



Biodegradation of Organophosphate Insecticide Malathion By Microorganisms Isolated From Soils of Solapur Region, India.

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ABSTRACT

In the present study efficiency of bacterial strain S1 (*Staphylococcus spp.*) and fungal strains F1 (*Aspergillus niger*) and F2 (*Penicillium spp.*) for degradation of Malathion (organophosphorus insecticide) was investigated. The microorganisms were isolated from wine yard soil samples near Solapur region, Maharashtra, India, which was found to be heavily contaminated with Malathion. The isolated organisms were inoculated in desired broth medium supplemented with Malathion (20 mg ml⁻¹⁰⁰ i.e. 2 ppm) for specific interval of time to degrade. Degradation of Malathion was estimated by Fiske Subbarow method. From the above study, it reveals that *Aspergillus niger* showed maximum potential to degrade Malathion than *Penicillium spp.* and *Staphylococcus spp.*

KEYWORDS : Malathion, bacterial and fungal strains, degradation, Fiske Subbarow method.

INTRODUCTION

Malathion, [S-(1, 2-dicarboethoxyethyl)-O, O-dimethyldithiophosphate], (C₁₀H₁₉O₆PS₂), is a compound belongs to organophosphate insecticides [2]. It is often used to resist the impact of insects on fruits and vegetables with killing insects by direct contact or through vapour action [7]. Water and soil receive relatively huge amounts of Malathion even from handling, direct application or else which lead to infrequent contamination besides accumulation lead to many health hazards associated with it [8,10]. Hence, in ecosystem the degradation process of pesticides universally takes an immense importance. Recently, use of microbes for effective detoxifying, degrading and removal of toxic compounds from contaminated soil and water samples has emerged as an efficient technique to clean up polluted environments [12]. Microorganisms are potentially used in bioremediation of environmental pollutants. Bacteria [8,10], fungi [3] and some plants have potency to degrade Malathion. Malathion can be degraded or detoxified using physical, chemical or biological methods [5,11]. Use of microorganisms for degradation or detoxification of Malathion is natural process, very effective beyond other methods and applicable for in-situ bioremediation. The process of biodegradation of organophosphate compounds releases phosphate as one of the end product [6]. The main objective of our study was isolation, characterization of bacterial & fungal strains from Malathion contaminated soils samples from Solapur region as well as to determine its Malathion degradation potential.

MATERIALS AND METHODS Collection of Soil sample

Malathion contaminated soil samples were taken from two different sites of Solapur region in the June 2014 used for screening and isolation of bacterial and fungal strains. Samples were stored into autoclaved plastic bags, then air dried for 3-5 day at 18°C and sieved through 2 mm sieve to be representative and homogeneous then kept at 4°C until use.

Isolation and identification of microorganisms

The collected soil sample is serially diluted, dilution series was made up to 10⁶, and then aliquots (0.5 ml) were spread on Czapek-dox agar medium, HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India and incubated at 33±2 °C for 2 days. Bacterial and Fungal strains isolation was carried out using nutrient agar and Sabouraud agar medium respectively. The inoculated plates were subsequently incubated at 37 °C for 48hrs. Microbial colonies obtained were sub-cultured for 4-6 times to obtain pure cultures. To identify the isolated bacterial culture morphological analysis and cultural characteristics on agar plates were performed. The morphological analysis comprises shape, motility & Gram staining (for bacteria) and Lactophenol, cotton, blue. staining (for fungi). The cultural characteristics include nutrition type, col-

ony size, form, growth on agar medium, and optimum temperature for their identification according to Bergey's Manual of Determinative Bacteriology were used.

Degradation activity Biodegradation by Bacteria

The inoculum was prepared to get sufficient quantity and desired growing phase of the selected bacterial strain S1. The pure culture of bacterial strain S₁ was inoculated into the nutrient broth for 2-3 days at 37°C in shaking incubator to get heavy growth. The process of degradation was performed by inoculating the inoculum in medium containing Malathion (20 mg ml⁻¹⁰⁰ i.e. 2 ppm) then microbe will degrade the Malathion with the liberation of inorganic phosphate ions, which was estimated after specific interval by using Fiske Subbarow method [4].

Biodegradation by Fungi –

The inoculum was prepared to get sufficient quantity and desired growing phase of the selected fungal strains F1 or F2. The pure culture of fungal strains F1 or F2 was inoculated into the Sabouraud broth for 5-6 days at 25°C in incubator to get heavy sporulation. The process of degradation was performed by inoculating the inoculum in Sabouraud broth containing Malathion (20 mg ml⁻¹⁰⁰ i.e. 2 ppm) then microbe will degrade the Malathion with the liberation of inorganic phosphate ions, which was estimated after specific interval by using Fiske Subbarow method [4].

RESULTS

Bacterial and fungal strains identification

In the present study total five bacterial strains were isolated from soil samples out of which Bacterial Strain S1 (*Staphylococcus spp.*) showed positive results which was characterized as Gram positive, cocci, non-motile. Similarly, Fungal strain F1 (*Aspergillus niger*), globose, dark brown and Fungal strain F2 (*Penicillium spp.*), Conidia are globose, greenish, smooth- or rough- walled were successfully isolated from soil samples.



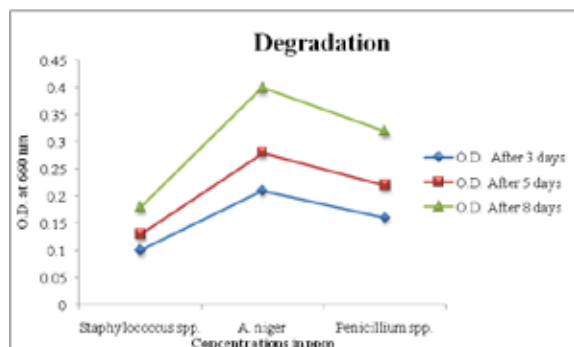
Biodegradation

Data in Table 1 and Figure 2 show the amount of Malathion in mg found after the calibration of analysed value from Optical Density to Volume with help of Standard values of Malathion obtained from Fiske Subbarow method.

Table 1 – Biodegradation of Malathion by using selected microorganisms in (mg/100 ml).

Incubation period	<i>Staphylococcus spp.</i> (mg/100ml)	<i>A. niger</i> (mg/100ml)	<i>Penicillium spp.</i> (mg/100ml)
3 days	4.2	9	6.8
5 days	5.5	12	9.3
8 days	7.6	17.2	13.4

Fig. 2 – Degradation of Malathion by using selected microorganisms.



This graph reveals that *A. Niger* shows maximum i.e. 17.2 mg/100 ml while *Staphylococcus spp.* shows minimum degradation potential i.e. 7.6 mg/100 ml. In addition, *Penicillium spp.* shows intermediate i.e. 13.4 mg/100 ml type of degradation among both.

Fig. 3 - Malathion Degradation by *A. niger*



Sabourauds Broth During Degradation After Degradation

DISCUSSION

Biodegradation of pesticides by using microbes have more advantages over the conventional methods. In biodegradation process, the agents like bacteria, fungi and plants were frequently used. Baljinder Singh *et al.* (2013) reported that 77.7% of Malathion was degraded after 7 days of incubation with mixed culture of *Bacillus spp.* Similarly, Akilandeswari *et al.*, (2013), reported that 59.76% of Malathion was degraded after 10 days of incubation with *Staphylococcus aureus*. Similar results were drawn from our reports. In addition, Chalamala *et al.* (2012) worked on My codegradation of Malathion by using *A.niger* and got 86.72 % of degradation. Similar to above reports we compared the degradation potential of bacterial species to fungal species. It reported that *A. niger* showed maximum degradation potential i.e. 86% than *Staphylococcus spp.* i.e. 38%.

CONCLUSION

The study has elucidated that the fungal species *A.niger* are effective organophosphorus pesticide biodegrading species as compared to above studied fungal and bacterial strains. Because, *A. niger* may produce certain enzymes that can modifies or degrades organophosphorus pesticides *A. niger* can grow efficiently in the high concentration of organophosphorus pesticide as compared to bacteria, even though bacteria has rapid cell division potential than fungi. So we strongly recommend that *A. niger* effectively used to remove Malathion from contaminated soil and water samples on large scale.

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