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Short Communication

Effect of Phosphate Solubilization on Biodegradation Efficiency of Organophosphorus Pesticide (Dimethoate)

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Dimethoate being grouped into organophosphorus insecticides category and is majorly used in agricultural and local fields to control the menace of interfering insects. Dimethoate is potent to control wide range of insect's right from flies, aphids, mites and plant hopper which are generally infecting standing crops [1]. In most of the fields of vegetables, fruits, grains and ornamental plants, dimethoate applied on a regular basis. Not only is that, it is regularly spread on non-agricultural establishments such as landscaping for its maintenance and pest control practices. As per the estimation, 816,466 Kg of dimethoate is utilized to spread annually over number of agricultural sites especially growing grains, wheat, cotton, corn and alfalfa [2].

Every year use of pesticide in India is growing by 12 to 13 percent per year. Out of which domestic requirement would be 8 to 9 percent and export requirement of 15 to 16 percent. As per pesticides industry analysis in India compound annual growth rate (CAGR) is 14.7 percent reaching Rs. 2,29,800 million by 2018. A market of pesticide in India would grow at rate of 2.38 percent per year [3]. Dimethoate being grouped into organophosphorus insecticides category and is majorly used in agricultural and local fields to control the menace of interfering insects. Dimethoate is potent to control wide range of insect's right from flies, aphids, mites and plant hopper which are generally infecting standing crops [4]. In most of the fields of vegetables, fruits, grains and ornamental plants, dimethoate applied on a regular basis.

Organophosphorus pesticides are highly toxic and easily absorbed through the skin. Poisoning may also occur through the mouth. There are many effects when inhaled. The first effects are usually respiratory and may include bloody or runny nose, coughing, chest disorder, difficult or short breath. These may include vomiting, diarrhea, abdominal cramps, headache, eye pain and blurred vision. Severe poisoning will affect the central nervous system lack of co-ordination and eventually paralysis of the body extremities & respiratory muscles [5-6].

In the present study attempt has been made to isolate and screen dimethoate degrading microorganisms from dimethoate exposed soils. In an application part, these isolates will be screened for plant growth promoting features along with dimethoate degrading ability which can make these microorganisms commercial valuable for further studies.

Sample collection

Soil sample were collected from the fields of five different locations in Maharashtra namely, Theur, Lonikalbhor, Shirval, Urulikanchan. Dimethoate and Parathion pesticides are commonly used in the fields of these areas. B. Pesticide used: Dimethoate (Anu Products Pvt. Ltd.).

Primary screening

Soil samples collected are diluted in sterile distilled water upto10-9. Minimal medium with 1gm% of respective pesticide was used for spread plate technique. Plates were incubated at 37°C for 48-72 hours.

Secondary screening

Individual isolate obtained at primary screening was inoculated on minimal medium with different pesticide concentration (1gm% to 30gm%). Plates were incubated at 37°C for 48-72 hours.

Identification of dimethoate degrading isolates

Dimethoate degrading isolates obtained in pure culture and it was further used for biochemical studies [7]. Those tests were Oxidase, Catalase, Methyl red, Voges Proskauers, Urease, Nitrate reduction, Glucose fermentation, Maltose fermentation, Mannitol fermentation, Lactose fermentation, Gelatinase. Further isolates were targeted for 16S rRNA gene by using universal primer and conditions suggested by [8]. Lactophenol cotton blue staining was performed for identification of fungi.

Phosphate solubilization [9]

The ability of bacteria to solubilize phosphate was determined by plating the bacteria on Pikovaskya's agar medium containing dimethoate. The presence of clear zone

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around the colonies following one week of incubation at R.T. indicates phosphate solubilization.

Two bacterial isolates, *P. fluorescens* and *A. pulmon* is successfully isolated from agricultural soil polluted with dimethoate and those found to capable of degrading dimethoate. Isolate *P. fluorescens* (AM1) and *Achromobacter pulmonis* (AM2) analyzed for the 1% dimethoate degradation in a nutrient medium; it is recorded that both isolates (AM1 and AM2) able to utilize the 1% dimethoate [10].

In the present study an attempt has been made to isolate and understand the mechanism of action of pesticide degradation (Organophosphorous) by microbial community present in the soil. Study also put forward the combined activity of microbes to showcase the ability to degrade pesticides as well as it can produce plant growth promoting features which could be used in altogether for better plant growth and in controlling environment pollution by pesticides use. Keeping in view in the present study, agricultural fields of Pune denoted as Urulikanchan and Fursungi has been selected for the study remained pesticide exposed and that can provide opportunity to isolate adapted microbiota from the soil. Present study has selected these fields by considering its contamination with dimethoate as a pesticide as they regularly using the same for pest control.

In the present study, once the dimethoate contaminated soil has been selected and sampled they were successfully processed for the detection of microbiota able to tolerate 1% dimethoate when cultured in a medium Davis Mengole's. This medium along with 1% dimethoate stress compound allowed only bacteria to grow capable of sustaining the given concentration of pesticides and indirectly provided an opportunity to allow restricted growth of bacteria capable of utilizing dimethoate as a carbon\nitrogen source for survival [10].

Fig 1 Zone of phosphate solubilization by *A. pulmonis* (AM2)

Isolate AM1 and AM2 when allowed grow on Pikovaskys agar plate added with dimethoate, in a result phosphate solubilization was recorded as per formula only in *A. pulmonis* (AM2) isolate plate with 75% of phosphate solubilization having solubilization zone (3.5mm) as well as colony diameter 2mm (Fig 1). In case of isolate AM1 (*P. fluorescens*) test was recorded to be negative for phosphate solubilization. The calculation for isolate AM1 has been given below:

$$SE\ (\%) = [\text{Solubilisation zone (mm)} - \text{Colony diameter (mm)}] / \text{colony diameter} \times 100$$

$$= [(3.5 - 2) / 2] \times 100$$

$$SE = 75\%$$

In a related study, where we have proposed that our isolate AM1 and AM2 able to degrade dimethoate and also remain capable of supporting plant growth by producing plant growth promoters; [11] reported a similar finding that chlorpyrifos pesticide degrading *Achromobacter xylosoxidans* strain JPC4 and *Ochrobactrum sp* strain fcp1 able to produce indole acetic acid, ammonia production and can also solubilize phosphate in presence as well as absence of chlorpyrifos. This report confirms our finding also that soil borne isolate remain capable of dual features as stated above and should be useful in bioremediation and plant growth simultaneously. In the present study, plant growth promoting feature as phosphate solubilization has been found positive on Pikovaskys agar plate by only isolate AM2 while AM1 found to be non-producer.

SUMMARY

In the present increasing problem of pesticide pollution in soil, water and animal body has been tackled by investigating soil micro biota capable of degrading one type of

pesticide 'dimethoate' belonged to organophosphorus category. Study recorded that soil harbours bacterial as well as fungal species capable of utilizing pesticide as a substrate and can convert it into metabolites less toxic in the environment. This study put forward that *P. fluorescens* and *Achromobacter pulmonis* isolates remained capable of degrading dimethoate effectively and due to their metabolism, formed metabolite found to non-lethal to the environment. In the present increasing problem of pesticide pollution in soil, water and animal body has been tackled by investigating soil micro biota capable of degrading one type of pesticide 'dimethoate' belonged to organophosphorus category. Study recorded that soil harbours bacterial as well as fungal species capable of utilizing pesticide as a substrate and can convert it into metabolites less toxic in the environment. During investigation, it has been recorded that not only the bacterial species *P. fluorescens* AM1 and *Achromobacter pulmonis* AM 2 able to degrade dimethoate by involving esterase enzyme but also it carries ability to supply plant growth promoting biomolecule like phosphate solubilization. This feature is well documented in plant growth promotion, when co-inoculated bacteria (AM1 or AM2).

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