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AGROCHEMISTRY. SOIL SCIENCE

Assessment of Rhizospheric Arbuscular Mycorrhizae Spores in Relation to Soil Characters in the Rice Fields of Malda District, India

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Abstract—Arbuscular mycorrhizal fungi (AMF) show symbiotic associations with the roots of plants and facilitates nutrients uptake through the hyphae-plant root-soil interface. Many edaphic and anthropogenic activities greatly influence this mutualistic association. Among which soil parameters are of great importance. The major objective of this study is to assess the soil colonization of AM fungi in different textural and nutritional conditions in agricultural rice fields of different locations of Malda district, West Bengal, India. Fifty rhizospheric soil samples of rice fields were collected from Jadupur (25.080° N, 88.1255° S) and Mohadipur (24.8525° N, 88.1255° E) areas of Malda district, India and the mycorrhizal spores were isolated and quantified according to adhesion and floatation techniques and identified morphometrically through the INVAM database. Soil texture was determined by conventional pipette method and soil pH, conductivity, nitrogen, potassium, phosphorus, and organic carbon contents were quantified according to standard methods. It was observed that the soil samples had a wide diversity of genera of mycorrhizal spores such as *Aculospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. The texture of the sample sites are mostly sandy loamy to loamy and have variations in soil chemical properties. The clay and phosphorous contents are inversely proportional to mycorrhizal spore content whereas it was directly proportional to the organic carbon and nitrogen content in the soil. The AM fungal diversity was more in Jadupur soil while it was less in Mohadipur. The study concludes that low nutrient content soil had more spore numbers than high nutrient content, reflecting their role in mineral acquisition for better plant nutrient supply.

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF), a type of mycorrhiza which shows symbiotic association with the roots of angiosperms, belong to subphylum Glomeromycota under Mucoromycota [1]. It is an obligate biotroph which expands hyphae in the rhizosphere to facilitate uptake of nutrients (Phosphorus, Sulphur, and Nitrogen) by increasing the hyphae-plant root-soil interface. In this perspective, the rhizospheric arbuscular mycorrhizal fungal spores and glomalin related soil proteins (GRSPs) are considered as the biological indicators of soil quality. Biochemically glomalin is a glycoprotein molecule, which helps in colonization, nutrients capture and increases the tolerance to different environmental conditions including the agroecosystems. The present agroecosystem is being overpowered by excess application of inorganic fertilizers comprise of nitrogen, potassium, and phosphorus [2]. This over-application of nitrogen fertilizers affects soil fertility and soil microbiome that includes bacteria, fungi, and especially arbuscular mycorrhuzae. Apart from fertilizers application, many management practices like tillage, crop rotation, soil quality influence mycorrhizal infestation, phosphorus mobilization. Establishment of mycorrhizal colonization in the soil, as well as plant roots, increases the efficiency of nutrient mobilization and control diseases [3].

Rice (*Oryza sativa* L.) is a staple food crop across the globe. In the tropical and subtropical region of South Asia and southeastern Africa where the population blast is a common emerging issue, majority of inhabitants consume rice as a prime source of food for more than 700 yr [4]. In this concern, yield enhancement of this crop requires to mitigate the food crisis. In the world, rice production has increased by only 1.1% whereas in India, the second-highest rice producer after China, had increased the production of 4.33% [5]. Only in India, the predicted enhancement of yield in the last 5 yr became 1.46% only [5]. These predictions indicate that some innovative strategies should be taken to increase the crop yield and thus to mitigate the crisis of food soon. Conceptually rice production can be increased by near sustainable way through the application of biofertilizers such as plant growth-promoting AMF, rhizobacteria, etc. AMF effectively mobilize nutrients (mainly Phosphorus and Nitrogen) to the plant system for their enhanced growth and metabolism. Apart from that AMF also increase the root area for higher absorption of nutrients. Similarly, AMF effectively decreases the heavy metal (Pb, As, Hg) stresses to the plant [6]. In this perspective, the objective of this study is to document the soil colonization of AMF fungi in agricultural rice fields of different locations of Malda district, W.B., India and assess the preferences of colonization in the different textural and nutritional parameter of the soil.

MATERIALS AND METHODS

Sample Site and Collection of Soil Samples

The soil samples were collected from the fifty different rhizospheres of a rice field of Jadupur (25.080 0° N, 88.1255° S) and Mohadipur (24.8525° N, 88.1255° E) areas of Malda district, India at November and December 2018. This agricultural land has a rotation of three cropping seasons, like Rainy, Winter, and Summer crops, like rice; wheat, mustard and vegetables, and rice, respectively. The samples were assigned as by the average of five replicates each of rice fields. The fields were near to the wetlands and large ponds and the selected fields were frequently infected with several weed plants during and after the cropping seasons. The study area receives an average annual rainfall of 1349 mm with temperature ranges of 28 to 33°C.

Isolation and Morphometric Identification of Spores from Soil

Isolation and quantification of spores from soil samples were performed according to adhesion and floatation technique with some minor modification. Briefly, the dried soil samples were passed through the 500 micron and 250-micron sieves. After that 10 g powdered soils were again passed through 45-micron sieves under running water. Then the remnant fraction was collected and suspended in a saturated sucrose solution (1 ml). The suspension then centrifuged at 112 × g for 10 min to sediments the soil particles. The floating fraction was collected and was resuspended in 100% methanol (v/v). Then the methanol suspension again centrifuged at 11200 × g for 10 min and the floated spores were observed in dissecting microscope (Olympus, Magnus MLX, Japan). The mycorrhizal spores isolated by wet sieving and decanting method from the different soil sampling sites of two different areas and the spore count were computed manually. The spores were morphometrically identified by comparing the observed salient features of the INVAM database (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html).

Determination of Soil Texture Properties

Soil texture is an important parameter for the determination of soil quality. It is the determinant factor of aeration, water content, organic matter in the respective soil. Soil texture was determined by conventional pipette method [7]. Briefly, 20 g of soil was taken and dissolved in 100 ml of water in a glass beaker. Add 5 ml of 30% hydrogen peroxide solution (w/v) was added and heated the content until effervescence stops. Then 10 ml of 5% solution (w/v) of sodium hexametaphosphate (w/v) solution was added. The whole content was transferred to a mixer. Next, the cup to the stirrer was attached. The top of the cup should be raised straight upwards and hooked securely under the switch finger, with base hooked over the lower finger support. The soil sample was transferred to the cylinder using the wash bottle. The volume was made up to 1000ml and mixed the content with a plunger. After 12 h, carefully pipette out 25 ml of solution from a depth of 10 cm below the suspension surface. Then, the solution was dried with the help of an oven and the dry weight was taken. After that, the relative amounts of sand, silt, clay in the <2 mm fraction of the component layer was estimated using the principal outlined in the protocol.

Determination of Chemical Properties of Soil

The chemical parameters such as soil pH, conductivity, nitrogen content, potassium, phosphorus, organic carbon content were quantified according to the standard protocol [7, 8]. Soil pH and electrical conductivity were determined according to the method described methods [8]. Soil nitrogen, potassium, phosphorus, and organic carbon contents were done according to standard protocols [7].

Extraction and Estimation of Glomalin Content in the Soil Sample

Glomalin is a glycoprotein that is the indicator of the presence of mycorrhiza in soils. The glomalin extract and estimation was done to confirm the presence of mycorrhiza infestation in soil samples. The extraction of glomalin was done according to the standard protocol [9]. The extracted glomalin was estimated through the Folin-Ciocalteu method with bovine serum albumin (BSA) as standard. The glomalin contains in the samples was graded as good, moderate and poor with ranges of above 0.1, 0.05–0.1 and 0–0.05 respectively.

Statistical Analysis

All the estimation was determined in triplicate trials. The principal component analysis and standard deviation were done through PAST3 and SPSS software with a 95% confidence level.

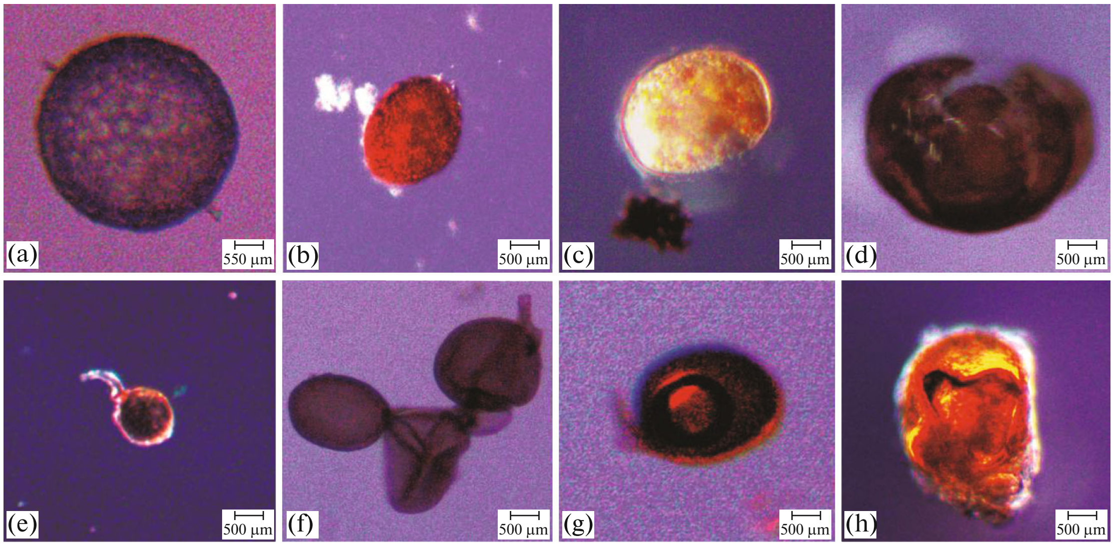

Fig. 1. Photomicrograph of different spore types present in the rice fields under the light microscope (10×). (A)–(C) *Aculospora* sp.; (D) *Entrophospora* sp.; (E) *Gigaspora* sp.; (F, G) *Glomus* sp., and (H) *Scutellospora* sp. Here, the bar in the figure indicates the scale value.

RESULTS

Sample Site and Collection of Soil Samples

The soil samples were collected from the rhizospheric region of rice plant of two different sites viz. Jadupur and Mohadipur of Malda, India. The earlier site was an air polluted area beside the national highway (NH 34) and the later one was a less polluted site located away from the highway and surrounded by large water lands and small ponds.

Isolation and Morphometric Identification of Spores from Soil

The mycorrhizal spores were isolated by wet sieving and decanting method. From the different 50 sampling sites of two different areas and the spore count were computed manually. The community of mycorrhizal spores was similar in both the study area. In this study, it was inferred that the soil samples carried a wide diversity of genera of mycorrhizal spores such as *Aculospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. (Fig. 1) with diverse colors and size variations.

Determination of Soil Texture Properties

The soil textures i.e. sand, silt, and clay are the most influencing factor for mycorrhizal growth and the texture of the sample sites are mostly sandy loamy to loamy. The soil samples from the Jadupur area contain 48% sand-rich soils whereas, in Mohadipur, there were 64% samples, which were sandy (<50% sand). There was no dominated soil in Mohadipur area whereas only 4% of samples of Jadupur were clay-rich (Table 1). The silt dominated soils of both the area were either nil or negligible (4% in Jadupur area).

Determination of Chemical Properties of Soil

Soil nutrients are the essential parameters for all the living organisms associated with soils. The soil samples from Jadupur area (52.28/g soil) contain a higher amount of spore count than Mohadipur area (44.56/g soil). The average pH of both soil sample was slightly acidic (Table 1). The average electrical conductivity (EC) values were higher at Mohadipur (0.2776 ds/m) than Jadupur (0.2496 ds/m). The electrical conductivity of the soil sample is non-saline and the organic carbon amount was high in both the study sites. The organic carbon content of both the area (Jadupur area = 0.8844%, Mohadipur area = 0.8692%) were higher compared to the standard value (>0.75% = high). The soil samples of Jadupur area contain a higher amount of phosphorus pentoxide (P_2O_5) (Average 29.88 kg/ha) than Mohadipur area (Average 23.8 kg/ha). On other hands, the K_2O content of Mohadipur area was higher (347.28 kg/ha) but the Jadupur area had medium range (315.68 kg/ha) compared to the standard (value 36–337.5 kg/ha = medium and >337.5 kg/ha = high). The value of Nitrogen was marginally low in Jadupur area (381.9 kg/ha) and medium in Mohadipur area (384.39 kg/ha) compared to the standard limit (272–544 kg/ha = medium) [7, 8].

Glomalin Content of the Soil Samples

Glomalin is an indicative factor of mycorrhizal investigation on the soil. Glomalin is a molecular marker glycoprotein which helps to intake the nutri-

Table 1. The textural and nutritional properties and mycorrhizal spore count content of 50 soil samples

Study sites	Sampling no.	pH, mol/L	E.C., ds/m	% of *C*	K_2O, kg/ha	Glomalin conc.	Total spore count, 10 gm soil
Jadupur	1	6.3 ± 0.31	0.13 ± 0.01	0.92 ± 0.04	241 ± 12.05	Moderate	59 ± 2.95
	2	6.2 ± 0.31	0.14 ± 0.01	0.67 ± 0.03	268 ± 13.4	Moderate	4.14 ± 2.05
	3	6.4 ± 0.32	0.13 ± 0.01	0.64 ± 0.03	295 ± 14.75	Moderate	56 ± 2.80
	4	6.4 ± 0.32	0.21 ± 0.01	0.82 ± 0.04	335 ± 16.75	Good	66 ± 3.30
	5	6.6 ± 0.33	0.22 ± 0.01	0.75 ± 0.03	402 ± 20.1	Good	77 ± 3.85
	6	6.4 ± 0.32	0.23 ± 0.01	0.75 ± 0.03	281 ± 14.05	Good	65 ± 3.25
	7	6.4 ± 0.32	0.23 ± 0.01	0.64 ± 0.03	255 ± 12.75	Moderate	36 ± 1.80
	8	6.4 ± 0.32	0.25 ± 0.01	1.1 ± 0.05	308 ± 15.4	Moderate	54 ± 2.70
	9	6.4 ± 0.32	0.25 ± 0.01	0.75 ± 0.03	348 ± 17.4	Good	70 ± 3.5
	10	6.4 ± 0.32	0.3 ± 0.01	1.1 ± 0.05	335 ± 16.75	Moderate	40 ± 2
	11	6.6 ± 0.33	0.17 ± 0.09	1.1 ± 0.05	268 ± 13.4	Poor	25 ± 1.25
	12	6.2 ± 0.31	0.18 ± 0.01	1.1 ± 0.05	402 ± 20.1	Moderate	50 ± 2.50
	13	6.2 ± 0.31	0.21 ± 0.01	1.1 ± 0.05	348 ± 17.4	Moderate	40 ± 20
	14	6.7 ± 0.33	0.33 ± 0.02	1.1 ± 0.05	241 ± 12.05	Poor	17 ± 0.85
	15	6.5 ± 0.32	0.41 ± 0.02	0.82 ± 0.04	268 ± 13.4	Poor	18 ± 0.90
	16	6.6 ± 0.33	0.3 ± 0.01	0.92 ± 0.04	268 ± 13.4	Moderate	52 ± 2.60
	17	6.2 ± 0.31	0.26 ± 0.01	0.92 ± 0.04	308 ± 15.4	Good	97 ± 4.85
	18	6.6 ± 0.33	0.32 ± 0.02	0.94 ± 0.04	255 ± 12.75	Good	71 ± 3.55
	19	6.8 ± 0.34	0.32 ± 0.02	0.64 ± 0.03	295 ± 14.75	Moderate	55 ± 2.75
	20	6.6 ± 0.33	0.47 ± 0.02	0.97 ± 0.04	322 ± 16.1	Moderate	37 ± 1.85
	21	6.6 ± 0.33	0.27 ± 0.01	1.1 ± 0.05	335 ± 16.75	Moderate	40 ± 2.00
	22	6.5 ± 0.32	0.22 ± 0.01	0.94 ± 0.04	375 ± 18.75	Good	98 ± 4.90
	23	6.2 ± 0.31	0.26 ± 0.01	0.75 ± 0.03	402 ± 20.1	Poor	29 ± 1.45
	24	6.5 ± 0.32	0.21 ± 0.01	0.75 ± 0.03	389 ± 19.45	Moderate	48 ± 2.40
	25	7.1 ± 0.35	0.22 ± 0.01	0.82 ± 0.04	348 ± 17.4	Good	66 ± 3.30
Mohadipur	26	6.5 ± 0.32	0.21 ± 0.01	1.1 ± 0.05	348 ± 17.4	Moderate	39 ± 1.95
	27	6.6 ± 0.33	0.19 ± 0.01	0.82 ± 0.04	295 ± 14.75	Poor	19 ± 0.95
	28	6.3 ± 0.31	0.26 ± 0.01	0.82 ± 0.04	255 ± 12.75	Poor	27 ± 1.35
	29	6.4 ± 0.32	0.51 ± 0.02	1.1 ± 0.05	308 ± 15.4	Poor	26 ± 1.30
	30	6.3 ± 0.31	0.21 ± 0.01	1.1 ± 0.05	469 ± 23.45	Poor	31 ± 1.55
	31	6.4 ± 0.32	0.3 ± 0.01	0.92 ± 0.04	375 ± 18.75	Good	76 ± 3.80
	32	6.7 ± 0.33	0.16 ± 0.01	0.92 ± 0.04	268 ± 13.4	Good	82 ± 4.10
	33	6.5 ± 0.32	0.33 ± 0.01	0.92 ± 0.04	429 ± 21.45	Good	82 ± 4.10
	34	6.3 ± 0.31	0.31 ± 0.01	0.75 ± 0.03	402 ± 20.1	Poor	27 ± 1.35
	35	6.6 ± 0.33	0.31 ± 0.01	0.94 ± 0.04	335 ± 16.75	Moderate	42 ± 2.10
	36	6.5 ± 0.32	0.37 ± 0.02	0.94 ± 0.04	469 ± 23.45	Moderate	37 ± 1.85
	37	6.7 ± 0.33	0.37 ± 0.02	0.94 ± 0.04	469 ± 23.45	Moderate	53 ± 2.65
	38	6.5 ± 0.32	0.22 ± 0.01	0.75 ± 0.03	375 ± 18.75	Poor	18 ± 0.90
	39	6.8 ± 0.34	0.23 ± 0.01	0.75 ± 0.03	415 ± 20.75	Poor	27 ± 1.35
	40	6.5 ± 0.32	0.22 ± 0.01	0.75 ± 0.037	335 ± 16.75	Good	78 ± 3.90
	41	6.5 ± 0.32	0.37 ± 0.02	0.82 ± 0.041	375 ± 18.75	Poor	11 ± 0.55
	42	6.5 ± 0.32	0.15 ± 0.01	0.9 ± 0.045	335 ± 16.75	Poor	19 ± 0.95
	43	6.5 ± 0.32	0.22 ± 0.01	0.75 ± 0.037	308 ± 15.4	Poor	31 ± 1.55
	44	6.4 ± 0.32	0.17 ± 0.01	0.75 ± 0.037	255 ± 12.75	Poor	24 ± 1.20
	45	6.5 ± 0.32	0.28 ± 0.01	0.94 ± 0.047	268 ± 13.4	Good	87 ± 4.35
	46	6.5 ± 0.32	0.23 ± 0.01	0.67 ± 0.033	281 ± 14.05	Poor	10 ± 0.50
	47	6.4 ± 0.32	0.25 ± 0.01	0.82 ± 0.041	348 ± 17.4	Good	68 ± 3.40
	48	6.5 ± 0.32	0.32 ± 0.02	0.92 ± 0.046	322 ± 16.1	Good	85 ± 4.25
	49	6.4 ± 0.32	0.33 ± 0.02	0.82 ± 0.041	335 ± 16.75	Moderate	49 ± 2.45
	50	7.1 ± 0.35	0.42 ± 0.02	0.82 ± 0.041	308 ± 15.4	Good	66 ± 3.30

Here, the values are the average of triplicate trials ± SD.

ents by the plants through mycorrhizal hyphae. In this study, glomalin content was significantly high in all the soil samples of the two different sites (Table 1).

Statistical Analysis

The PCA analysis is an essential tool to statistically justify the relationship between two parametric components in a numerical study. In this study, the principal component analysis (PCA) was done between sand and clay component with spore counts of all the soil samples (Fig. 2) along with spore count with nitrogen and phosphate contents (Fig. 3) with two different study sites. It was shown that among all the 50 soil samples, only 1% soil sample did not belong to 95% confidence levels (Fig. 2), where the rest of the samples were distributed according to the resultants of the two components. Whereas all the soil samples were within the confidence limit when spore counts and soil nutrient properties were correlated in PC analysis (Fig. 3). The observations of all three PCAs showed that the greater clay content had fewer spore counts while high sand percentage and high phosphorus pentoxide had greater the mycorrhizal spore counts (Fig. 3).

DISCUSSION

Arbuscular mycorrhizal fungi (AMF) are important soil microbes that influence the soil ecosystem functioning through nutrient cycling, crop productivity, plant diversity and competition between the ecosystem habitats [10]. In this study, it was found the mycorrhizal diversity and richness in soil system of agriculturally important staple crop rice in two different study sites of agriculturally rich sandy loam to loamy soil types of Malda, India. The rice fields of both the study area i.e. Jadupur and Mohadipur were dominated by five mycorrhizal genera viz. *Aculospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. based on the morphometric study of the spore morphologies. The earlier study also reported that these five genera were most common in an agroecosystem in temperate climate irrespective of tillage practices [11]. It was also reported that *Aculospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. were the most abundant in the tropical shrubland throughout the year [12]. Previous reports also illustrated the high abundance of *Aculospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. in both tropical and temperate ecosystems such as forests, grasslands, and agricultural lands [13].

The soil studies also strongly suggest that the soil samples were mostly sandy loam to loamy. Among these soils, the high clay nature of soils has a strong impact on the spore density of both the area. The relationship between clay content in soil and spore count were inversely proportionate. In this connection, it was reported that soil texture highly influences the mycorrhizal spore density and concluded that the differential centrifugation can effectively separate *Glomus* sp. in clay soils with water-sucrose gradient centrifugation procedure [14]. In our study water-sucrose gradient centrifugation also gave effective results in mycorrhizal spore isolation in sandy loam to loamy soils. The soil property studies stated that the mycorrhizal infestation or spore content does not dependent on pH variations, electrical conductivity, and potassium contents in the soil. Whereas, the organic carbon, nitrogen contents and available phosphorus contents had a significant influence in mycorrhizal infestation in soils. Here, the relation of organic carbon and nitrogen with mycorrhizal spores were directly proportionate. The efficacy of mycorrhizae to improve the available phosphate mobilization is increased in *Zea mays* L. [15]. It was reported the increasing content of organic carbon and glomalin content indicate the increasing nature of mycorrhizal infestation and these parameters can be used in long term assessment [7]. In our observation, the organic carbon and glomalin content were similar to the reported literature. The statistical and experimental data also confirms that mycorrhizal spore densities are highly dependent on soil textural and nutritional properties, especially available phosphorus and organic carbon contents. The clay content is a determining factor for spore density in soil. High clay content increases the compactness of soil with less aeration and thus inversely regulate the AMF population [16]. It was reported that variation soil phosphate content can effectively modulate the mycorrhizal population in the soil. In our experimental and statistical (PCA) analysis, similar observations were found that soil phosphate is an important indicator of mycorrhizal populations. Whereas soil nitrogen content and mycorrhizal spore contents were directly proportionate in the principal component analysis which reflects the non-essentiality of nitrogen in the soil for effective mycorrhizal infestation [17].

The presence of glomalin in soil samples indicates the presence of mycorrhizal spores in the study sites. Glomalin is a stable molecule with 30–40% carbon contains especially found in *Glomus intraradices.* Although there are some controversies about the glomalin and soil organic carbon intercept [9]. The presence of glomalin in this study indicates that the soil samples collected have mycorrhizae that correlates with the soil nutrient parameters.

CONCLUSIONS

In this study, the relation between soil characters and mycorrhizae spores abundance were deciphered in rice fields of Malda district, W.B., India. The mycorrhizae spore densities were highly dependent on soil clay content and organic carbon, phosphorus contents in soil. The clay content is inversely proportional to mycorrhizal spore content whereas spore content is directly proportional to the organic carbon and phos-

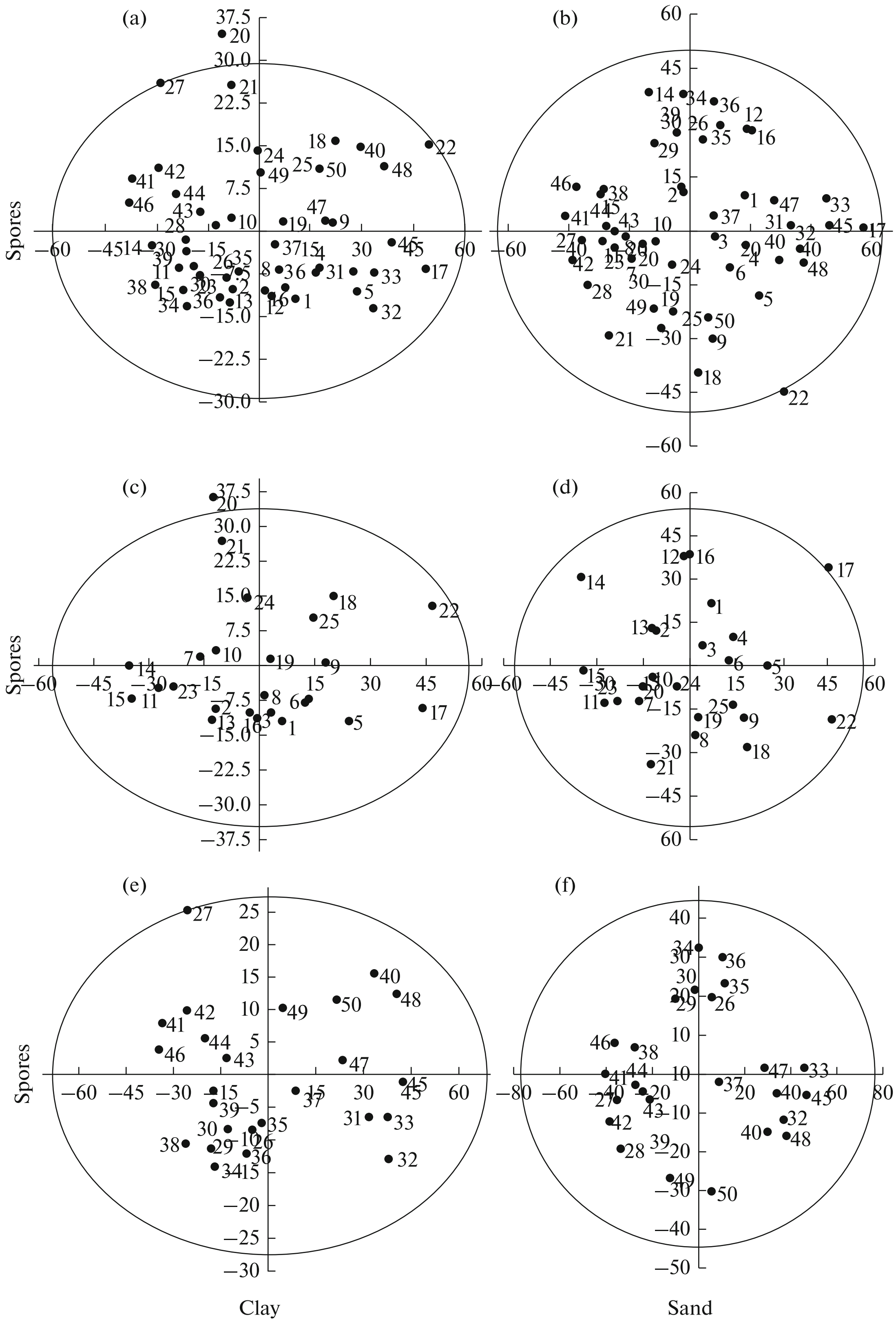

Fig. 2. Principal component analysis (PCA) between soil clay contents and mycorrhizal spore counts. (A, B) PCA of all 50 samples of two study sites; (C, D) PCA of all 25 samples of Jadupur study site; (E, F) PCA of all 25 samples of Mohadipur study site.

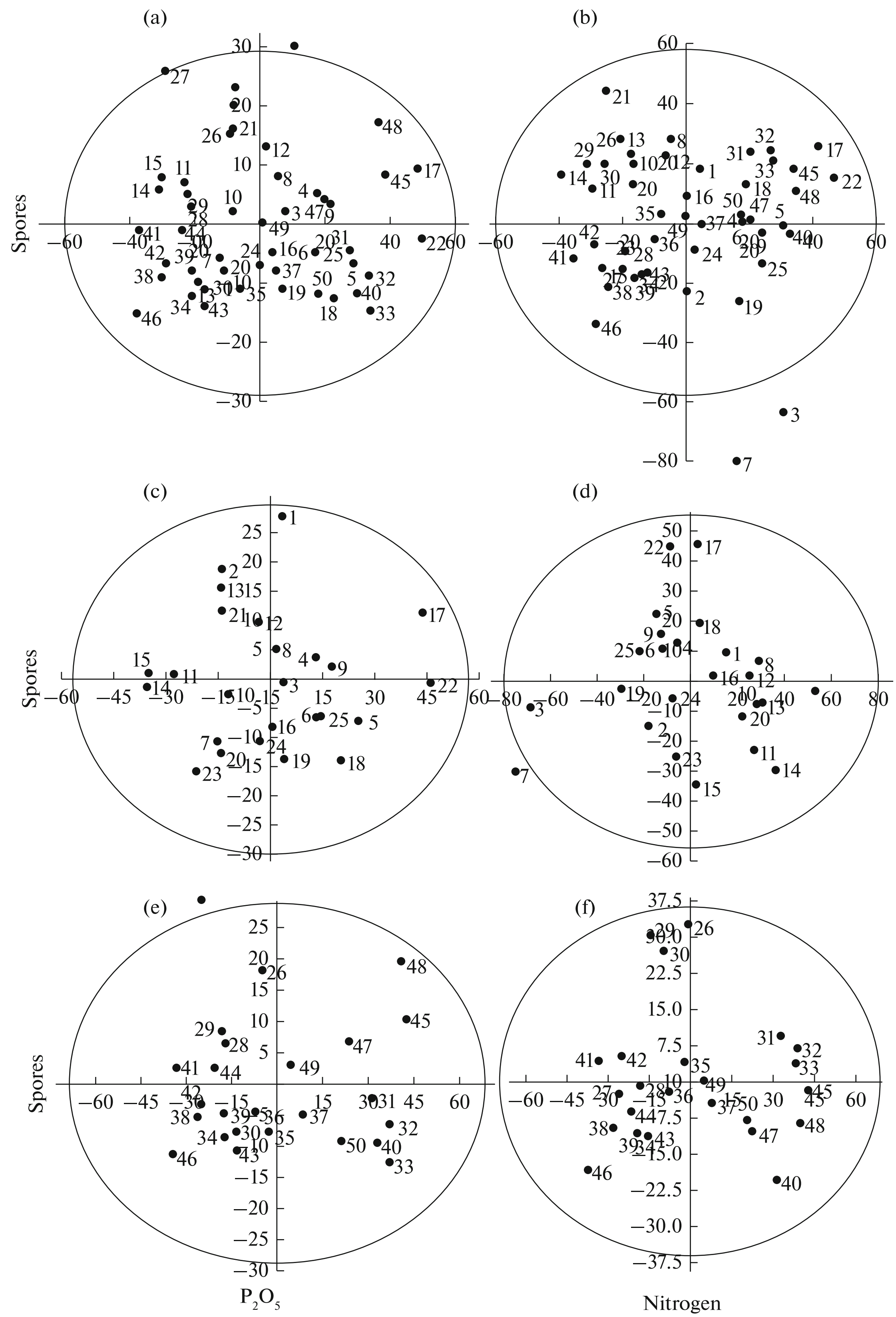

Fig. 3. Principal component analysis (PCA) between soil phosphorus and nitrogen contents with mycorrhizal spore counts. (A, B) PCA of all 50 samples of two study sites; (C, D) PCA of all 25 samples of Jadupur study site; (E, F) PCA of all 25 samples of Mohadipur study site.

phorus content in the soils, respectively. So, from these observations, it can be concluded that phosphate-based fertilizers application in sandy loam soil could improve mycorrhizae (AMF) population in agricultural crop fields and specifically contribute to higher rice production. Therefore, maintenance of proper soil texture and nutritional quality are very much important for higher crop productivity through AMF based sustainable agricultural practice to mitigate the food scarcity throughout the world.

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AUTHOR'S CONTRIBUTION

Vivekananda Mandal designed the work plan, prepared the figures and corrected the manuscript for article submission and communication.

Abhishake Karmakar, Prithwish Mandal, Rajsekhar Adhikary did the experimental works, statistical analysis and wrote the article.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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