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EFFECT OF TEMPERATURE, PH, CARBON AND NITROGEN SOURCES ON THE GROWTH OF PESTICIDE DEGRADING BACTERIAL ISOLATES

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ABSTRACT

A pH of 7.0, a temperature of 30 degrees Celsius, an initial bacterial concentration of 0.25%, and a rotation speed of 150 revolutions per minute were shown to be ideal for degradation. Bacillus sp. YB-10, under ideal circumstances, may decrease an initial omethoate concentration of 1,000 mg/L to undetectable levels in 5 days. The soil sample was taken from a location with a long record of pesticide use: Agricultural University Gwalior in the Indian state of Madhya Pradesh. Staphylococcus sp., a kind of bacteria, was shown to be capable of decomposing Malathion and Dichlorvos. Strains of Micrococcus. Several species of the bacteria Entrobacte, Bordetella, Pseudomonas, and Klebsella.

KEYWORDS Pesticides, Degrading, Bacterial Isolates,

INTRODUCTION

It was previously thought that only a tiny portion of the millions of tons of pesticides sprayed manually really reaches the intended organisms and the rest are just deposited on the soil. Soil pesticides may have a long-lasting impact on weather patterns, including how much rain falls and when it falls. Pesticides and the bacteria they kill may be affected by the current climate change. Even though pesticides have been shown to increase crop yields, harming non-target species with careless application is a major source of environmental and human health concerns. It is widely documented that bacteria may breakdown organophosphates, and plausible degradation processes have even been postulated. Research shows that up to 500 mg/L of profenofos may be digested by bacteria in about 96 hours without the need for an external carbon source. Soil samples contaminated with old pesticides will be used to identify the bacteria with the greatest potential to break down the profenofos. Also, the optimal pH, growth pattern, and selection of a single strain with the best profenofos-degrading capacity were determined.

LITERATURE REVIEW

Paulina Książek-Trela et.al (2022) For a long time, people have relied on chemical means of protecting plants from harm. The risks to human health from using too many chemical protection items have received widespread attention in recent years. Loss of biodiversity and the proliferation of plant-harming organisms resistant to plant protection agents are only two of the many ways in which plant protection agent residues in crops alter the natural environment. Biological plant protection strategies, such as the use of biological active compounds comprising microorganisms, and

natural substances, have been given importance under the principles of integrated plant protection, which aim to preserve the health of people, animals, and the environment. Agricultural and environmental chemical plant protection products may be broken down more rapidly by microbes and other naturally occurring substances. This literature review examines how synthetic insecticides break down, which focuses on the role played by natural and biological pesticides. Bacillus spp. and Trichoderma spp. are the most significant and promising microorganisms for use in IPM systems due to their efficiency in pesticide breakdown and the abundance of commercial preparations on the market that include them. IPM systems advocate for the use of biological pesticides, which have the potential to improve soil quality, reduce environmental damage, and protect human health.

Phatcharida Inthama et.al (2021) Thai culture is deeply rooted on agriculture. Herbicides, notably paraquat, are crucial to agricultural output. Paraquat buildup is becoming an issue in an expanding region of farmland. Plants, animals, and aquatic species are all negatively impacted by paraquat wastes. Agricultural chemical pollutants may be reduced using the biological remediation method. Bacteria are a particularly intriguing bioremediator. Certain soil bacteria not only have the ability to degrade paraquat, but also to stimulate plant growth, which is a distinct practical benefit when using these bacteria in the field. Soil microorganisms that breakdown paraquat and stimulate plant development were the focus of this investigation. The chosen bacteria, according on morphological and 16S rDNA sequence analysis, is Bacillus aryabhattai strain MoB09. It may thrive in low- or no-nitrogen environments. At pH 7 and 30°C, B. aryabhattai thrives and degrades paraquat most effectively. The selected strain had all of the features that helped plant development, such as the ability to synthesize indole, produce siderophores, dissolve phosphate, and produce 1-aminocyclopropane-1-carboxylic acid deaminase. Degradation of paraquat in cowpea was also measured in the cooker. This strain proved successful in removing paraquat residue from soils that had been sterilized and those that had not. Cowpea plants inoculated with B. aryabhattai and cultivated on paraquat-contaminated soil had significantly larger root and shoot lengths than control plants. In addition, B. aryabhattai enhanced cowpea development even when the plant was subjected to artificial dry conditions. Based on these findings, B. aryabhattai may be used to reduce the amount of paraquat left in soil and increase crop yields in organic farming.

Noreen Asim et.al (2021) Background. Pollution of natural resources is particularly severe in underdeveloped nations because of a lack of infrastructure for the disposal of industrial effluents. These contaminants, when added to solid waste, are especially harmful to plant and animal life. There was evidence that native microbial communities were responsible for naturally attenuating these contaminants. Bacteria capable of decomposing pesticides were isolated from soil near factories producing such chemicals. Methods. Biodegrading microorganisms have been isolated and characterized. The chosen pesticide-degrading bacteria were isolated from industrial waste using an enrichment culture approach. Results. Over 20 unique strains were identified, with 6 exhibiting considerable biodegradation activity toward pesticides. Two of the bacteria were found to be Acinetobacter baumannii (5B) and Acid thiobacillus ferroxidans using 16S rRNA analysis, while the other four were shown to be distinct strains of Pseudomonas aeruginosa (1A, 2B, 3C, 4D). Their shared ancestry was verified by phylogenetic research. Up to the 36-hour mark, all strains shown their own unique degrading abilities. The selected strains were shown to be

able to use the given insecticides at concentrations of 50 mg/mL or less, as seen by the degradation trend. The results of this research show promise for soil treatment and restoration by using these strains to degrade persistent residual herbicide.

Madhushree T (2017) This research expands on previous work that used microorganisms to remove cypermethrin from polluted fields for bioremediation purposes under optimal conditions, with the goal of lowering environmental toxicity caused by pyrethroids. The bacteria responsible for breaking down cypermethrin have been identified and isolated. Thin-layer chromatography coupled with a spectrophotometric analysis was used to calculate the pesticide's rate of degradation. Transformation was used to introduce the pesticide-degrading plasmids that had been previously identified.

Magda M. Aly et.al (2017) Using the enrichment culture method, three soil isolates were successfully grown on a minimum growth medium containing 60 mg/l of Diazinon as the sole carbon source. Diazinon degradation was tested on a total of three bacterial strains. Isolate BMNF7 showed the most activity, degrading a total of 33 percent. The deterioration rates for BMRF3 and BMTF 8 were much lower, coming in at 21% and 30%, respectively. Morphological and biochemical analysis of the BMNF7 isolate suggested that it belonged to the genus Bacillus, and 16s rRNA sequencing verified this. After 10 days incubation at pH 7 and 45oC with a 4x106 CFU/ml inoculum size, the most destruction was achieved. It was found that 37 degrees Celsius was optimal for growth, whereas 45 degrees enhanced the deterioration process to its maximum. Bacterial strains isolated from farmland soil decomposed the pesticide diazinon. These strains belonged to the genus Bacillus, and the degradation rate was increased by the modification of growing conditions.

METHODS

Effect of Degradation Conditions

In this work, we varied the starting bacteria concentration (0.1%-0.5%), pH (5.0-8.0), temperature (15°C-35°C), rotation speed (100-250 r.p.m.), and rotational speed (100-250 r.p.m.) to examine the impact of degrading conditions. For each test, bacteria were suspended in degradable medium and grown for 5 days. All studies began with omethoate values of 1,000 mg/L. There were three sets of each experiment.

Soil

The top five to ten centimeters of soil were dug out and sampled in triplicate. Partial air drying allowed for the characterization of soil samples. Table1 displays the characteristics of the soil sample used.

Pesticide

Gwalior, Madhya Pradesh's local pesticide store provided us with commercial-grade Malathion (50%) and Dichlorvos (76%). The structures of these insecticides, which are all part of the organophosphate family, are seen in Fig. 1.

Isolation of Pesticide Degrading Bacteria

Bacteria capable of breaking down the pesticides were isolated by inoculating each sample with 100 mg/lit of Malathion and Dichlrovose and then enriching the samples with MSM, Sucrose 2.0, and Yeast extract 3.0. The culture flasks were incubated at 30°C with the help of an orbital shaker operating at 120 rpm. Two milliliters of cultures were then cultured in a new medium containing 100 milligrams of insecticide. 200 L of the fifth passage was cultured in an incubator at 30°C for 24 hours after being plated on nutrient agar. Struck into a nutrient agar slant, Bacterial stain pure culture colonies were preserved by refrigeration at 4 degrees Celsius.

Table 1. Characteristics of soil sample collected

Sample No.	Depth	pH	Moisture contain (%)	Clay (%)	Silt (%)	Sand (%)
1	10	7.3	4.71	65.0	8.16	26.8
2	15	6.8	4.71	70.2	8.30	21.4
3	10	6.9	7.54	69.1	6.80	24.0
4	15	7.3	11.32	59.0	8.80	32.0
5	10	6.3	7.54	54.0	10.80	35.1
6	15	6.7	7.54	59.1	9.00	26.1
7	10	6.5	5.66	63.3	7.60	29.0
8	15	6.7	7.54	51.8	10.30	37.6
9	10	6.8	12.26	60.0	15.00	25.0
10	15	7.2	20.75	56.8	14.60	142.1
11	10	7.1	9.43	62.0	9.16	28.8
12	15	7.3	15.09	55.5	10.30	34.1

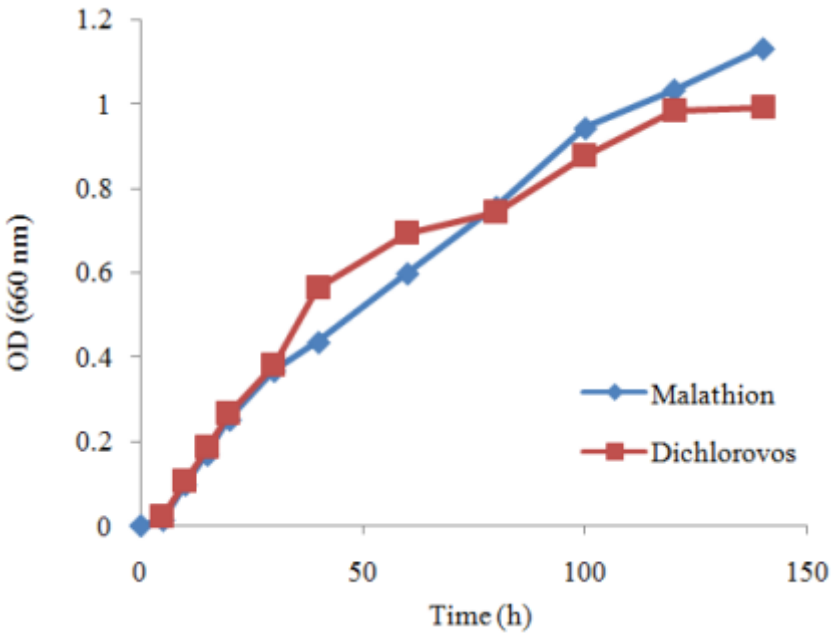

Fig. 1. Bacterial growth by Staphylococcus sp. AUG6

Degradation Studies of Pesticide

Each bacterial colony was first cultivated in 10 mL of LB medium at 37 degrees Celsius. The bacterial inoculum utilized had an optical density (OD) at 660 nm of 1. Degradation was studied using 100 mL of MSM containing sucrose (10.0 glit) and peptone in a 250 mL conical flask (2.0 glit). To guarantee sterility, 100 mg/liter of Malathion and Dichlorvos were added to the MSM medium after it was autoclaved.

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The media was seeded with 5 mL of lit biomass and then incubated at 30°C for 7 days on an orbital shaker (100 rpm). The cell-cultivation experiments were done twice. Using a UV-Visible spectrophotometer, we measured the optical density of samples in 1 cm cuvettes at 660 nm to provide a rough estimate of the biomass content.

RESULT

Effect of initial pH

We tested the impact of pH 5.0-8.0 at 30°C, with an initial bacterial concentration of 0.25%, and a rotation speed of 150 r/min since omethoate might be hydrolyzed and lose effectiveness in an alkaline environment. The results were shown in Fig. 2. (a). Bacillus sp. YB-10 was able to decompose omethoate at pH values between 5 and 8, as seen in Fig. 2(a). At a pH of 7.0, the breakdown rate rapidly increased with time (77.11% at 2 d), yielding the highest effectiveness. The growth of Bacillus sp. YB-10 was inhibited by the acidic and alkaline environments. When the pH was 5.0 during the breakdown of omethoate, the rates of degradation were less than 40%. Degradation rates were likewise reduced (56.11 percent after 2 days) when the pH was 8.0. The optimal pH for omethoate degradation, as found by this research, is 7.0.

Effect of temperature

In these conditions, the effects of temperature were studied. Temperature clearly had an impact on omethoate degradation, as seen in Fig. 2(b). The deterioration rate was higher when the temperature was raised from 15 degrees Celsius to 30 degrees Celsius. Around 30 degrees Celsius, the rate of deterioration was maximum. The process of organic biodegradation, which involves biochemical processes catalyzed by enzymes, was responsible. An increase in temperature may deactivate an enzyme, whereas a decrease in temperature can reduce its effectiveness. Temperature also has an indirect effect on bacterial proliferation. At 30 degrees Celsius, the bacteria flourished, leading to a rise in organophosphate-degrading enzyme activity.

Effect of initial bacteria concentration

Fig. 2 displays the obtained outcomes. The degradation rate was greatest when the starting bacterial concentration was less than 0.25 percent. More bacteria at the outset means a faster breakdown rate on day one, as seen in Fig. 2(c). When the initial concentration of bacteria was higher than 0.25 percent, the degradation rate rose gradually over the following days. These findings imply that degrading capability is independent on the concentration of bacteria at the outset. There wasn't enough biomass for omethoate degradation at lower bacterial concentrations. Yet, since there was an excessive amount of germs to begin with, the bacteria were starved. Hence, bacterial competition existed, which might slow their expansion. The best starting bacterium concentration found in this research was 0.25 percent.

Effect of rotation speed

Clearly, the deterioration rate accelerated from 100 to 150 revolutions per minute. Except for the first day, all degradation rates decreased when rotation speeds were greater than 150 r/min. With a slower rotation speed, there wouldn't be enough oxygen in the water to support bacterial development. Yet, at slower rotation speeds,

the bacteria were able to congregate, limiting their exposure to the media. The combined effects of these conditions stifled bacterial growth. The natural development of bacteria might be destroyed with greater rotation speeds due to the mechanical impact. The greatest deterioration rate in the present investigation was 77.24% after 1 day when the rotation speed was 150 r/min. The rate of omethoate breakdown by Aspergillus niger was 77.59%, which was comparable with previous research. This research used a pure microbia culture of Bacillus sp. YB-10 to determine its degrading potential. Yet, environmental factors were quite intricate. When bacteria are utilized in bioremediation or bioaugmentation, it is important to take into account the impact of environmental factors. Yet, the findings of this research have the potential to aid in the biodegradation of omethoate in the natural world.

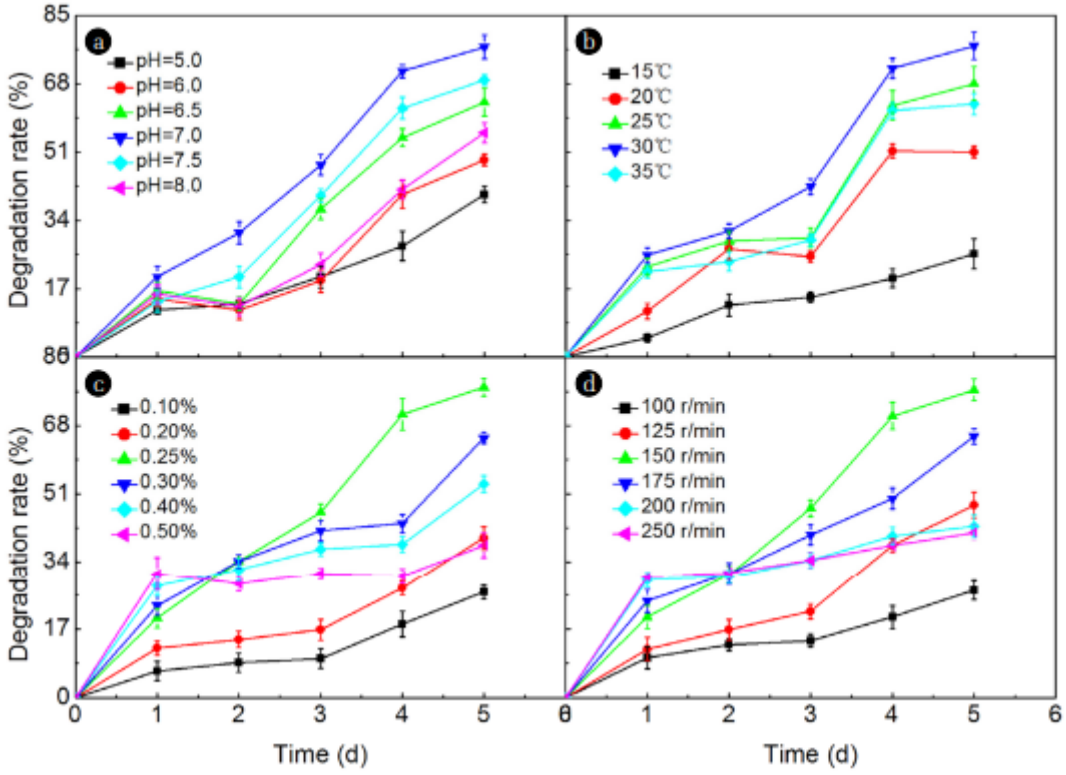

Fig. 2. The effect of different conditions on omethoate degradation by Bacillus sp.YB-10.

Isolation and Identification of Pesticide Degrading Bacteria

Using the enrichment culture approach, six unique bacteria were extracted from soil samples contaminated with pesticides. For subsequent study, all of the isolates that showed growth in the presence of the pesticide were stored in glycerol stock with the names AUG 6, 8, 9, 11, 12, and 13.

Table 2. Colony characteristics, Morphological characteristics of six different Malathion and Dichlorvos

	Colony characterization							
Isolates	Size	Shape	Margin	Elevation	Surface texture	Consistency	Opacity	Pigmentation
AUG-6	M	Round	Entire	Convex	Smooth	Gummy	OP	LY
AUG-8	S	Irregular	Lobed	Low convex	Smooth	watery	TL	NP
AUG-9	M	Irregular	Uneven	Low convex	Smooth	Gummy	OP	NP
AUG-11	M	Round	Even	Convex	Rough	Gummy	OP	DW
AUG-12	S	Uneven	Entire	Flat	Smooth	Gummy	OP	LY
AUG-13	M	Irregular	Lobed	Flat	Rough	Gummy	OP	LC

Table 3. Biochemical characteristics of six different Malathion and Dichlorvos degrading isolates grown on nutrient agar at 30°C for 24 h

Biochemical Ch1aracterization	AUG6	AUG8	AUG9	AUG11	AUG12	AUG13
Indole production	-	-	+	-	-	-
Urea hydrolysis	-	-	-	-	-	-
Catalase	+	+	+	+	+	+
Gelatin hydrolysis	-	-	-	-	-	-
Citrate utilization	-	+	+	+	+	+
Starch hydrolysis	-	+	+	-	-	+
Motility test	+	-	+	+	+	-
MR test	-	-	-	-	-	-
VP test	-	-	-	+	-	-
Arginine hydrolysis	-	-	-	-	-	-
Casein utilization	+	-	-	-	-	-
TSI	Y	R/Y	R/Y	Y	R/Y	R/Y
Sugar test	+	+	+	+	+	+
H_2S test	-	-	-	-	-	-
Co_2 test	-	-	-	-	-	-
Gram staining	+	-	-	-	-	-
Identified cultures	*Staphylococcus sp.*	*Micrococcus sp.*	*Entrobacte sp.*	*Bordetella sp.*	*Pseudomonas sp.*	*Klebsella sp.*

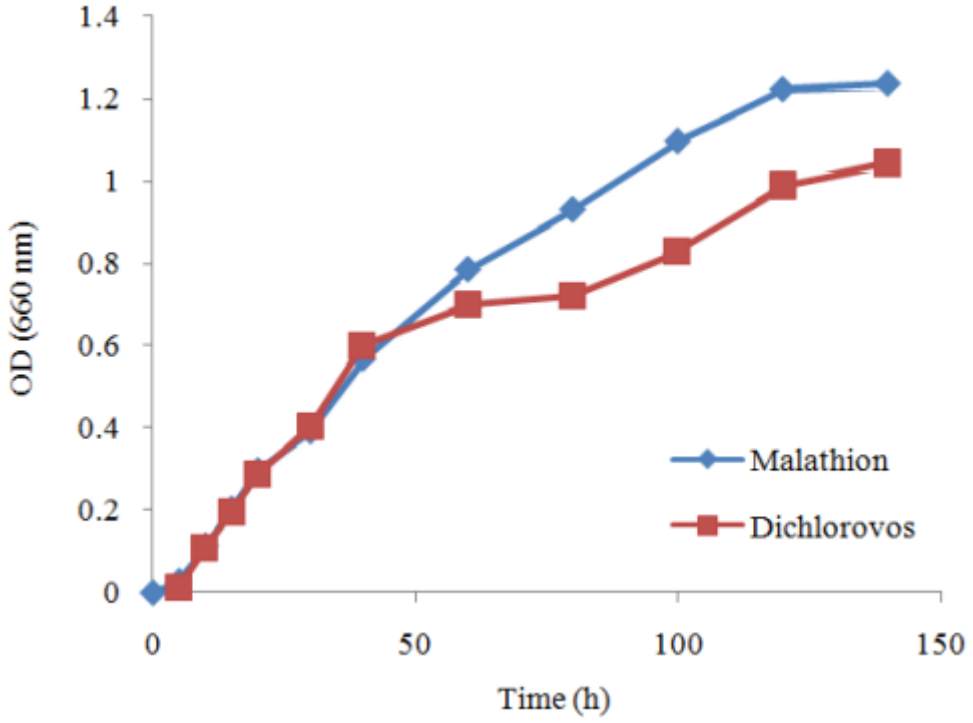

Fig. 3. Bacterial growth by Micrococcus sp. AUG8

CONCLUSION

pH 7.0, 30 degrees Celsius, an initial bacterial concentration of 0.25 percent, and a rotational speed of 150 revolutions per minute were found to be optimal for deterioration. Bacillus sp. YB-10 could break down 1,000 mg/L of omethoate in about 5 days under optimal conditions. This study's findings may help us better understand how omethoate biodegrades in different environments. Sucrose and peptone were added to promote the development of Pseudomonas sp. AUG12. In addition to chemical processes, the favorable growth findings of the isolates revealed that microbial degradation is a crucial mechanism of Malathion and Dichlorvos dissipation in water.

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