Study the Effect of Pesticide Amended Media on Selected Bacterial Isolates from Forest Area.

Josna V K Johnson, Vimala K John

Research and Post Graduate Department of Zoology, St. Thomas' College, Thrissur, Kerala

ABSTRACT

Pesticide is any substance used to kill, repel or control certain forms of plant or animal life that are considered to be pests. Basically all chemical pesticides are poisons and pose deep rooted danger to the environment and humans through their persistence in nature and human tissue. Bioremediation is the evolving method for the removal and degradation of many environmental pollutants. Neonicotionoid and Organophospahate insecticides are the fastest growing classes of insecticides used in modern crop production. Thiamethoxam and Quinalphos are the two selected insecticides. Microorganisms play an important role in degrading synthetic chemicals in soil. Selected bacterial colonies such as Bacillus and Pseudomonas were isolated from the forest area (virgin soil) from Valparai, TamilNadu. These bacterial isolates are cultured on insecticides amended agar media and NA broth at different concentrations to know the bacteria population at different concentrations of pesticides. The bacterial tolerance activity is observed.

Keywords: Quinalphos, Thiamethoxam, *Bacillus sp., Pseudomonas sp.*, Tolerance, Zone of Inhibition

1. INTRODUCTION

Pesticides include an array of chemicals used to destroy crop pests, household pests and vermin, and they have played an indispensible role both in increasing agricultural production and in ensuring a stable food supply of high quality. Continues use of pesticides may cause the unconscious transfer of them to the environment where they can impact non-target organisms. Various biological strategies are generally devised to remove such harmful pesticides from the environment through a process known as bioremediation. The biological strategies where microorganisms are used to act upon these pesticides remain the most efficient and cost effective option to clean up pesticide contaminated sites.

2. AIM AND OBJECTIVE

- To know the tolerance level of selected bacteria on different concentrations of pesticides.

3. MATERIALS AND METHODS

Collection of soil samples: Soil samples were collected from a forest area at Valparai, Tamil Nadu region of Western Ghats. Approximately, 1 to 2kg of soil samples was collected between 0-15cm surface depths of earth. Lumps in soil samples were broken and all stones and debris were removed. Soil samples were then air dry, crushed and sieved through mesh. A serial dilution of the soil samples were done and pour plate method was done.

Selection, Isolation and Purification of isolates

Isolation of two different bacterial colonies were done on the basis of morphological difference of the colonies. Through several biochemical tests they were identified as *Bacillus sp.* and *Pseudomonas sp.*

Pesticides selected

Quinalphos and Thiamethoxam are the two pesticides selected. These were diluted to five different concentrations (15ppm, 20ppm, 25ppm, 50ppm and 100ppm) with hexane and acetone respectively.

Disc diffusion assay

Different concentrations of pesticide containg disc were prepared and placed on nutrient agar media (Dilara & Mithun 2013) with streaked with different test organisms. The plates were then incubated at 37^0C overnight.

4. RESULTS AND DISCUSSION

Soil samples from forest area was collected for the study, because it is considered as the virgin soil, where no pesticide residue was found. Two different bacterial colonies were randomly selected and the biochemical tests were done. (Nawab et.al.2003). The selected colonies are *Bacillus sp.* and *Pseudomonas sp*. Quinalphos and Thiamethoxam were selected as the pesticides to be tested.

Results from disc diffusion assay (Fig) shows that *Bacillus sp.* shows no zone of inhibition at two concentration (15 and 20ppm) but shows zone of inhibition at other three concentrations (25,50 and 100ppm) in the Thiamethoxam pesticide. *Bacillus sp* is resistant to the toxic effect of Quinalphos pesticide at low concentrations (15,230 and 5ppm) and shows small zone of inhibition at 50ppm and 100ppm

Pseudomonas sp. is resistant to Thiamethxam upto 50ppm, with no zone of inhibition and showing small inhibition at 100ppm. In the case of Quinalphos zone of inhibition increases with high concentrations indicating that *pseudomonas sp.* To be susceptible to increased concentration of Quinalphos(20ppm to 100ppm).

Bacillus sp is more resistant to pesticide Quinalphos than Thiamethoxam. *Bacillus sp*. form zone of inhibition at three different concentration (25ppm – 100ppm) of Thiamethoxam. *Pseudomonas sp.* is highly resistant to Thiamethoxam upto 50ppm and susceptible to Quinalphos. The tolerance level of bacteria towards different concentration of selected pesticides is known. Further studies on degradation ability of the selected bacteria can be conducted.

Table 1: Quinalphos media

Sl.No	Strains	Zone of inhibition(mm)				
		15ppm	20ppm	25ppm	50ppm	100ppm
1	*Pseudomonas Sp.*	No zone of inhibition	13	15	18	23
2	*Bacillus Sp.*	No zone of inhibition	No zone of inhibition	No zone of inhibition	15	19

Table 2: Thiamethoxam Media

Sl.No	Strains	Zone of inhibition(mm)				
		15ppm	20ppm	25ppm	50ppm	100ppm
1	*Pseudomonas Sp.*	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	17
2	*Bacillus Sp.*	No zone of inhibition	No zone of inhibition	8	14	20

REFERENCES

1. Dilara Islam Sharif, M. M. (2013). Selective isolation of a gram negative carbamate pesticide degrading bacterium from brinjal cultivates soil. *American journal of agricultural and biological sciences.*, 249-256.

2. 2.Nawab A, A. A. (2003). Determination of organochlorine pesticides in agricultural soil with special reference to y-HCH degradation by pseudomonas strain. *Bioresource technology*, 41-45.

3. 3. Gordon W. Robertstad, L. S. McClung and Leroy R. Maki (1968) *The American Biology Teacher,* Vol. 30, No. 6, pp. 557-565.
4. 4.S. Sadhu and T. K. Maiti, (2013) Cellulose production by bacteria: a review," *British Microbiology Research Journal*, vol. 3, no. 3, pp. 235–258.
5. 5. R. N. Wadetwar and A. T. Patil, (2013) Isolation and characterization of bioactive actinomycetes from soil in and around Nagpur, *International Journal of Pharmaceutical Sciences and Research*, vol. 4, no. 4, p. 1428.
6. 6 N. Abdul Malek, A. J. K. Chowdhury, Z. Zainuddin, and Z. A. Z. Abidin (2014) Selective isolation of actinomycetes from mangrove forest of Pahang, Malaysia, in *Proceedings of the International Conference on Agriculture, Biology and Environmental Sciences (ICABES'14)*, pp. 8-9, Bali, Indonesia.