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ENUMERATION OF MICROBIAL POPULATION FROM RICE FIELDS AND THEIR DEHYDROGENASE ACTIVITIES IN TWO CROPPING SEASONS IN EASTERN INDIA

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ABSTRACT
A field experiment was carried out to study the different microbial population in rice field with respect to their dehydrogenase(respiratory enzyme)activity during *Kharif*(July- Oct) (wet) and *rabi*(Jan-may) (dry)in the year 2013. The rice cultivar *Lalat* was taken for cultivation. Different micro flora was observed under both the cropping system. The population like heterotrophs, anaerobic population, fungal population and actinobateria population was isolated under different stages of crop. During *Kharif* seasons the heterotrophic bacterial population was significantly higher under panicle initiation stage of rice crop (cvlalat). The fungal population was not significant amongthe different stages of rice crop.. Dehydrogenase activity was significantly higher under panicle initiation stage of both *rabi*(dry)and *kharif*(wet)rice crop. Dehydrogenase activity was 20% more under PI stage than maximum tillering stage in *kharif* seasons. In *kharif*seasons the anaerobic bacteria population was significantly higher as compare with *rabi*seasons. Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles thereby regulating the microbial diversity.Environmental sustainability point of view, different micro flora present in rhizospheric soil under rice-rice cropping system in tropical low land soil could be adopted to maintain soil health, increased yield rate and increased carbon pools and dehydrogenase enzyme which is good indicator of fertile soil.

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 (Le is ack *et al.,* 2000).Soil microorganisms control aspects of nutrient cycling; some of which are pivotal in the response of ecosystems to climate change. Examples include soil organic matter (SOM) transformations that may result in carbon (C) storage instable soil pools and mobilization of nutrients that are required for C assimilation into plant biomass (Das *et al*., 2014).Rhizo sphere microbes have a significant role to play in the ecology of rice being exclusively regulating the pest population in rice .Several biotic and a biotic factors affected soil dehydrogenase activity such as the incubation time and temperature, soil aeration status and soil moisture content and is also often used as a measure of any disruption caused by pesticides, trace elements or management. Enzyme activity in soil results from the activity of accumulated enzymes and from enzymatic activity of proliferating microorganisms (Kiss *et al.,* 1975). Dehydrogenase activity can be considered to be a good measure of microbial oxidative activity in soils.Soil contains a variety of microorganisms included bacteria that can be found in any natural ecosystem. Microorganisms play an important role on nutritional chains that are an important part of the biological balance in the life in our planet. Where, bacteria are essential forthe closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulfur and phosphorous cycle.Without bacteria, soil would not be fertile and organic matter such as straw or leaves would accumulate with in a short time (Kummerer, 2004). Fungi are similar to algae, but they do not contain chlorophyll and require pre-formed organic matter as energy and carbon sources (e.g., sugars, fat, protein, and other carbohydrates). Such organisms are called heterotrophs. Fungi, ranging in size from a few microns to several centimeters, grow either role in supplying essential soil nutrients. The relation ship between soil organic matter, microbial biomass and microbial activity have been proposed as indicators of soil maturity (Nayak *et al*., 2015).

Heterotrophic bacteria associated to the root system of rice could contribute efficiently to the nitrogen fixation (Sen, 1992)Soil microorganisms constitute a source and sink for nutrients and are involved in numerous activities, such as transformation of C, N, P and S, degradation of xenobiotic organic compounds, formation of soil physical structure and enhancement of plants nutrient uptake (Gregorich *et al*.,1994; Seklemova *et al*., 2001). Soil enzyme activities are often used as indices of microbial growth and activity in soils. Quantitative information concerning which soil enzymes most accurately reflect microbial growth and activity is lacking. Rice (*Oryza*

sativa L) is being an important staple food crop in India and this study was intended to find out the different micro flora present in soil and how they are associated with soil respiration.The objective of this research was to study the presence of different microorganisms in rice field and their reflection towards dehydrogenase activity.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from rice field from *rabi* (Jan-May) and *kharif* (July -Oct) 2012 seasons. Soils were collected from rhizo spheric region with three replicates within 0-15 cm depth. After collection drying of soil takes place and sieved through 2mm of sieve. For microbiological experiment samples was kept inside freeze.

Crop Cultivation

The field experiment was laid by two consecutive seasons under intensive rice-rice cropping system. The wet seasons of *kharif* was (July-Oct) under irrigated condition. The field was ploughed thoroughly and flooded 2-3 days before transplanting for puddling and leveling. Seedlings were raised in seed trays kept under the respective treatments. Over the *kharif* cropping season the sowing of the seedlings were done in the month of June. The important growth stages included active tillering (30-35) days after transplanting (DAT), maximum tillering (40-45) days after transplanting (DAT), panicle initiation (50-60) DAT and harvesting (83-88 DAT) under the various treatments of the study. Rice plants (cv. *Lalat*) were transplanted at a spacing of 15 cm × 15 cm (i.e. 45 plants in 1 m^2) with one seedling per hill.

Enumeration of microorganisms from soil

Total number of population was estimated by using standard protocol. Standard procedure of serial dilution methods was using. To find out the total number of aerobic heterotrophic heterotrophs, anaerobic bacteria population, fungus and action bacteria population microbiological media are used like nutrient hiveg agar, anaerobic hiveg agar, rose Bengal chloramphenicol agar and action mycetes isolation agar was used for isolation of different microbes were procured from Hi Media, Mumbai and prepared as per the manufacturer's instruction. After plating incubated the plate under incubator having temp $30 \pm 2^{o}C$ and for fungi $28 \pm 2^{o}C$. The number of colony forming unit per gram of soil obtained from the valid plate count was calculated by using the formula of

$$\text{CFU/g of soil} = \frac{\text{No of colonies (with replication)}}{\text{quantity taken} + \text{dilution factor}} \quad \ldots\ldots\ldots\ldots (1)$$

Dehydrogenase activity study

Len hard (1956) introduced the concept of determining the metabolic activity of microorganisms in soil and measuring of dehydrogenase activity. Freshly collected soil sample was for the dehydrogenase test. First homogenize the sample in mortar and sieve through 2-mm-sieve. Determine pH of the tested sample and the dry matter content of the sample. After adding substrate 2,3,5-triphenyltetrazolium chloride (TTC) to the sample for overnight incubation and one set kept for blank. Microbial dehydrogenase activity during this incubation results in reduction of the water soluble, colorless TTC to the water-insoluble red coloured 2,3,5-triphenyltetrazolium for mazan. Optical density was taken with the help of spectrophotometer with 475nm.

RESULTS AND DISCUSSION

During *rabi* season, the heterotrophic population was significantly different among the crop growth stages and it show highest (7.42 log cfu) under panicle initiation stage of the rice (*cv lalat*) at ($p < 0.05$). Heterotrophic population was also significantly more among all the different microbial population. Anaerobic bacteria population was found no significant among the different crop growth stages of the rice crop (Table1). Fungal population was found less compare to

Table 1: Microbial population (log cfu) under different growth stages under rice ecosystem (*cvlalat*) in *rabi* seasons. Here, AT, MT, PI and H represent active tillering, maximum tillering, panicle initiation and harvesting, respectively. In each column means followed by common letter are not significantly different ($p < 0.05$) by Duncan's Multiple Range Test (DMRT) at different plant growth stages with different treatments

Different microbial population	*Rabi 2013* Different Growth stage AT	MT	PI	H
Heterotrophicbacterial population	7.41d	7.38d	7.42d	7.03d
Anerobic bacterial population	6.09c	5.64c	5.64c	5.02c
Fungal population	5.20b	5.28b	5.28b	5.02b
Actinobacterial population	4.60a	4.85a	4.91a	4.87a

Table 2: Microbial population under different growth stages under rice ecosystem (*cvlalat*) in *kharif*seasons. Here, AT, MT, PI and H represent active tillering, maximum tillering, panicle initiation and harvesting, respectively. In each column means followed by common letter are not significantly different ($p < 0.05$) by Duncan's Multiple Range Test (DMRT) at different plant growth stages with different treatments.]

Different microbial population	*Kharif2013* Different Growth stage AT	MT	PI	H
Heterotrophicbacterial population	7.35d	7.48d	7.60b	7.02b
Anerobic bacterial population	6.09c	6.06c	6.25c	6.05c
Fungal population	5.11b	5.28b	4.52a	5.50a
Actinobacterial population	4.54a	4.92a	5.99a	4.77a

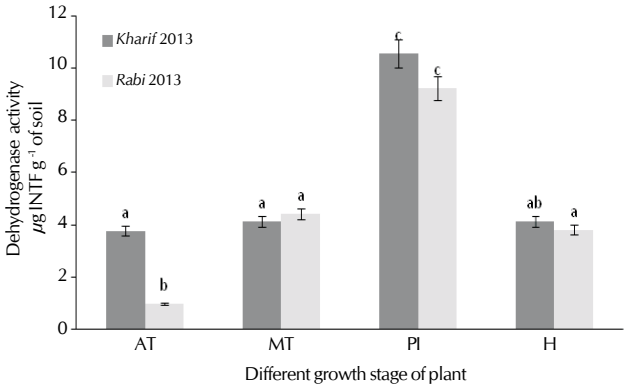

Figure 1: Dehydrogenase activity *μ*g INTF g^{-1} of soil under *rabi* and *kharif*2013 in different growth stage of rice (*cv lalat*). Here, AT, MT, PI and H represent active tillering, maximum tillering, panicle initiation and harvesting, respectively. In each column means followed by common letter are not significantly different (p < 0.05) by Duncan's Multiple Range Test (DMRT) at different plant growth stages with different treatments.]

other microbial population and also found highest in PI stage of rice. Actino bacterial population was significantly different among the stages and highest was found in PI stage of rice (Table 1). During *kharif*2013, the heterotrophic population was significantly different among the crop growth stages and it show highest (7.60 log cfu) under panicle initiation stage of the rice (*cvlalat*) at ($p < 0.05$). Heterotrophic population was also significantly more among the population. Anaerobic bacterial population was found highest (6.25 log cfu) under panicle initiation stage of the rice crop. (Table2). Anaerobic bacterial population was significantly different among the treatments and it was found in the order of PI > MT > AT > H. Fungal population was found less compare to other microbial population and also found highest in PI stage of rice. An increase in fungi as a percentage of the microbial community could be significant to ecosystem function, as fungi play important roles in organic matter degradation, nutrient cycling, and the formation of soil aggregates. Belowground microbial processes play an essential role in nutrient cycling and organic matter turnover, and influence the growth of the plants by competing for nutrients(Das et al. 2014).Actino bacterial population was not shown any significant different among the stages (Table 2). Population was found highest under panicle initiation stage of the crop growth stage.During both the cropping seasons DHA was significantly different among the growth stage of rice (cvlalat). DHA was found more in PI stage of crop growth (Fig.1). Dehydrogenase enzyme was found in the order of PI > MT > AT > H in both *kharif* (wet) and *rabi* (dry) seasons. In *kharif* (wet) season's dehydrogense activity was significantly high among all the stages (10.55*μ*g INTF g^{-1} of soil).Dehydrogenase enzyme is a respiratory chain enzyme that plays an important role in the energy production of organisms. This enzyme works as an essential component for enzyme system. DHA could be due to the activity going on in the rhizo spheric region during the cropping system such as release of root exudates, mineralization, decomposition etc.

Dehydrogenase activity isused as an indicator of biological redox systems and as measure of microbial activity insoil. The microbiological activity of a soil directly influences the ecosystem stability and fertility and it is widely accepted that a good level of microbiological activity is essential for maintaining soil quality (Dick et al., 1993). Soil enzyme activities are more sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes (Dick, 1997).

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