Int.J.Curr.Res.Aca.Rev.2017; **Special Issue-4***: 180-188*

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Special Issue-4 (October-2017)

Journal home page: http://www.ijcrar.com

Pesticides Resistant and Utilization Profile of *Pseudomonas* and *Bacillus* Soil Isolates

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KEYWORDS

Xenobiotic, Pesticide, *Pseudomonas*, *Bacillus*, Endosulfan, Dimethoate, Fenvalerate

A B S T R A C T

Soil contamination by xenobiotic chemicals has become a serious worldwide problem, not only because it reduces the value of land for crop production and habitation, but also because these chemicals are sources of water pollution. Indiscriminate use of these chemicals especially in excess often poses a serious threat to the environment. In this background, native bacterial isolates were screened for their resistance to commonly used farm chemicals. Heterotrophic bacterium isolated from an active farm land soil were characterized and tested for their pesticides resistance and utilization pattern against commonly used pesticide namely, endosulfan, dimethoate and fenvalerate. *Pseudomonas* sp. and *Bacillus* sp. were able to utilize the chosen pesticides as a sole nutrient at lower concentrations. Concentration dependent tolerance can be found through growth pattern analysis. Among the three pesticides, endosulfan was toxic to both the test bacterial isolates. The data presented in this study demonstrates the bioremediation potentials in native soil bacterium.

Introduction

The global population explosion is exerting a phenomenal pressure on the food and fodder requirement. This has resulted in an increase in the fertilizer consumption. In terms of nitrogen alone, the world demand has gone up several million tons. Industrial ammonical N_2 production by Haber-Bosch process depends heavily on fossil fuel. Unfortunately, no major breakthrough is in sight to minimize the energy input for industrial ammonia production. Among the biological N_2 fixation *Azolla* sp and *Anabena* sp symbiosis appear significant and promising for exploitation in agriculture and forestery. In rice ecosystem phototrophic blue green algae and heterotrophic rhisosphere bacteria play a joint role in meeting the nitrogen needs for low land rice cultivation.

Bacteria fungi and algae are frequently employed as biofertilizers in crop fields

(Gaur, 2006). They alter the soil condition favourable for plant growth, which lead to an increased yield. BGA enrich the soil fertility by various ways. These beneficial N_2 fixing organisms face a challenge for survival in the soil habitat as they are exposed to an array of manmade chemicals in the form of pesticides and herbicides (Vilchez and Manzanera, 2011). The competing weeds, destructive insects and other pests consume a large part of the agricultural production. This demands an intensive application of xenobiotic compounds like pesticides and herbicides. These pesticides are posing serious pollution threats to the agricultural land, irrigational water etc., (Saikia and Jain, 2007). Continuous use of agrochemical leads to the accumulation of residues and ultimate pollution of soil and aquatic habitat (Gowrisankar *et al.,* 2002). A large number of chemical fertilizers and pesticides used in various crops have been shown to have adverse effect on the non-target microorganisms, which are of great relevance to the crop ecosystem. (Venkataraman, 1981; Hill and Wright, 1978; Gyaneshwar *et al.,* 2002).

These pesticides and their residues not only affect the target pest but also severely destroy the non-target biota (Hill and Wright 1978). Most of these pesticides inhibit the metabolic activities and metabolites in microorganisms. In order to protect the soil fertility and to ensure the continuance of various micro- and macro-flora, constant monitoring of the fate of these chemicals in soil and water is required (Weller and Thomashow, 1994; Glick, 1995; Probanza *et al.,* 1996). If detected, an early and efficient bioremedial measure needs to be initiated to prevent a large scale, long term and irreversible damage to the ecosystem. In this background, native bacterial isolates were screened for their resistance to select farm chemicals. Furthermore, their metabolic ability to use these chemicals as substrates for their energy metabolism was analyzed.

Materials and Methods

Pesticides employed in this study

The pesticides endosulfan (35%AI) dimethoate (30%AI) fenvalerate (20%AI) were used in this study. These herbicides are used in crop fields more specifically paddy fields to control varieties of weeds including *Cyperus* sp. and *Echinochloa* sp.

Enumeration and characterization of heterotrophic bacterial population

Soil samples from the paddy fields were mixed thoroughly and 25gms soil sample was serially diluted upto 10^{-7} using sterile saline solution (0.85% NaCl). Each dilution was spread plated on sterile air dried nutrient agar (NA) plate. The plates were incubated for 24-48 hrs at 37^{o}C. Morphologically discrete bacterial colonies were streaked for purity and the individual isolates were stored in NA slants at -20^{o}C till further use. The soil heterotrophic bacterial isolates were characterized based on their microscopic, morphological, biochemical and physiological characteristics in accordance to the recommendations of Bergey's manual of systemic bacteriology (1st edition).

Pesticide tolerance pattern of the bacterial isolates

The identified bacterial isolates were tested for their ability to tolerate different concentration of the chosen pesticide *viz.* endosulfan, dimethoate and fenvalerate. A loopful of the fresh broth culture was streaked on an air dried pesticide incorporated (at different concentration of

20 ppm, 40 ppm.... 100ppm) nutrient agar plate. Plates were incubated at 37°C for 24 to 48 hrs. Ability of the bacteria to grow in this indicates their tolerance towards the test chemical at that concentration. Pesticide tolerance pattern was also evaluated in broth culture to obtain a semi-quantitative assessment. For this, the bacterial cultures were inoculated in pesticide amended MSM broth and the growth response recorded spectrophotometrically at OD_{580}.

Evaluation of pesticide utilization by the native bacterial isolates

The pesticide resistant bacterial isolates were tested for their ability to utilize the chosen pesticides *viz.* endosulfan, dimethoate and fenvalerate, either as a sole source of carbon or nitrogen or both.

The overnight broth cultures were streaked on the air-dried mineral salt media (MSM) plates containing 20ppm, 40ppm..... 100ppm of the chosen pesticide as a sole carbon source. In another set, the nitrogen source from the MSM was removed, dextrose 1% (v/v) added as carbon source and this was plated along with 20ppm, 40ppm..... 100ppm of the chosen pesticide. In another set MSM was prepared without both carbon (dextrose) and nitrogen (ammonium nitrate) and the chosen pesticide was incorporated as the sole carbon and nitrogen source. Appropriate positive and negative controls were included.

Pesticide resistant bacterial isolates were inoculated into 10ml nutrient broth, incubated at 37°C with agitation (120 rpm) for 24hrs. One ml of this broth culture was transferred to sterile eppendorf tube and centrifuged at 10,000 rpm for 15 minutes at room temperature. The supernatant was drained completely and the bacterial pellet was resuspended in 1.0 ml of sterile saline (0.85% NaCl). This was washed twice with sterile saline through intermittent centrifugation. After saline wash, bacterial cells were suspended in 1.0 ml sterile saline. Pesticide incorporated MSM agar was air-dried and the saline suspension of the chosen bacterial isolate was streaked on this. Inoculated plates were incubated at 37°C for 2-5 days. Presence of bacterial growth with zone of clearance reveals their ability to utilize the test chemical as the sole carbon/nitrogen or both.

Results and Discussion

A large part of agricultural input goes towards the control of competing weeds, destructive insects and other pests. Pesticides and herbicides unambiguously are a boon to agriculture. These chemicals save the crop plants, there by enhance the overall agricultural produce. Owing to their economic feasibility and easy application methods, they are extensively used. In spite of all these advantages there is one main handicap about their usage, environmental pollution. Constant use of these chemicals tends to have negative impact on the beneficial microflora and in turn on the soil productivity. They not only affect the target pests but also severely destroy the non-target biota. The extensive use of pesticide may lead to their accumulation in plant cells and affect metabolic activities as well as secondary systems involved in food chain.

In this background, bacterial species isolated from crop field were evaluated in this study for their ability to resist and utilize commonly used pesticides. The population of heterotrophic bacteria was recorded at 10^4 CFU/gm. This is considerably lower than expected considering the constant agricultural activity in the farm land with tons of nutrients applied in soil. Possible indication of the deleterious impact of

pesticides on soil microflora. The isolated bacterial strains were identified to belong to the genus *Pseudomonas* and *Bacillus*. The isolated organisms were evaluated for their ability to tolerate 3 most commonly used pesticides namely, endosulfan, dimethoate and fenvalerate. The data presented in table 1 reveals the pesticide resistance pattern of both *Pseudomonas* sp. and *Bacillus* sp. isolates.

Table.1 Pesticide resistance pattern of the chosen soil bacterial isolates

Pesticide tested	Concentration (ppm)	*Bacillus*. sp	*Pseudomonas*. sp
Endosulfan	20	+	+
	40	+	+
	60	+	+
	80	+	+/-
	100	-	-
Dimethoate	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	-	-
Fenvalerate	20	+	+
	40	+	+
	60	+	+
	80	+	-
	100	-	-

+ = Presence of growth; - = Absence of growth

Table.2 Pesticide utilization pattern of the soil bacterial isolates – as a sole carbon source

Pesticide tested	Concentration (ppm)	*Bacillus*. sp	*Pseudomonas*. sp
Endosulfan	20	+	+
	40	+	+
	60	-	-
	80	-	-
	100	-	-
Dimethoate	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	-	-
Fenvalerate	20	+	+
	40	+	+
	60	+	+
	80	-	-
	100	-	-

+= Presence of growth; - = Absence of growth

Table.3 Pesticide utilization pattern of the soil bacterial isolates – as a sole nitrogen source

Pesticide tested	Concentration (ppm)	*Bacillus*. sp	*Pseudomonas*. sp
Endosulfan	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	-	+
Dimethoate	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	+	-
Fenvalerate	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	-	-

+ = Presence of growth; - = Absence of growth

Table.4 Pesticide utilization pattern of the soil bacterial isolates – as a sole carbon and nitrogen source

Pesticide tested	Concentration (ppm)	*Bacillus*. sp	*Pseudomonas*. sp
Endosulfan	20	+	+
	40	+	+
	60	+	+
	80	-	-
	100	-	-
Dimethoate	20	+	+
	40	+	+
	60	+	+
	80	-	+
	100	-	-
Fenvalerate	20	+	+
	40	+	+
	60	+	+
	80	-	+
	100	-	-

+ = Presence of growth; - = Absence of growth

Table.5 Pesticide utilization pattern of the soil bacterial isolates – with carbon and nitrogen supplement

Pesticide tested	Concentration (ppm)	*Bacillus*. sp	*Pseudomonas*. sp
Endosulfan	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	-	+
Dimethoate	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	+	-
Fenvalerate	20	+	+
	40	+	+
	60	+	+
	80	+	+
	100	+	+

+ = Presence of growth; - = Absence of growth

Fig.1 Survival pattern of soil bacterial isolates in the presence of endosulfan

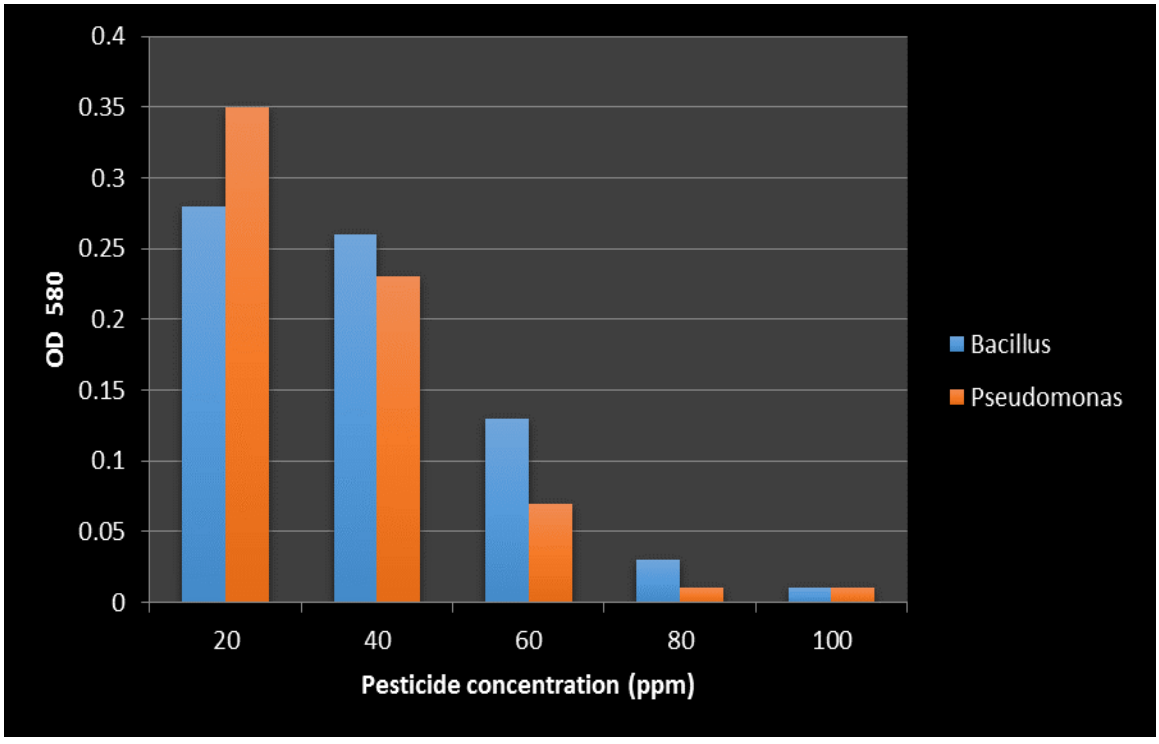


Fig.2 Survival pattern of soil bacterial isolates in the presence of Dimethoate

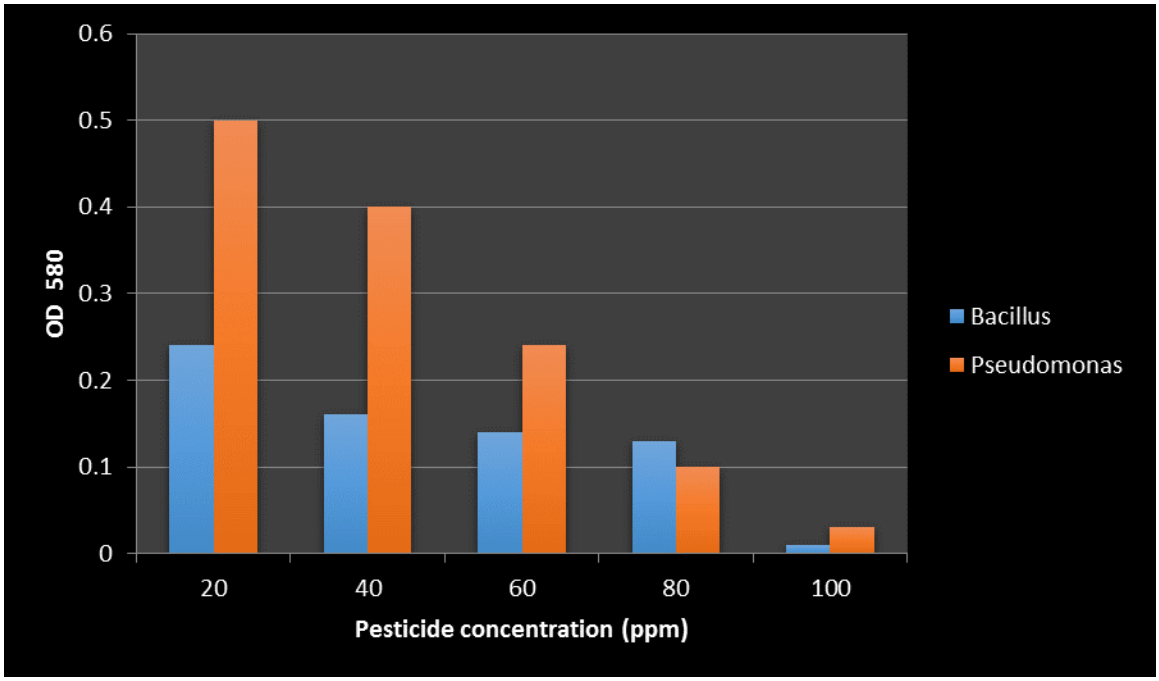
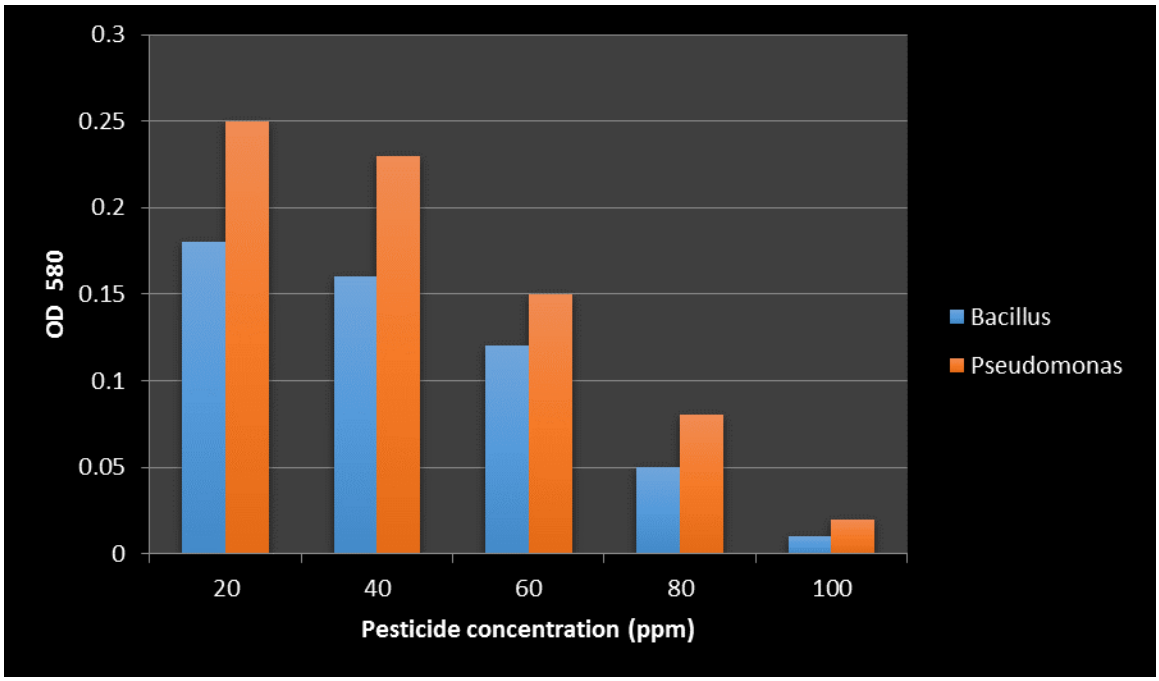


Fig.3 Survival pattern of soil bacterial isolates in the presence of Fenvalerate



The pesticide resistance ability of *Pseudomonas* sp. was recognized and reported by many researchers. Grover *et al.*, (1993) have reported the incidence of chlorobenzoate resistant *Pseudomonas putida*. Entry *et al.*, (1994) have remarked the pesticide resistance and utilization pattern of various *Pseudomonas* strains

exposed to atrazine and 2,4, -D. Analyzing our data, it can be inferred that *Pseudomonas* sp. and *Bacillus* sp. can tolerate the farm chemicals, endosulfan and dimethoate up to 80ppm. *Bacillus* sp. can tolerate the test chemical namely, fenvalerate up to 80ppm concentration, compared to that of the *Pseudomonas* sp. up to 60ppm of concentration (Table 2-5)

In the present investigation, both the test bacterial isolates were found to be very effective in utilizing test chemicals as the sole carbon and nitrogen source. Hence, the organisms can be considered as promising candidate for decontamination of pesticide polluted habitat. Utilization of pesticide is often considered as an index of the ability of the bacterium to degrade the target pesticide. Utilization of farm chemicals is by means of catabolic metabolism or co-metabolism. Entry *et al.,* (1995) reported catabolic transformation of 2,4 –D in riparian forest. *Pseudomonas* sp. and *Bacillus* sp. can utilize target chemicals as a sole carbon and nitrogen sources at low concentrations through catabolic pathways. Ability of microbes to degrade chemicals through carbolic processes was recognized by many workers. Javanjal and Deopurkar (1994) have reported the biodegradation of p-nitrophenol by indigenous isolates through catabolic processes. Perich and Lockwood (1978) have reported the pesticide utilization and transformation by soil microbes.

In growth pattern analysis, concentration dependent tolerance can be noted among the test bacterial strains in the present study (Fig 1 to 3). Inverse relationship was found between growth pattern and herbicide concentration in both the strains. Pesticides are often reported to be highly recalcitrant with the tendency of exerting non-target effects on micro and macro flora. Persistence of their chemicals and their metabolites were often reported to change the ecology of a particular ecosystem (Percich and Lockwood 1978). This observation necessitates the use of proper bioremediation procedures in order to safeguard the ecosystem.

This investigation reiterates that the continuous exposure of pesticides in an ecosystem would result in bioaccumulation there by damage the wild varieties of microorganisms due to their persistence in the environment. Hence, it becomes the responsibility of the scientific community to frame out suitable bio-remedial strategies to overcome pesticide pollutions.

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How to cite this article:

Sumithira P. and Gowrisankar R. 2017. Pesticides Resistant and Utilization Profile of Pseudomonas and Bacillus Soil Isolates. *Int.J.Curr.Res.Aca.Rev.* Special Issue-4: 180-188.