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RESEARCH ARTICLE

Efficiency of Bacterial Isolates in the Degradation of Malathion and Parathion

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ABSTRACT

The soil sample was collected from the paddy field of Annamalai Nagar which is having a history of repeated pesticide applications. The isolation of pesticide degrading bacteria was carried out and the isolated bacterial isolates were identified as *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella sp*. The growths of these three pesticide degrading isolates were assessed in Minimal salt broth containing 50ppm of pesticides. Two different pesticides *viz*., Malathion and Parathion were used in this study. Among the three bacterial isolates, the bacteria *Klebsiella sp.* utilized the pesticides effectively and showed maximum growth. The growth of these three pesticide degrading isolates *viz*., *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella sp.* was assessed in Minimal salt broth containing 50ppm of pesticides at different temperature levels *viz*., 25°C, 35°C, 45°C, 55°C and different pH levels *viz*., pH 4, pH 5, pH 6, pH 7, pH 8 and different carbon sources *viz*., Dextrose, Fructose, Lactose, Galactose, Mannose and different nitrogen sources *viz*., Malt extract, Peptone, Yeast extract, Casein, Beef extract respectively. The maximum growth rate of bacteria was recorded at 35°C and pH 6. The growth of bacteria was maximum in the presence of Dextrose followed by Fructose, Lactose and Galactose. The least growth was recorded in Mannose. The growth of bacteria was maximum in the presence of Malt extract followed by Peptone, Yeast extract and Casein. The least growth was recorded in Beef extract. The bacterial isolates showed maximum growth in the Minimal salt broth containing Parathion followed by Malathion.

Key words: Parathion, Malathion, *Pseudomonas fluorescens, Bacillus subtilis* and *Klebsiella sp.*

INTRODUCTION

1. INTRODUCTION

Environment preservation is one of the aims of the sustainable development. Environmental pollution has increased in many regions due to industrialization. Pesticides, herbicides and polychlorobiphenyls are PCB's that are widely distributed in the environment. In recent years, pesticides, herbicides and PCBs have been detected in aquatic systems in India.

In India, alarming levels of pesticides have been reported in air, water, soil as well as in foods and biological materials [1]. Some of these pesticides have also been reported to be toxic [2], mutagenic, carcinogenic and tumorogenic [3,4]. The most important pollutants among the toxicants in India are organochlorine and organophosphorus pesticides. For the past several decades, organochlorine pesticides have been widely used for both agricultural and public health purposes, but there is always a tendency to use them in excess. Microorganisms are important in maintaining soil fertility and are also important agents which detoxify pesticides in soil. Thus chemicals which seriously affect the soil microflora may harm soil fertility and crop production [5,6].

Increase in the consumption of pesticide is likely to be atleast two to three times more in the years to come. However, there has been a considerable qualitative and quantitative change in pesticide use in the last few years worldwide and in India as well. Chlorinated compounds and carbamates are being phased out, while organophosphorus pesticides are becoming the major backbone for pest control. The consumption of technical grade pesticides in India during 2000-2001 was 43,580 MT [7]. Among the insecticides, monocrotophos, quinalphos and chlorpyriphos top the list of organophosphorus insecticides in the Indian market. The estimated consumption of technical

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grade chlorpyriphos in India during 2002-2003 was 5,000 MT [7].

Pesticide biodegradation is a ubiquitous environmental process. Pesticide biodegradation has been documented in a wide range of habitats, including soils, sediments, surface and ground water and sewage sludges etc. The ubiquity of pesticide degradation suggests that bioremediation strategies can play an important role in the treatment of pesticide wastes [8].

Insecticides and their degradation products generally get accumulated in the top soil and influence not only the population of various groups of soil microbes but also their biochemical activities like nitrification, ammonification, decomposition of organic matter and nitrogen fixation [9]. Microorganisms play an important role in degrading synthetic chemicals in soil [10]. They have the capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source. The metabolism of chlorpyriphos by microorganisms in soil has been reported [11] with 3, 5, 6-trichloro-2-pyridinol (TCP) as the primary breakdown product. Use of pesticide degrading microbial systems for bioremediation, thus, receives attention because of its cost effectiveness and ecofriendly nature.

2. MATERIALS AND METHODS

2.1. Collection of soil sample

The soil samples were collected from the different places of paddy fields of Annamalai Nagar which is having a history of repeated pesticide applications. The collected soil samples were ground, passed through 2mm sieve and stored at 4°C.

2.2. Pesticides used

Pesticides used in this present study are

a) Malathion
b) Parathion

2.3. Isolation and identification of bacterial isolates

Pour plate technique was used for the isolation of pesticide degrading bacteria in Nutrient agar and King's B agar plate.Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar and King's B agar slants and stored at 4°C. Identification of these three different bacterial isolates were carried out by the routine bacteriological methods i.e., By the colony morphology, preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Catalase and Oxidase, Plating on selective media and by performing biochemical tests.

2.4. Determination of the growth of pesticide degrading bacterial isolates on Minimal salt broth

The suspension of 24 hours old cultures of *Pseudomonas fluorescens, Bacillus subtilis* and *Klebsiella* sp. were used to prepare the bacterial inoculum. They were prepared in saline solution (0.85% sodium chloride). A loopful of *Pseudomonas fluorescens, Bacillus subtilis* and *Klebsiella* sp. cultures were inoculated into 50 ml of saline and incubated at 37°C for 3 hours.

The growth of pesticide degrading bacterial isolates (*Pseudomonas fluorescens, Bacillus subtilis* and *Klebsiella* sp.) was determined by using Minimal Salt Broth. For this, 2 ml of the bacterial inoculum was inoculated into 100 ml of Mineral salt broth containing 50 ppm of two different pesticides. The flasks were then incubated at 37°C for 7 days in a microbial shaker at 150 rpm. Five ml of culture was drawn and centrifuged at 5000rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria. The growth of the pesticide degrading bacterial isolates was assessed by using UV – spectrophotometer at 560nm.

2.5. Effect of temperature for the growth of pesticide degrading bacterial isolates

To study the stability of the bacterial isolates for the biodegradation of pesticides, an experiment conducted in a Erlenmeyer flask containing 50ppm of pesticides in 100 ml Minimal salt broth. After sterilization by autoclaving the flasks were cooled and inoculated with the bacterial cultures and maintained at different temperatures (25°C, 35°C, 45°C and 55°C). After 24 hours, 5ml of culture was drawn and centrifuged at 5000rpm for 10 minutes and the pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria. The optical density was taken at 560nm using UV – spectrophotometer.

2.6. Effect of pH for the growth of pesticide degrading bacterial isolates

To study the stability of the bacterial isolates for the biodegradation of pesticides, an experiment conducted in an Erlenmeyer flask containing 50ppm of pesticides in 100ml Minimal salt broth. After sterilization by autoclaving the flasks were cooled and inoculated with the bacterial cultures and maintained at different pH (4, 5, 6, 7 and 8). After 24 hours, 5ml of culture was drawn and centrifuged at 5000rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading

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bacteria and the optical density was taken at 560nm using UV – spectrophotometer.

2.7. Effect of carbon sources for the growth of pesticide degrading bacterial isolates

The effect of various carbon sources for the maximization of pesticide biodegradation was tested. The bacterial isolates were cultivated in 100 ml of Mineral salt broth with 50ppm of pesticides and 1g of various carbon sources (Lactose, Dextrose, Fructose, Mannose and Galactose) and incubated at 37°C. After 24 hours, 5ml of culture was drawn and centrifuged at 5000rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria and the optical density was taken at 560nm using UV – spectrophotometer.

2.8. Effect of nitrogen sources for the growth of pesticide degrading bacterial isolates

The effect of various carbon sources for the maximization of pesticide biodegradation was tested. The bacterial isolates were cultivated in 100ml Minimal salt broth with 50ppm of pesticides and 1g of various nitrogen sources (Peptone, Beef extract, Yeast extract, Malt extract and Casein) and incubated at 37°C. After 24 hours, 5ml of culture was drawn and centrifuged at 5000rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria and the optical density was taken at 560nm using UV – spectrophotometer.

3. RESULTS AND DISCUSSION

The widespread use of these pesticides over the years has resulted in problems caused by their interaction with the biological systems in the environment [12]. Considering the toxic effect of these pesticides, it is essential to remove these chemo-pollutants from the environment. Biological removal of chemo-pollutants becomes the method of choice, since microorganisms can use a variety of xenobiotic compounds including pesticides for their growth and mineralize and detoxify them. Many scientists have isolated microorganisms from nature which have the capacity to degrade chlorpyriphos and obtained good degradation yields [13]. Data are also available on the possible use of microorganisms for bioremediation of pesticide contaminated soil [14,15].

In this present study, the pesticide degrading bacteria were isolated and identified from the pesticide contaminated soil samples. The isolated isolates were identified as *Pseudomonas fluorescens, Bacillus subtilis* and *Klebsiella* sp. based on staining technique, plating on selective media and biochemical tests. The isolates were preserved on agar slants at 4°C for further use.

It was interesting to note the ability of the bacterial strains isolated from pesticide enriched soils to tolerate and grow on higher concentration of pesticides. Many bacterial strains were isolated and grew on Mineral agar medium as well as in broth medium containing 50 ppm pesticides as the sole carbon source. Similarly, a number of scientists isolated bacteria following enrichment culture technique capable of degrading organophosphorus pesticides such as chlorpyriphos. These included *Enterobacter* sp. [14], *Flavobacterium* sp. [16], *Arthrobacter* sp. [17] and *Pseudomonas putida* [18,19].

Numbers of reports concerning the bacterial degradation of chlorobenzoates had already been published, such as *Alcaligenes*, *Burkholderia*, *Pseudomonas*, *Bacillus*, *Ralstonia*. Most of these strains are Gram-negative and mesophilic microorganisms. However, in cold climatic regions, temperature often decreases to 20°C at most time of the year [20].

In this present research, the growth of the three pesticide degrading isolates *viz*., *Pseudomonas fluorescens, Bacillus subtilis* and *Klebsiella* sp. was assessed in Minimal salt broth containing50ppm of pesticides. Two different pesticides *viz*., Malathion and Parathion were used in this study. Among the three bacterial isolates, the bacteria *Klebsiella* sp. utilized the pesticides effectively and showed maximum growth followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. The bacterial isolates showed maximum growth in the Minimal salt broth containing Parathion (0.333) followed by Malathion (0.270) (**Fig 1& Fig 2**).

Fig 1: Growth of pesticide degrading bacterial isolates on Minimal Salt broth containing Malathion

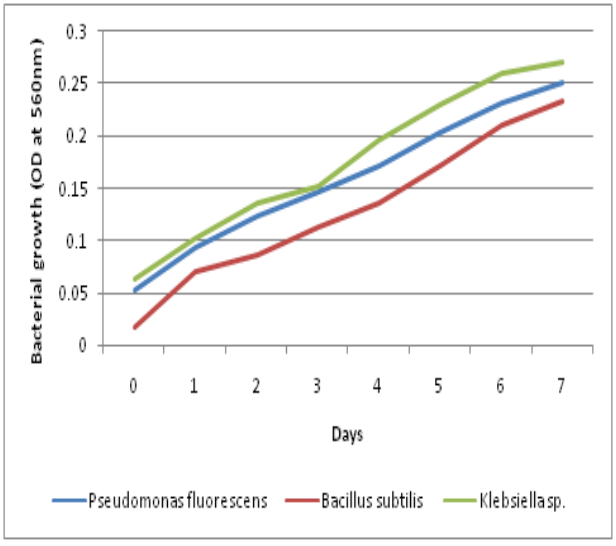

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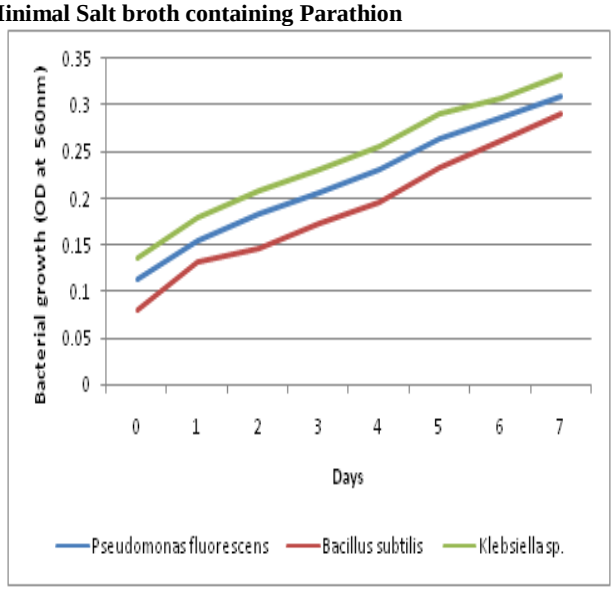
Fig 2: Growth of pesticide degrading bacterial isolates on Minimal Salt broth containing Parathion



Recently, Murugesan *et al*. [21] studied the ability of five bacterial isolates (*Pseudomonas aeruginosa*, *Klebsiella* sp., *Escherichia coli*, *Bacillus* sp. and *Corynebacterium*) to degrade cypermethrin. It was confirmed that these isolated organisms were able to utilize and degrade cypermethrin. On that five different bacterial colonies, *Pseudomonas aeruginosa*, *Klebsiella* sp., *Escherichia coli* were found active in utilizing cypermethrin (1%) where as *Bacillus* sp. and *Corynebacterium* were moderately active in utilizing cypermethrin (0.1%). The growth curve experiment was performed at 0.1 and 1% dose of cypermethrin to analyze the viable count of *Pseudomonas aeruginosa*.

In this research, the growth of the three pesticide degrading isolates *viz*., *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella* sp. was assessed in Minimal salt broth containing 50ppm of pesticides at different temperature levels *viz*., 25°C, 35°C, 45°C and 55°C. Two different pesticides *viz*., Malathion and Parathion were used in this study. The maximum growth rate of bacteria was recorded at 35°C followed by 25°C and 45°C. The least growth rate was recorded at 55°C. Among the three bacterial isolates, the bacteria *Klebsiella sp.* utilized the pesticides effectively and showed maximum growth followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. The bacterial isolates showed maximum growth in the Minimal salt broth containing Parathion (0.588) followed by Malathion (0.470) (**Fig 3 & Fig 4**).

Fig 3: Effect of temperature for the growth of pesticide degrading bacteria in Minimal Salt broth containing Malathion

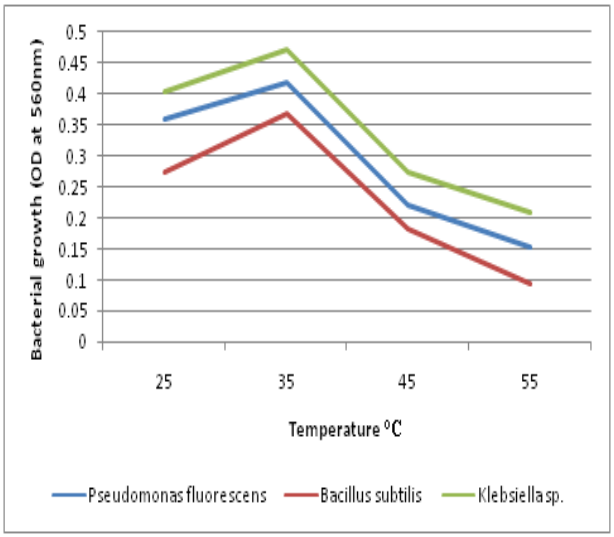
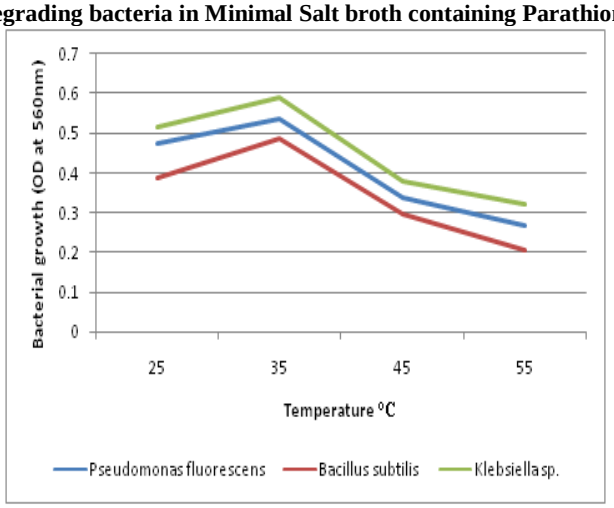


Fig 4: Effect of temperature for the growth of pesticide degrading bacteria in Minimal Salt broth containing Parathion



In this study, the growth of the three pesticide degrading isolates *viz*., *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella* sp. was assessed in Minimal salt broth containing 50ppm of pesticides at different pH levels *viz*., pH 4, pH 5, pH 6, pH 7 and pH 8. Two different pesticides *viz*., Malathion and Parathion were used in this study.The maximum growth rate of bacteria was recorded at pH 6 followed by pH 7, pH 8 and pH 5. The least growth rate of *Klebsiella* sp. was recorded at pH 4. Among the three bacterial isolates, the bacteria *Klebsiella* sp. utilized the pesticides effectively and showed maximum growth followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. The bacterial isolates showed maximum growth in the Minimal salt broth containing Parathion (0.556) followed by Malathion (0.532) (**Fig 5 & Fig 6**).

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Fig 5: Effect of pH for the growth of pesticide degrading bacteria in Minimal Salt broth containing Malathion

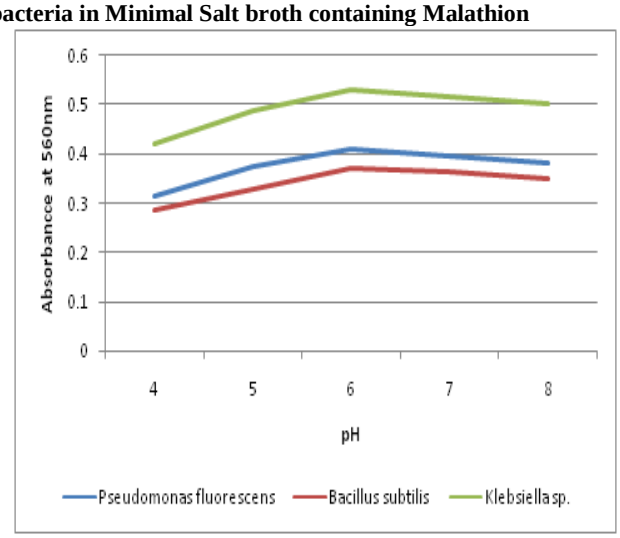
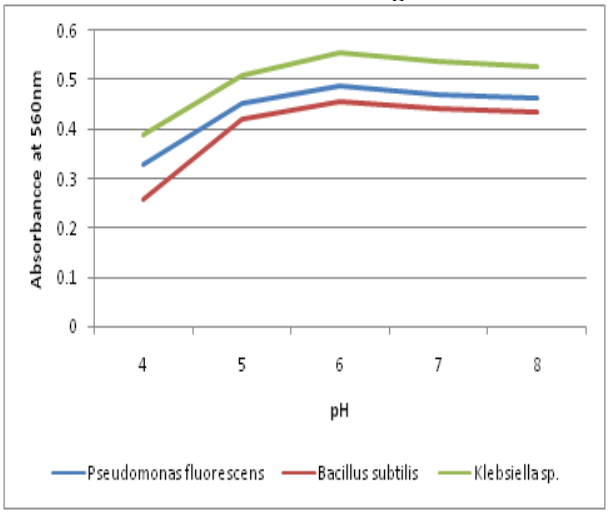


Fig 6: Effect of pH for the growth of pesticide degrading bacteria in Minimal Salt broth containing Parathion



Degradation of parathion in flooded acid soils was reported by [22]. A *Bacillus* sp. capable of readily decomposing 20 ppm p-nitrophenol as the sole carbon source was isolated from parathion amended flooded alluvial soils. Accelerated parathion degradation by inoculation with parathion utilizing bacteria was observed by [23]. Bourquin [24] reported the degradation of malathion by salt marsh organisms. Numerous bacteria were found to degrade malathion when supplied with additional nutrients like peptone as energy and carbon sources.Singh *et al.* [14] separated *Enterobacter*, a strain could degrade chlorpyrifos [25,26]. Yang *et al.* [25] and Li *et al.* [26] separated *Stenotrophomonas* sp. and *Sphingomonas* sp. respectively, which could utilize chlorpyrifos as the only source of carbon and phosphorus.

In the present study, the growth of the three pesticide degrading isolates *viz.*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella sp.* was assessed in Minimal salt broth containing 50ppm of pesticides using different carbon sources *viz.*, Dextrose, Fructose, Lactose, Galactose and Mannose. Two different pesticides *viz.*, Malathion and Parathion were used in this study. The growth of bacteria was maximum in the presence of Dextrose followed by Fructose, Galactose and Mannose. The least growth was recorded in Lactose. Among the three bacterial isolates, the bacteria *Klebsiella sp.* utilized the pesticides effectively and showed maximum growth followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. The bacterial isolates showed maximum growth in the Minimal salt broth containing Parathion (0.574) followed by Malathion (0.555) (**Fig 7 & Fig 8**).

Fig 7: Effect of carbon sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Malathion

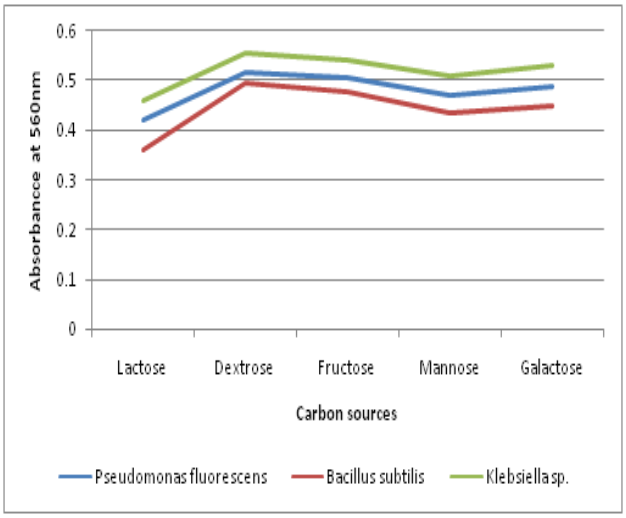
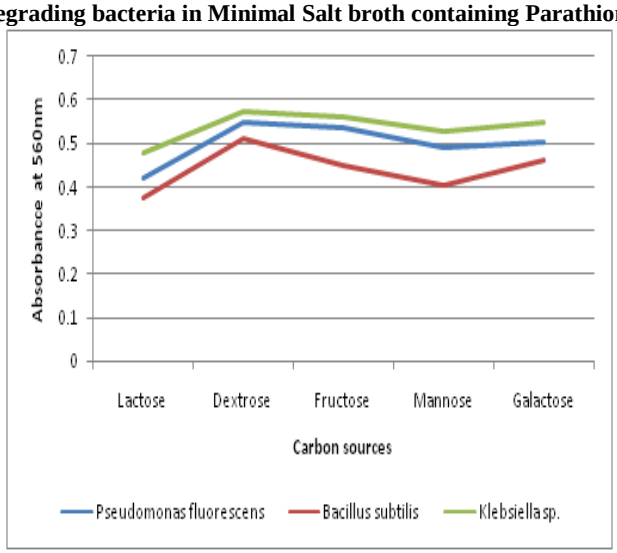


Fig 8: Effect of carbon sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Parathion



Eleni Chanika *et al.* [27] identified two bacterial isolates as *Pseudomonas putida* and *Acinetobacter rhizosphaerae* to degrade the organophosphate (OP) fenamiphos (FEN). Both strains hydrolyzed FEN to fenamiphos phenol which was further transformed, only by *Pseudomonas putida*. The two strains used FEN as Carbon and Nitrogen source as a nutrient.

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In this present research, the growth of the three pesticide degrading isolates *viz*., *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella* sp. was assessed in Minimal salt broth containing 50ppm of pesticides using different nitrogen sources *viz*., Malt extract, Peptone, Yeast extract, Casein and Beef extract. Two different pesticides *viz*., Malathion and Parathion were used in this study. The growth of bacteria was maximum in the presence of Malt extract followed by Peptone, Yeast extract and Casein. The least growth was recorded in Beef extract. Among the three bacterial isolates, the bacteria *Klebsiella* sp. utilized the pesticides effectively and showed maximum growth followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. The bacterial isolates showed maximum growth in the Minimal salt broth containing Parathion (0.541) followed by Malathion (0.525) (**Fig 9 & Fig 10**).

Fig 9: Effect of nitrogen sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Malathion

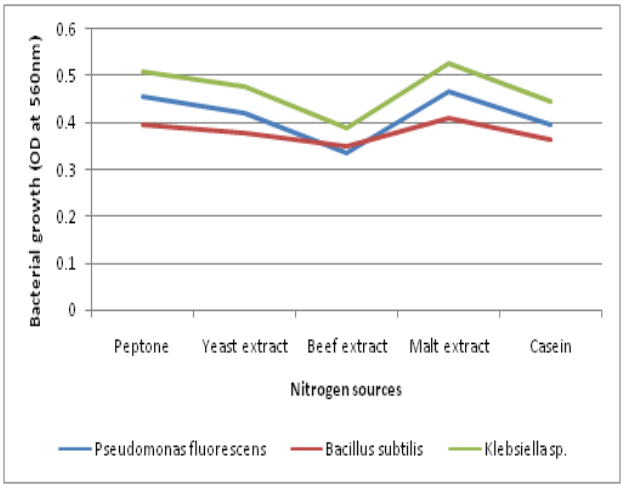
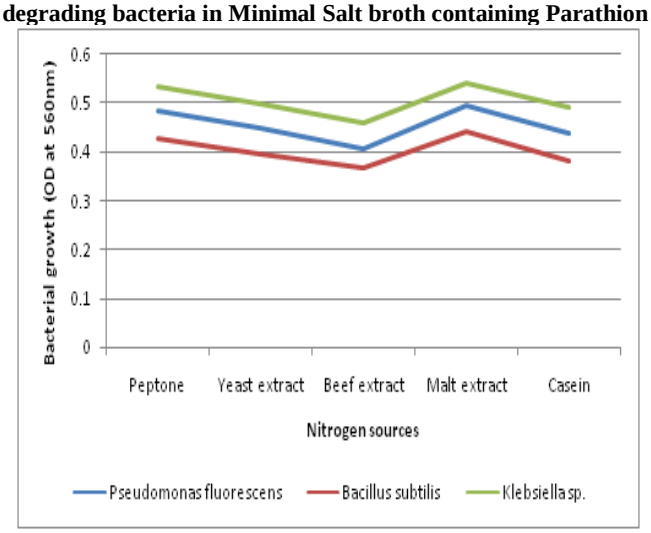


Fig 10: Effect of nitrogen sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Parathion



4. CONCLUSION

From this study, it was concluded that the bacterial isolates like *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella* sp. have the capacity to utilize the pesticides and grow well in the medium supplemented with pesticides. Among the isolated bacterial isolates, *Klebsiella sp.* grows well in the presence of pesticides followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. These bacterial isolates have the capacity to utilize the pesticides and use of these bacterial isolates in the biological treatment of pesticide contaminated soil will give fruitful results. The isolates *Pseudomonas fluorescens* and *Bacillus subtilis* have the capacity to control many diseases. Replacement of chemical pesticides with biopesticides like *Pseudomonas fluorescens* and *Bacillus subtilis* will minimize the pesticide contamination in agricultural soil.

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