Bioremediation of Monocrotophos and Malathion by Bacillus sp.

Bini Samal[1], Parul Bhatt Kotiyal[2]

[1]Ecology and Environment Division, Forest Research Institute, Dehradun, Uttarakhand, India

ABSTRACT

The use of chemical pesticides has brought benefits such as the increment of agricultural production, soil productivity and products quality, which is reflected in economical benefits, vector disease control and in general, in public health. There are some bacteria in the soil which can help in solving the problem of Pesticide Pollution. The isolation of pesticide degrading bacteria was carried out using selective media (Bacillus cereus medium) and the isolated bacterial isolate were identified as Bacillus sp. The growth of the pesticide degrading isolate was assessed in Bushnell haas broth containing different (0.5%, 1%, 1.5%) of Malathion and Monocrotophos.

Growth was observed in all the three concentrations of Malathion and Monocrotophos by the bacteria. But the best degradation potentials were observed by Bacillus sp. at 0.5% concentration of pesticides. Colonies of bacillus sp. were seen in minimal medium agar (Bushnell Haas Agar) inoculated with different concentrations of Malathion and Monocrotophos also. The bioremediation potential of bacteria was confirmed by inoculating pesticide and bacteria in chickpea plants (pot experiment). The growth of the plants showed a positive growth when both pesticide and bacteria were inoculated as compared to only pesticide inoculation. Bacillus sp. has bioremediation potential for the degradation on Malathion and Monocrotophos at all the three concentrations (0.5%, 1%, 1.5%). But the maximum values of absorption were obtained at 0.5% concentration. Bacillus sp. has shown better results for Malathion.

Keywords: Bacteria, bioremediation, malathion, monocrotophos.

1. INTRODUCTION

Bioremediation uses living systems or biological products to biodegrade anthropogenic waste, with the objective being reduction of waste to chemical forms that can be assimilated into natural cycles. It leads to actual reduction and detoxification of wastes, ideally degraded to CO2 and water, when there are organic compounds. The goal of bioremediation is to biotransform toxic materials into nontoxic ones and to make accumulating anthropogenic waste enter natural biogeochemical cycles

more quickly. Biodegradation of organic chemicals is accomplished enzymatically. Either the enzymes work internally or externally. The waste can be absorbed and broken down internally or it is treated by the secreted enzymes outside the microorganism.

Therefore, co metabolism plays a role when microbial enzymes, produced for breaking down normal food sources may fortuitously degrade certain wastes that are present as well. Specificity is a great characteristic of enzymes, but some of them are not very specific. For example, oxygenases often fortuitously oxidize methane or toluene. We take advantage of co metabolism to biodegrade compounds, that otherwise would remain persistent in the environment. Some toxic compounds can be used as primary electron donors for energy and growth and some are transformed as electron acceptors. Halogenated organic compounds were recognized as potential electron acceptors in energy metabolism.

Monocrotophos is one of the most toxic pesticides to birds and is extremely toxic other wildlife, highly toxic to bees. It is toxic to shrimps and crabs, moderately toxic for fish and non-toxic for microorganisms. The acute LD50 for birds ranges from 0.9-6.7 mg/kg body weight and for honey bees 33-84 µg/bee. Monocrotophos is mobile in soil, and although it degrades rapidly it may possess potential for groundwater contamination. Malathion is an organophosphate parasympathomemetic which binds irreversibly to cholinesterase. Malathion is an insecticide of relatively low human toxicity. In the former USSR, it was known as carbophos, in New Zealand and Australia as maldison and in South Africa as mercaptothion

Microbial degradation process involves biochemical reactions such as dehalogenation, oxidative reactions such as epoxidation, dealkylation, reduction, ester hydrolysis and condensate or conjugate formation. Most pesticide-degrading microorganisms have been isolated from soil. The types and rates of microbial degradation are determined by the pH, temperature, redox potential, nutrient availability and the general microbial ecology of a given system. If the pesticide can be used as an energy or nutrient source, it will disappear from the soil slowly or rapidly, the rate depending upon the compound, method of application, degree of adsorption, rate of growth of the active species, various environmental factors and possible toxicity of substrate to microorganisms using it.

2. MATERIAL AND METHODS

2.1 Collection of Soil Samples

Soil samples were collected from a field near IGNFA (Forest Research Institute), Dehradun.

Selection of cultures for isolation of pesticide degrading organisms: Bacterial species (Bacillus) were isolated from (IGNFA site) soil sample.

Isolation and Purification of bacteria from soil: Bacillus species were isolated using selective media (Bacillus cereus media), colonies were observed the next day. Pure cultures were picked up and transferred onto the selective media again. The plates were then incubated at 26^0C for 24 hrs.

Growth of bacteria in Nutrient Broth: One colony each from the purified cultures of *Bacillus* were transferred into nutrient broth and incubated for 24 hrs at 33 degree centigrade.

Malathion Degrading Capacity: To verify whether the isolates have pesticide endurance or degradation capacities, they were subjected to grow using Malathion as a sole source of carbon. The overnight grown fresh cultures were inoculated on Minimal medium i.e. Bushnell Haas Medium containing pesticide in various concentrations i.e. 0.5%, 1% and 1.5 %. The cultures were then incubated at 22 deg. C. on a rotary shaker at 100rpm/m. The turbidity in these cultures indicated growth and thus Malathion resilience and Malathion degrading capacity of the isolated.

Monocrotophos Degrading Capacity: To verify whether the isolates have pesticide endurance or degradation capacities, they were subjected to grow using Monocrotophos as a sole source of carbon. The overnight grown fresh cultures were inoculated on Minimal medium i.e. Bushnell Haas Medium containing Monocrotophos in various concentrations i.e. 0.5%, 1% and 1.5% v/v. The cultures were then incubated at 22±3 ^{0} C on a rotary shaker at 100rpm/m. The turbidity in these cultures indicated growth and thus, Monocrotophos resilience and Monocrotophos degrading capacity of the isolated.

Degradation of Malathion under chickpea: Minimal medium agar (Bushnell Haas agar) was inoculated with chickpea and incubated at 28 degree centigrade. After 24 hrs it was inoculated with Malathion at concentrations (0.5%, 1%, 1.5%) and 1ml of the bacterial cultures (Bacillus) at dilutions (-3, -4) respectively. The plates were incubated for 2 days and colonies were observed on the media. Growth of bacterial colonies shows that they are able to resist and degrade Malathion.

Degradation of Monocrotophos under chickpea: Minimal medium agar (Bushnell Haas agar) was inoculated with sprouted chickpea and incubated at 28 degree centigrade. After 24 hrs it was inoculated with Monocrotophos at concentrations (0.5%, 1%, 1.5%) and 1ml of nutrient broth containing bacterial cultures (Bacillus) at dilutions (-3, -4) respectively. The plates were incubated for 2 days and colonies were observed on the media. Growth of bacterial colonies shows that they are able to resist and degrade Monocrotophos.

2.2 Pot Experiment for Malathion

Soil Collection: Soil was collected from New Rosewood Hostel (Forest Research Institute), Dehradun

Sterilization of soil: It was sterilized by autoclaving at 121 degree centigrade and 15 lbs pressure, for not allowing the growth of any other bacterial species.

Growth of Plants: Chickpea was grown in root trainers until the seedling stage, for growing them sterile soil was used. Then it was inoculated with 1ml of nutrient broth containing bacterial cultures of Bacillus sp. at dilutions (-3, -4) respectively and Malathion at concentrations (0.5%, 1%, 1.5%) and incubated for 4 days. Growth of the plants was observed. Plants which were inoculated with bacterial cultures showed at better growth performance as compared with plants containing only Malathion devoid of bacterial cultures.

Pot Experiment for Monocrotophos: Chickpea was grown in sterile soil in root trainers until the seedling stage, Then it was inoculated with 1ml of nutrient broth containing bacterial cultures of Bacillus sp. at dilutions (-3, -4) respectively and Monocrotophos at concentrations (0.5%, 1%, 1.5%) and incubated for 4 days. Growth of the plants was observed. Plants which were inoculated with bacterial cultures showed at better growth performance as compared with plants containing only Monocrotophos devoid of bacterial cultures.

Growth Curves: Overnight grown cultures were transferred to minimal medium and at the time of inoculation, 0 hr. Reading was noted. The pesticide (Monocrotophos and Malathion) were added to the cultures and were then allowed to grow. Readings were taken every 24 hrs. And the growth rate was thus observed.

2.3 Degradation Potential: Overnight grown cultures were transferred to minimal medium and at the time of inoculation, 0 hr. Reading was noted. The pesticides (Malathion and Monocrotophos) were added to the cultures and were then allowed to grow. Readings were taken every 24 hrs. And the growth rate was thus observed.

Degradation potential was determined by the formula –

Degradation potential = Initial peak-Final peak/Initial peak x 100

3. RESULTS AND DISCUSSION

Growth was observed in all the three concentrations of Malathion and Monocrotophos by the Bacillus sp. Colonies of bacteria were seen in minimal medium agar inoculated with different concentrations of Malathion and Monocrotophos also. The bioremediation potentials of bacteria were confirmed by inoculating pesticide and bacteria in chickpea (pot experiment). The growth of the plants showed a more growth when both pesticide and bacteria are inoculated as compared to only pesticide inoculation. But the maximum values of absorption were obtained at 0.5% concentration. Hence Bacillus sp. has the capacity to utilize the pesticides and use of this isolate in the biological treatment of pesticide contaminated soil will give fruitful results. But in order to improve the use of microbe–based processes some questions still have to be answered, such as the

long term impact of introducing microorganisms into the environment, as well as the narrow range of applications.

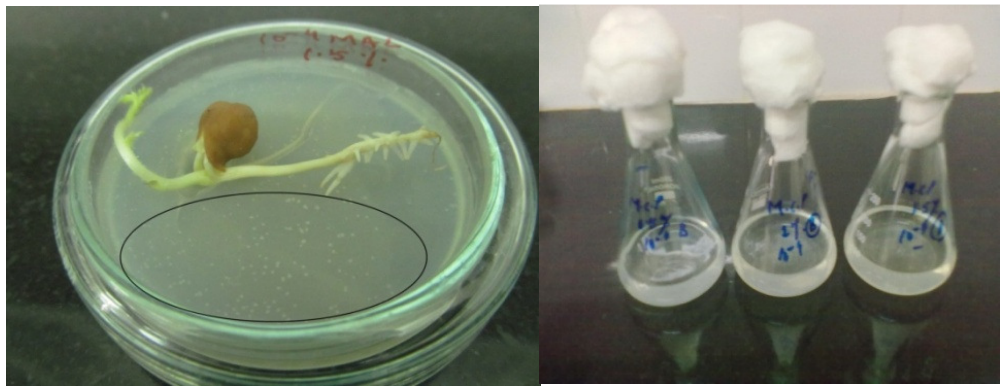

Fig no. 1 Growth of bacillus on Bushnell haas agar and broth

Table no. 1 Degradation potentials of Bacillus sp at dilutions (-3 & -4)

For Bacillus 3rd dilution (Malathion)	Day 1	Day 2	Day 3	Degradation Potential
Abs at (0.5%)	0	0.185	0.18	92.70%
Abs at (1%)	0	0.171	0.166	78.12%
Abs at (1.5%)	0	0.05	0.01	47.91%
For Bacillus 3rd dilution (Monocrotophos)	Day 1	Day 2	Day 3	Degradation Potential
Abs at 0.5%	0	0.19	0.14	35.41%
Abs at 1%	0	0.19	0.14	35.41%
Abs at 1.5	0	0.115	0.105	7.7%
Bacillus dilution 4 (For Malathion)	Day 1	Day 2	Day 3	Degradation Potential
Abs at 0.5%	0	0.2	0.15	21.80%
Abs at 1%	0	0.17	0.12	9.30%
Abs at 1.5%	0	0.17	0.12	9.30%
Bacillus dilution 4 (For Monocrotophos)	Day 1	Day 2	Day3	Degradation Potential
Abs at 0.5%	0	0.2	0.15	45.80%
Abs at 1%	0	0.17	0.15	14.50%
Abs at 1.5%	0	0.17	0.05	14.50%

4. CONCLUSION

Colonies of bacteria were seen in minimal medium agar inoculated with different concentrations of Malathion and Monocrotophos also. The bioremediation potentials of bacteria were confirmed by inoculating pesticide and bacteria in chickpea (pot experiment). The growth of the plants showed a more growth when both pesticide and bacteria are inoculated as compared to only pesticide inoculation.

Bioremediation technique for removal of Malathion and Monocrotophos is nothing but a very effective and interesting field with the advantages like it is a natural process with less cost requirement compared to other methods and this process can be applicable at the place where the problem is located. Although, this technique has some disadvantages like formation of undesirable degraded products, desorption of Malathion and Monocrotophos and disposal of it, disposal of cells containing Malathion and Monocrotophos in absorbed/ adsorbed condition. These problems can be solved with further monitoring of the process and there are lots of scopes for research work in this field.

Acknowledgements: Author would also like to thank Mr. Santosh Kumar Samal and Mrs. Vijaya Samal for their constant support and inspiration

REFERENCES

[1] D. Arthur Bloom, A Biological Approach to Environmental Remediation (1995).

[2] Wei S. H., Clark A. G. & Syvanen M. (2001).Identification and cloning of a key Insecticide metabolizing glutathione S-transferase (MdGST-6A) from a hyper insecticide resistant strain of the housefly *Muscadomestica.Insect Biochemistry and Molecular Biology*. 31(12), 1145-1153.

[3] Wenk M., Baumgartner T., Fuchs T. D., Kuesera J., Zopfi J. and Stuchi G. (1998). Rapid atrazine degrading *Pseudomonas* sp. Strain, *Applied Microbiology and Biotechnology,* 49:624-630.

[4] Xie, S., J. Liu, L. Li and C. Qiao, (2009). Biodegradation of malathion by Acinetobacterjohnsonii MA19 and optimization of cometabolism substrates. J. Environ. Sci., 21:76- 82.

[5] Zayed A., Gowthaman S., Terry N. (1998). Phytoaccumulation of trace elements by wetland plants: I. Duck weed. J Environ Qual 27: 715-721.

[6] Zhang C. and Bennett G.N. (2005).Biodegradation of xenobiotics by anaerobic bacteria. Appl. Microbiol. &Biotechnol., 67 (5): 600–618.

[7] KumariRatna, Sobha K, Mounika K, Jageswara G and Ashok M (2012) Molecular characterization of bacteria capable of organophosphate degradation Vol. 1, No. 3, July, ISSN 2250-3137.