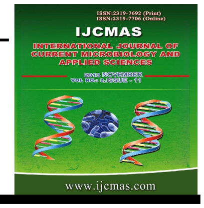

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Original Research Article

Effect of induced amendments on microbial enzyme activity in the rhizosphere of rice

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A B S T R A C T

Rice (*Oriza sativa*), a member of family *Poaceae,* is one of the important cereal crops in the world. As a result of the indiscriminate use of chemicals, microbial enzymatic activity of soil has decreased significantly. Hence, the addition of organic matter to restore and sustain the soil microflora is highly essential. An experiment in the rhizosphere of rice was conducted to study the effect of malathion, bavistin and coir compost on the microbial status of the soil during different growth stages. The impact of soil amendments on rhizosphere was assessed in laboratory and field conditions. The microbial enzyme assay (dehydrogenase, urease and invertase) was employed as indirect method of microbial activity. The experimental results showed that the treatment with coir compost recorded the highest activity of dehydrogenase, invertase and urease where as the treatment with malathion recorded the lowest activity. Diversity indices revealed that the application of insecticides and fungicides significantly lowered the microbial enzyme activity of the rhizosphere. This study focuses on the importance of organic farming by utilizing various natural compounds and the ill effects of pesticides.

Keywords

Dehydrogenase;
avistin;
Coir compost;
Invertase;
Malathion;
Tetrazolium;
Urease.

Introduction

Rice ecosystems remain flooded through a major part of the cropping period and are distinctive from upland soils in several physicochemical and biological properties. Therefore, flooded rice fields became a model system to study soil microbial ecology (Leisack et al, 2000). Rhizosphere is an important region of soil that is directly influenced by root secretions and associated soil microorganisms. Soil which is not the part of the rhizosphere is known as bulk soil. Plant rhizodeposition in the rhizosphere results in increased microbial population size and community structures distinct from that in bulk soil (Bais et al, 2006). They play lots of role like disease control, increase uptake of nutrients, etc. Rhizosphere soil of many crop plants, especially rice, is affected badly by various improper cultural practices like the addition of chemical pesticides and fertilizers.

Rhizosphere microbes have a significant role to play in the ecology of rice by being exclusively regulating the pest population in rice. The objectives of this study were therefore to evaluate the effect of induced amendments (malathion, bavistin and coir compost) on microbial enzyme activity (dehydrogenase, invertase and urease) in the rhizosphere of rice.

Materials and Methods

Sample collection

Rhizosphere soil of paddy field (Jaya variety) of Kallur of Thrissur district of Kerala during the period of seedling emergence was used for this study and an experimental set up was constructed which consist of 3 soil treatments. (1) fungicide treatment (Bavistine 3%) (2) insecticide treatment (Malathion 3%) (3) organic treatment (coir compost 30 gm/ m^2). Enzymatic assay was carried out for all the soil treatments taking untreated rhizosphere soil as the positive control and bulk soil as the negative control.

Enzyme activity assay

Enzyme activities were determined on fresh moist sieved (< 2 mm) soils within 15–20 days after soil treatment. Two enzymatic activities (invertase and urease) were analyzed according to the protocols described by Gu et al (2009). In case of invertase, 5 g of soil was mixed with 0.2 mL of toluene and 5 mL of phosphate buffer (pH 5.5) in 50-mL erlenmeyer flasks, then 15 mL of the 8% sucrose solution was added, and the flasks were swirled for a few seconds. The flasks were covered with stopper and placed in an incubator at 37°C for 24 h. After incubation, solution contents were passed through filter paper. The 1 mL of filtrate was pipetted into 50 mL test tube and 3 mL 3, 5-dinitrosalicylic acid monohydrate solution was added. Then all tubes were placed into a boiling water bath for 5 min and allowed to cool at room temperature with tap water. Further, the final volume of the each solution was made up to 50 mL with double distilledwater. The glucose produced was determined colorimetrically at 508 nm and was determined colorimetrically and expressed as mg glucose/g soil/24 h.

Urease activity was determined using 5 g of soil (< 2 mm) that was mixed with 1 mL of toluene in a 50-mL erlenmeyer flask, and allowed to stand for 15 min. Then 10 mL 10% urea solution and 20 mL citrate buffer (pH 6.7) were added and mixed well. The flasks were covered and placed in an incubator at 37°C for 24 h. After incubation, soil solution contents were passed through filter paper. Moreover, 3 mL of aliquot from filtrate was added into a 50-mL test tube along with 4 mL sodium phenol solution and 3 mL sodium hypochlorite solution and tubes were swirled well for mixing. After 20 min, the final volume of each solution was made to 50 mL with distilled water. The released ammonium was measured colorimetrically at 578 nm and expressed as mg NH4-N produced/g soil/24 h.

In case of dehydrogenase enzyme, 20 g of fresh soil at 90% water holding capacity mixed with 200 mg $CaCO_3$ and 2ml of 1% solution of triphenyl tetrazolium chloride (TTC). The mixture is inoculated for 24 hours. In the absence of oxygen TTC serve as a terminal accepter for the H_2 evolved and through the action of microbial dehydrogenase TTC get reduced to triphenyl formazan (red) at 485 nm. Optical density calculated calorimetrically. All the experiments were done in triplicates and the mean value of optical density was determined and the standard deviation and standard error was calculated. The optical density for the 3 enzymes plotted graphically for all soil treatments.

Results and Discussion

The results indicated that rhizosphere soil and soil treated with coir compost gives an increased enzyme activity for the three enzymes (table 1). Invertase and urease activity were higher in rhizosphere soil and soil treated with coir compost. Urease and invertase activity were drastically decreased following fungicide and insecticide treatment. Higher dehydrogenase activity was shown by rhizosphere soil followed by coir compost treated soil. Dehydrogenase activity drastically decreased for the soil samples treated with fungicide and insecticide (Figure.1).

Soil enzyme activities are often used as indices of microbial function and soil fertility and are greatly affected by quality and quantity of root exudates depending on the plant species, cultivar, plant growth stage and physiological status of the plant (Saxena et al, 1999). Invertase catalyses the hydrolysis of sucrose into glucose and fructose. Enzyme activity was expressed as the amount of glucose formed from the substrate. Invertase activity can be correlated with total organic nitrogen and carbon present in soil. Urease enzyme is responsible for the hydrolysis of urea fertilizer applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH (Byrnes and Amberger, 1989). Since urease plays a vital role in the hydrolysis of urea fertilizer, it is important to uncover other unknown factors that may reduce the efficiency of this enzyme in the ecosystem. A better understanding of this enzyme would provide more effective ways of managing urea fertilizer especially in high rainfall areas, flooded soils and irrigated lands as well as where urea fertilizer is vulnerable to urease enzyme.
Biological oxidation of soil organic compounds is generally a dehydrogenation process carried out by specific dehydrogenase involved in the oxidative energy transfer of microbial cells. This activity is a measure of microbial metabolism and thus of the oxidative microbial activity in soils. A good correlation has been shown between microbial biomass and soil dehydrogenase activity (Chander *et al*., 1977). The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978). Since these processes are part of respiration pathways of soil micro-organisms, studies on the activities of dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility. Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil as well as a direct measure of soil microbial activity. It can also indicate the type and significance of pollution in soils.

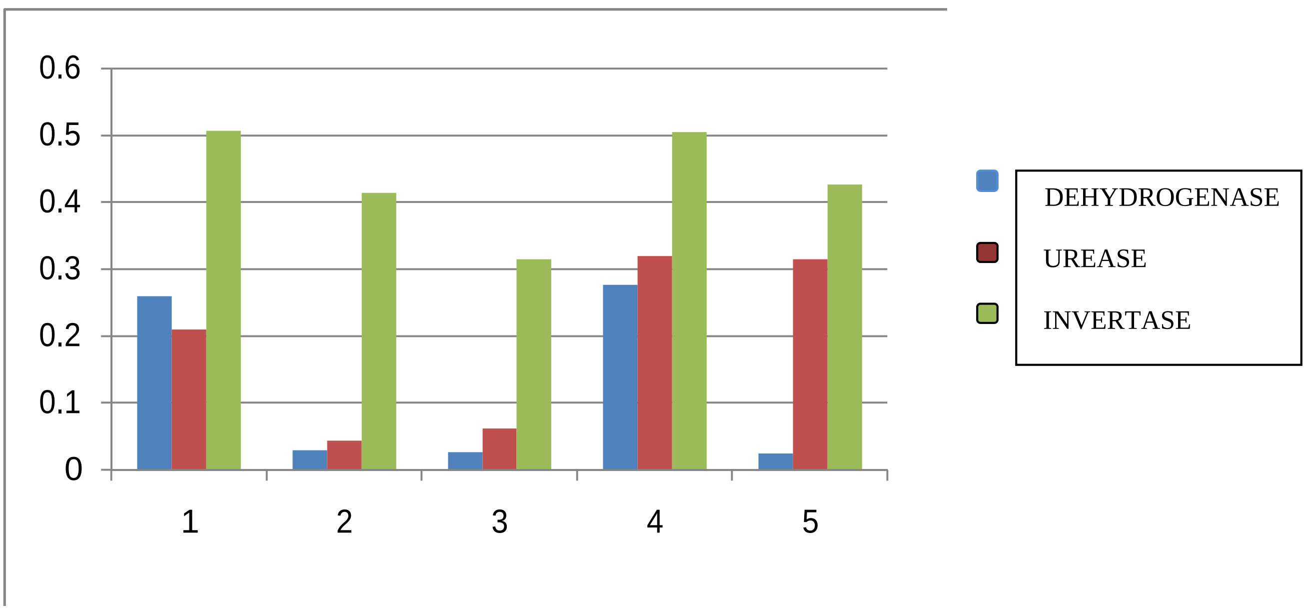
The experimental results showed that the treatment with coir compost recorded the highest activity of dehydrogenase, invertase and urease where as the treatment with malathion recorded the lowest dehydrogenase activity. Soil enzyme activities may also be increased by the addition of organic materials (Nannipieri et al, 1983; Zantua and Bremner 1976; Balasubramanian et al,

Table.1 Enzyme activity assay in different soil amendments.

Treatments	Optical density								
	Dehydrogenase			Urease			Invertase		
	MEAN	S.D*	S.E	MEAN	S.D	S.E	MEAN	S.D	S.E
Rhizosphere soil	0.26	0.014	0.007	0.21	0.0141	0.0063	0.507	0.01	0.005
Fungicide	0.03	0.0089	0.004	0.044	0.0005	0.00022	0.415	0.01	0.005
Insecticide	0.027	0.272	0.136	0.062	0.0118	0.0052	0.315	0.18	0.09
Biofertilizer	0.277	0.276	0.138	0.32	0.028	0.0125	0.505	0.114	0.0057
Bulk soil	0.025	0.026	0.013	0.315	0.011	0.0055	0.4275	0.0148	0.0074

*SD –standard deviation S E – standard error

Figure.1 Graphical representation of enzyme activity assay in different soil amendments



x axis - 1.rhizosphere soil, 2.fungicide treatment, 3.insecticide treatment, 4.biofertilizer treatment, 5.bulk soil; **y axis –** optical density

1972). This increased activity has generally been attributed to the increased microbial biomass although additional evidence has shown that plant materials and sludges may directly contribute enzymes to soil (Zantua and Bremner, 1976; Franken and Tabatabai, 1980).

Diversity indices revealed that the application of insecticides and fungicides significantly lowered the microbial enzyme activity of the rhizosphere. Excessive use of pesticides and fungicides has decreased rhizosphere microbial population affecting the ability of natural microbial population to enhance the soil health. The addition of organic nutrients increased the activity of rhizosphere microbes and thereby they will definitely promote crop productivity without causing any harm to environment and human health. The present study clearly showed that the microbial enzyme activities in rhizosphere soil was greatly reduced upon the addition of fungicide and insecticide, and promoted by the addition of organic manure.

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