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ORIGINAL RESEARCH ARTICLE

Biological Degradation of Chlorpyrifos and Monocrotophos by Bacterial Isolates

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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 growth of the three pesticide degrading isolates was assessed in Minimal salt broth containing 50ppm of pesticides. Two different pesticides *viz*., Monocrotophos and Chlorpyrifos 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 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, 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 Chlorpyrifos followed by Monocrotophos.

Key words: Monocrotophos, Chlorpyrifos, *Pseudomonas fluorescens, Bacillus subtilis* and *Klebsiella sp.*

1. INTRODUCTION

India is an agriculture based country. About 60-70% of its population is dependent on agriculture. A huge portion of arable land already under cultivation is being rapidly depleted by industries and urban encroachments. On the other hand, the demand for agricultural crops is increasing day by day due to the rapidly increasing population. Hence, there is a need for a huge increase in the quantity of agricultural produce as well as improvement in its quality. To meet these objectives; agrochemicals like insecticides, fungicides, pesticides, and herbicides and also; use of better quality seeds are being used on a large scale in agricultural lands. About 30% of agricultural produce is lost due to pests. Hence, the use of pesticides has become indispensable in agriculture [1].

The term "pesticide" covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscacides, nematocides, plant growth regulators and others. Thus far, more than 1000 active substances have been incorporated in the approximately 35,000 preparations, which are known as pesticides. Insecticides represent the greatest proportion of pesticides used in developing countries, whereas herbicide sales have been greater than those of other pesticides in industrialized countries.

In recent years, plant protection has become one of the essential inputs in crop production. In the context of changing cropping patterns, introduction of high yielding varieties, application of high doses of fertilizers, with enhanced irrigation facilities, pests have assumed a special significance and more and more pesticides are being applied. Large complex of insect pests ranging from borers to root feeding insects are responsible for heavy losses of commercial crops, both quantitatively and qualitatively. In order to reap maximum yields, the farmers resort to pesticidal application to combat the pest problem.

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Although some persistent organochlorine pesticides have been banned from agricultural and public health use during the past few decades, high concentrations of pesticides and its metabolites have been found in soil, water, and sediment samples [2,3,4]. Furthermore, other insecticides, such as endosulfan and lindane, are currently in use throughout the world [5] and their presence in air, water, and soil is a problem of great concern. Reducing their levels in the environment has therefore become an important goal.

Microbial degradation of pesticides applied to soil is the principle mechanism which prevents the accumulation of these chemicals in the environment. Yet, when pesticides are degraded too rapidly, pest control may be less effective. One factor that has been shown to increase the rate of microbial degradation of pesticides in soil is one or more previous applications of the same pesticide or another pesticide with a similar chemical structure. This phenomenon is known as accelerated or enhanced degradation [6] and can result in economic losses to farmers.

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) Chlorpyrifos
b) Monocrotophos

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 the three different bacterial isolates was 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 flask were cooled and inoculated with the bacterial cultures and maintained at different temperature (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 a Erlenmeyer flask containing 50ppm of pesticides in 100ml Minimal salt broth. After sterilization by autoclaving the flask 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 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

Pesticides constitute the key control strategy for pest management and have been making significant contribution towards improving crop yields. Currently, among the various groups of pesticides that are being used world over, organophosphates form a major and most widely used group, accounting for more than 36 per cent of the total world market [7]. Quinalphos, monocrotophos, chlorpyriphos, malathion, parathion are some of the widely used organophosphorus pesticides. Amongst these, chlorpyriphos dominates. It is a synaptic poison having broad spectrum of insecticidal activity and is used to control insects attacking corn, cotton, citrus, fruits, nut crops, potato, beets, pulses *etc*.

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.

Perclich and Lockwood [8] observed that incidence of pesticide utilizing bacterial genera such as *Bacillus*, M*icrococcus*, *Pseudomonas* and *Vibrio* in the water and sediment samples of irrigational channel. Walker *et al*. [9] investigated that, Pesticide is mainly degraded by *Pseudomonas* and *Bacillus* and this versatility might be due to the presence of wide range of enzymes.

Microorganisms are involved in soil processes such as recycling of essential plant nutrients, humus formation and soil structure stability. The addition of pesticides may disturb the equilibrium and thus fertility of the soil. The chances of isolating microbial strains from polluted soils, with high ability to metabolize a particular xenobiotic are brighter [10]. During enrichment with a xenobiotic compound, the natural selection of microorganisms which have been adapted to the presence of that xenobiotic and its rapid biodegradation are known to take place [11].

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. Two different pesticides *viz*., Monocrotophos and Chlorpyrifos 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 Chlorpyrifos (0.580) followed by Monocrotophos (0.460) (**Fig 1 & 2**).

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

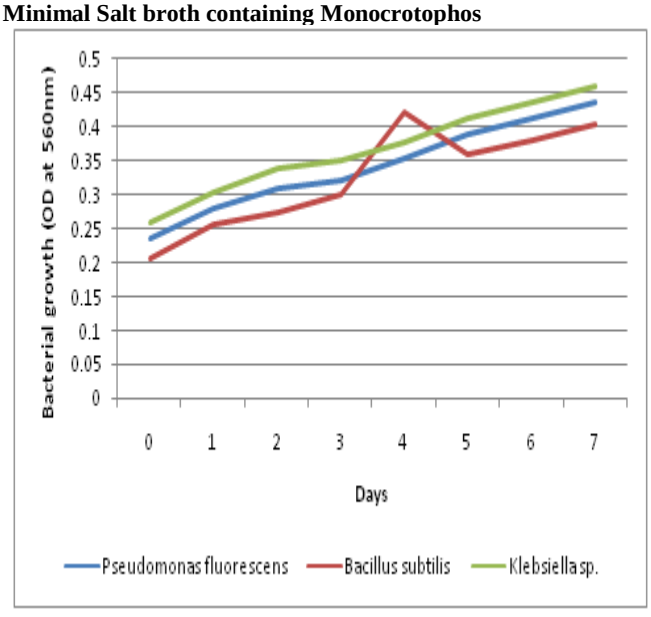
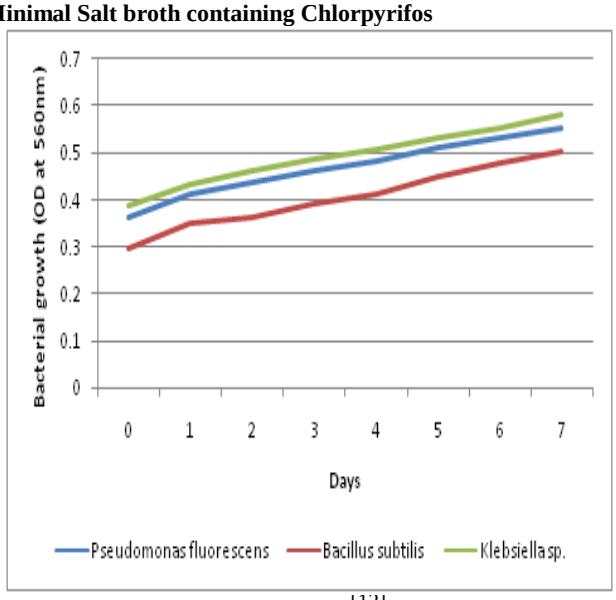


Fig 2: Growth of pesticide degrading bacterial isolates on Minimal Salt broth containing Chlorpyrifos



Recently, Murugesan *et al.* [12] 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*., Monocrotophos and Chlorpyrifos 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 Chlorpyrifos (0.785) followed by Monocrotophos (0.677) (**Fig 3 & Fig 4**).

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

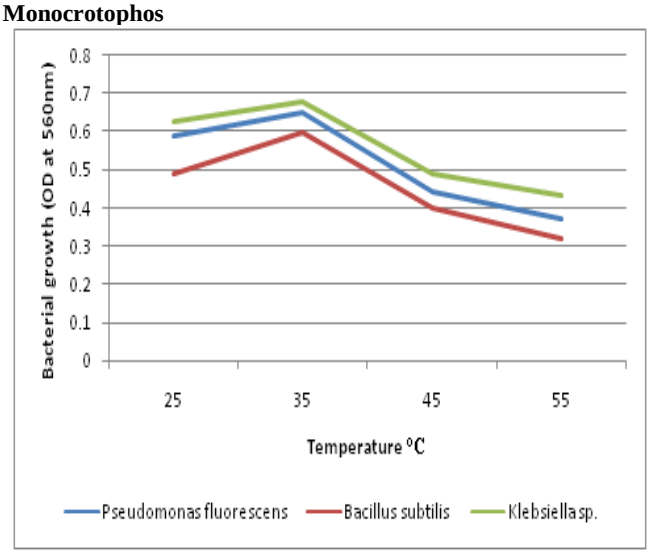
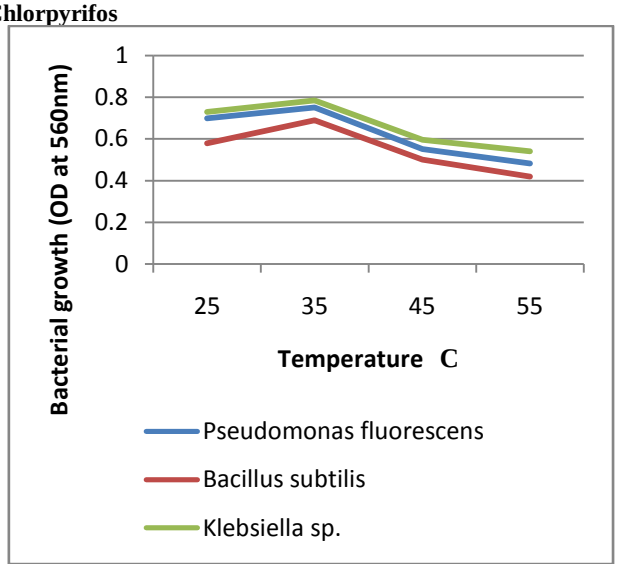


Fig 4: Effect of temperature for the growth of pesticide degrading bacteria in Minimal Salt broth containing Chlorpyrifos



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*., Monocrotophos and Chlorpyrifos 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 Chlorpyrifos (0.778) followed by Monocrotophos (0.667) (**Fig-5 & Fig-6**).

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

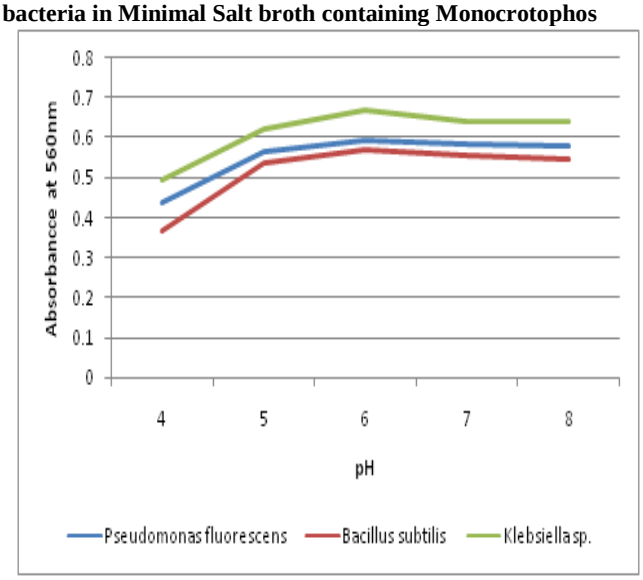
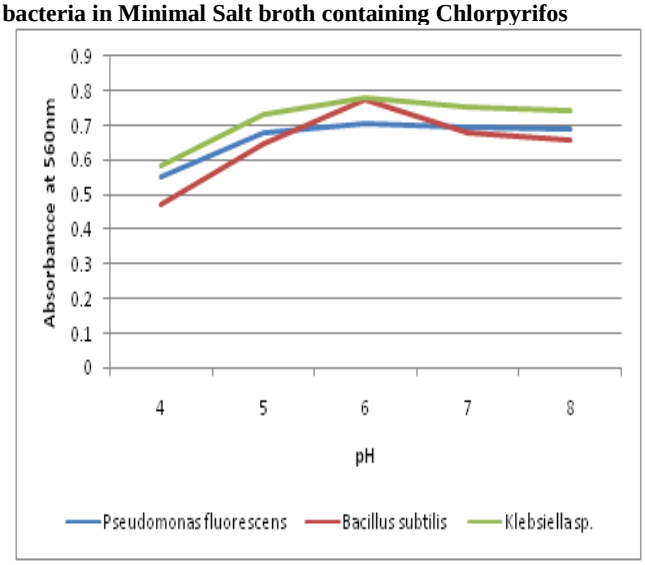


Table 6: Effect of pH for the growth of pesticide degrading bacteria in Minimal Salt broth containing Chlorpyrifos



Enhanced degradation of chlorpyrifos by *Enterobacter* strain was reported by [13]. Yang *et al*. [14] isolated *Alkaligenes faecalis*, which is capable of degrading chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol (TCP). Six chlorpyrifos-degrading bacteria were isolated using chlorpyrifos as the sole carbon source by an enrichment procedure [15].

Choi *et al.* [16] isolated three parathion-degrading bacteria and eight pairs of bacteria showing syntrophic metabolism of parathion from rice field soils, and investigated their genetic and phenotypic characteristics. The three isolates and eight syntrophic pairs were able to utilize parathion as a sole source of carbon and energy, producing p-nitrophenol as the intermediate metabolite during the complete degradation of parathion.

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*., Monocrotophos and Chlorpyrifos 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 Chlorpyrifos (0.786) followed by Monocrotophos (0.685) (**Fig 7 & Fig 8**)

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

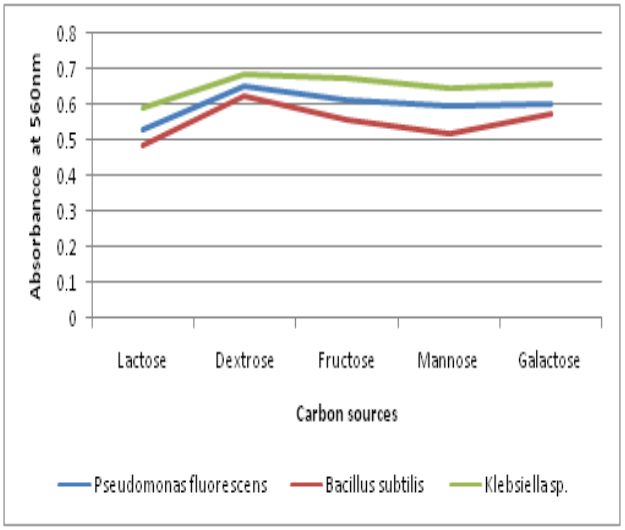
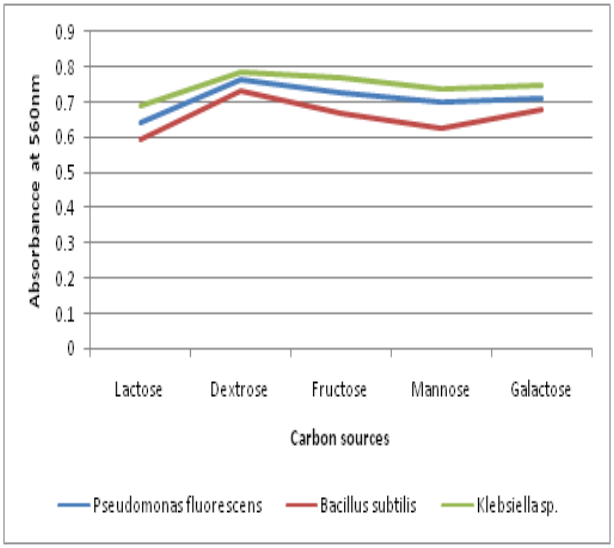


Fig 8: Effect of carbon sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Chlorpyrifos



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*., Monocrotophos and Chlorpyrifos 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 Chlorpyrifos (0.763) followed by Monocrotophos (0.652) (**Fig 9 & Fig 10**).

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

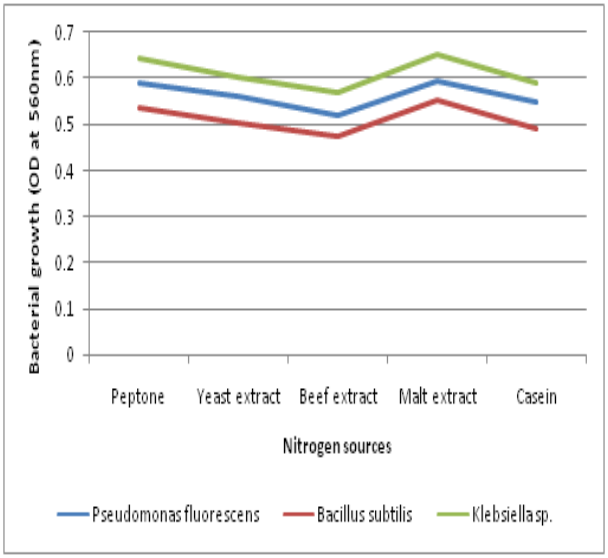
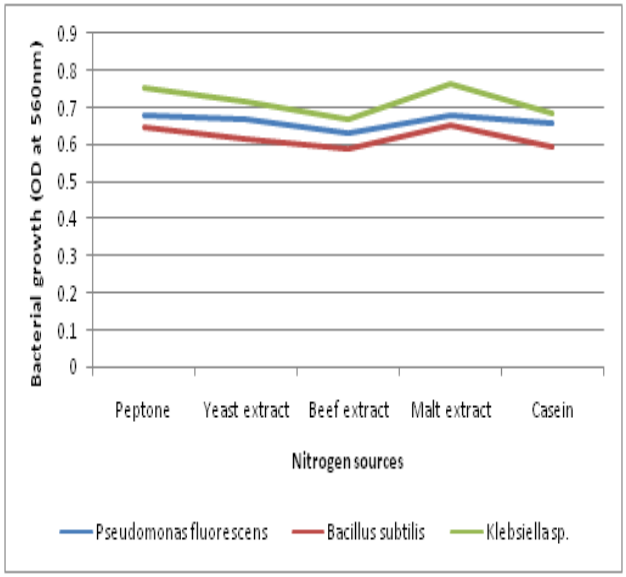


Table 10: Effect of nitrogen sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Chlorpyrifos



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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