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Comparative Study of Effects of Synthetic Pesticide and Biopesticide on Rhizospheric Bacteria

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Abstract: *With incremental implementation of synthetic pesticides in agriculture sectors, non-target rhizosphere microbial communities are affected largely by this that are critical to soil health maintenance. Rhizosphere microbes are well known for their ability to promote plant growth and control of phytopathogens that are capable of causing disease in plants. Hence the objective of work was to evaluate the effects of synthetic pesticide and biopesticide on the rhizospheric microbial community of Oryza sativa at different concentrations. Two common rhizospheric bacteria isolated from young and healthy roots of Oryza sativa were considered. Azotobacter is a free living nitrogen fixer and Agrobacterium that acts as natural microbial Pesticide for plants and inhibits the growth of pathogenic bacteria.*

Keywords: *Pesticides, Rhizospheric bacteria, Oryza sativa, Azotobacter, Agrobacterium.*

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Introduction:

In a natural soil environment, a cooperative relationship exists between plants and microbes. Plants like trees, grass and food crops rely upon microorganisms within the soil to solubilize nutrients, get water, stop nutrient loss, defend from pests and pathogens and break down compounds that could inhibit growth. These soil microorganisms in return get benefit from the plants growing within the soil and substances secreted by the plants root system. This relationship creates a strong living system that's easily broken by conventional systems that use synthetic fertilizers, herbicide and pesticides. The synthetic pesticides that we often use to boost plant growth and production can actually destroy the soil biodiversity, killing or inflicting mutation pressure on the soil microorganisms.

Rhizosphere microbes are well known for their ability to promote plant growth and in the control of phytopathogens that are capable of causing disease in plants. Beneficial soil microorganisms in rhizosphere region of plant like *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Rhizobium*, *Bacillus*, *Serratia, Burkholderia*, *Caulobacter*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Chromobacterium* etc (Bhattacharyya and Jha, 2012) have high capability to produce various classes of well-known phytohormones, including cytokinins, auxins, gibberellins, abscisic acid and ethylene. Plants respond well to these hormones in the rhizosphere which can mediate various processes, such as plant cell enlargement, division, extension in roots, plant growth promotion, nutrition and interactions of disease. Beside this, other microbes also play crucial role in soil fertility and productivity like organic matter biodegradation, humus formation, nutrients recycling, plant growth promotion, soil structural stability, disease biocontrol, biological process, and different biochemical transformation like phosphorus solubilizing, ammonification and nitrification. (Reddy *et al.*, 1984; Husain *et al.*, 2003).

Indiscriminate, prolonged and over-application of synthetic pesticides has become a matter of environmental concern probably because of the adverse effect on physio-chemical properties of the soil like salinity, pH and alkalinity leading to infertility of soil. Eroded soils lose their ability to sustain and enhance the production of crops on the same land. Aktar *et al.*, 2009 have reported harsh effects on soil ecology which will result in alterations or the depletion of useful plant probiotic soil microflora. Pesticides in the soil affect the non-target and helpful microorganisms and their activities. A perfect pesticide ought to be toxic solely to the target organism, biodegradable and should not reach into groundwater. All of these qualities are found in biopesticides also known as biological pesticides. Biopesticides are pesticides obtained from natural materials including animals, plants, bacteria, and certain minerals. Biopesticides typically target a specific range of pests or diseases while non-target organisms, such as bees, fish, birds, humans and beneficial soil microorganisms like PGPR remain unaffected. These are often effective in very small quantities and decompose rapidly, resulting in less exposures and highly avoiding the problems of pollution caused by synthetic pesticides. (Sharma *et al.*, 2018).

Two most common rhizospheric bacteria, *Agrobacterium* and *Azotobacter* were considered and the effect of synthetic pesticide and biopesticide on it were studied. *Agrobacterium* is a gram negative soil bacterium that has immense plant growth promoting properties and inhibit the growth of pathogenic bacteria i.e. *Agrobacterium radiobacter* (Stockwell *et al.*, 1993; Bernard Glick 2012) and *Azotobacter* is a gram negative free living nitrogen fixing bacteria.

Materials and Methods:

Sample collection : A young and healthy root sample of paddy plant, *Oryza sativa,* was taken from a paddy field from the area of Phulwari Sharif, Patna, Bihar, and kept in a labelled sterile bag to avoid any contamination.

Isolation of bacteria: Plant root was cut into small pieces after removing extra soil and weighed 1 gm, mixed in 9 ml normal saline solution and vortex under aseptic conditions. Isolation of bacteria was done by serial dilution (10^{-1} to 10^{-7}) and spread-plate technique (Aneja, 2018). Sample dilution was spread on Congo red yeast extract mannitol agar media plate for *Agrobacterium* and

Ashby's mannitol agar media plate for *Azotobacter*. Congo-red YEMA media Plates were kept at 28°C for 48 hours for incubation, Ashby's mannitol agar media Plates were kept at 30°C for 72 hours for incubation, and growth was observed after incubation period.

Purification of isolates: Isolates from the root sample were re-streaked on Congo red yeast extract mannitol agar media for *Agrobacterium* and Ashby's mannitol agar media for *Azotobacter* to obtain pure colonies.

Preparation of stock solution of biopesticide and synthetic Pesticide: Synthetic Pesticide was filtered by syringe filter sterilization through a membrane filter of pore size 0.45μm. Stock solutions of synthetic pesticide "Syngenta Ampligo" a combination of Chlorantraniliprole (10 %)+Lambda Cyhalothrin (5%) and biopesticide (*Metarhizium anisopliae*) were prepared separately. Synthetic pesticide of concentration 0.005 mg/l and biopesticide of concentration 0.1 mg/ml were prepared under aseptic conditions.

Antimicrobial assay: Agar dilution method was used for antimicrobial assay. Yeast extract mannitol agar media and Ashby's mannitol agar media were prepared separately of concentration 50ppm, 100ppm, 200ppm, 400ppm and 800ppm of synthetic pesticide and biopesticide of concentration 1000ppm. Prepared concentrated media were poured and spot inoculation was done with pure culture of *Agrobacterium* and *Azotobacter* on respective plates.

Results and Discussion:

Two most common rhizospheric bacteria i.e., *Azotobacter* and *Agrobacterium were considered in this study.* After 48 hours of incubation two different colonies were observed on congo-red YEMA plates. YEMA medium containing congo red is absorbed by *Agrobacterium* forming red stained colonies which is similar to the work done by (Allen and Allen 1950) in contrast to *Rhizobium* strains that did not utilize congo red (Shetta *et al.*, 2011; Agarwal *et al.,* 2012). Slimy, flat, translucent colonies were observed on Ashby's mannitol agar media plate after 72 hours of incubation (Beijerinck, 1901). The most commonly used synthetic Pesticide "Syngenta Ampligo" was considered (that has recommended dosage for field application of 0.5-1ml per liter) and Biopesticide "*Metarhizium anisopliae*" (that has recommended dosage for field application of 1ml per liter). After 72 hours of incubation period, growth of *Azotobacter* and after 42 hours of incubation period, growth of *Agrobacterium* on different Pesticide concentrated plates were noted respectively. We represent the growth of *Azotobacter* and *Agrobacterium* in terms of positive (+) and (-). The symbol (++++) indicates best growth, (+++) indicates moderate growth, (++) indicates slight growth, (+) indicates least growth, (-) indicates no growth at all as shown in (Table 1 and Table 2).

This study shows that at 50 ppm concentration of synthetic pesticide "Syngenta Ampligo", growth of both *Azotobacter* and *Agrobacterium* were best and with gradually increase in concentration growth of both the rhizospheric bacteria decreases heavily, at 400 ppm no growth observed on *Azotobacter* Plates while there was a least growth on *Agrobacterium* plates. At 800 ppm no growth was observed on either *Azotobacter* or *Agrobacterium* plates. This shows that apart from causing environmental pollution, synthetic pesticides also inhibit the growth of useful soil microbiota even at recommended concentration/dosage also studied by (Shao and Zang, 2017), Rhizosphere microbes are well known for their ability to promote plant growth and soil fertility in addition to control of phytopathogens that are capable of causing disease (Mehjin *et al.,* 2019). It is observed that even at 1000 ppm concentration of biopesticide (*Metarhizium anisopliae)* the growth of *Azotobacter* and *Agrobacterium* were best. Biopesticides are eco-friendly, specific for their host and do not inhibit non-target beneficial soil microflora as studied by (Meena *et al.,* 2020).

Table 1. Growth of *Azotobacter* and *Agrobacterium* on different concentrations of synthetic pesticide (Syngenta Ampligo)

S. No	Concentration	*Azotobacter*	*Agrobacterium*
1.	50 ppm	++++	++++
2.	100 ppm	+++	+++
3.	200 ppm	++	++
4.	400 ppm	–	+
5.	800 ppm	–	–

Table 2. Growth of *Azotobacter* and *Agrobacterium* on concentration 1000 ppm of biopesticide (*Metarhizium anisopliae*)

S. No	Concentration	*Azotobacter*	*Agrobacterium*
1.	100 ppm	++++	++++

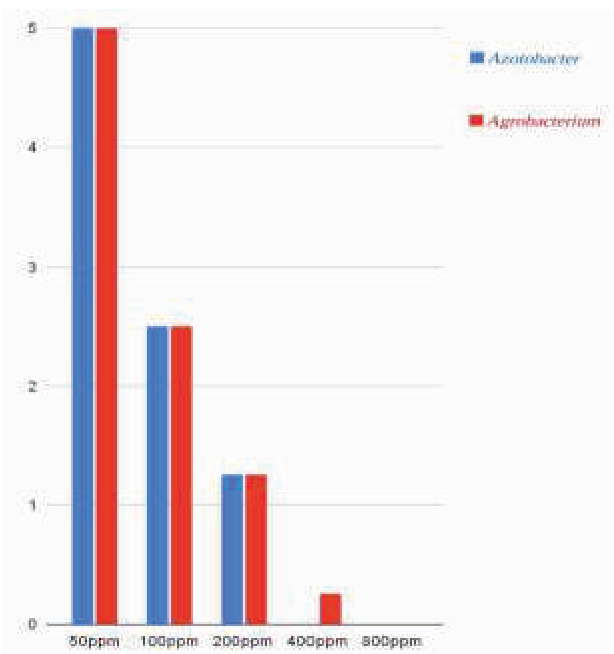

Fig. 1. Growth of *Azotobacter* and *Agrobacterium* at different concentrations of synthetic pesticide

(a) (b)

Table 2. Growth of *Azotobacter* and *Agrobacterium* on concentration 1000 ppm of biopesticide (*Metarhizium anisopliae*)

Conclusion:

Present study of biopesticide and synthetic pesticide that is commonly used for *Oryza sativa* indicates that synthetic pesticide (Syngenta Ampligo) inhibits the growth of rhizospheric Microbial community of *Oryza sativa* that is needed to maintain soil fertility and provides essential nutrition to plants like nitrogen, phosphorus, sulfur etc. It is observed that synthetic pesticide even at low concentration inhibit the growth of *Azotobacter* that is a free living nitrogen fixer in plants and *Agrobacterium* that is a prominent rhizospheric bacteria. Many species of *Agrobacterium* acts as natural microbial pesticide and inhibits the growth of pathogenic bacteria i.e. *Agrobacterium radiobacter*. Biopesticide (*Metarhizium anisopliae)* even at 1000 ppm concentration the growth of *Azotobacter* and *Agrobacterium* were best indicating that biopesticide did not inhibit the growth of rhizospheric microbial community of *Oryza sativa.* Rhizosphere microbes are well known for their ability to promote plant growth and control of phytopathogens that are capable of causing disease in plants. The synthetic pesticides that we often use to boost plant growth and production can actually destroy the soil biodiversity, killing or inflicting mutation pressure on the soil microorganisms. Thus, According to the study conducted it is advisable to use pesticide that is toxic solely to the target organism, biodegradable and should not reach into groundwater. All of these qualities are found in biopesticides.

References:

Agrawal P. K., Agrawal S., Singh U., Katiyar N and Verma S. K. (2012). Phenotypic characterization of rhizobia from legumes and its application as a bioinoculant. *Journal of Agricultural Technology* 8: 681-92.

Aktar W., Sengupta D and Chowdhury A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology'.* Mar; 2(1): 1-2.

Allen E. K & Allen O. N. (1950). Biochemical and symbiotic properties of the rhizobia. *Bacteriol Rev* 14: 273–330.

Aneja K. R. (2018). Experiments in Microbiology, Plant Pathology and Biotechnology, 2nd Edition.

Beijerinck M. W. (1901). On oligonitrophilous bacteria Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen 3: 586–595.

Bernard R. Glick. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications, *Scientifica.* Volume 2012, Article ID 963401, 15 pages.

Bhattacharyya P. N. & Jha D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture, *World Journal of Microbiology and Biotechnology*. Volume 28: 1327–1350.

Husain S., Siddique T., Salem M., Arshad M and Khalid A. (2003). Impact of pesticides on soil microbial diversity, *Enzyme and biochemical reactions, Advances in Agronomy*, 102: 159 – 200.

Meena R. S., Kumar S., Datta R., Lal R., Vijayakumar V., Brtnicky M., Sharma M. P., Yadav G. S., Jhariya M. K., Jangir C. K., Pathan S. I., Dokulilova T., Pecina V and Marfo T. D. (2020). Impact of Agrochemicals on Soil Microbiota and Management. *Land.* vol. 9, issue 2: 1-21.

Mehjin A M AL-Ani., Rawaa M Hmoshi., Ibtiha A Kanaan and Abdullah A Thanoon. (2019). *Journal of Physics, Conference Series.* Volume 1294, p.7.

Reddy P. B., Dhanaraj P and Narayana R. V. (1984). Effects of Insecticides on Soil Microorganisms. *In Insecticide Microbiology* (R. Lal ed.), Springer-Verlag Berlin Heidelberg, pp.169–201.

Shao H & Zang Y. (2017). Non-target effects on soil microbial parameters of the synthetic pesticide carbendazim with the biopesticides cantharidin and norcantharidin, *Scientific Reports.* 7(1), pp. 1-12.

Sharma K.R., Raju S.V.S and Thakur D.K.J.S. (2018). Biopesticides: An effective tool for insect pest management and current scenario in India, *Indian Agric. Allied Sci.* 4, pp. 59-62.

Shetta N.D., Al-Shaharani T.S and Abdel-Aal M. (2011). Identification and Characterization of *Rhizobium* Associated with Woody Legume Trees Grown under Saudi Arabia Condition. *American-Eurasian J. Agric. Environ. Sci.* 10(3): 410-418.

Stockwell V. O., Moore L. W. and Loper J. E. (1993). Fate of Agrobacterium radiobacter k84 in the Einvornment, *Applied and Environmental Microbiology*, 59(7): 2112-20.