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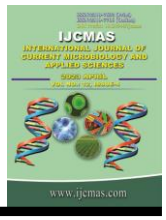

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Biodegradation of an Organophosphate Pesticide Monocrotophos by Bacterial Isolates from Soil

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A B S T R A C T

The article is to identify the monocrotophos-degrading bacteria. There were around 32 different strains that were isolated, and out of those, 14 isolates showed considerable pesticide biodegrading activity. Following examination with 16S rRNA, the two bacteria that had been isolated were determined to be *Serratia marcescens* and *Stenotrophomonas maltophilia*. Up to ten days after the experiment, each strain showed a distinctive capacity to degrade the monocrotophos. *Stenotrophomonas maltophilia* strains have shown a degradation percentage of 90%, while *Serratia marcescens* strains demonstrated a degradation percentage of 91%. Even at 100 ppm, the degradation trend demonstrated that all of the selected strains were able to use the provided pesticides as single-carbon energy sources.

Introduction

Monocrotophos is a widely used organophosphate insecticide that toxic a significant threat to the environment and human health due to its toxicity and persistence. Biodegradation of monocrotophos by bacterial species has been identified as a potential solution to mitigate its negative impact (Bhadbhade *et al.*, 2002a). Various bacterial species have been identified to efficiently degrade monocrotophos by producing enzymes such as organophosphorus hydrolase (OPH), which breaks down the chemical bonds in monocrotophos, leading to its degradation into less toxic compounds (Bhalerao and Puranik, 2009).

The degradation of monocrotophos is necessary to minimize its harmful effects on the environment and human health. Monocrotophos is a highly toxic organophosphate insecticide used to control a wide range of pests in agriculture, including insects, mites, and nematodes. It is a systemic insecticide that is absorbed by the plant's roots and distributed throughout the plant, providing long-lasting protection against pests (Mishra *et al.*, 2021).

However, monocrotophos is also highly toxic to humans and other non-target organisms. It can cause acute poisoning through inhalation, ingestion, or skin contact, leading to symptoms such as nausea, vomiting, diarrhea, muscle tremors, convulsions,

and respiratory paralysis (Zhan *et al.,* 2018). Chronic exposure to monocrotophos has been linked to neurological damage, developmental disorders, and cancer.

Due to its high toxicity, many countries have banned or restricted the use of monocrotophos. In addition, alternative pest control methods such as integrated pest management (IPM) and biological control are being promoted as safer and more sustainable alternatives to chemical pesticides (Sariwati and Purnomo, 2018).

Monocrotophos is an organophosphate insecticide that can be degraded by soil bacteria. Several species of soil bacteria such as Pseudomonas, Bacillus, Arthrobacter, and Alcaligenes have been reported to be capable of degrading monocrotophos (Rizqi *et al.,* 2021).

The degradation of monocrotophos by soil bacteria occurs through the process of biodegradation, which involves the enzymatic breakdown of the chemical compound into simpler compounds that are less harmful to the environment (Regueiro *et al.,* 2015). The degradation process can be influenced by various factors such as temperature, pH, soil moisture content, and the presence of other contaminants (Kaya *et al.,* 2014).

The degradation of monocrotophos by soil bacteria is an important mechanism in reducing the environmental impact of this pesticide. However, the effectiveness of this process can vary depending on the conditions of the soil and the types of bacteria present. Therefore, it is important to carefully manage the use of pesticides to minimize their impact on the environment (Grewal *et al.,* 2016).

Materials and Methods

Isolation of biodegrading bacteria

The MSM agar media with 25 ppm monocrotophos plates were first inoculated with 0.1 mL of each enhanced culture for the isolation of pesticide-degrading bacteria and then incubated at 30 °C until colonies emerged. Single colonies were selected, streaked aseptically on solid media plates, and then incubated for 48 hours at 30 °C. Until pure cultures developed, different colonies were sub cultured onto MSM agar plates. The strains from the pure culture were then injected into a 100-mL MSM broth that was the only carbon source and contained 100 ppm of the insecticide monocrotophos. To make sure that sources mentioned in the media were not tainted, negative controls were always conducted. An ultraviolet-to-visible-to-near-infrared (UV-Vis) spectrophotometer was used to quantify the absorbance (Roy *et al.,* 2018). The MSM agar slants containing the cultures with the highest capacity for degradation were kept at 4 °C.

Identification and characterization of isolates

Biodegrading isolates were identified and characterized using morphological, biochemical, and molecular testing. The conserved isolates with the highest degrading activity were selected and cultivated on MSM medium using the streak plate method, followed by 48 hours of culture at 30 °C (Jiang *et al.,* 2019). The colony features and morphological structure of the cells were examined using a microscope. The bacteria that were capable of breakdown were then identified by gram staining. Catalase, oxidase, casein hydrolysis, Indole synthesis, Methyl Red, citrate utilization, and nitrate reduction tests were among the biochemical tests performed.

Molecular characterization

The DNA from the isolated strains was extracted using the CTAB-phenol-chloroform-isoamyl alcohol extraction method (Zhan *et al.,* 2018). The isolated strains' DNA was used as a template for PCR amplification with 16S rDNA primers. The bacterium's forward primer was 16sF (5' GGATGAGCCCGGCCTA 3'). 16sR (5' CGGTGT GTACAAGGCCCGG 3') was the reverse primer. To examine sequences, the Basic Local Alignment Search Tool (BLAST) was used (Theriot *et al.,* 2010). The 16S rRNA gene sequences of selected strains were obtained from GenBank was used to

compare them with the gene sequences of our isolates. Using the aligned sequences, a distance matrix was created (Somtrakoon and Pratumma, 2012).

Statistical analysis

Experiments were carried out in triplicates, and the results presented are the mean ± standard error of the three replicates.

Results and Discussion

Isolation and screening of pesticide-degrading bacteria

Many indigenous pesticide-tolerant bacteria with pesticides as their sole carbon source were found in polluted soil through enrichment. On MSM agar plates containing monocrotophos pesticides, 32 morphologically different organisms were recovered from these enrichment cultures. The pesticide breakdown capacity of these strains was tested on MSM agar plates containing monotrophos at various concentrations (figure 1).

Pesticide degradation by selected isolates

On preliminary examination of degradation by spectrophotometry, Degradation percentages were recorded for 14 isolates (S1MoA, S1MoB, S2MoA, S3MoA, S4MoA, S5MoA, S6MoA, S7MoA, S8MoA, S9MoA, S9MoB, S10MoA, S11MoA, and S12MoA) when exposed to 100 ppm of monocrotophos. The observed degradation percentages were 77%, 82%, 91%, 82%, 81%, 77%, 92%, 82%, 83%, 82%, 89%, 78%, 77%, and 82%, respectively(figure 2).

The degradation of Monocrotophos by two of the fourteen isolates, namely S2MoA and S6MoA, which demonstrated the highest spectrophotometric degradation, was assessed using GC-MS analysis. The degradation rates of monocrotophos were found to be 91% and 90% for S2MoA and S6MoA, respectively (figure 3 and 4).

Identification and characterization of isolates

The bacterial strains were chosen based on an analysis of their morphology and colony characteristics, which provided insight into the microbial diversity present in the collected samples. The observation of different pigmented colonies, characterized by colors of yellow, cream, and white, was determined based on the physical appearance of the isolates. Upon observation through a phase-contrast microscope, it was noted that the isolates exhibited varying cellular morphologies.

The majority of the isolates were observed having a rod-shaped morphology, with the exception of three isolates which displayed a coccus shape. The majority of isolates were found to be gram-negative and motile, while a minority were non-motile. Endospores were not detected in any of the isolates, and there was no indication of exospores in any of the isolates.

Molecular identification

A partial sequence of the 16S rRNA showed that two isolates had 99.86% homology with *Serratia marcescens* Simultaneously, the other isolate were 99.76% identical to *Stenotrophomonas maltophilia* (Table 1).

All these sequences are deposited in the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/).

Table.1 Bacterial strains with GenBank accession numbers

Isolates code	Isolates strain	GenBank accession no.
S2MoA	*Serratia marcescens*	OQ619148
S6MoA	*Stenotrophomonas maltophilia*	OQ619149

Fig.1 Screening of degrading bacterial isolates on 100 ppm

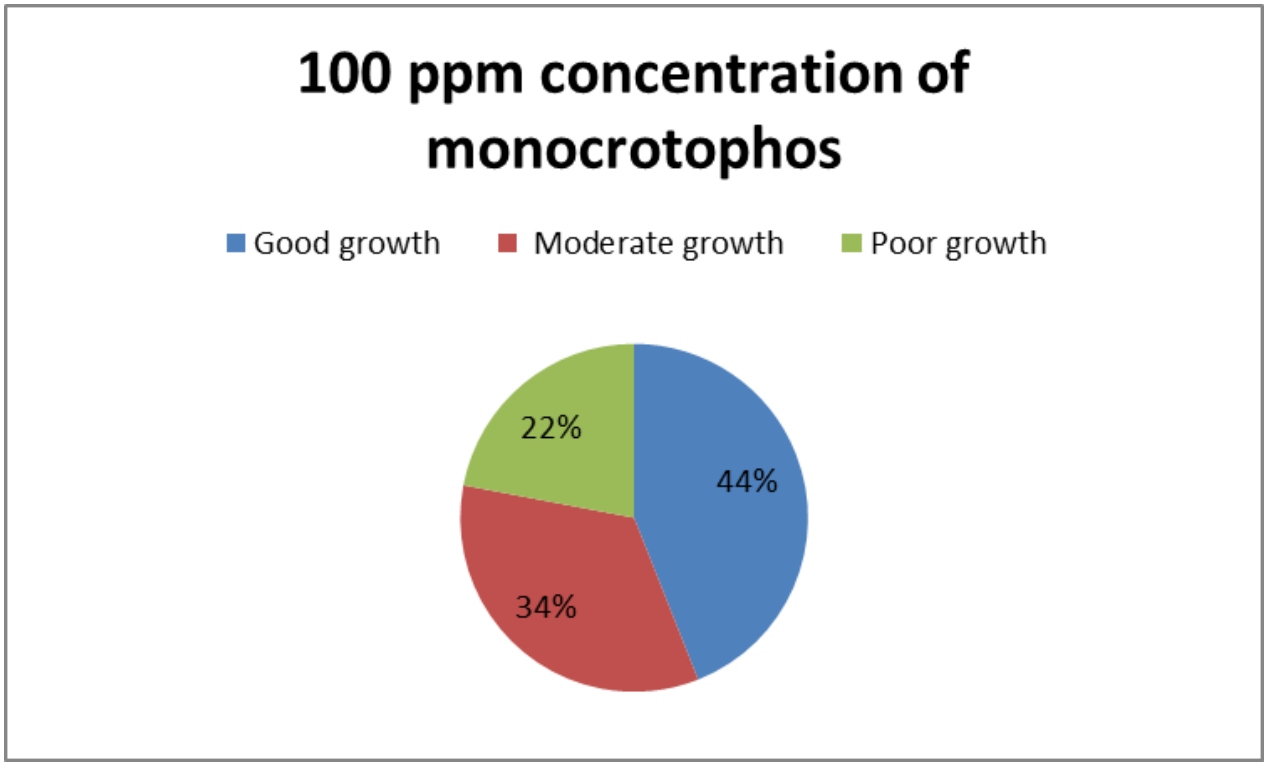


Fig.2 Monocrotophos degradation by spectrophotometry analysis

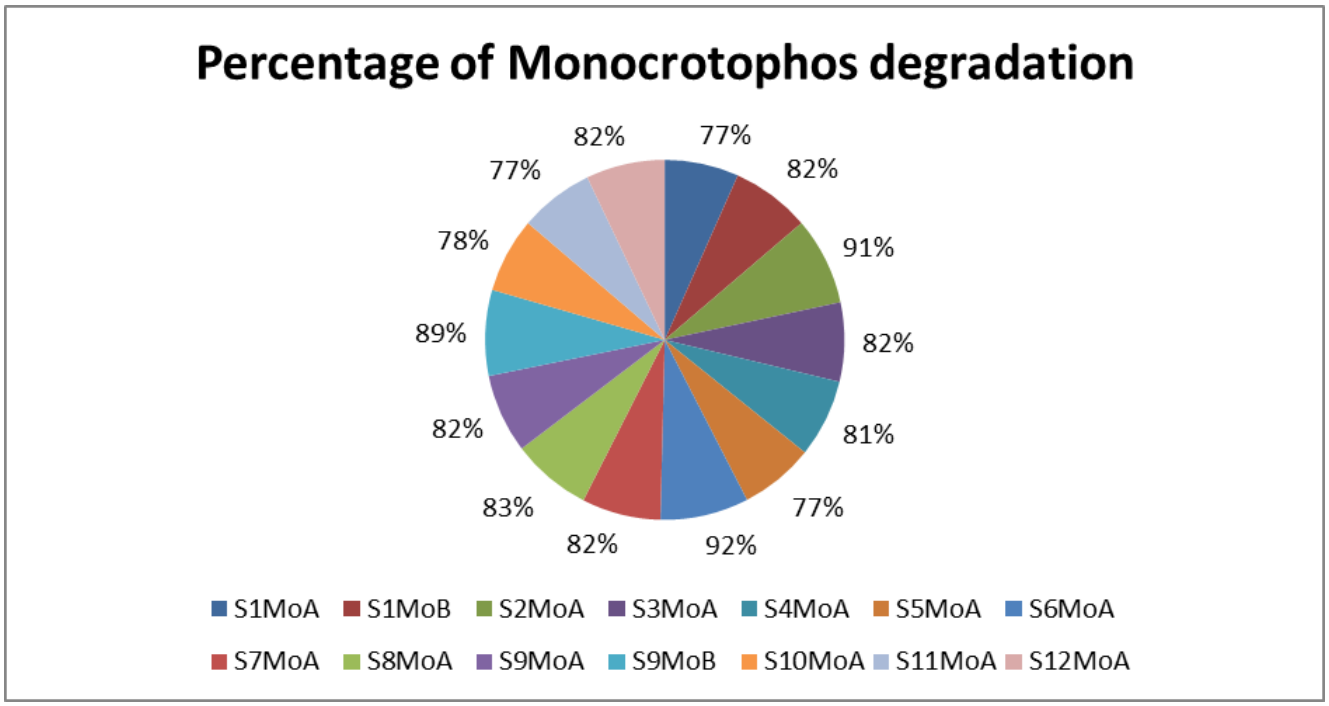


Fig.3 Degradation of Monocrotophos by S2MoA isolate

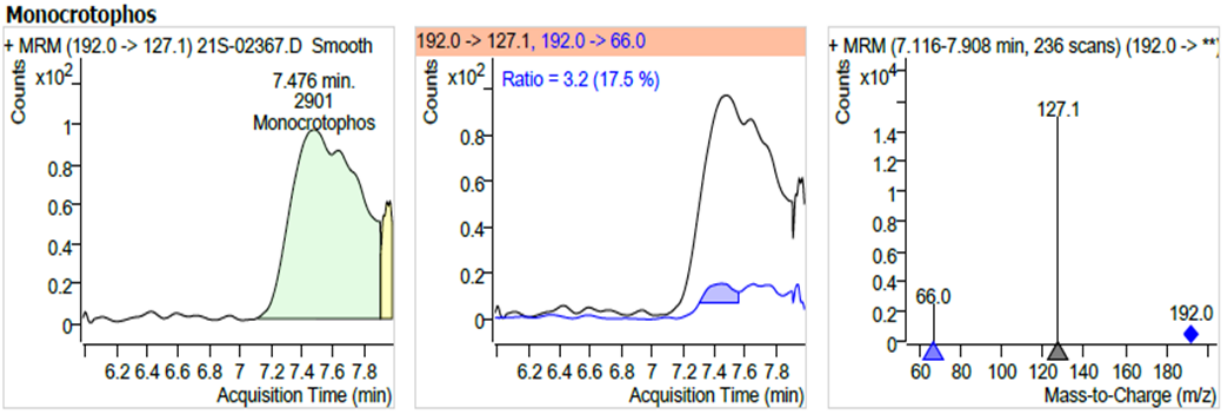
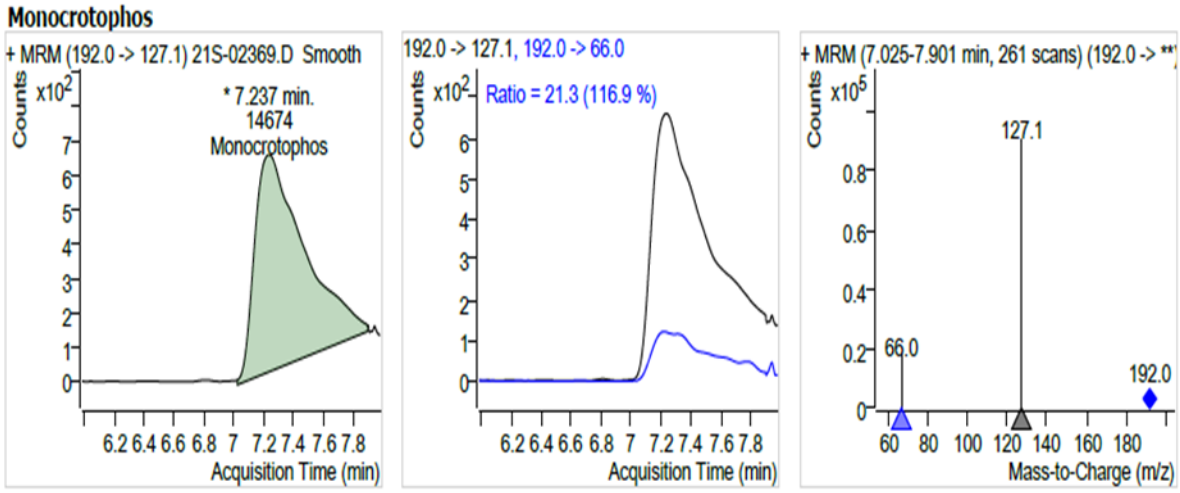


Fig.4 Degradation of Monocrotophos by S6MoA isolate



In conclusion, the present investigation shows two microorganisms exhibiting different degradation capabilities. The majority of previous studies have indicated that degradation occurs at 30^0C within a period of approximately 7 to 10 days of incubation. However, the present study has identified microorganisms that show a greater capacity for degradation at the same temperature within a shorter time frame of 72 hours. One possible explanation for this phenomenon is that these organisms were obtained from environments that were contaminated with pesticides, which may have resulted to their enhanced adaptability. This trend has demonstrated that specific strains can be utilized based on the type of pollutant found in a given environment to eliminate harmful chemicals. The present study reveals that, of the two strains obtained from a contaminated pesticide environment, *Serratia marcescens* S2MoA exhibited a greater capacity to degrade monocrotophos, with a degradation rate of 91%. Meanwhile, *Stenotrophomonas maltophilia* demonstrated the highest percentage of degradation, reaching 90%.

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