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Pesticides Induced Alternations in Plant Growth Hormone (IAA) in RHIZOBACTERIA

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Abstract: Four pesticides Endosulfan, Lambda cyhalothrin, Monocrotophos and Macnozeb were applied at recommended and higher rates to examine their effects on the plant growth promoting activities of the pesticides tolerant Pseudomonas sp, Klebsiella sp and Azospirillum under in vitro conditions. Pesticides at recommended dose had little effect while the dose higher than the recommended one adversely affected the physiological traits, like indole acetic acid synthesis. Among all pesticides, Lambda cyhalothrinand Macnozeb in general, showed maximum toxicity to plant growth promoting activities. Endosulfan-tolerant Pseudomonas sp and Azospirillum sp have inherent ability to produce growth regulators. It can be used as a bio-inoculant to increase the productivity of crops grown in pesticide stressed soils.
Keywords: Indole acetic acid, pesticides, Rhizobacteria

I. INTRODUCTION

Soil microbes (Rhizosphere and non-rhizosphere) are play an important role for plant growth. Plant growth promoting rhizobacteria accounts for about 2-5% of total rhizobacteria involved in plant growth promotion [1]. These bacteria use one or more direct or indirect mechanisms to improve the growth and health of plants. The phosphate solublization, nitrogen fixation, nutrient uptake and phytohormones production like IAA are some of the regulators that profoundly influence plant growth [2]. Biofertilizers are attention to be a safe alternative to chemical fertilizers to minimize the ecological disturbance. *Azotobacter* and *Azospirillium* are non-symbiotic bacteria; it produces growth promoting substances which improve seed germination and growth of saprophytic and pathogenic microorganisms near the root system of crop plants. *Rhizobium* sp for IAA production and effect of tryptophan concentration, carbon sources and pH on IAA production was assessed [3]. Nowadays many pesticides, herbicides were applied in the crop plant for controlling the pests. Unfortunately, many pesticides can kill more than just their intended targets, namely the necessary micro-organisms in the soil. When chemicals are used for a period of time on plants in an area, they will eventually leach into the soil. The present study was designed to evaluate the effect of pesticides such as Endosulfan, Lambda cyhalothrin, Monocrotophos and Macnozeb on *Azospirillium sp. Pseudomonas sp.* and *Klebsiella sp* for IAA production

II. MATERIALS AND METHODS

A. Isolation of IAA producing bacteria

For enrichment of IAA producing bacteria, 0.1 gm of soil sample was added in 250ml capacity conical flask containing 100ml of sterile nutrient broth supplemented with 0.1 % (w/v) tryptophan. The flask was then incubated at 37^0C for 72 hrs on rotary shaking incubator at 200 rpm. After incubation sample from the incubated flask was further streaked on sterile nutrient agar (N.A.) plates containing 0.1 % (w/v) Tryptophan. These plates were incubated at 37^0 C for 24 hrs. The isolated colonies were inoculated in 10 ml of sterile nutrient broth tubes supplemented with 0.1 % (w/v) of tryptophan and incubated 37^0 C for 24 hrs. 2 ml broth from each tube was centrifuged at 7000 rpm for 10 minutes at 40C. 1 ml of supernatant was mixed with 4 ml of Salkowaskey reagent and the tubes were incubated at room temperature in dark for 30 minutes and observed for development of pink colour.

B. Identification of isolates

Isolated bacteria were identified by morphology and the biochemical tests were carried out with standard methods (IMViC. urea utilization, ammonia production, nitrate utilization, H2S production, catalase, oxidase activity and various carbohydrates utilization) [4].

C. Standardization of IAA

Different concentrations of IAA were prepared in nutrient broth ranging from 1 to10 µg/ml. For estimation of IAA, 1 ml of broth

was mixed with 4 ml of Salkowskaya reagent and incubated at room temp in dark for 30 mins. After incubation the absorbance of the mixture was measured at 530 nm using UV/Visible spectrophotometer. Standard graph of concentration of IAA against absorbance was plotted and used for estimation of unknown concentration of IAA during subsequent studies.

D. Effect of Pesticide concentration for IAA production

Sterile nutrient broth tubes supplemented with 0.1 % (w/v) tryptophan were prepared and pesticide was added to the individual tubes at varying concentration viz., 0.25%,0.50%, 0.75%, 1.0% and 1.25%. The tubes were inoculated with culture and incubated at 370C for 4 days. After incubation the concentration of IAA was determined using Salkowskaya method as mentioned above.

III. RESULTS AND DISCUSSION

In the present study, the non-rhizosphere bacteria were isolated from garden soil sample and it was identified as *Azospirillum sp, Pseudomonas sp* and *Klebsiella sp* by Bergy's manual of systematic bacteriology [4]. The isolated strains were exposure to various concentrations (0.25% to1.25%) of four different pesticides in well assay method. A process of developing tolerance to pesticides is a complex physiological and genetic character. It may be temporary or permanent tolerance. Microorganisms that developed resistance to xenobiotics, such as pesticides, are frequently capable of biodegrading them and are able to bioremediate the soils [5]. In our study, the isolates were tolerated up to 1.25% of pesticides.

Physiological changes inducing temporary resistance can establish a new metabolic pattern, which tends to bypass a biochemical reaction inhibited by a specific toxin. However, it may also increase the potential of bacterial metabolites or enzymes which inhibit the action of the pesticides through detoxification [6]. On the other hand, permanent tolerance (resistance) occurs due to genetic modifications, inherited by the subsequent generation of microbes. The rate of genetic variations is particularly high in microbes and the frequency of evolved resistance depends on the gene mutation frequency [7].

The production of indole acetic acid was varies from bacteria to bacteria and pesticide to pesticide (Table 1-4). *Pseudomoas* sp and *Azospirillum* sp are higher resistant to endosulfan than other pesticides and these bacteria were produced IAA up to 1.25% concentration of pesticide. *Klebsiella* sp did not produce IAA because the endosulfan may be inhibited or change IAA producing metabolic pathway. Normally endosulfan is frequently and more used in the crop field compared to others. Hence *Pseudomonas sp* and *Azospirillum sp* were resistant to endosulfan and produce IAA (Table 1). The results of endosulfan degradation indicate that the enzyme responsible was probably a monooxygenase, converting endosulfan into endosulfan sulfate. Endosulfan is converted in to endosulfan sulfate, endosulfan diol, endosulfan ether, endosulfan hydroxyl ether and endosulfan lactone [8]. It was found that the concentration of IAA decreased with increase in concentration of pesticides. If the concentration is further increased the IAA production was completely inhibited. IAA production by the two bacteria (PBS-2 and PBS-4) was inhibited at the concentration of 200 µl/100ml. IAA productions by the four bacteria (PBS-1, PBS-3, PBS-5 and PBS-6) was inhibited at the concentration of 250 µl/100ml [9]. our results also coincides this findings, but in our findings the isolated bacteria (*Pseudomonas* and *Azospirillum*) was produce IAA up to 1250 µl/100ml of endosulfan. *Azospirillum* sp only tolerate and produced IAA up to 1250 µl/100ml in Monocrotophos pesticides (Table 3). It is evident from data that pesticide residue persisted in the agricultural soil may enhance the plant growth promoting ability of bacteria. Table 2 and 4 shows that the isolated three strains tolerate the two (Lambda cyhalothrin and Macnozeb) pesticides but did not produce the IAA compound.The medium unsupplied with pesticides, the *Mesorhizobium* sp. strain MRC4 produced significant amount ($p \leq 0.05$) of IAA (44 µg/ml). In contrast, the quantity of IAA released by the *Mesorhizobium* sp. strain MRC4, however, decreased progressively with graded-increment of each pesticide in LB broth. Of herbicides, insecticides and fungicides, most severe effect on IAA synthesis was evident in the presence of glyphosate, imidacloprid and hexaconazole, respectively [10]. For example, glyphosate decreased IAA significantly ($p \leq 0.05$) by 14%, 18% and 25%, imidacloprid by 9%, 16% and 20% and hexaconazole by 36%, 43% and 52% at 1·, 2· and 3·, respectively. While comparing the concentrations and types of pesticides, hexaconazole in general had the most toxic effect on IAA biosynthesis by the *Mesorhizobium* sp. strain MRC4.

IV. CONCLUSION

The present study gives some indication of the toxicological consequences and careless application of pesticides in crop plants. The strain *Pseudomonas sp* and *Azospirillum* sp with the inherent ability to produce growth regulators even in the presence of pesticides can be exploited as a bio-inoculant to increase the productivity of crops grown in pesticide contaminated soils.

Table 1.Effect of various concentration of endosulfan on Rhizobacteria for the production of IAA (µg/ml)

S.No	Organisms/ Conc.	control	0.25%	0..50%	0.75%	1.0%	1.25%
1	*Pseudomonas sp*	36	38	33	30	38	29
2	*Klebsiella sp*	22	-	-	-	-	-
3	*Azospirillum sp*	43	40	37	37	38	31

Table 2. Effect of various concentrations of Monocrotophos on Rhizobacteria for the production of IAA (µg/ml)

S.No	Organisms/ Conc.	control	0.25%	0..50%	0.75%	1.0%	1.25%
1	*Pseudomonas sp*	30	31	24	-	-	-
2	*Klebsiella sp*	25	26	-	-	-	-
3	*Azospirillum sp*	37	40	36	29	24	25

Table 3. Effect of various concentration of Lambda cyhalothrin on Rhizobacteria for the production of IAA (µg/ml)

S.No	Organisms/ Conc.	control	0.25%	0..50%	0.75%	1.0%	1.25%
1	*Pseudomonas sp*	36	-	-	-	-	-
2	*Klebsiella sp*	15	9	4	-	-	-
3	*Azospirillum sp*	10	5	-	-	-	-

Table 4. Effect of various concentration of Macnozeb on Rhizobacteria for the production of IAA (µg/ml)

S.No	Organisms/ Conc.	control	0.25%	0..50%	0.75%	1.0%	1.25%
1	*Pseudomonas sp*	22	19	10	-	-	-
2	*Klebsiella sp*	26	18	-	-	-	-
3	*Azospirillum sp*	19	11	-	-	-	-

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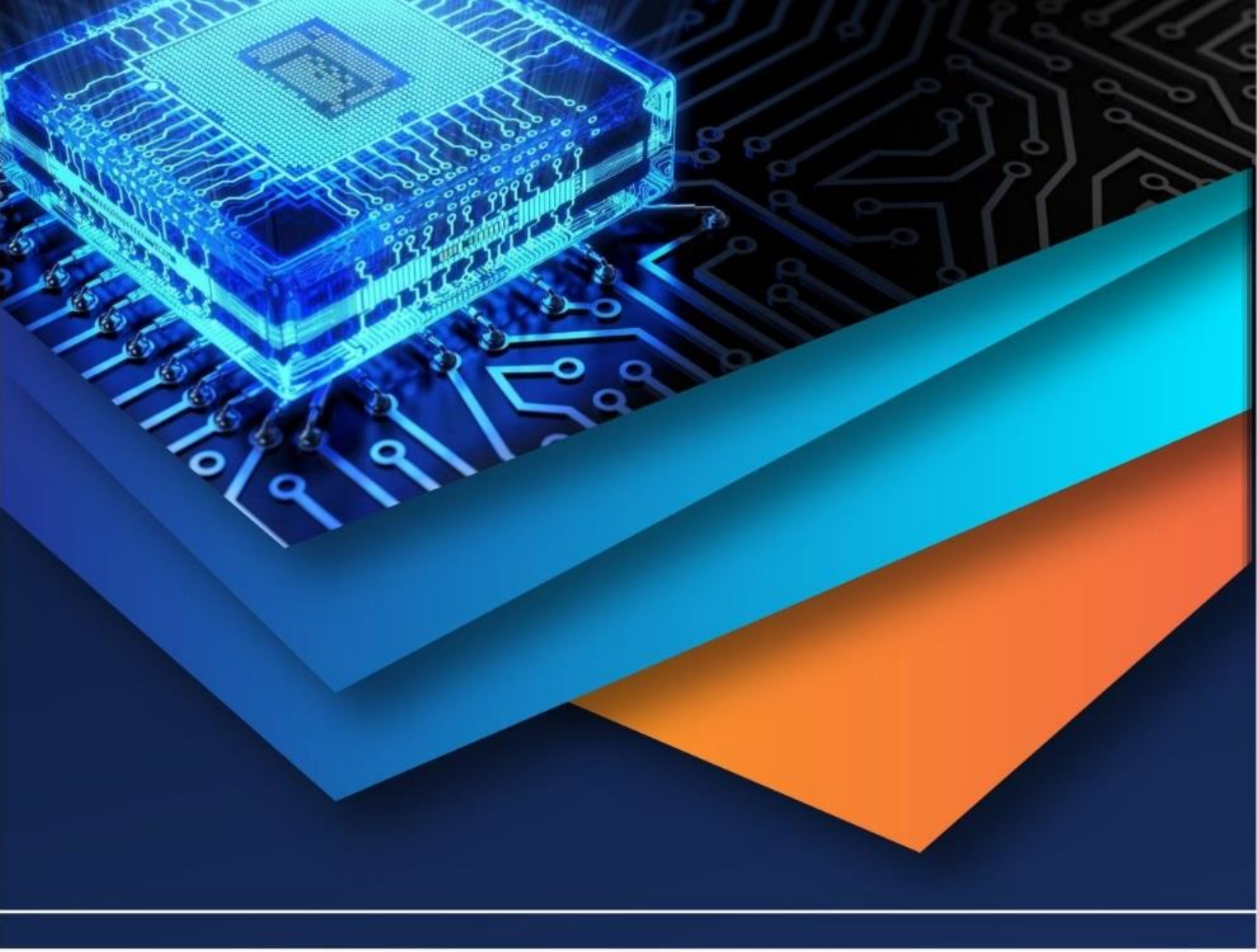

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