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Research Article

RESPONSES OF SOIL ENZYMES TO PESTICIDES AND BIOPESTICIDES IN SOIL

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ABSTRACT

Soil enzymes play a vital role in catalyzing several important reactions necessary for life processes of microorganisms in soil. Soil enzymes play an important role in catalyzing reactions for the decomposition of organic matter and nutrient recycling in ecosystems. Microorganisms are the producers of enzyme activities in soil. Pesticides and Biopesticides, was assessed for its effect on different enzymatic activities (Protease, Cellulase, Amylase, Alkaline phosphatase, Glucosidase and Invertase) in soil for definite period of incubation. Variations in activity were independent upon the period of incubation. An increasing trend in the activity of microbial population and soil enzyme activity were observed during 14th - 28th day of incubation in both control and treated soil. These fluctuations in activities were in accordance with soil pH and organic matter.

INTRODUCTION

In practice, different agrochemicals are used in modern agriculture as important tools that help the farmer to minimize economic losses caused by weeds, pathogens and insect pests. The economy of India is largely dependent on agriculture but about 15-20% agricultural production is negatively influenced by pests (Bhalerao and Puranik, 2007). Application of individual pesticides particularly with many cash crops like groundnut, cotton and sugarcane to minimize the crop loss. Pesticides are recognized as a source of potential adverse environmental impacts and their persistence in soil and ground water has grown considerably (Tejada, 2009). When a pesticide is environment about 0.1% reaching the target organism while the remaining 0.99% reaches the soil causing not only trouble local metabolism or enzymatic activities (Liu *et al*., 2008) but also disturb soil ecosystem and thus, may affect human health by entering in the food chain, have raised considerable public concern.

Although pesticide have been restrictively used or even banned their persistence and bioaccumulation still be found in soils. Thus it is required to estimate soil biological responses to the pesticides. To date, many efforts have been made to understand the effect of pesticides on soil enzyme activities, amylase and cellulose but little is known about the effect of pesticides and biopesticides. So from past 10 decades more specific prominence has been given to soil enzymes because these are indicators of biological equilibrium (Frankenberger and Tabatabai, 1991), fertility (Antonius, 2003), quality (Bucket and Dick, 1998) and changes in the biological status of soil due to pollution (Trasar-Cepeda *et al*.,2000). When compared with enzymes from different sources, soil enzymes commonly show particular and peculiar feature. Soil enzymes are involved in energy transfer, nutrient cycling, environmental quality and crop productivity. Negative impact of pesticides on soil enzyme activities has been widely reported in the literature (Menon *et al*., 2005). Hence the present study has been undertaken to investigate the effect of pesticides and biopesticides on enzymatic activities in soil.

MATERIALS AND METHODS

Soil sampling: Top soil (up to 5 cm depth) samples were collected with no prior pesticide treatment. The soil samples were sieved through a 2.0 mm mesh size to remove stones and plant debris.

Pesticides: The pesticides used in this study were obtained from a local agricultural dealer store in Jaipur, India. The pesticides used were Cypermethrin, Malathion, Victor, Monocil and Tafgor.

Biopesticides: The biopesticides used in this study were obtained from Dharma Biotech Company, Hyderabad, India. The biopesticides used were Folicon (neem based), *Paeciliomyces lilacinus, Bacillus subtilis, Pseudomonas florescens and Beauveria bassiana*

Enzyme Assay

The protease activity of soil enzyme was estimated by the Ladd and Butler method (1972). The Cellulase activity of soil enzyme was estimated by the Deng and Tabatabai method

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(1994). The amylase activity of soil enzyme was estimated by the Ross method (1966). The invertase activity of soil enzyme was estimated by the Ross method (1966). The alkaline phosphatise activity of soil enzyme was estimated by the Tatabai and Brenner method (1969). The β-glucosidase activity of soil enzyme was estimated by the EIviza and Tatabai method (1981).

RESULTS AND DISCUSSION

Bacteria have a large contact interfaces with their surrounding environment because of their small size with a high surface area to volume ratio. Soil bacteria thus have high potential as sensitive bioindicators of perturbations of soil quality by pesticide treatments. Pesticides toxic effects are often evaluated by measuring overall rates of microbial activity. Thus, measurements of several, relatively specific enzymatic activities (amylase, invertase and cellulase), or a combination of them, may contribute to characterize more precisely the response of soil bacterial communities to pesticides.

This response may include pesticide degradation capacities and/or overall community tolerance to toxic bioactive chemicals. If a pesticide-dependent response may often be detected, however, demonstration of its dose-dependence is more complex and often seems to depend on the experimental methods used the characteristics of the investigated compounds, and soil properties. It often remains unclear to what extent such observed changes in enzyme activity result from an adaptative shift of soil communities towards more pesticide-tolerant types of bacteria, rather than from a lower nominal exposure to pesticides owing to dissipation or sorption of bioactive compounds to soil organic matter or minerals through time. The enzymatic response of soil bacteria to pesticides is also influenced by soil physico-chemical characteristics (Gevao *et al*., 2000) and/or agricultural practice (Alletto *et al*., 2010). These factors strongly affect the fraction of contaminant that causes an effect on soil microorganisms. The bioavailable fraction of pesticides is controlled by soil properties, in particular by organic matter content, and by the physico - chemical properties of the pesticide molecule itself (its hydrophobicity in particular). The toxicity of pesticides to soil microorganisms may be markedly reduced in soils containing large amounts of organic matter or amendments.

Our investigation has revealed that protease activity has more drastically decreased at higher concentrations of all pesticides treated soils than the untreated controls throughout the experiment, suggesting that the enzyme is sensitive to pesticides. On the other hand application of Bio pesticides stimulates the protease activity within soil up to 14th day and the decreases up to 28th day of incubation. (Fig 1).

Soil enzyme protease is excreted by the soil microorganisms, plants and animals by means of their metabolic activities. This is an extracellular enzyme secreted by soil microorganisms, including bacteria and fungi widely available, where the protein rich effluents dislodge into the soil increases proteolytic activity in the test soil is due to the presence of high organic wastes (amino acids) discharged in the effluents (Nagaraju *et al*., 2007). The protease activity increased up to 14th day and then declined at 21st day probably due to exhaustion of the readily available substrates. Similarly, soil protease activity in soils treated with dairy shed effluents, dairy factory effluents increased at first and then decreased with the time. In contrast, soils polluted with herbicides, chlorothionil, decreased soil protease (Katayama and Kuwatsuka, 1991).

Similar to protease, cellulase also follows the same trend, a inhibitory effect of pesticides was observed at concentrations with pesticidal treatments on the control, after 14 days of incubation. Also similar trends were observed on the application of Bio pesticides, the cellulose activity within soil decreases from14th to 28th day of incubation. (Fig 2).

Cellulases play an important role as a group of enzymes in global recycling of the most abundant polymer, cellulose in nature. Hence the impact of selected pesticides on cellulase activity in soil was assessed. Similarly, an anthraquic fluvisol soil incubated with the formulated fungicide, hymexazol, for 4 weeks remained unchanged in cellulolytic activity. However, quintozene resulted in initial decrease under flooded soil conditions, whereas under upland conditions, the activity was recovered to control value (Singh *et al*., 1988). On the other hand, captafol significantly inhibited mineralization of cellulose in a sandy loam soil (Vincent and Sisler, 1968). A distinct depression was observed with chlorothalonil, under all conditions tested in both flooded and non-flooded soil. Further Petkar and Rai (1992) demonstrated that five fungicides, captan, cosan, thiram, zineb and sandolex inhibited the cellulose activity, with greater inhibition with increasing fungicidal concentrations.

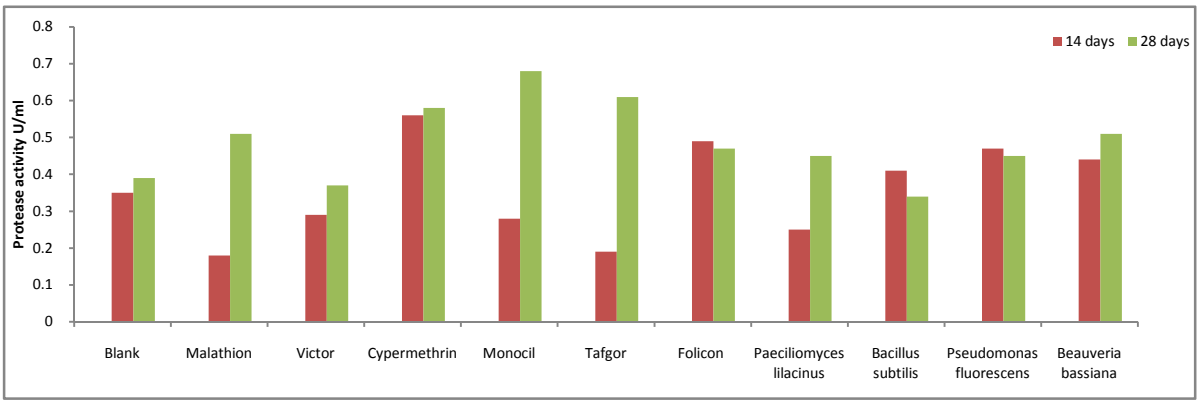

Fig 1 Effect of Pesticides and Biopesticides on Soil protease activity

On the application of Chemical pesticides the amylase activity within soil increases from14th to 28th day of incubation while on the application of Bio pesticides the amylase activity within soil decreases from14th to 28th day of incubation (Fig 3).

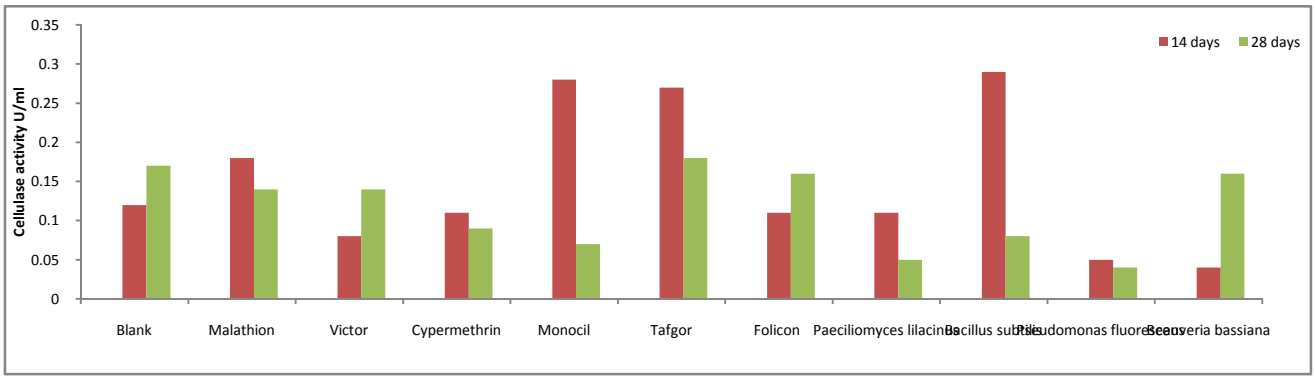

Fig 2 Effect of Pesticides and Biopesticides on Soil cellulase activity

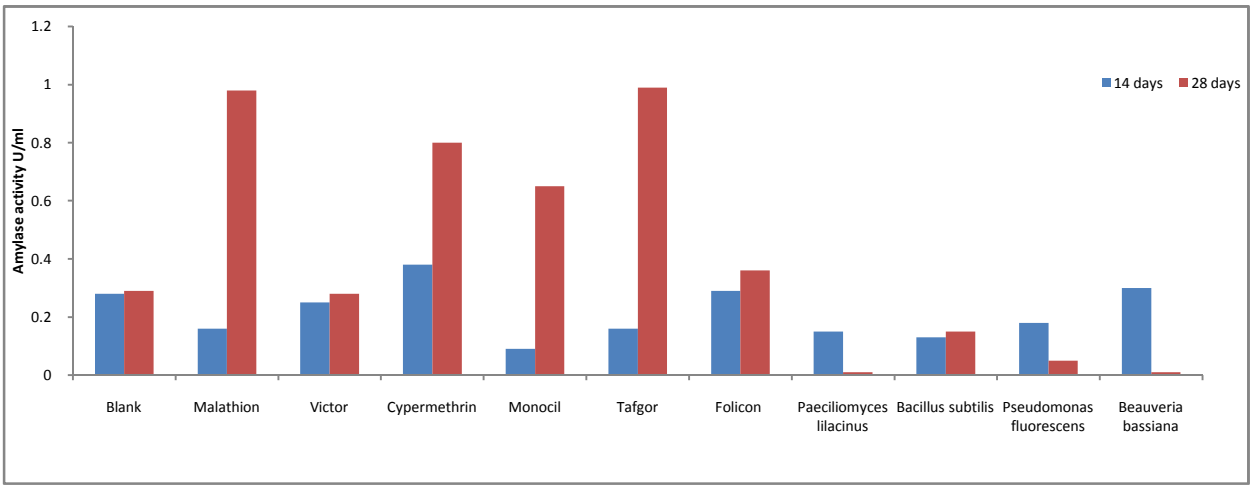

Fig 3 Effect of Pesticides and Biopesticides on Soil amylase activity

Earlier reports are divided in that they show that herbicides may either enhance or inhibit soil enzyme activity (Voets *et al*., 1974). A marked suppression of amylase activity in groundnut oil treated by such insecticides as monocrotophos, quinalphos, cypermethrin, and fenvarelate was shown by Rangaswamy and Venkateswarlu (1992). Similarly, Tu (1988) reported that 11 herbicides used in his study inhibited amylase activities after 1 day of incubation.

On the application of Chemical pesticides the Invertase activity within soil increases from14th to 28th day of incubation. On the application of Bio pesticides the Invertase activity within soil increases from14th to 28th day of incubation. (Fig 4.)

Invertase is a ubiquitous enzyme that occurs in plant tissues and soil organisms. It hydrolyze sucrose to fructose and glucose. As previously reviewed by so many researchers, several studies have been performed on the impact of pesticides on soil microbial activity, and application of pesticides increased, decreased, or did not affect the activities of these enzymes in soils, depending upon the nature and concentrations of pesticides used, incubation period, status of enzymes in soil, and soil condition. Srinivasulu and Rangaswamy (2006) reported decrease in the invertase activity at higher concentrations (7.0 and 10.0 kg^{-1} ha) by the application of tridemorph and captan.

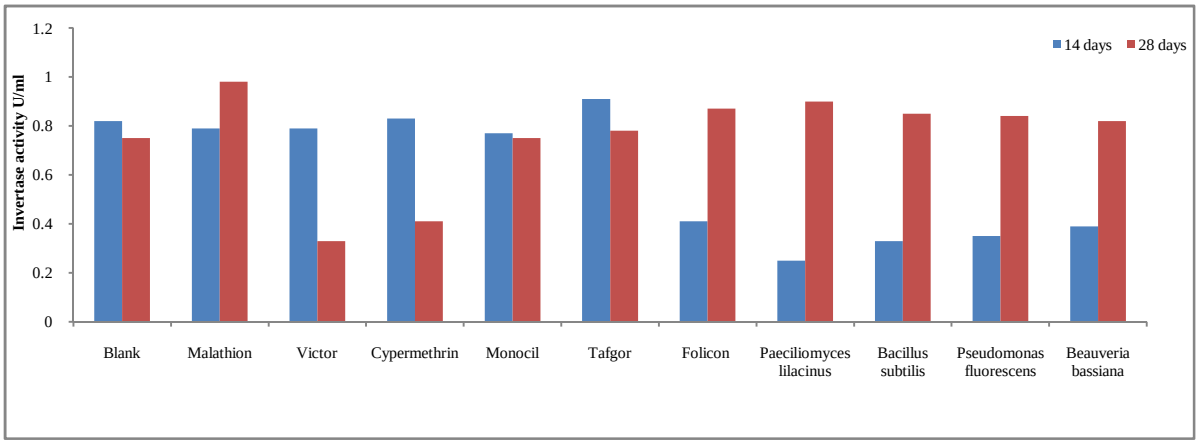

Fig 4 Effect of Pesticides and Biopesticides on Soil invertase activity

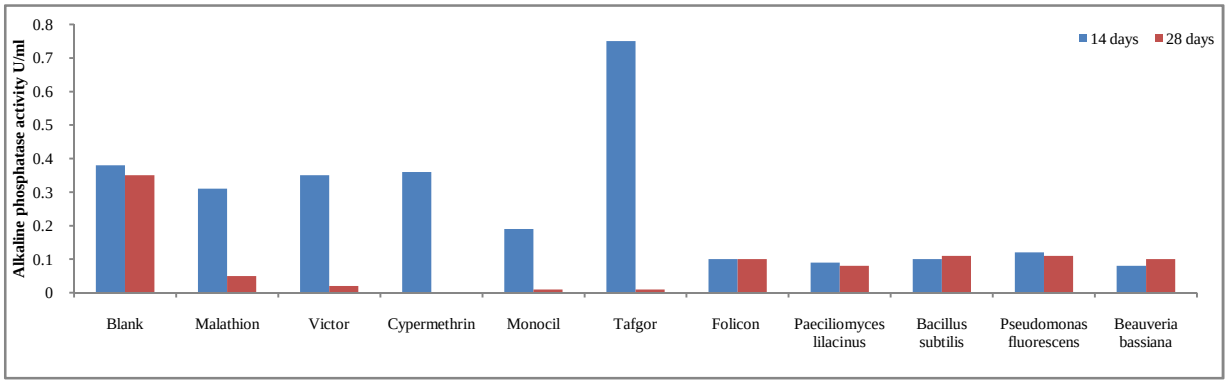

Fig 5 Effect of Pesticides and Biopesticides on Soil Alkaline phosphatase activity

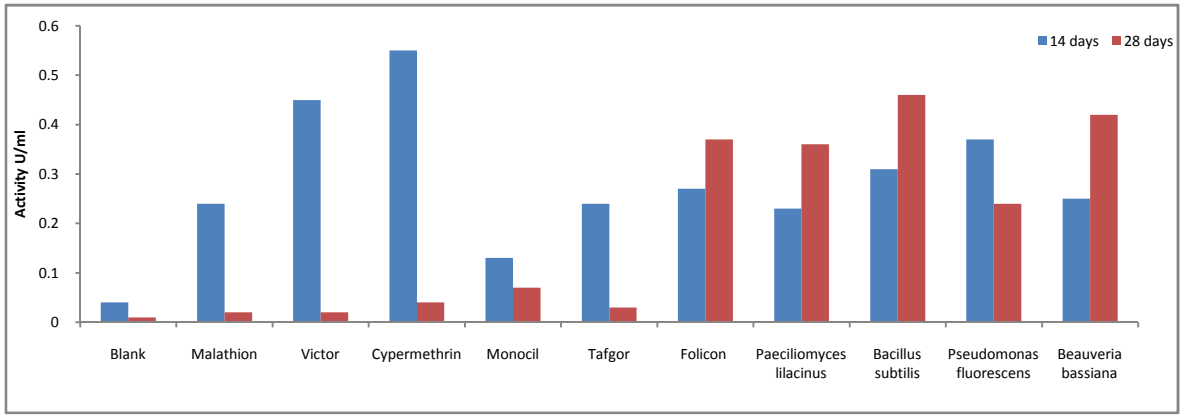

Fig 6 Effect of Pesticides and Biopesticides on Soil Glucosidase activity

On the application of Chemical pesticides the Alkaline Phosphatase activity within soil increases from14th and decreases up to 28th day of incubation**.** On the application of Bio pesticides the Alkaline Phosphatase activity within soil increases from14th and decreases up to 28th day of incubation. (Fig 5).

On the application of Chemical pesticides the β - Glucosidase activity within soil decreases up to 28th day of incubation. On the application of Bio pesticides the β – Glucosidase activity within soil increases upto 28th day of incubation. (Fig 6).

A lower enzyme activity indicates reduced redox intensity in the soil and the reduction degree depended on the concentration and duration of pesticides activity. The enzyme activity was reduced by the harmful activity of pesticides but enhanced in the presence of biopesticides. As pesticides are to some degree environmental toxicants, their noxious activity may be described being manifested mostly through a disturbance of the natural balance.

Soil enzymes are a sensitive indicator of unique biological and biochemical ineractions in soil resulting from anthropogenic, agronomic, chemical and weather changes. In this experiment, soil contamination with insecticides modified the biochemical parameters of the soil. Even the lowest insecticide doses affected soil enzyme activities. However, the effects were positive or negative, depending on the enzyme tested and the applied pesticide dose.

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