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Microbial population response exposed to Different pesticides

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Abstract

Growth curve experiment of various microorganisms was studied with different Pesticides (Alpha Cyhalothrin, Miticide and Fungicide). Alpha Cyhalothrin and Miticide showed drastic inhibitory effect on the growth of all the bacteria which include *E.coli, Bacillus subtilis, Serratia marscens, Enterobacter sp and Kurthia sp.* But the application of Fungicide to the bacterial cultures proliferates the growth of all the bacterial cultures.

INTRODUCTION

Pesticides are defined as "any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (insects, rodents, nematodes, fungus, weeds and other forms of terrestrial or aquatic plant or animal like bacteria or other microorganisms)" [1]. The extensive use of pesticides in agriculture to eliminate pest, such as grasshoppers, has resulted in the accumulation of pesticide residues in soil and has altered the soil microbial communities by favouring the growth of those pesticide degrading organisms. Due to the xenobiotic features of pesticides, pesticides in soil can be persistent in the environment and eventually enter the food chains, which cause reproductive failure in birds and even cancer in humans [2].

There is also concern about the increased use of pesticides, which may cause (i) environmental hazards such as watertable and water body contamination, (ii) biological imbalance in ricefield populations, and (iii) reduced efficiency because of shifts toward soil microorganisms more efficient in pesticide degradation.

The pesticides that are used frequently eventually reach the soil from the crop plants and are accumulated usually in top 0-15 cm layer of soil, where the activities of microbes are found to be maximum. Pesticides in the soil affect the non target and beneficial microorganisms [3] and their activities which are essential for maintaining soil fertility [4].

Microorganisms form a vital part of the soil food web, therefore microbial biomass is considered to be a measure of potential microbiological and ecosystem functioning. However for proper understanding of ecosystem functioning and determining soil disturbances because of various agricultural management practices, microbial activities must also be determined [5, 6] along with microbial biomass.

Insecticidal residues are generally degraded and degradation products as similated by soil microorganisms resulting in increased population sizes and activities of microorganisms which in turn influences the transformations of plant nutrient elements in soil [7]. On the other hand, there are some insecticides which are not utilizable by soil microorganisms and these types of insecticides are degraded in soil by microorganisms through cometabolism. Other insecticides exert deleterious effects on microorganisms. Therefore, no definite conclusion can be made on the effect of insecticides on microorganisms and their associated transformations of nutrients in soil, since different groups of insecticides exhibit manifold variations in toxicity.

A study was conducted to investigate the effects of different pesticides including Lambda Cyhalothrin, Myticide and Fungicide on the growth and distribution of soil microorganisms.

MATERIALS AND METHODS

In laboratory experiment, soil samples were taken with the help of sterilized spatula at a depth of 15-20 cm from different agricultural fields. Soil organic matter and moisture was determined then other plant debris was removed manually and soil was sieved with 4mm mesh. 1 gram of soil was mixed with 9 ml of sterilized water and shook it thoroughly. 1 ml from the solution was then mixed in 9 ml sterilized water to make 10^{-2} dilution of this solution and in the same pattern dilutions up to 10^{-7} were prepared to determine the appropriate

dilutions to count bacterial population conveniently. A stock solution of each pesticide was made for further dilutions and different volumes i.e. 50, 100, and 200 µl was used.

The bacterial cultures were enumerated with and without adding Pesticides. 0.2ml aliquot of 24h culture grown in nutrient broth was inoculated into 10ml nutrient broth flask containing different concentrations of each pesticide and incubated in incubator shaker. Control flasks of equal volume of nutrient broth medium containing culture but no pesticide were run in parallel. Growth of each microorganism was determined Spectrophotometrically immediately after inoculation up to the stationary phase of microorganism at different time intervals (30 min, one hr, Two hr upto 24hr for bacteria). With the exhaustion of nutrients and build-up of waste and secondary metabolic products, the growth rate has slowed to the point where the growth rate equals the death rate. Effectively, there is no net growth in the population after the stationary phase.

RESULTS AND DISCUSSION

The results of present study revealed that different concentration of all pesticides brought reduction on the growth of all the bacterial populations which were significantly different from control including *E.coli, Bacillus subtilis, Enterobacter sp, Serratia marcescens and Kurthia* and this effect was offset during exponential phase of treatment but at slow rate.

Alpha Cyhalothrin brought reduction on the growth of all the bacterial populations which were significantly different from control including E.coli, *Bacillus, Serratia, Enetrobacter and Kurthia* and this effect was offset during exponential phase of treatment but at slow rate (Figure 1-5 a) which is in conformity with Azaz *et al* (2005) [8]who reported that above cited effect of cypermethrin and monocrotophos on bacteria while fenvalerate had very low effect on soil microbes approx 25% (Figure 1A-1E).

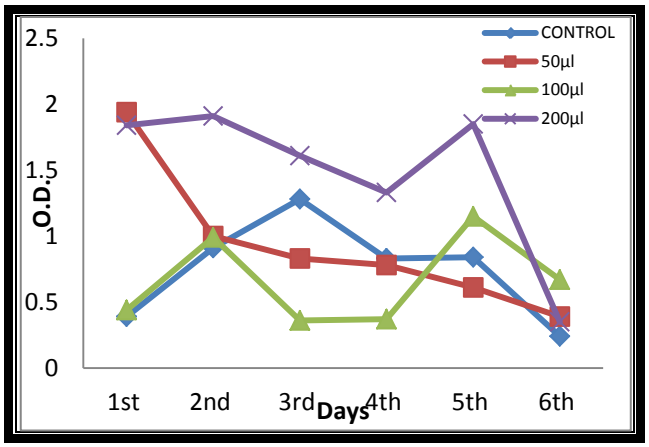

Fig 1 (a)- Effect of Different concentrations of Lambda Cyhalothrin on *E.coli*

Fig 1 (B)- Effect of Different concentrations of Lambda Cyhalothrin on *B.subtilis*

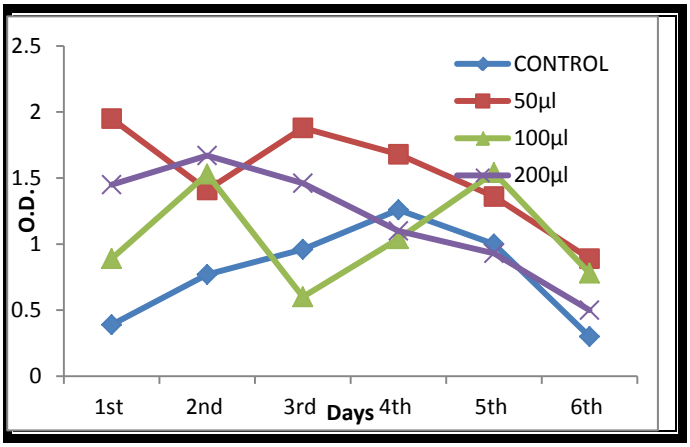

Fig 1 (C)- Effect of Different concentrations of Lambda Cyhalothrin on Enterobacter sp

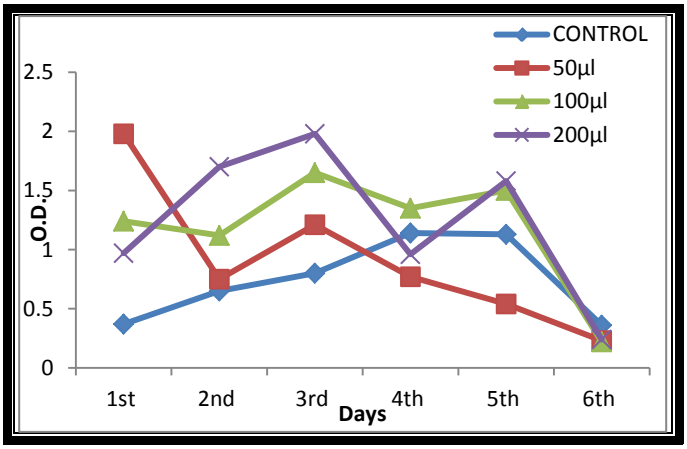

Fig 1 (D)- Effect of Different concentrations of Lambda Cyhalothrin on *Serratia sp*

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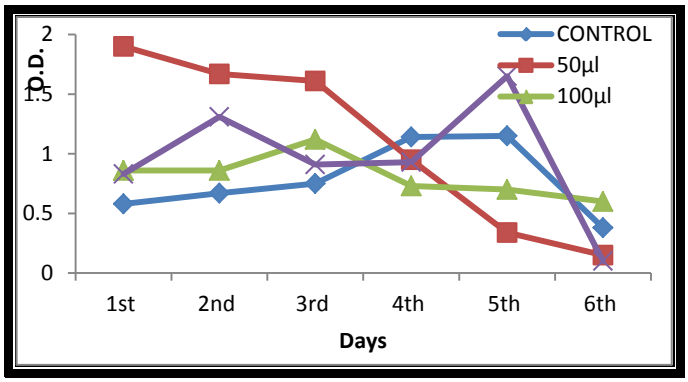
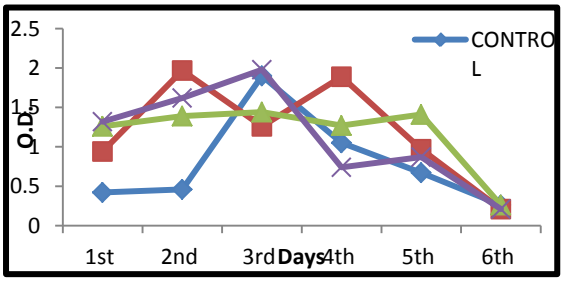


Fig 1 (E)- Effect of Different concentrations of Lambda Cyhalothrin on *Kurthia*

These findings support earlier workers [9] who reported that numbers of soil microoganisms were stimulated through the utilization of chlorinated hydrocarbon, organophosphate and synthetic pyrethroid insecti-cides. Deltamethrin and Alpha cyhalothrin had demonstrated little change on the growth of all the bacterial populations when compared at different concentrations and control which indicated greater utilization of insecticidal residues as well as their degraded products [10] by the soil micro organisms.



Further Miticide brought reduction on the growth of all the bacterial populations with increase in the concentrations which were significantly different from control including *E.coli, Bacillus, Serratia, Enterobacter and Kurthia* and this effect was offset during exponential phase of treatment but at slow rate (Figure 2A-2E)

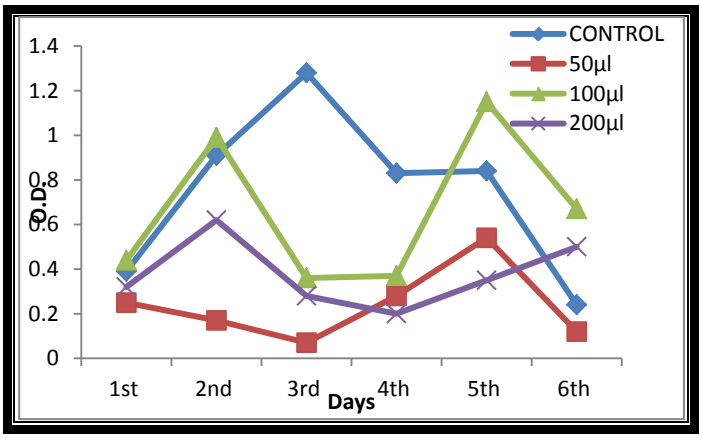


Fig 2 (A)-Effect of Different concentrations of Miticide on *E.coli*

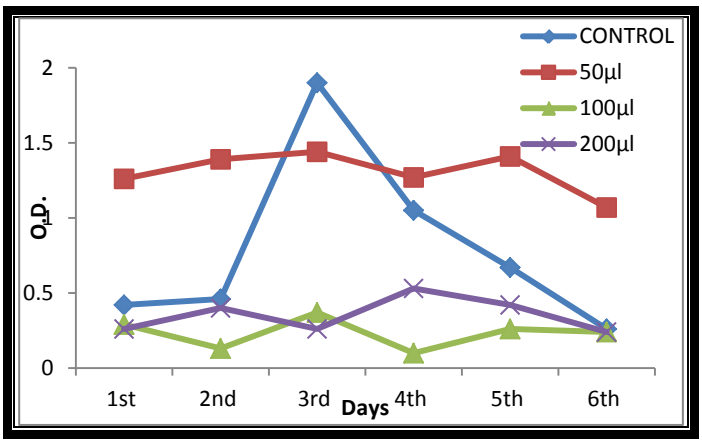


Fig 2 (B)-Effect of Different concentrations of Miticide on *Bacillus subtilis*

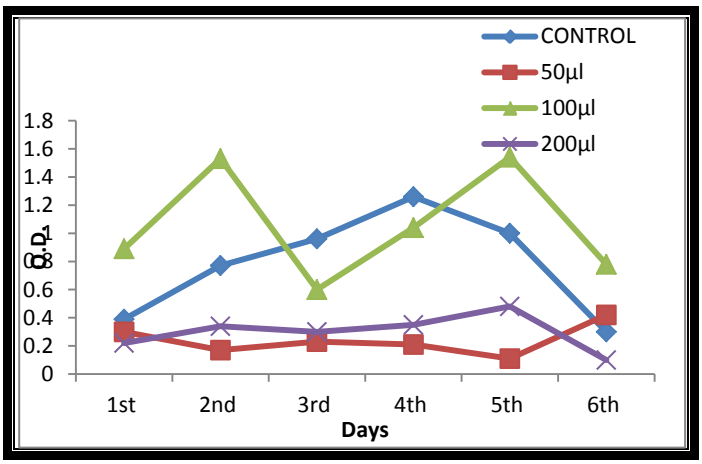


Fig 2 (C)-Effect of Different concentrations of Miticide on *Enterobacter*

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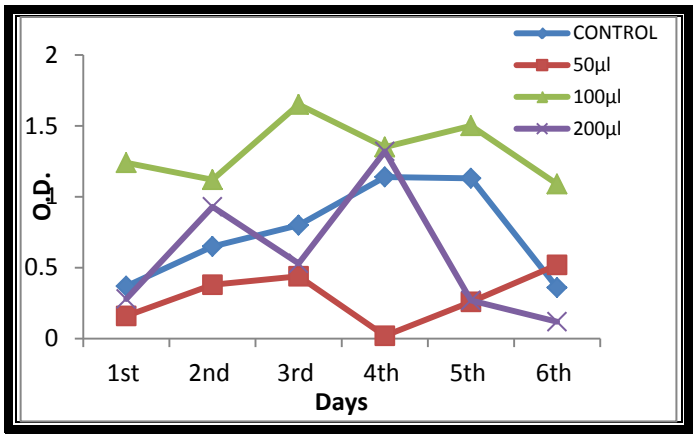

Fig 2 (D)-Effect of Different concentrations of Miticide on *Serratia*

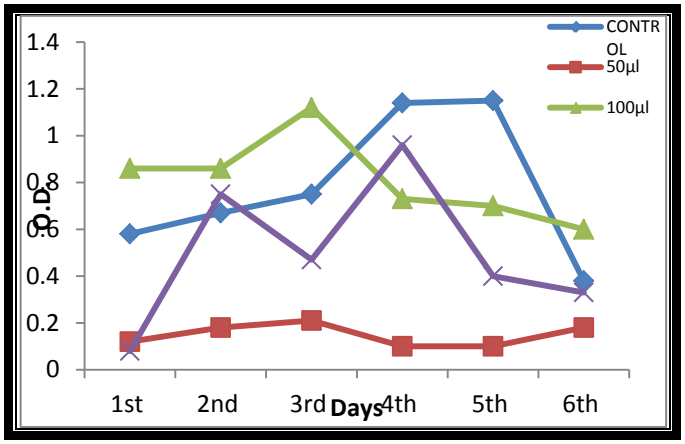

Fig 2 (E)-Effect of Different concentrations of Miticide on *Kurthia*

Whatever the mechanism of this effect was, with the concentration of insecticide applied, it could not have a significant impact on the total biomass synthesis. These facts, suggest that among the species of microorganisms some of them can be selected to be used for the controlled degradation of this insecticide. Probably for the intensification of this process a concomitant carbon source is necessary to serve as a co-substrate [11]. It is assumed that for the full degradation of insecticides makes sense not pure (mono) cultures, but a mixture of populations sequentially metabolizing the intermediates to be used [12]. Such method of combining co-metabolism with comensalism, i. e. the coordinated interaction of cellular and population levels open great opportunities for intensification of the treatment processes in biosphere as well as under controlled conditions.

Further the effect of different concentrations of fungicide on soil microorganism was studied and found that on application of fungicide enhances the growth of microorganisms as compared to the control. All the bacterial cultures *E.coli*, *Bacillus subtilis*, *Serratia marscens*, *Enterobacter sp* and *Kurthia sp* showed positive effect on the application of fungicide (Fig 3A-3E)

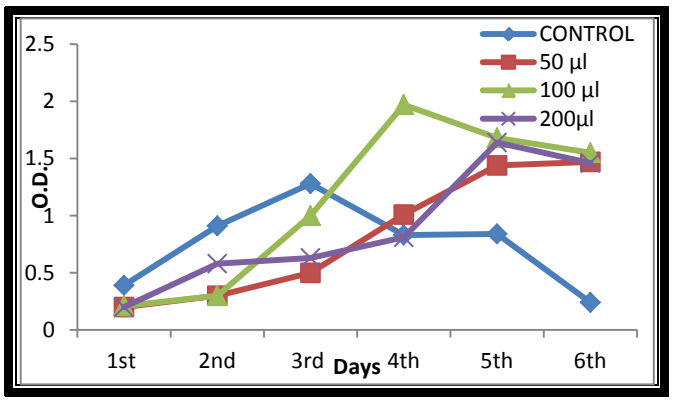

Fig 3 (A)-Effect of Different concentrations of Fungicide on *E.coli*

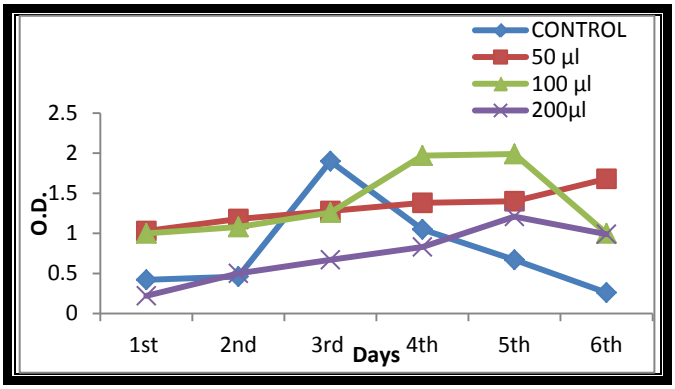

Fig 3 (B)-Effect of Different concentrations of Fungicide on *Bacillus subtilis*

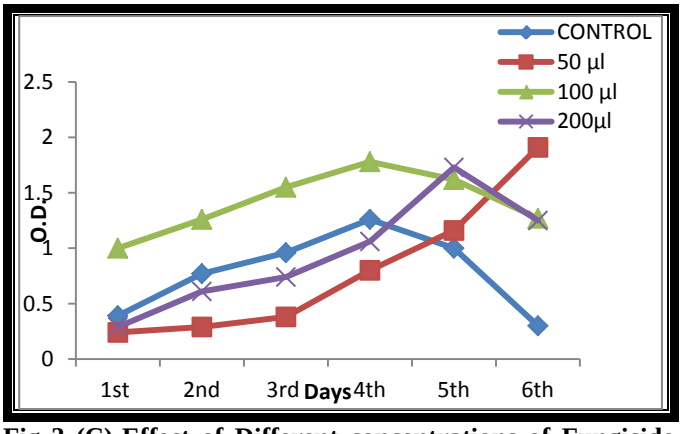

Fig 3 (C)-Effect of Different concentrations of Fungicide on *Enterobacter sp*

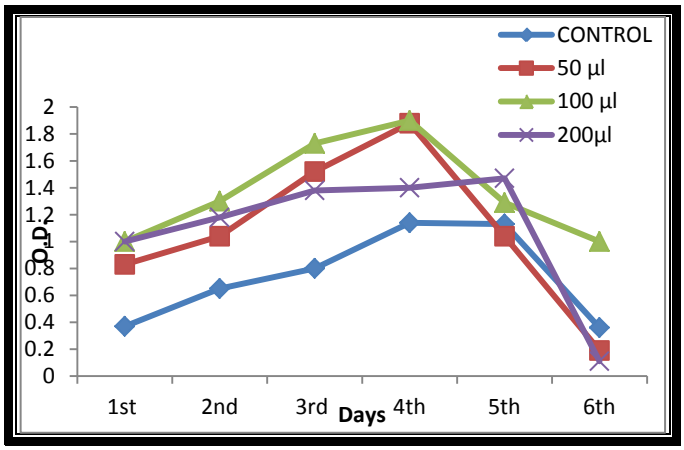

Fig 3 (D)-Effect of Different concentrations of Fungicide on *Serratia sp*

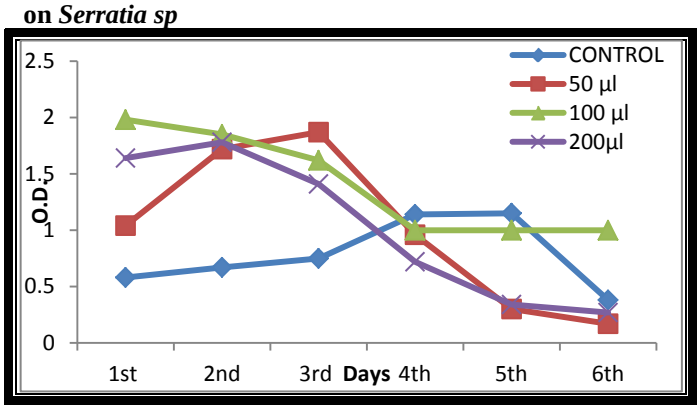

Fig 3 (E)-Effect of Different concentrations of Fungicide on *Kurthia*

The proliferation of the bacteria at low concentrations was augmented due to the application of insecticides in the medium and the augmentation was highly significant when the medium was treated with pesticides. This clearly indicates that organisms preferentially utilize insecticides to derive energy and other nutrients resulting in increase in their populations in medium [13, 10].

Microorganisms are a vast heterogeneous group that occurs in substantial numbers in soil where they are active agents of mineralization of organic matter. These organisms possess a variety of diverse catabolic pathways that enables them to metabolize an equally diverse number of low molecular weight compounds, including insecticides. The growth responses revealed the degree of bacterial sensitivity or resistance and the amount of growth stimulation when exposed to different type and concentration of insecticides.

Some studies report increased populations of actinomycetes and fungi after treatment with glyphosate [14] increased soil microbial biomass [15] or no long-term change in microbial populations [16]. There was significant reduction in percentage organic matter after the pesticides were applied to soils, although organic matter increased after continuous application from the second to the sixth week of treatment.

Ayansina and Oso (2006) [17] reported that soil treatment with atrazine resulted in significant changes in percentage organic matter measurements. Ali (1990) [18] had shown that the fate of pesticides in soils is greatly affected by the presence of organic matter in the soil by aiding their disappearance.

CONCLUSION

Pesticides are indispensible in agriculture and public health programs. Among pesticides, insecticides are the principle pollutants of our environment. It has been recognized that microbes possess remarkable spectrum of activities and are resilient to most natural and anthropogenic perturbations. However, indiscriminate use of insecticides might affect microbial populations and their micro-organisms also play a vital role in the movement, transport and biomagnifications of insecticides residues in various component of our environment. It is, therefore a fundamental necessity to have a deeper understanding of 1)the quality and quantity of insecticides 2)their interaction with micro-organism in order to maintain a stable ecosystem.

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