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Analysis of Pesticide Residues in Agricultural Soil via GC-MS and Enhancement of Degradation Efficiency by Bacillus velezensis SKRB5

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ABSTRACT

Overuse of pesticides negatively impacts soil fertility and ecosystems. This study aimed to screen agricultural soils in Mahabubabad, Telangana, India, for pesticide residues using GC-MS techniques and to identify bacteria capable of degrading these pesticides. The findings revealed three major pesticides: Monocrotophos, Atrazine, and Mancozeb. Among ten bacterial isolates, *Bacillus velezensis* SKRB5 demonstrated significant pesticide degradation ability. The growth and biodegradation capacity of *Bacillus velezensis* SKRB5 varied significantly across different pH values, temperatures, and pesticide concentrations. Optimal growth conditions were identified as pH 7, 37°C, and a pesticide concentration of 250 mg/L. Enzyme activity analysis over a week showed peak degradation on the fourth day, with Phosphotriesterases activity reaching 750 U/mg for Atrazine and Phosphatases activity reaching 600 U/mg for Mancozeb and Monocrotophos. This research highlights the importance of enzymatic activity in biodegradation and supports the future application of *Bacillus velezensis* SKRB5 in environmental remediation.

INTRODUCTION

The escalating global population, projected to reach 9 to 10 billion by 2050, poses a significant challenge in meeting the rising food demand. Agriculture, crucial for the economic development of many nations, involves crop cultivation and livestock rearing to provide food, fiber, and essential products (Searchinger et al., 2019). However, over the past four decades, agricultural productivity has surged, driven by increased use of fungicides, synthetic chemical fertilizers, and improved irrigation systems. This heightened food demand has led to environmental pollution and adverse effects on soil microbes and insect pollinators due to the overuse of chemical fertilizers and pesticides (Bisht and Singh Chauhan 2021). Chemical fertilizers have also increased crop susceptibility to diseases and reduced soil fertility. Organophosphorus compounds, known for their extensive pest control activity, biodegradability, and effectiveness, have garnered significant attention in the agricultural industry worldwide. Despite their widespread use, only about 0.1% of pesticides achieve their intended purpose, with the remainder ending up in the environment, causing a range of issues from reduced crop yields and degraded soil quality to lowered agricultural output and contaminated water, posing risks to animal and human health (Kaushal et al., 2021).

Biodegradation, defined by the International Union of Pure and Applied Chemistry as the breakdown of a substance catalyzed by enzymes, is a naturally occurring process where organisms break down pesticides or xenobiotic compounds. This process helps renovate pollutants at a discernible rate, restoring contaminated ecosystems (Prathap et al., 2022). Various studies have identified sources of organochlorine pesticide contamination in India's tropical coastal environment, leading to reports providing scientific data on future pesticide use and management.

Pesticide use can have detrimental effects on various living forms and the environment, impacting non-target species and altering biological processes (Pathak et al., 2022). The present study identified Monocrotophos, Atrazine, and Mancozeb as major pesticide residues in contaminated soils. Monocrotophos, initially registered by the US Environmental Protection Agency in 1965, has seen a significant increase in industrial output in India over the past three years (Singh et al., 2021). The Ministry of Chemicals and Fertilizers in India reports that biological detoxification is an economical and reliable method for eliminating pesticides (Ministry of chemical and Fertilizer India, 2020). Microbes play a significant role in the mineralization of pesticides, rendering them less toxic or non-toxic. Selecting highly effective and specific degrading strains that maintain soil viability is crucial for the bioremediation of soils contaminated with pesticides (Dash and Osborne, 2020; Singh et al., 2021). Atrazine, the most widely used herbicide in the forestry and agricultural sectors, is used to control broadleaf and grassy weeds in crops such as sorghum, sugarcane, and maize. However, atrazine has been shown to be harmful to fish, algae, insects, aquatic plants, and mammals (Cai et al., 2003; Ghosh et al., 2006). Various physical, chemical, and biological approaches are beneficial for removing atrazine from soil and water. Mancozeb, a dithiocarbamate compound used to manage weeds, presents challenges in removal or degradation due to its persistence and toxicity towards biological forms, necessitating the development of bioremediation technologies (Singh et al., 2021).

Studies have shown that mancozeb degradation is higher in inoculated non-sterile soils compared to sterile soils, suggesting that mancozeb-degrading bacteria could be effectively used in bioremediation (Hong et al., 2007). The increased catabolic potential of soil resulting from the introduction of degrading bacteria can lead to enhanced mancozeb utilization. Previous studies have demonstrated that in polluted soils, pesticide degradation is accelerated by the multiplication of potentially decomposing bacterial strains. Several investigations have revealed the impact of pesticides on various soil microorganisms and their metabolic activities, including respiration, nitrification, ammonification, nitrogen fixation, and dehydrogenase activity, among others (Cycoń et al., 2014).

Pesticides can be metabolized, excreted, stored, or bioaccumulated in the body fat of humans and animals. Chemical pesticides have been linked to detrimental health effects in several organ systems, including the reproductive, neurological, gastrointestinal, respiratory, and endocrine systems (Zhang et al., 2020; Badr, 2020). Additionally, high levels of unintentional, deliberate, or occupational pesticide exposure can lead to hospitalization and even death. While individual microorganisms are less likely to achieve complete mineralization of pesticide residues than mixed populations, bacterial species play significant roles in pesticide metabolism (Meena et al. 2020). Several studies have documented the ability of naturally occurring microbial consortia extracted from polluted soils to break down various pesticides and pesticide blends. For instance, a consortium of *Streptomyces* sp. capable of eliminating organochlorine pesticides has been studied (Briceño et al. 2020; Saez et al., 2018). The current study aimed to identify and isolate bacteria that can break down pesticides under optimal conditions. This paper describes how *Bacillus velezensis* breaks down monocrotophos, atrazine, and mancozeb. The *Bacillus velezensis* SKRB5 strain, found in pesticide-contaminated soil from vegetable farms in Mahabubabad, Telangana, India, was shown to degrade pesticides more effectively. This study also emphasizes optimizing the growth characteristics and enzyme activities of the *Bacillus velezensis* SKRB5 strain.

1. Methodology:

2.1 Soil samples collection and processing:

Soil samples were collected from the top layer of soil, specifically from depths of 0 to 20 cm, using a soil auger. These samples were taken from vegetable fields in Mahabubabad, Telangana, India, after removing leaves, roots, and stones. Prior to sampling, five sub-sites were randomly selected. From each sub-site, five soil samples were randomly collected and then combined to form composite samples. The soil was sieved through a 2 mm filter to remove stones and other foreign materials. The samples were then placed in polyethylene bags or plastic and glass containers, transported to the laboratory, and stored in sterile polythene bags at 4°C.

2.2 Extraction of pesticide residues from soil samples:

To extract certain pesticides from soil samples, 10 g of soil was placed in a 50 mL centrifuge tube. Then, 7 mL of deionized water was added to the tube, and the mixture was vigorously mixed and allowed to absorb water for 25-30 minutes. Next, 10 mL of acetonitrile was added to each sample and shaken for 5-6 minutes to extract the pesticide residues. Citrate salts from a Mylar pouch were then added to a portion of the soil sample in the centrifuge tube. The samples were vortexed for a minimum of 120 seconds and centrifuged at a speed of at least 3500 relative centrifugal force for 5 to 6 minutes. After centrifugation, 1.5 mL of the liquid remaining was transferred to a 2 mL tube containing C-18 solid-phase extraction material. The samples were mixed vigorously for 2 minutes and then centrifuged at a high speed for 2 minutes (e.g., > 5000 revolutions per minute). Finally, the presence of pesticides in the soil samples was analyzed using GC-MS (EL-Saeid et al., 2019).

2.3 Analysis by GC-MS:

The soil samples were analyzed using a gas chromatograph manufactured by Agilent, equipped with a mass detector. The injector temperature was set to 270°C, and the transfer line temperature was set to 280°C. The initial temperature of the column was 35°C, maintained for 5 minutes, then increased by 10°C per minute for the next 10 minutes. Subsequently, the temperature was ramped up rapidly by 50°C per minute until reaching a final temperature of 285°C. Helium gas was used as the carrier with a flow rate of 1.25 mL/min. The Agilent 5973 mass selective detector operated with an ion source temperature of 250°C and a quadrupole temperature of 180°C. The ion source temperature was maintained at 230°C. An accelerated energy of 70 electron volts (eV) was employed for electron impact (EI) to produce mass spectra. The sample extract was injected using an autosampler in 1.0µL aliquots. Fragment ion analysis was conducted in the mass range of 40–550 m/z during the complete scan, with a filament delay time of 3.3 minutes (A.H. Jagaba et al., 2023).

2.4 Degradation of pesticides by selected isolates

Three 250ml Erlenmeyer flasks were prepared, each containing 100ml of mineral salt media with concentrations of 100mg/L for Atrazine, Mancozeb, and Monocrotophos. Bacterial isolates were then added to the flasks, which were subsequently incubated at 30°C with continuous shaking at 150rpm for 7 days. A control flask without any bacterial inoculation was also included. The percentage of pesticide degradation was calculated using the method described by Akbar et al. 2016 (EL-Saeid et al., 2020).

$$Pesticide\ degradation\ (\%) = \frac{\text{Initial value} - \text{Final value}}{\text{Initial value}}\ X\ 100$$

2.5 Optimization of temperature

This study aimed to investigate the impact of temperature on the growth of pesticide-degrading bacteria in mineral salt media (MSM). Mineral salt media in 250 mL Erlenmeyer flasks (100 mL) were supplemented with 250 mg/L of Atrazine, Mancozeb, and Monocrotophos, and inoculated with isolate 5, in triplicate. The flasks were then subjected to different temperatures (20°C, 25°C, 30°C, 35°C, and 40°C) with continuous shaking at 120 rpm and pH 7.0. Bacterial growth under these varied temperature conditions was monitored over 7 days. A control flask without inoculation was included. Optical density at 600 nm was used to assess bacterial growth.

2.6 Optimization of pH

In this experiment, 250 ml Erlenmeyer flasks with 100 ml of mineral salt media were used in triplicate. Each flask was supplemented with 250 mg/L of Atrazine, Mancozeb, and

Monocrotophos, and inoculated with isolate 5. The flasks were then incubated at different pH levels (5.0, 6.0, 7.0, 8.0, and 9.0) with continuous shaking at 120 rpm and a temperature of 37°C. The effect of these varying pH levels on bacterial growth was monitored over 7 days. A control flask without inoculation was included. Bacterial growth was assessed by measuring the optical density at 600 nm.

2.7 Analysis of degradating enzymes of *Bacillus velezensis* SKRB5.

2.7.1 Phosphotriesterases enzyme assay

To perform the phosphotriesterase enzyme assay, start by preparing a 50 mM Tris-HCl buffer at pH 8.0, containing 1 mM MgCl2. Prepare a 10 mM stock solution of the organophosphate substrate methyl parathion in a suitable solvent. In a 1 ml cuvette, mix 970 µl of the buffer, 10 µl of 10 mM MgCl2, 10 µl of the substrate solution (from isolate 5 supernatant) to achieve a final concentration of 100 µM, and 10 µl of the enzyme solution. Immediately after mixing, measure the optical density at 412 nm using a UV-Vis spectrophotometer. Conduct control experiments without the enzyme to correct for non-enzymatic hydrolysis and with heat-inactivated enzyme to validate enzymatic activity. Calculate the enzyme activity in units, which represents the amount of enzyme needed to convert one µmol of substrate per minute in the assay. Adjustments may be necessary based on specific experimental requirements (Thomas et al., 2020).

2.7.2 Phosphatase enzyme assay

To conduct the phosphatase enzyme assay, first prepare a reaction buffer using 50 mM Tris-HCl (pH 8.0), supplemented with 1 mM MgCl2 or other necessary cofactors. Prepare a substrate solution by dissolving p-nitrophenyl phosphate (pNPP) in the reaction buffer to a concentration of 5-10 mM. In a cuvette or microplate well, combine the buffer, substrate solution, and enzyme preparation (supernatant of isolate 5) to a final volume of 1 ml (or appropriate volume for microplates). Add the enzyme solution last and mix thoroughly to initiate the reaction. Measure the absorbance at 405 nm. Include a control without the enzyme to correct for non-enzymatic substrate hydrolysis. Calculate the enzyme activity in units, which represents the amount of enzyme needed to convert one µmol of substrate per minute under assay conditions. Adjust experimental conditions as necessary for specific experimental requirements (Wu et al., 2020).

2. Results:

2.1 Screening of pesticides residues present in agriculture soil by using GCMS:

The study identified several pesticides in the agricultural soil samples, including Dimethyl (2E)-4-(methylamino)-4-oxobut-2-en-2-yl phosphate (Monocrotophos), 1,2-Ethaznediybis(carbamodithio)(2-)]manganese zinc salt (Mancozeb), and 12-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine (Atrazine), through mass spectra and fragmentation pattern analysis. Monocrotophos exhibited a retention time (RT) of 0.119 and a peak area of 16109910. Mancozeb showed a retention time and peak area of 1.108 and 4178914, respectively, while Atrazine had a retention time of 0.325 and a peak area of 8393683. The degradation efficiency (%) was calculated using the provided formula, and the area under the curve (AUC) was determined using the equation y = 0.2133x. *Bacillus velezensis* SKRB5 was able to degrade 55–70% of the pesticides, as shown in Table 1 & Fig.1, demonstrating its effectiveness in pollutant degradation, consistent with findings from EL-Saeid,M.H et al., 2019.

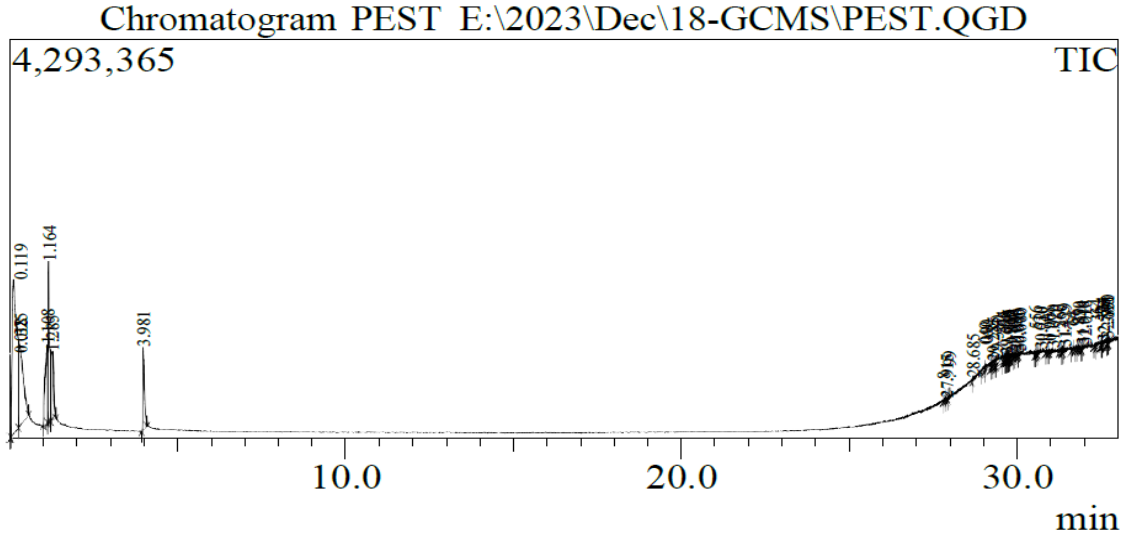

Figure 1: GC-MS chromatogram of pesticides compounds in soil

Table 1: Major compounds identified in soil by GC-MS screening.

Peak	R.Time	Area	Area%	Height	A/H	Base m/z	Name
1	0.018	870552	1.79	904290	0.96	41.1	E,E-1,9,17-Docasatriene
2	0.119	16109910	33.15	1653582	9.74	67.1	Dimethyl (2E)-4-(methylamino)-4-oxobut-2-en-2-yl phosphate **(Monocrotophos)**
3	0.325	8393683	17.27	821012	10.2	41.1	2-chloro-4-(ethylamino)-6-(isopropylamino)- 1,3,5-triazine **(Atrazine)**
4	1.108	4178914	8.6	852642	4.9	44.05	1,2-Ethaznediybis(carbamodithio)(2-)]manganese zinc salt **(Mancozeb)**

5	1.164	5358276	11.03	1731250	3.1	45.1	Isopropyl Alcohol
6	1.285	3911801	8.05	744646	5.25	44.05	5-cis-Methyl-1R,3-cis-cyclohexanediol
7	3.981	3055385	6.29	892832	3.42	55.05	Cyclohexanone
8	29.09	524298	1.08	79142	6.62	281.05	1,4-Benzenedicarboxylic acid, bis(4-butylphenyl) ester
9	29.574	849899	1.75	103301	8.23	207	d-Mannitol, 1-decylsulfonyl-

3.2 Optimization studies of degradation of pesticides by using potent *Bacillus velezensis* SKRB5

3.2.1 Degradation capacity of *Bacillus velezensis SKRB5* against pesticides

An experiment was conducted to evaluate the degradation efficacy of isolate 5 when exposed to varying concentrations (100 mg/L, 250 mg/L, and 500 mg/L) of three pesticides: Atrazine, Mancozeb, and Monocrotophos. Each pesticide concentration was added separately to flasks containing the isolate and then incubated for 7 days. Following the incubation period, the percentage of pesticide degradation was determined. The findings indicated that isolate 5 exhibited notably high degradation activity at the 100 mg/L and 250 mg/L concentrations for all three pesticides. This heightened activity was particularly prominent between the third and fourth days of incubation, suggesting a gradual enhancement in the isolate's capacity to degrade the pesticides during this interval.

Table 2: pesticide degradation capability of the isolate 5 (*Bacillus velezensis* SKRB5)

Pesticide with concentration mg/L	Incubation period in days						
	1	2	3	4	5	6	7
	Percentage of degradation (%)						
Atrazine 100	12±(0.8)^{f}	21±(0.2)^{e}	38±(0.4)^{d}	52±(0.4)^{a}	55±(0.2)^{b}	57±(0.8)^{c}	58±(0.1)^{c}
Atrazine 250	10±(0.2)^{f}	18±(0.4)^{e}	35±(0.2)^{d}	50±(0.2)^{a}	54±(0.4)^{b}	55±(0.6)^{c}	56±(0.2)^{c}
Atrazine 500	01±(0.4)^{f}	05±(0.2)^{e}	12±(0.1)^{d}	16±(0.2)^{a}	17±(0.2)^{b}	18±(0.4)^{c}	20±(0.6)^{c}
Mancozeb 100	10±(0.1)^{f}	15±(0.4)^{e}	34±(0.2)^{d}	51±(0.1)^{a}	53±(0.2)^{b}	55±(0.2)^{c}	55±(0.4)^{c}
Mancozeb 250	9±(0.2)^{f}	16±(0.4)^{e}	35±(0.2)^{d}	52±(0.1)^{a}	54±(0.4)^{b}	55±(0.6)^{c}	55±(0.2)^{c}
Mancozeb 500	1±(0.1)^{f}	04±(0.1)^{e}	10±(0.4)^{d}	16±(0.2)^{a}	17±(0.4)^{b}	18±(0.2)^{c}	18±(0.1)^{c}
Monocrotophos 100	14±(0.4)^{f}	22±(0.2)^{e}	38±(0.2)^{d}	62±(0.1)^{a}	68±(0.4)^{b}	69±(0.6)^{c}	70±(0.2)^{c}
Monocrotophos 250	12±(0.4)^{f}	24±(0.6)^{e}	40±(0.1)^{d}	65±(0.2)^{a}	67±(0.2)^{b}	68±(0.4)^{c}	69±(0.6)^{c}
Monocrotophos 500	5±(0.2)^{f}	11±(0.4)^{e}	16±(0.2)^{d}	21±(0.1)^{a}	24±(0.6)^{b}	26±(0.4)^{c}	27±(0.6)^{c}

Values superscribed by a-g indicate importance in descending order; values with the same alphabet are not significant by Fischer's least significant difference test ($p<0.05$). numbers in the column indicate the mean of two separate trials with three replications; numbers in brackets are the standard error.

The degradation of Atrazine, Mancozeb, and Monocrotophos by isolate 5 was examined at varying concentrations (100 mg/L, 250 mg/L, and 500 mg/L) over a 7-day incubation period. For Atrazine, degradation percentages at 100 mg/L increased from 12% on day 1 to 58% on day 7, and at 250 mg/L, from 10% to 56% over the same period. At 500 mg/L, degradation was lower, starting at 1% on day 1 and reaching 20% by day 7. Similarly, Mancozeb degradation at 100 mg/L increased from 10% on day 1 to 55% on day 7, and at 250 mg/L, from 9% to 55%. At 500 mg/L, degradation ranged from 1% on day 1 to 18% on day 7. Monocrotophos degradation at 100 mg/L increased from 14% on day 1 to 70% on day 7, and at 250 mg/L, from 12% to 69%. At 500 mg/L, degradation started at 5% on day 1 and reached 27% by day 7. Overall, isolate 5 showed the highest degradation rates for all three pesticides at the 100 mg/L and 250 mg/L concentrations, with degradation increasing over the incubation period.

3.3 Optimization studies of *Bacillus velezensis* SKRB5 for pesticide degradation

3.3.1 Optimization of temperature

Among all the temperatures tested, 37°C was found to be the most effective for the degradation of all three pesticides. *Bacillus velezensis* SKRB5 demonstrated significantly higher degradation activity at 37°C compared to the other temperatures. This optimal temperature allowed the bacterium to effectively break down Atrazine, Mancozeb, and Monocrotophos, indicating that 37°C is the ideal condition for maximizing the pesticide-degrading capabilities of *Bacillus velezensis* SKRB5. The results suggest that temperature is a critical factor influencing the biodegradation efficiency of *Bacillus velezensis* SKRB5. The higher degradation activity observed at 37°C can be attributed to the enhanced metabolic and enzymatic activities of the bacteria at this temperature. Conversely, temperatures lower or higher than 37°C resulted in reduced degradation efficiency, likely due to suboptimal conditions for the bacterial enzymatic processes involved in pesticide degradation.

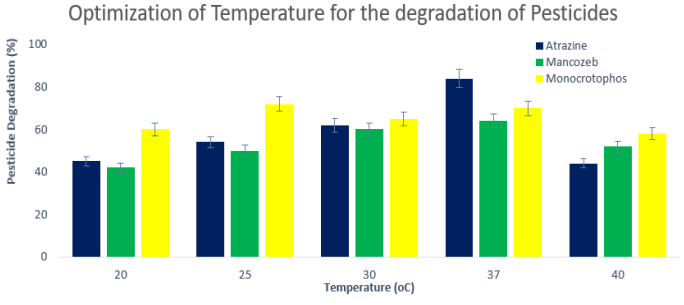

Figure 2: Optimization of temperature for the degradation of pesticides

The percentage degradation of three pesticides Atrazine, Mancozeb, and Monocrotophos across different temperatures (20°C, 25°C, 30°C, 37°C, and 40°C). The degradation percentages vary notably with temperature changes. At 20°C, the degradation percentages for Atrazine, Mancozeb, and Monocrotophos are approximately 45%, 50%, and 55% respectively. At 25°C, the degradation percentages for the pesticides are around 50%, 55%, and 60% respectively. At 30°C, all three pesticides exhibit similar degradation percentages,

each around 60%. A significant increase in degradation is observed at 37°C, with Atrazine showing the highest degradation percentage at approximately 85%, while Mancozeb and Monocrotophos display degradation percentages around 70%. At 40°C, the degradation percentages slightly decrease, with Atrazine, Mancozeb, and Monocrotophos showing around 65%, 60%, and 65% respectively. These results indicate that the optimal temperature for maximum pesticide degradation varies, with 37°C being particularly effective for Atrazine.

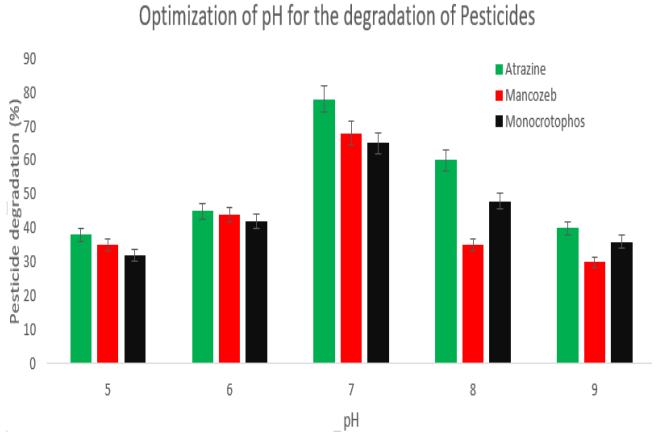

Figure 3: Optimization of pH for the degradation of pesticides.

The percentage degradation of three pesticides Atrazine, Mancozeb, and Monocrotophos across different pH levels (5, 6, 7, 8, and 9). The degradation percentages demonstrate significant variation with changes in pH. At pH 5, the degradation percentages for Atrazine, Mancozeb, and Monocrotophos are approximately 45%, 40%, and 50% respectively. At pH 6, the degradation percentages for all three pesticides are similar, around 50%. A notable increase in degradation is observed at pH 7, with Atrazine showing the highest degradation percentage at approximately 80%, Mancozeb at 75%, and Monocrotophos at 70%. At pH 8, the degradation percentages decrease to about 55% for Atrazine, 60% for Mancozeb, and 55% for Monocrotophos. At pH 9, the degradation percentages further decline, with Atrazine, Mancozeb, and Monocrotophos showing around 35%, 40%, and 40% respectively. These results indicate that pH 7 is the most effective pH level for the maximum degradation of these pesticides, particularly for Atrazine.

3.4 Analysis of degradating enzymes of *Bacillus velezensis* SKRB5 on Atrazine, Mancozeb, and Monocrotophos.

3.4.1 Phosphotriesterases and Phosphatase enzyme assay:

An analysis was conducted to evaluate the degrading enzymes produced by *Bacillus velezensis* SKRB5 when exposed to the pesticides Atrazine, Mancozeb, and Monocrotophos. Specifically, the study focused on the activity of Phosphotriesterases for Atrazine degradation and Phosphatases for the degradation of Mancozeb and Monocrotophos. The enzyme activity was monitored over a period, and the results revealed a peak in degradation enzyme activity on the fourth day of the experiment. On this day, the activity of Phosphotriesterases, responsible for breaking down Atrazine, reached a high of 750 U/mg. Similarly, the activity of Phosphatases, which degrade Mancozeb and Monocrotophos, was recorded at 600 U/mg.

3.3.2 Optimization of pH:

Among all the pH values tested, pH 7.0 was found to be the most effective for the degradation of all three pesticides. *Bacillus velezensis* SKRB5 exhibited significantly higher degradation activity at pH 7.0 compared to the other pH levels. This optimal pH condition allowed the bacterium to efficiently break down Atrazine, Mancozeb, and Monocrotophos, suggesting that a neutral pH environment is ideal for maximizing the pesticide-degrading capabilities of *Bacillus velezensis* SKRB5.

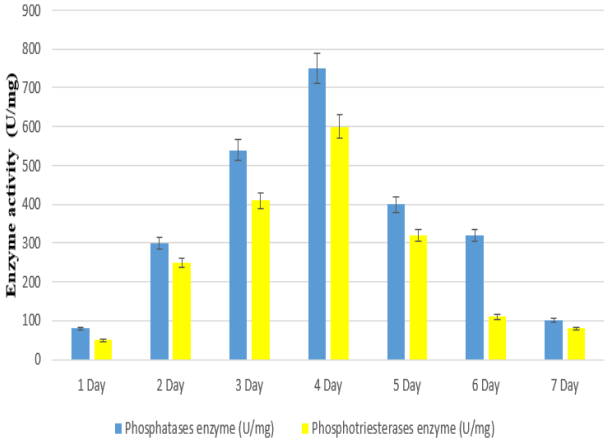

Figure 4: Analysis of degradating enzymes of *Bacillus velezensis* SKRB5.

These findings indicate that *Bacillus velezensis* SKRB5 is highly effective at producing degrading enzymes, with maximum enzymatic activity occurring on the fourth day of incubation. The high activity levels of Phosphotriesterases and Phosphatases suggest that these enzymes play a crucial role in the biodegradation process, breaking down the complex chemical structures of the pesticides into less harmful components. The peak enzyme activity observed on the fourth day suggests an optimal timeframe for biodegradation, which could be critical for designing bioremediation strategies. This timeframe allows for the maximum efficacy of the degrading enzymes, ensuring the most efficient breakdown of contaminants.

The enzyme activity of Phosphatases and Phosphotriesterases, measured in units per milligram (U/mg), over a period of seven days. On Day 1, the Phosphatases enzyme activity is around 150 U/mg, while the Phosphotriesterases enzyme activity is slightly lower, at approximately 120 U/mg. On Day 2, there is a notable increase in both enzyme activities, with Phosphatases reaching about 300 U/mg and Phosphotriesterases around 250 U/mg.By Day 3, enzyme activities continue to rise, with Phosphatases at approximately 550 U/mg and Phosphotriesterases at about 500 U/mg. The peak enzyme activities occur on Day 4, where Phosphatases reach the highest value of around 800 U/mg and Phosphotriesterases at about 700 U/mg. Following this peak, a decline in enzyme activities is observed. On Day 5, Phosphatases decrease to approximately 500 U/mg, and Phosphotriesterases drop to about 450 U/mg. By Day 6, the activities of both enzymes further decrease, with Phosphatases at around 300 U/mg and Phosphotriesterases at approximately 250 U/mg. On Day 7, the enzyme activities reach their lowest point since the start, with both Phosphatases and Phosphotriesterases around 150 U/mg and 120 U/mg respectively. These results indicate that the enzyme activities of both Phosphatases and Phosphotriesterases initially increase, peak on the fourth day, and then progressively decline over the subsequent days.

DISCUSSION

Bioremediation offers an environmentally friendly method for eliminating various pollutants from the environment (Gupta et al., 2016; Deng et al., 2006). The degradation of pesticides through microbial action is controlled by the entry of pesticide molecules into a population of microbes that consume them, along with the activity of these microbial populations (Seeger et

al., 2011). Nature regulates the concentration of pesticides in soil by enabling native microbial communities to ingest these hazardous molecules, thus benefiting both ecology and agriculture (Geed et al., 2018; Singh et al., 2017; Geed et al., 2017b). However, due to their highly resistant molecular structures, pesticides exhibit slow natural biodegradation kinetics, resulting in their persistent presence in soil. Consequently, microbiological research is essential for developing novel and advanced biotechnological tools that employ highly selective microbial species to detoxify pesticides (Geed et al., 2018b).

It is challenging to cultivate certain bacteria that interfere with the processes of pesticide breakdown in natural environments in a lab setting (M. Shahid et al., 2020). Nevertheless, isolating bacteria that can be used for eliminating pesticide-contaminated areas is crucial. Much research has focused on the genera *Paracoccus* and *Pseudomonas* due to their ability to degrade monocrotophos using it as the sole carbon source in various ways (Kim et al., 2020; Buvaneswari et al., 2017; N.S.A. Yaro et al., 2023). In this study, the pesticide compounds Monocrotophos, Atrazine, and Mancozeb were identified in contaminated soils, and 10 bacterial strains were isolated to evaluate their degradation capacity. The results revealed that *Bacillus velezensis* SKRB5 (Isolate 5) could utilize 55% to 70% of the studied pesticides. Overall, isolate 5 showed promising potential for bioremediation applications at lower pesticide concentrations, effectively degrading 100 mg/L and 250 mg/L of the tested chemicals within a week. Although *B. megaterium* has been reported to degrade monocrotophos in other published studies (Buvaneswari et al., 2017), this is the first report of a strain of *B. subtilis* degrading monocrotophos. Essential factors influencing the use of pesticides by bacteria as substrates for environmental growth include soil pH, agricultural practices, and the amount of pesticide applied (Acharya et al., 2015).

The current research identified a highly effective strain of *Bacillus* species for the degradation of atrazine and factor optimization. The findings indicated that the optimal conditions for achieving maximum atrazine breakdown with the bacterial isolate were a pH of 7.00, a temperature of 37°C, and an atrazine concentration of 250 mg/L. These results are consistent with previous research (K.J. Abioye et al., 2023; H.A. Muhammed et al., 2023), which utilized species such as *Rhizobium rhodococcus* (72%), mixed microbial consortium (50%), *Pseudomonas aeruginosa* (80%), *Pseudomonas alcaligenes* (>80%), *Klebsiella* sp., and *Comamonas* sp. (83.3%) for degradation. Consequently, the *Bacillus velezensis* SKRB5 strain, obtained from contaminated soil, proved to be effective in enhancing the atrazine degradation process. Mancozeb, a widely used fungicide, is popular for controlling fungal diseases in fruits and vegetables. Due to its known hazards to mammals and its potential to contaminate soil and water sources, it is crucial to remove Mancozeb from the environment promptly. Bacterial strains capable of degrading Mancozeb were extracted from polluted agricultural soil samples. *Bacillus velezensis* SKRB5, one of the promising isolates, was observed to degrade 55% of Mancozeb (250 mg/L) within seven days.

Prior studies have shown that pesticide degradation in polluted soils is accelerated by the proliferation of potentially degrading bacterial strains. For instance, *Burkholderia* sp. FDS-1, when inoculated with indigenous microbiota in fenitrothion (FT)-contaminated soil, enhanced the utilization of fenitrothion (Hong et al., 2007). Similarly, Serratia marcescens degraded deltamethrin more quickly in non-sterile soils than in sterile soils (Cycoń et al., 2014). Research on traits that promote plant growth has identified significant activities such as phosphate solubilization, indole acetic acid synthesis, and ammonia utilization by mancozeb-degrading bacteria. The literature indicates that the *Pseudomonas aeruginosa* strain isolated from tomato plants exhibits several plant growth-promoting characteristics, including high levels of in vitro and in vivo phytopathogen growth inhibition and pathogenicity prevention, suggesting its potential as a biocontrol agent (Ghadamgahi et al., 2022). Recent research also indicates that the *Rhizobium pusense* strain exhibits all plant growth-promoting rhizobacteria (PGPR) characteristics, produces siderophores, and promotes maize growth (Amezquita-Aviles et al., 2022).

The use of naturally occurring microbial populations for in-situ biodegradation of hazardous substances has garnered significant attention from researchers in recent decades (Kim et al., 2020). Pesticide degradation has been achieved using strains such as Acinetobacter johnsonii, *Lysini bacillus*, *Bacillus* sp., and *Pseudomonas* sp., isolated from polluted soils and sludge from industrial and agricultural sites (Li R, Wang et al.,2021). For instance, *Bacillus* sp. (consortia) isolated from an agricultural area was utilized in a two-stage integrated aerobic treatment plant (IATP) to biodegrade pesticides such as atrazine, malathion, and parathion (Geed et al., 2017d). The process involved the influent stream containing these pesticides (with an initial chemical oxygen demand COD of 123 mg/L) being fed into the first reactor, and the effluent from the first reactor then being processed in the second reactor. This setup achieved pesticide removal levels exceeding 90%. Additionally, investigations have aimed to biodegrade atrazine in synthetic wastewater using isolated bacteria, such as Alcaligenes sp. S3 from agricultural areas, in an alternating aerobic-anoxic lab-scale pilot plant (Geed et al., 2018a).

CONCLUSION

This study focused on using the *Bacillus velezensis* SKRB5 strain to optimize the effects of specific environmental parameters on pesticide breakdown. These parameters included pH, temperature, enzyme activity, and pesticide concentration. Through rigorous investigation, the ideal conditions for maximizing pesticide degradation were identified as a pH of 7.0, a temperature of 37°C, and a pesticide concentration of 250 mg/L. Under these optimized conditions, *Bacillus velezensis* SKRB5 demonstrated a significant capacity for pesticide breakdown. The success of this isolated bacterial strain suggests its potential to be further developed into a microbial consortium for enhanced pesticide degradation both in laboratory settings and field applications. Such consortia could leverage the synergistic interactions between different microbial species, potentially improving the efficiency and scope of biodegradation. Future research should aim to identify and characterize bacterial consortia that can achieve the most effective pesticide biodegradation under various environmental conditions. This includes exploring different combinations of microbial strains, optimizing consortia composition, and testing their efficacy across a range of soil types and contamination scenarios. Additionally, studies should investigate the long-term stability and resilience of these consortia in natural environments, as well as their interactions with native microbial communities.

REFERENCES

- Jagaba, AH. et al., Trend and current practices of coagulation-based hybrid systems for pulp and paper mill effluent treatment: mechanisms, optimization techniques and performance evaluation, J. Clean. Prod. (2023) 139543.
- Acharya KP, Shilpkar P, Shah MC, Chellapandi P. Biodegradation of insecticide monocrotophos by *Bacillus subtilis* KPA-1, isolated from agriculture soils. Appl Biochem Biotechnol (2015). 175(4):1789–1804. https ://doi.org/10.1007/s1201 0-014-1401-5.
- Amezquita-Aviles, CF., C. B. Coronel-Acosta, S. D. L. Santos-Villalobos, G. Santoyo & F. I. Parra-Cota. Characterization of native plant growth-promoting bacteria (PGPB) and their effect on the development of maize (Zea mays L.). Biotecnia, 2022, 24(1): 15-22.
- Cai, B., Y. Han, B. Liu, Y. Ren, S. Jiang, Isolation and characterization of an atrazine-degrading bacterium from industrial wastewater in China, Lett. Appl. Microbiol. 36 (2003) 272–276.

- Badr, AM. Organophosphate toxicity: updates of malathion potential toxic effects in mammals and potential treatments. Environ. Sci. Pollut. Res. 2020. 27 (21), 26036–26057. https://doi.org/10.1007/s11356-020-08937-4.
- Bisht N and Singh Chauhan P. Excessive and disproportionate use of chemicals cause soil contamination and nutritional stress Soil Contamination - Threats and Sustainable Solutions. 2021 (https://doi.org/10.5772/intechopen.94593).
- Briceño G, Lamilla C, Leiva B, Levio M, Donoso-Piñol P, Schalchli H, et al. Pesticide tolerant bacteria isolated from a biopurification system to remove commonly used pesticides to protect water resources. PLoS ONE (2020).15(6): e0234865. https://doi.org/10.1371/journal. pone.0234865.
- Buvaneswari G, Thenmozhi R, Nagasathya A, Thajuddin N. Screening of efficient monocrotophos degrading bacterial isolates from paddy field soil of Sivagangai district, Tamil Nadu, India. J Environ Sci Technol 2017;10:13-24.
- Cycoń, M. Żmijowska A & Piotrowska-Seget Z. Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of Serratia marcescens. International journal of environmental science and technology, 2014. 11: 1305-1316.
- Dash, DM., Osborne, JW. Biodegradation of monocrotophos by a plant growth promoting *Bacillus aryabhattai* (VITNNDJ5) strain in artificially contaminated soil. Int. J. Environ. Sci. Technol. 2020. 17, 1475–1490. https://doi.org/10.1007/s13762-019-02432-1.
- Deng X, Huang J, Rozelle S, Uchida E. Cultivated land conversion and potential agricultural productivity in China. *Land Use Policy* 2006;23(4):372-384.
- EL-Saeid, Mohamed & Alghamdi, Abdulaziz. Identification of Pesticide Residues and Prediction of Their Fate in Agricultural Soil. Water, Air, & Soil Pollution (2020). 231. 10.1007/s11270-020-04619-6.
- EL-Saeid, MH, Majjami, AY, Modaihsh AS, Al-Barakah NI, Fahad, G, Adel and Bazeyad, A. Impact of QuEChERS and GC-MS/MSTQD as multiresidues techniques for determination of 74 pesticides in olive farm soil. International Research Journal of Pure & Applied Chemistry, (2019). 17(4): 1–11, 2018; Article no. IRJPAC.46264 ISSN: 2231– 3443, NLM ID: 10164766.
- Geed SR, Prasad S, Kureel MK, Singh RS, Rai BN. Biodegradation of wastewater in alternating aerobic-anoxic lab scale pilot plant by Alcaligenes sp. S3 isolated from agricultural field. *J. Environ. Manage.* 2018a;214:408-415.
- Geed SR, Raj A, Kureel MK, et al. Singh RS. Studies on removal of Atrazine by coupling Fenton reaction with bioreactor in series. *Indian J. Exp. Biol.* 2017d;55:55(7):498-505.
- Ghadamgahi, F, Tarighi, S. Taheri, GV. Saripella, A. Anzalone, PB. Kalyandurg. Vetukuri RR. Plant growth-promoting activity of Pseudomonas aeruginosa FG106 and its ability to act as a biocontrol agent against potato, tomato and taro pathogens. Biology , 2022, 11(1): 140.
- Gupta M, Mathur S, Sharma TK, et al. A study on metabolic prowess of Pseudomonas sp. RPT to degrade imidacloprid, endosulfan and coragen. *J. Hazard. Mater.* 2016;301:250-258.
- Muhammed, HA. et al., Mitigating water contamination by controlling anthropogenic activities of organochlorine pesticides (OCPs) for surface water quality assurance, Case Studies in Chemical and Environmental Engineering (2023) 100474.
- Hong, Q., Zhang, Z. Hong Y. & Li, S. 2007. A microcosm study on bioremediation of fenitrothion-contaminated soil using Burkholderia sp. FDS-1. International biodeterioration & biodegradation, 59(1): 55-61.
- Abioye KJ, Harun NY, Saeed A.A. A brief review of solving biomass ash deposition with aluminum-silicate based additives and future perspective of kaolin, Chem. Eng. Trans. 98 (2023) 27–32.
- Kaushal, J, Khatri, M, Arya, SK. A treatise on organophosphate pesticide pollution: current strategies and advancements in their environmental degradation and elimination. Ecotoxicol. Environ. Saf. 2021. 17, 1299–1324. https://doi.org/10.1016/j.ecoenv.2020.111483.
- Kim SK. Trophic transfer of organochlorine pesticides through food-chain in coastal marine ecosystem. *Environ. Eng. Res.* 2020;25(1):43-51.
- Li R, Wang, J, Li, S. Eleftherohorinos IG. Biodegradation of omethoate by Bacillus sp. YB-10: optimization of culture conditions and degradation characteristics. *Environ. Eng. Res.* 2021;26(4):1-9.
- Shahid M, Khan MS and Zaidi A. Fungicide toxicity to legumes and its microbial remediation: A current perspective, Pesticides in Crop Production: Physiological and Biochemical Action, 2020, pp. 15–33.
- Meena RS et al 2020 Impact of Agrochemicals on Soil Microbiota and Management: A Review Land 9 34.
- Ministry of chemical & Fertilizer India, 2020. Annual Report 2019-20 [WWW Document]. URL https://chemicals.nic.in/sites/default/files/English Annual Report date 24-2-2020.
- Yaro NSA. A comprehensive review of biochar utilization for low-carbon flexible asphalt pavements, Sustainability 15 (8) (2023) 6729.
- Ghosh PK, Ligy P, Environmental significance of atrazine in aqueous systems and its removal by biological processes: an overview, Global Nest. 8 (2006) 71–90.
- Pathak VM. Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review Front. Microbiol. 13 962619. Environ. Res. Commun. 5 (2023) 075011.
- Prathap Somu, Saranya Narayanasamy, Levin Anbu Gomez, Saravanan Rajendran, Yong Rok Lee, Deepanraj Balakrishnan (2022). Immobilization of enzymes for bioremediation: A future remedial and mitigating strategy, Environmental Research, 212, (Part D), 113411.
- Saez JM, Bigliardo AL, Raimondo EE, Briceno GE, Polti MA, Benimeli CS. Lindane dissipation in a biomixture: Effect of soil properties and bioaugmentation. Ecotoxicol Environ Saf. 2018; 156: 97–105.https://doi.org/10.1016/j.ecoenv.2018.03.011.
- Seeger EM, Kuschk P, Fazekas H, Grathwohl P, Kaestner M. Bioremediation of benzene-, MTBE-and ammonia-contaminated groundwater with pilot-scale constructed wetlands. *Environ. Pollut.* 2011;159(12):3769-3776.
- Singh K, Giri BS, Sahi A, et al. Biofiltration of xylene using wood charcoal as the biofilter media under transient and high loading conditions. *Bioresour. Technol.* 2017;242:351-358.
- Singh, S, Kumar, V, Kanwar, R, Wani, AB, Kaur Gill, JP, Ramamurthy, PC, Singh, J, Garg, VK. Toxicity and detoxification of monocrotophos from ecosystem using different approaches: a review. Chemosphere 2021. 275, 130051. https://doi.org/10.1016/j.chemosphere.2021.130051.
- T. Searchinger, R. Waite, C. Hanson, J. Ranganathan, P. Dumas, E. Matthews, and C. Klirs Creating a sustainable food future: A menu of solutions to feed nearly10 billion people by 2050. Final report, 2019.

- Thomas, P, Atassi, M, Leprince, J, Balieu, S, Nègre, V, & Gobin, E. A novel bacterial phosphotriesterase with high catalytic efficiency for the degradation of organophosphates. Journal of Biological Chemistry (2020)., 295(9), 2695-2708.
- Wu, S, Guo, X, Gao, Y, Zhang, Z, & Chen, S. A high-throughput assay for screening phosphatase inhibitors based on enzyme-catalyzed precipitation. Analytical Biochemistry (2020). 609, 113984.
- Zhang, Q, Yu, C, Fu, L, Gu, S, Wang, C. New insights in the endocrine disrupting effects of three primary metabolites of organophosphate flame retardants. Environ. Sci. Technol. 2020. 54 (7), 4465–4474. https://doi.org/10.1021/acs.est.9b07874.