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Empirical Study on Urease Activity and Nitrogen Fixing Capacity in Organic and Conventional Status- A Case of Nadia District, India

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Abstract The current research was conducted to analyze the urease enzyme activity and nitrogen fixing capacity under organic and conventional production systems during whole scale of incubation of 84 days to consider the mineralization potential of soil. Withanoutlook to have knowledge on soil parameters like pH, EC, organic carbon, moisture content soils was sampled from established organic farms of New Alluvial Zone at different locations of Nadia district. Soils of conventional plots of these regions were also collected to understand the level of different soil parameters and also edge of organic husbandry. The results showed that soil urease activity nitrogen fixing capacity and population dynamics of nitrogen fixing bacteria (*Azotobactor* sp.) is lower in conventional system. But however, application of organic amendment like Vermicompost, edible oil cakes, organic manure can enhance these activities. Higher Organic carbon (OC), Nitrogen (N), Phosphorus (P) content was also observed from experimental organic plots as compared with conventional system. Significantly high capacity of nitrogen fixing and urease enzyme activity was reported in strawberry plot at Sahadev Jaib Krishi Farm treated with Vermicompost and edible oil cake among the organic farms considered. Collectively the results indicate that the application of organic amendments may improve the urease enzyme activity and nitrogen fixing capacity in soil rhizosphere. Present effort is a contribution to have a long term productivity of soil and sustainability of agro ecosystems.

Keywords Urease enzyme activity, Nitrogen fixing capacity, *Azotobactor* sp.

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Introduction

Nitrogen fixation is the most important biological process on earth encompassing reduction of atmospheric nitrogen to two molecules of ammonia. Nitrogen fixation is carried out various genus of bacteria, cyno-

Table 1. Physical and chemical properties of the soils used in the experiment.

Sites	Treatments	pH	EC (μSm^{-1})	OC (%)	Av. N (kg/ha)	Av. P (kg/ha)	Av. K (kg/ha)	Textural class	Moisture content (%)
Sahadev Jaib Krishi farm (Strawberry)	Vermicompost+ edible oilcake	7.92	152.9	0.916	219.5	25.85	274.19	Loam	25
Sahadeb Jaib Krishi farm (Rice)	Vermicompost+ organic manure	8.19	197.4	0.861	165.8	21.73	133.39	Silt loam	21.5
Sahadev Jaib Krishi farm (Rice)	Conventional	8.11	105.5	0.653	134.8	13.94	92.4	Silt loam	21.8
Central research farm (Gayeshpur)	Vermicompost	8.08	177.4	0.902	213.6	24.57	102.48	Loam	27.38
Central research farm (Gayeshpur)	Conventional	8.32	124.9	0.51	112.8	8.96	145.34	Silt loam	27.07

bacteria and actinomycetas [1]. Nitrogen-fixing microbes can exist as independent, free-living organisms or it associates with other microbes and plants with different degrees of complexation [2]. Among the non symbiotic heterotrophic bacteria, *Azotobactor* sp. has found very promising result producing growth promoting substances and are antagonistic to some pathogens. It increases crop growth and yield through biosynthesis of biologically active substances and producing phytopathogenic inhibitors [3]. Modification of nutrient uptake ultimately enhances biological nitrogen fixation [4]. Depending upon physicochemical and microbial properties *Azotobactor* sp. are found in rhizosphere soil lying in the range from negligible to 10^5/g of soil. In soils, *Azotobactor* population are affected by organic matter content, pH, soil moisture, temperature and other microbial properties. *Azotobactor* sp. are non-symbiotic organisms capable of fixing an average 20 kg N/ha/year [5]. Since soil biological properties are indicators for soil quality, soil health and fertility, examining the relationship between these parameters and *Azotobactor* spp. population can be considered to play a vital role in agriculture and its management practices.

Soil enzymatic activity is a potential indicator of soil fertility and soil health. It depends upon soil moisture, aeration, organic carbon, nitrogen content. Among various soil enzymes, urease activity largely depends on soil types [6], cultivated crop species [7]. Urea is the cheapest and most commonly used nitrogenous fertilizer in India and in urea, nitrogen is present in amide form. When urea is added to soil, it undergoes hydrolysis, where amide form of nitrogen is converted to ammonium carbonate by an enzyme urease. Urea hydrolysis is buffered by warm and moist soil conditions, by which most of the urea is transformed to NH_4^+ in several days. The productivity in cultivated crops can significantly declined by reduction in urease activity. It has been reported organic and natural fertilization is positively correlated with the activity of enzymes [8]. It is therefore a prerequisite to understand the dynamics of urease enzyme activity and its response to different soil status. Current study made use of steam distillation method which is a simple and rapidly used technique for assaying urease activity, which was also deployed by Tabatabai and Bremner earlier [9]. In steam distillation ammonia released by urease activity is quantified when soil is incubated with THAM buffer (pH 9.0), urea solution and toluene at 37°C for 2 h.

The present study made an attempt to determine the Urease enzyme activity, nitrogen fixing capacity and population dynamics of nitrogen fixing bacteria (*Azotobactors* spp.) in organic and inorganic soil. Efforts were also made to explore the relationship between N fixing capacity and urease activity.

Table 2. Population of nitrogen fixing bacteria (CFU×10^5/g) of different organic and conventionally managed plots against days of incubation. Mean values followed by same letter are not significantly different (p<0.05) by Duncan's multiple ranges test.

Sites	Type of farm	Treatments	Incubation period				
			0 days	21 days	42 days	63 days	84 days
Phulia	Organic	Vermicompost+edible oilcake	150.66^b	120^a	107.33^a	98.45^a	82.05^a
Phulia	Organic	Vermicompost+organic manure	131.5^c	110.82^b	96.45^b	74^b	68.75^b
Phulia	Conventional	Conventional	120.45^d	107.33^b	90.36^c	68.9^b	45.12^d
Gayeshpur	Organic	Vermicompost	167.35^a	120.45^a	110.19^a	55.32^c	50.36^c
Gayeshpur	Conventional	Conventional	133.86^c	106.84^b	90.44^c	52.05^c	35.38^c

Materials and Methods

The entire investigation was planned and executed in two sites in Nadia district of West Bengal. Soil samples were collected from Different organic plots of strawberry and rice in Sahadev Jaib Krishi Farm at Phulla, which is situated at 23.23°N latitude and 88.49°E longitude at an elevation of 10.25 m MSL. Different organic and inorganic plots of Rice in Central Research Farm at Gayeshpur, which is situated at 23°N latitude and 89°E longitude at an elevation of 9.75 m MSL. Soils of conventional plot of above mentioned regions were also been collected for evaluation of various soil parameters.

The evaluation of urease activity was done based on steam distillation method [9]. In the laboratory, 5 g of fresh moist collected soil samples was taken along with 0.2 ml toluene and 9 ml of THAM buffer (pH 9.0, 0.05 M) in 50 ml volumetric flask. The flask was then swirled for few seconds to mix the contents. Subsequently, 1 ml of 0.2 M urea solution was added and swirled for few seconds. The flask were stoppered and placed in an incubator at 37°C for 2 h. The stopper was removed after 2 h and approximately 35 ml of KCL-Ag_2SO_4 solution was made by dissolving 100 mg of reagent grade Ag_2SO_4 and 188 g of reagent grade KCL in 700 ml water and diluted to 1 liter. The estimation of ammonium nitrate (NH_4^+-N) was performed by pouring the 5 ml suspension of the soil samples in each replicate into the 250 ml distillation flask followed by addition of few pinch of MgO to it. The content of the flask was then distilled for 7 minutes in Kjeldal distillation unit in a 250 ml of conical flask containing 10 ml of boric acid mixture indicator solution (Bromocresol green).The distillate was titrated against 0.005 M H_2SO_4.

To study the population dynamics of *Azotobactor* sp. and its nitrogen fixing power, soil of 10 g in weight were shaken with 90 ml of sterile distilled water at 30°C for 15 min. After this, solutions were serially diluted in a proportion of 1:10 upto 10^5 in sterile distilled water. 1 ml of each dilution was planted on Jensen's nitrogen free medium (Sucrose 10g, $MgSO_4$ 0.5 g, Ferrous sulfate 0.1 g, Dipotassium hydrogen phosfate 1 g, Sodium chloride 0.5 g, Sodium Molybdate 0.005 g, Calcium Carbonate 2 g, Agar-agar 15 g with 1 lit of distilled water) at pH 7.0 to count population of *Azotobactor* sp. Non-symbiotic nitrogen-fixing capacity of a soil sample was determined by estimating total nitrogen in 50 ml. N-free sucrose-calcium carbonate broth containing 2% sucrose after incubating 1 g of soil in conical flask at 30±1°C for 15 days [9]; after 15 days of incubation, content of all the flasks were analyzed for total nitrogen following Kjeldhal's method [9]. The difference in the amount of total nitrogen in the sterilized and non-sterilized flasks was the nitrogen fixation capacity of the soil.

The observed data was subjected for standard CRD analysis (using SPSS software, Version 20) and testing for the significance of treatments is made. The multiple comparisonsis made using DMRT (Duncan

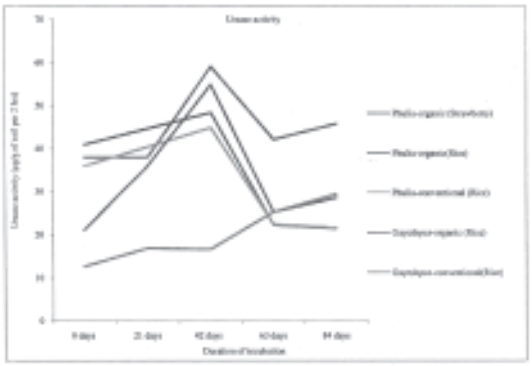

Fig. 1. Urease enzyme activity of different organic and conventionally managed soils over different days of incubation [in µg/g of soil per 2 h].

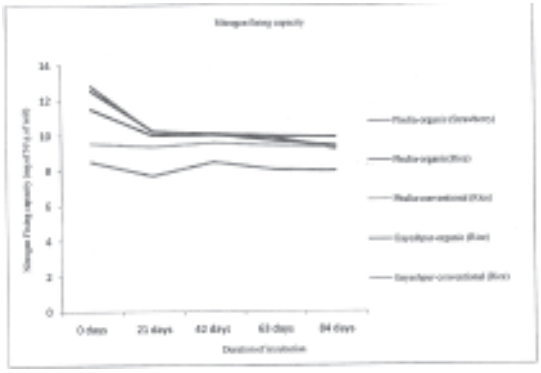

Fig. 2. Nitrogen fixing capacity (mg of N/g of soil) of different organic and conventionally managed plots against days of incubation.

Multiple Range Comparison) technique.

Results and Discussion

Physical and chemical properties of the soils taken at different regions are given in Table 1. The results showed that soil samples have alkaline in pH, medium to high organic carbon content, low available nitrogen content, low to medium available phosphorus and potassium content. Research results also showed that particle size distribution of soil has been ranged from loam to silty loam and adequate amount of moisture percentage.

Urease enzyme activity (µg of NH_4^+/g/2 h of different organic and conventionally managed plots over the days of incubation is represented in Fig. 1. We can observe from Figure 1 that in most of the farming sites urease activity was significantly higher under organic production systems over conventional counter parts in all the locations irrespective of management practices adopted and cropping sequences followed. The result indicates that having organic farming systems can improve urea utilizing microorganism population and hence their activities also. In many experiments there is close association is found between organic matter content and urease activity [10, 11]. The enzymatic activity was also found higher under organic production systems may be because of higher amount of organic matter present in the top soil layer. Soil microbial and enzyme activity are considered as indicators of soil quality as they are responsible for the degradation of organic substrates and release of plant nutrients [12].

In organic system of soils increased carbon and total nitrogen content could serve as a basis for increasing both biological and enzymatic activities rather than conventionally maintained plots. This increased activity indicates relatively higher degree of organic nitrogen mineralization process in organically produced farms. This is because urease activity is considered as a potential biomarker for organic nitrogen mineralization. Among the organic farms, Strawberry plot under Sahadev Jaib Krishi Farm treated with Vermicompost and oil cake recorded the highest urease enzyme activity (Fig. 1). This is due to vermicompost treated soils provides long term positive effect on soil processes [13]. Increase in urease activity is observed upto 42 days after incubation and then slight decrease is noted (Fig. 1). It is reported that the carbon oxidation is directly related to N-Fixation and if carbon oxidation is decreased, the nitrogen fixation is also decreased and no energy available for survival of microbes. The same explanation can be extended in concomitant decrease in urease activity in soil.

To assess the nitrogen transforming microbial

Table 3. Correlation between nitrogen fixing capacity by *Azotobactor* spp. and ureae enzyme activit.

Sites	Crop	Treatments	Mean urease activity [in µg/g of soil per 2 h]	Mean N fixing capacity of *Azotobactor* spp. [mg of N/g of soil]	Correlation coefficient
Phulia	Strawberry	Vermicompost+ edible oilcake	44.586	10.586	-0.428252177
Phulia	Rice	Vermicompost + organic manure	35.542	10.126	0.4933261
Phulia	Rice	Conventional	33.052	9.44	0.578835633
Gayeshpur	Rice	Vermicompost	33.148	10.384	-0.359287626
Gayeshpur	Rice	Conventional	20.178	8.134	-0.449019763

composition, nitrifying bacteria were enumerated. Under organic production systems Sahadev Jaibya Krishi Farm at Phulia recorded the higher level of organic carbon which in turn, augmented the highest population of nitrogen fixing bacteria (Table 2). Application of Vermicompost and edible oil cake in strawberry plot serves energy and carbon sources for microorganisms. Organic farms are relatively undisturbed as compared to conventional farm with regard to fertilizer and other input application resulting in differential stresses. Increase organic amendment in organic farms also increase microbial activity in soil between 16–20% as compared to inorganic fertilizer [14] and there by increases the population of nitrifying bacteria. Habitat alteration was noticed from application of chemical fertilizer, herbicides, insecticides and other intercultural operations which are indiscriminately used in conventional farms. The subsequent deterioration of population dynamics of nitrogen fixing bacteria during the period of incubation is due to declining available carbon sources for growth and development.

We can notice from Figure 2 that a substantial amount of nitrogen was fixed by the heterophic nonsymbiotic N fixing bacteria being stimulated by the organic practices irrespective to space and cropping sequences followed. It has been documented that, addition of organic manure under organic production system can optimize the microbial driven internal cycling of nutrients [15]. On the other hand, conventional farming depressed the nitrogen fixing capacity of soil in almost all cases. Among the organic farms, the magnitude of nitrogen fixation was higher in organic strawberry field at Phulia followed by organic rice field at Central Research Farm at Gayeshpur. Higher N fixation under organic system is in accordance with the higher organic carbon content of those soils. Organic carbon assures the N fixers with energy and sufficient C for growth and performance of diazotrophs. Conventional farms though harbored relatively higher number of nitrogen fixing bacteria, failed to secure higher level of nitrogen fixation. Moreover soluble nitrogen fertilizers used in conventional farming repressed the synthesis of nitrogenase enzyme that helps fixing atmospheric N. In general, maximum N found fixed in initial time and latter perioda progressive decline in nitrogen fixation was noticed in all cases during the course of incubation. This is due to lack of readily available carbon source for the growth of bacteria during incubation and the other group of microbes assimilate most of the readily available organic substrates [16].

In this study, the research results showed that there were significant negative correlation exists between urease enzyme activity and nitrogen fixing capacity in most cases (Table 3). This indicates increased nitrogen fixation can decrease the urease activity of soil either by feedback of inhibition or by repress the synthesis of urease enzyme.

Numerous studies revealed that urease activity and nitrogen fixing capacity is higher in organic sys-

tem rather than conventional mode of farming. The strawberry plot under Sahadev Jaib Krishi Farm treated with Vermicompost and edible oil cake recorded highest amount of nitrogen fixing capacity (*Azotobactor* spp.) and urease activity which may be due to higher amount of organic matter present on the top soil. Higher N fixation under organic system is due to presence of higher organic carbon content of those soils. Organic carbon assures the N fixers with energy and sufficient carbon for growth and performance of diazotrophs. In most cases N fixing capacity and urease enzyme activity decreased in the latter period of incubation which may be because of declined carbon source for the growth of microbes. The research results revealed that there exists a negative correlation between urease enzyme activity and nitrogen fixing capacity in most cases which emphasises that N fixation negatively influence the urease activity either by feedback of inhibition or by repress the synthesis of urease enzyme. Thus it may be concluded that organic strawberry plot under Sahadev Jaib Krishi Farm treated with Vermicompost and edible oil cake is putatively selected as best one among other plot.

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