Isolation of metribuzin and profenofos resistant bacteria from agricultural soil collected in Uva province of Sri Lanka

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1. Introduction

Pesticides are complex chemicals used to combat pests. They are extensively used in agricultural activities to protect crops from pest attacks. Although pesticides are important in increasing productivity and quality of yield, they can cause harmful effects to the natural ecosystem through accumulating in the environment. Indiscriminate usage of pesticides has triggered this issue causing serious environmental consequences including human health problems. A triazine herbicide metribuzin and an organophosphate insecticide profenofos are two commonly used pesticides in agricultural lands all over the world including Sri Lanka. They have also been identified as two of major pesticides which cause harmful impacts on the environment. They are known to have some degree of persistence in the environment. Surface and groundwater contaminations followed by entering into live biomass have been observed. Some microorganisms have been identified to show a great tolerance to pesticides with pesticide degrading ability. There is a potential of using them in bioremediation of pesticide contaminants. Scientists have been working on exploring such beneficial microbes worldwide. In Sri Lankan context it's hard to find such studies conducted on exploring pesticide resistant ability of soil microorganisms. Uva province is an extensively cultivated area having a long history of intensive pesticides application in Sri Lanka. Hence there is a potential of the survival of pesticide resistant, soil inhabitant microorganisms in this area. Objective of this study was to isolate metribuzin and profenofos resistant bacteria from agricultural soil collected from Uva province, Sri Lanka to mitigate the knowledge gap in the relevant discipline.

2. Materials and Methods

Having identified suitable sample collection sites using a baseline survey, 20 soil samples were collected from agricultural lands with a long history of profenofos and metribuzin applications in Uva Province of Sri Lanka. Collected samples were immediately transported to the laboratory.

The isolation method used in Malghani et al. (2009) and Zhang et al. (2014) was used with some modifications. Minimal salt medium (MSM) broth was prepared by dissolving 8.5 g of $Na_2HPO_4.2H_2O$, 3 g of KH_2PO_4, 0.5g of NaCl and 1 g of NH_4Cl in 1000 ml of distilled water. Media sterilization was performed by autoclaving at 121^oC and 15 lb/inch^2 for 20 minutes.

An aliquot (20 g) from each soil sample was separately placed in conical flasks containing 100 ml sterilized MSM broth supplemented with 100 ppm concentration of metribuzine and profenofos. Separate sets were prepared under each pesticide and were then incubated at 37^o C with continuous shaking at 100 rpm for 7 days. Then aliquots of 100 µl from each enriched culture was separately spread on MSM agar plates supplemented with each selected pesticide

in different concentrations (100 ppm, 200ppm, 300ppm, 500ppm, 1000ppm). Inoculated plates were incubated at 37^{o} C for 24 hours.

Prominently grown single colonies in different pesticide concentrations were selected. Loop-full of each selected colony was separately streaked on nutrient agar plates until pure single colonies could be isolated. Each isolate was overnight enriched in nutrient broths at 37^{o} C and separately transferred to 30% glycerol stocks and stored at -80^{o} C.

3. Results and Discussion

Total of 44 of well -grown single colonies were able to sort out/ isolate. Among the colonies, 25 were resistant for Metribuzin and 19 for Profenofos, whereas from different pesticide concentrations. The number of pesticide resistant bacteria isolated from different concentrations of selected pesticides are given at Table 1.

Minimal salt medium consists of only the minimal essential nutrients required for microbial growth (Jabeen et al., 2015). Added pesticides were the sole carbon source in the medium. Satisfactory growth of the isolated bacteria in the presence of added pesticides shows their affinity to these pesticides. These bacteria may utilize metribuzin and profenofos as their carbon source. Some bacteria grew well under high pesticide concentrations as higher as 1000 ppm. It proves that they can tolerate such a high concentration of selected pesticides. Indicating that those bacteria are not adversely affected by those pesticides.

Previous literature on related studies have revealed that pesticide resistant bacterial species of these kinds are capable of degrading toxic pesticide compounds and convert them into less or nontoxic metabolites (Zhang et al., 2014; Jabeen et al., 2015; Wahla et al., 2019). Metribuzin and Profenofos resistant bacterial isolates found during this study may be having such beneficial potentials. Biochemical characterization or molecular characterization of those isolates by 16S rRNA sequencing like technique will be advantageous in future research activities on this area. The biodegradation ability of those isolates can be measured using Gas Chromatography-Mass spectrometry (GC-MS) (Johnson & Pepperman, 1995; Malghani et al., 2009; Zang et al., 2014; Wahla et al., 2019). Further studies are required to confirm whether these isolates are able to degrade the pesticide compounds by means of their metabolism. Besides some more such beneficial bacterial strains may be available in Sri Lanka. Implementing further researches in order to isolate and characterize some more soil bacteria will be important in contributing to the knowledge and practical applications such as bioremediation of pesticide residues in Soil, water environments using bacteria having the capability of degrading pesticides.

Table 01. The number of pesticide resistant bacteria isolated from different concentrations of each selected pesticide.

	Concentration of pesticide				
Pesticide	100 ppm	200 ppm	300 ppm	500 ppm	1000 ppm
Metribuzine	8	6	4	6	1
Profenofos	3	4	4	4	4

4. Conclusions

There are some soil bacteria that can tolerate Metribuzin and Profenofos up to 1000 ppm concentration in some agricultural lands in Uva province Sri Lanka. These bacteria may be having some capabilities of degrading these pesticides. Implementing further researches in order to test their pesticide degrading ability and the potential of using them in the bioremediation of pesticide contaminants can be suggested as a further improvement of the study.

5. References

Jabeen, H., Iqbal, S., Anwar, S., & Parales, R. E. (2015). Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology. International Biodeterioration & Biodegradation, *100*, 89-97. https://doi.org/10.1016/j.ibiod.2015.02.022

Johnson, R. M., & Pepperman, A. B. (1995). Analysis of metribuzin and associated metabolites in soil and water samples by solid phase extraction and reversed phase thin layer chromatography. Journal of Liquid Chromatography & Related Technologies, 18(4), 739-753. https://doi.org/10.1080/10826079508009269

Malghani, S., Chatterjee, N., Hu, X., & Zejiao, L. (2009). Isolation and characterization of a profenofos degrading bacterium. Journal of Environmental Sciences, *21*(11), 1591-1597. https://doi.org/10.1016/S1001-0742(08)62460-2

Wahla, A. Q., Iqbal, S., Anwar, S., Firdous, S., & Mueller, J. A. (2019). Optimizing the metribuzin degrading potential of a novel bacterial consortium based on Taguchi design of experiment. Journal of Hazardous Materials, *366*, 1-9. https://doi.org/10.1016/j.jhazmat.2018.11.054

Zhang, H., Zhang, Y., Hou, Z., Wu, X., Gao, H., Sun, F., & Pan, H. (2014). Biodegradation of triazine herbicide metribuzin by the strain Bacillus sp. N1. Journal of Environmental Science and Health, Part B, *49*(2), 79-86. https://doi.org/10.1080/03601234.2014.844610

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