan Behera and Santi Lata Sahoo. Effect of cypermethrin, a pyrethroid insecticide on dynamics of soil microflora. J App Biol Biotech. 2015; 3 (05): 019-025. DOI: 10.7324/JABB.2015.3504