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Article

Impact of Insecticides and Fungicides on Bacterial Population in Groundnut (*Arachis hypogaea*. L.) Soils

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ABSTRACT

A field experiment was conducted at Sri Krishnadevaraya University, Anantapuramu, India to study the population of bacteria in groundnut soil. Most important annual oil seed crop is the groundnut. The yield of crop depends on various agronomic management practices. The groundnut bacterial composition in the rhizosphere is important for the performance of plant as bacterial species can have beneficial, neutral, or harmful relationships with the roots. Bacteria are important in process such as nitrification, denitrification, and nitrogen fixation. Soil bacteria play an important role in the global cycling of carbon and other elements. Soils containing a high microbial diversity are characteristic of a healthy soil-plant relationship. Soil samples (red sandy loam and black clay soils) were collected from groundnut (*Arachis hypogaea* L.) cultivated fields of Anantapuramu District of Andhra Pradesh, were treated with selected insecticides – bifenthrin, buprofezin and fungicides-dimethomorph, pyraclostrobin at different concentrations, that is, 10, 25, 50, 75, and 100 ppm which are equivalent to field application rates (1.0, 2.5, 5.0, 7.5, and 10.0 kg/ha) in the laboratory. Results of the study showed that the bifenthrin, buprofezin, dimethomorph, and pyraclostrobin significantly improved the bacterial population in 10 days incubated both red and black soil samples. Bifenthrin and buprofezin at concentrations ranging from 1.0 to 5.0 kg/ha gradually increased the population of bacteria and reached maximum at 5.0 kg/ha. Beyond 5.0 kg/ha the above pesticides shown negative effect on bacterial population at 10.0 kg/ha, whereas the bacterial population had decreased at concentration of 7.5–10.0 kg/ha. Whereas dimethomorph and pyraclostrobin at the concentrations of 1.0 and 2.5 kg/ha showed marked increase in bacterial populations, and beyond this concentration the bacterial population reached minimum at 10.0 kg/ha in both black and red soils.

Key words: Bacteria, Insecticides, Fungicides, Groundnut (*Arachis hypogaea L.*) Soils.

1. INTRODUCTION

Agriculture began thousands of years ago, currently the important role of the desirable interactions between microorganisms and plants in improving agricultural production. Groundnut (*Arachis hypogaea* L.) is famous sources of food occupies major position in the economy of developing nations. The area of soil around plant roots, known as the rhizosphere narrow region of substrate, contains higher populations and greater diversity of microorganisms than soil with no flora [1]. Plant-associated microbes such as endophytic bacteria and fungi may enhance plant growth and health by considerable activities such as fixation of nitrogen, synthesis of plant hormones and vitamins, and the upgrade of nutrient uptake. Biological control is a promising strategy for sustainable groundnut cultivation. This is because plant roots release exudates into the land that increases microbial activity by supplying nutrients to the organism [2]. Microbes present in soil include actinomycetes, fungi, algae, bacteria, archaea, and protozoa involved in many important roles. Soil microorganisms play a key role in maintaining soil structure, soil health, soil erosion control, ecosystem function, water holding capacity, and crop productivity. Many organisms are existed in the uppermost fragile layers of soil with organic matter, usually the top 2–3 cm where food sources are plentiful [3]. Groundnut is a leguminous edible oilseed crop. Both in worldwide as well as in India share of groundnut to total oilseed are considerable. The analysis of ecological, structural, and functional properties of soil microbial communities can allow a better understanding of the effect of pesticides on them, under field conditions [4]. To control plant diseases, biocontrol factors such as *Bacillus* and *Pseudomonas* have been used for a long time [5-7]. The organic matter consisting of plant and animal detritus of soil and plays an important role in maintenance and improvement of soil fertility and productivity. In the soil, micro biota plays major activity in the pesticides breakdown. Many microbes are capable of utilizing pesticides as sources of carbon. Using plenty of chemical nutrients and plant protection chemicals in agriculture showed negative effect on environment, soil, and water [8-10]. A complex matrix of organic and inorganic constituents of soil, particularly rhizosphere, creates a unique and dynamic environment for the microorganisms which affect plants and other associative microorganisms. The soil microbial biomass is the labile pool of organic matter and acts as both source and sink of

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plant nutrients. It plays a crucial role in nutrient cycling and is important in maintaining soil fertility and nutrient concentration. Groundnut is a major oil seed cash crop which is cultivating in lakhs of hectares in Andhra Pradesh region. Groundnut (*A. hypogaea* L.) is an important food crop with an excellent sources of proteins, carbohydrates, vitamins, and minerals contained within seeds [11]. Due to the importance of groundnut crop growing in Ananthapuramu District which is a semi-arid region of Andhra Pradesh, those soils were selected for the present investigation of our research work. The use of pesticides to protect crops may alter the biological ability either by direct or indirect action.

2. MATERIALS AND METHODS

2.1. Soils Used in the Present Study

Agricultural soil samples such as samples of black clay soil and red sandy loam soil collected from groundnut cultivated fields of Anantapuramu District of Andhra Pradesh, India in a semi-arid zone from the depth of 12 cm and mixed thoroughly to prepare a homogenate composite sample. Soil samples air dried at room temperature were cleaned by removing plant material and other debris and passed through a 2 mm sieve and stored at 4°C before analysis.

2.2. Analysis of Physicochemical Characteristics of Soil Samples

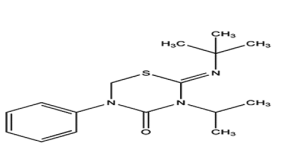
Mineral matter of soil samples such as sand, silt, and clay contents were analyzed with the use of different sizes of sieves by following the method of Alexander [12]. Water holding capacity of the soil samples was determined by adding distilled water up to the saturation point, and then, 60% water holding capacity of the soil samples was calculated by Johnson and Ulrich [13]. pH of soil samples was determined by mixing soil and water in the ratio of 1:1.25 using systronics digital pH meter with calomel glass electrode. Organic carbon content in soil samples was estimated by Walkey-Black method and the organic matter was calculated by multiplying the values with 1.72 [14]. Electrical conductivity of soil samples was measured by a conductivity bridge. Total nitrogen content in soil samples was determined by the method [14]. The inorganic ammonium nitrogen content in the soil samples after extraction of 1 M KCl by Nesslerization method [14] and contents of nitrite nitrogen [15] and the contents of nitrate-nitrogen by Brucine method [16] after extraction with distilled water were determined, respectively.

Physicochemical characters of the two soil samples are listed in Table 1.

2.3. Pesticides Used in the Present Study

2.3.1. Chemical structures

Insecticides



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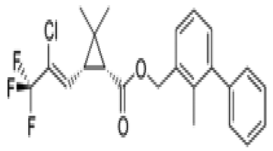
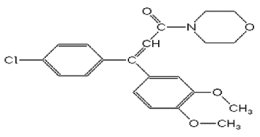
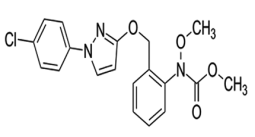


BIFENTHRIN

FUNGICIDES



DIMETHOMORPH



PYRACLOSTROBIN

Table 1: Physicochemical properties of soils used in the present study

Properties	Black clay soil	Red sandy loam soil
Sand (%)	64.4	52.5
Silt (%)	24.6	27.9
Clay (%)	9.2	19.6
pH[a]	7.5	6.4
Water holding capacity (mL/g soil)	0.45	0.32
Organic matter (%)[b]	1.44	0.74
Total nitrogen (%)[c]	0.089	0.048
NH4+ - N (µg/g soil)[d]	8.57	7.02
NO2 - - N (µg/g soil)[e]	0.45	0.66
NO3 - - N (µg/g soil)[f]	0.92	0.74

[a]1:1.25=Soil: Water slurry. [b]Walkley-Black method (Johnson and Ulrich, 1960). [c]Micro-Kjeldahl method (Johnson and Ulrich, 1960). [d]Nesslerization method (Johnson and ulrich, 1960). [e]Diazotization method (Ranney and Bartlett, 1972). [f]Brucine method (Barnes and Folkard, 1951)

Bifenthrin is a world renowned, new generation, the broad spectrum insecticide of pyrethroid group markar through its contact and stomach action controls different types of larvae, whitefly, mites, and jassids very effectively.

Buprofezin is an insecticide used for control of insect pests such as mealy bugs, leafhoppers, and whitefly.

Dimethomorph is a morpholine fungicide with systemic function. It is used for treating mildews and jassides very effectively.

Pyraclostrobin is a strobilurin which is a group of systemic fungicide used to control major plant pathogens.

2.4. Soil Incubation Studies

2.4.1. Population of bacteria

To determine the effect of selected pesticides, with concentrations of 10, 25, 50, 75, and 100 µg/g soil on the population of bacteria, 5 g portions of each soil samples were placed in 15 × 150 mm test tubes and were treated with different concentrations of pesticides, which were equivalent to 1.0, 2.5, 5.0, 7.5, and 10.0 kg/ha [17]. The soil samples receiving only distilled water served as controls. Soil samples were then homogenized to distribute the pesticide and sufficient distilled water was added to maintain at 60% water holding capacity (WHC) and incubated at room temperature (28 ± 4°C). After 10 days of incubation, triplicates of each treatment were withdrawn for the estimation of bacterial population. Aliquots were prepared from 10^{-1} to 10^{-7} from treated and untreated soil samples by serial dilution plate method on nutrient agar medium and subsequently incubated for 48 h in an incubator at 37°C. After incubation, bacterial colonies grown on nutrient agar medium were counted by Quebec colony counter. Bacterial populations were enumerated and expressed as number of colonies formed per gram of soil (dry weight basis) [18]. Once the stimulatory concentrations of pesticides were determined, the soil samples were, further, incubated for 20, 30, and 40 days for enumeration of bacterial populations.

2.4.2.Composition of nutrient agar medium

Peptone	:	5.0 g
Beef extract	:	3.0 g

Agar-Agar	:	20.0 g
Distilled water	:	1000 mL
pH	:	7.0

3. RESULTS AND DISCUSSION

3.1. Population of Bacteria

In the soil ecosystem, bacteria play a significant role in the global nitrogen cycling of carbon and other elements. The bacterial cell number was increased in all pesticides treated soils up to 5.0 kg/ha than the controls in 10 days incubated soil samples [Table 2]. The improvement in bacterial populations continued up to 20 days and, then, gradually decreased after 30 and 40 days of incubation [Figure 1a and b]. Bifenthrin, buprofezin, dimethomorph, and pyraclostrobin significantly improved the bacterial population in 10 days incubated both red and black soil samples. Bifenthrin and buprofezin at concentrations ranging from 1.0 to 5.0 kg/ha gradually increased the population of bacteria and reached maximum at 5.0 kg/ha. Beyond 5.0 kg/ha, the above pesticides shown negative effect on bacterial population at 10.0 kg/ha, whereas the bacterial population had decreased at concentration of 7.5–10.0 kg/ha. At the end of the 10 days of incubation period, about 27–120 and 30–144% and 47–160 and 56–193% increase in the population of bacteria was observed in black and red soil treated with bifenthrin and buprofezin compared to controls [Table 2], whereas dimethomorph and pyraclostrobin at the concentrations of 1.0 and 2.5 kg/ha showed marked increase in bacterial populations and beyond this concentration the bacterial population reached minimum at 10.0 kg/ha in both black and red soils. At the end of 10 days of incubation period, increased in bacterial populations were observed in black soil 13–116 and 20–104% and 17–139 and 28–150% in red soil treated with dimethomorph and pyraclostrobin in comparison to control soil samples [Table 2]. Lambda cyhalothrin applied at different concentrations; however, the stimulatory effect was observed at 5.0 kg/ha in soils on bacterial population [19].

Values plotted in the table are means of triplicates [20] observed no effect on the bacterial populations at concentrations of 60–400 ppm of captan. Ampofo *et al.* [21] noticed that there was a high population in cerox treated soils. Ismail *et al.* [22] showed that microbial

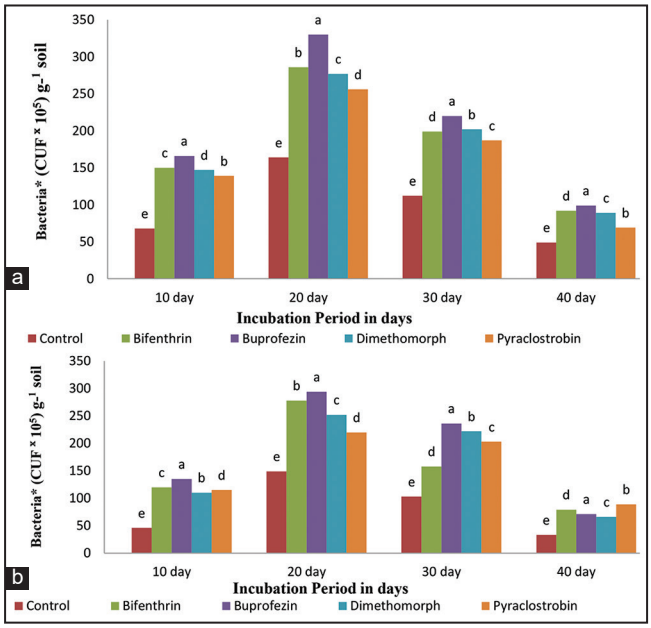


Figure 1: (a and b) Effect of bifenthrin and buprofezin at 5.0 kg/ha dimethomorph and pyraclostrobin, respectively, at 2.5 kg/ha on the population of bacteria* in (a) black and (b) red soil. Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to (DMR) test. *Values plotted in figure are means of triplicates.

Table 2: Effect of pesticides on population of bacteria* (CFU×10^{-5}/g soil) in black and red soil after 10 days

Pesticide concentration	Bifenthrin	Buprofezin	Dimethomorph	Pyraclostrobin
Black soil				
0.0	68e (100)	68e (100)	68e (100)	68e (100)
1.0	87d (127)	89c (130)	77c (113)	82d (120)
2.5	127b (186)	121b (177)	147a (216)	139a (204)
5.0	150a (220)	166a (244)	107b (157)	96b (141)
7.5	59c (86)	48d (70)	52d (76)	39c (57)
10.0	63f (92)	45f (66)	57f (83)	52f (76)
Red soil				
0.0	46e (100)	46e (100)	46e (100)	46e (100)
1.0	68c (147)	72c (156)	54d (117)	59c (128)
2.5	79b (171)	75b (163)	110a (239)	115a (250)
5.0	120a (260)	135a (293)	71b (154)	62b (132)
7.5	42d (91)	36d (78)	32c (69)	29d (63)
10.0	36f (78)	39f (84)	44f (95)	40f (86)

$$\text{*Number of colonies per gram soil} = \frac{\text{No. of. colonies} \times \text{Dilution factor}}{\text{Dry weight of soil}}$$

Figures, in parenthesis, indicate relative production percentages.
Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test

population decreased when the concentrations of metsulfuron-methyl increased during the first 3–9 days after application. Phorate and fenvelerate stimulated the populations of bacteria by influencing diversity of bacterial population in soil both under laboratory and field conditions [23] and [24]. Similarly, Zain *et al.* [25] reported a drastic inhibition of bacterial populations by paraquat to about 70–82% at recommended rate. Wainwright and Pugh [26] also observed an increase in bacterial number in laboratory incubated soils treated with captan. In contrast to these results, significant stimulation of soil bacteria by chlorpyrifos has also been reported [27-30]. Cyfluthrin appreciably increased the population of bacteria after 2 weeks of incubation [31].

4. CONCLUSION

The results of the present study clearly indicate that application of the insecticides and fungicides, in cultivation of groundnut, at field application rates (2.5–5.0 kg/ha) significantly enhanced the bacterial populations, in both black and red soils. However, higher concentrations (7.5 and 10 kg/ha) of the pesticides were either innocuous or toxic to the bacterial populations in soils. Little is known on the impact of insecticide and fungicide on the bacterial populations in groundnut cultivating black and red soils. Therefore, further study is needed to evaluate the influence of insecticide and fungicides on the bacterial populations in agricultural soils which are important and affect organic matter decomposition and nutrient cycling of soils.

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***Bibliographical Sketch**

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