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Organic management practices enhance Arbuscular Mycorrhiza biodiversity in tropical agricultural soil

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Introduction

Intensive agriculture practices and the continued degradation of natural resources have raised serious doubts whether the current input-intensive agricultural practices are sustainable. Yields have stagnated in several regions for 15–20 years (Dalgaard et al. 2003). Some of the problems related to the decline in soil fertility associated with intensive farming, especially the use of chemical fertilizers, can be overcome by using arbuscular mycorrhizal fungi (AMF), a practice the benefits of which are increasingly recognized. Many agricultural practices can negatively affect AM fungal population while organic agricultural practices may be most beneficial practices and increase number of AM spores in soil. This may be due to introduction of organic matter lead to increased porosity of soil which has a beneficial effect on AM colonization, mycorrhizal growth response and AM spore density (Giovanetti and Avio, 1985). Additionally, application of organic matter could also decrease the mechanical soil resistance which induced the growth of AMF hyphae (Joner and Jakobsen, 1995). AMF sporulation enhances when soil rich in organic amendments are suggested by Collins and McGraw (1988). Recent study by Gosling et al. (2010) examine eleven sites to test the hypothesis organic management increase AM fungal number and colonization potential of tilled agricultural soil. They conclude overall spore number in significantly higher, organically managed soil with no overall difference in soil physicochemical properties. However in conventional agricultural practices such as fertilization and tillage tend to decrease AMF spore abundance and alter community composition studied by Johnson et al. (1993) found that application of fertilization increased the abundance of *G. intraradices*, whereas other species like *Gi. gigantea*, *Gi. margarita*, *Scutellospora calospora* or *P. occultum* disappeared. With this background the bio diversity of AM fungal isolates found over a broad range of conventional and organically managed soil in tropical regions of India, to our knowledge, has not been investigated so far using morphological datasets. Moreover, only a few studies have described the diversity of AMF in the subtropical zone of northern India on the basis of morphology of spores found in soil samples collected directly from the field. Therefore, the present experiment sought to investigate the impact of chemical fertilizers in tropical soils of northern India on AMF grown under greenhouse trap culture. Our hypothesis, namely that AMF in soils under organic farming soils are more abundant and diverse than those in chemically managed soil was tested directly by quantifying the number of spores, species richness and Shannon-Wiener diversity index.

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Material and Methods

Two sites in northern India were selected for this study, Ghaziabad (28° 40' N, 77° 28' E) and Palwal (28° 9' 0" N / 77° 20' 0" E). Both sites are in the humid subtropics with annual precipitation of 700–1300 mm and three - five plots or fields were selected at each site. The site Ghaziabad, which is part of the western plains of Uttar Pradesh, comprised three fields (chemically managed land or CML, Zero tillage Land, or ZTL; and permanent raised beds, or RBP) supporting a rice–wheat production system under conventional management (120–150 kg/ha N, 40–60 kg/ha P_2O_5, 40–60 kg/ha K_2O, and 25 kg/ha Zn). The ZTL plot had been subjected to zero tillage for the last two rotations; and in the RBP plot wheat and rice had been grown on permanent raised beds with zero tillage for the last two years. Rhizosphere samples (soil and roots) were collected when wheat was harvested. The site near Palwal, in eastern Haryana, is dominated by rice and wheat and grown under organically managed soil (ORG) and comprised three plots, each with a different dose and mix of organic manure: PALF1 (poultry manure alone), PALF2 (poultry manure and 20 tonnes/ha of farmyard manure) and PALF3 (poultry manure and 40 tonnes/ha of FYM). Each sample contained soil as well as roots. The samples were used for (1) trapping and propagating the isolates of AMF (2) analysing fungal parameters, and (3) analysing the chemical properties of soil. After three successive trap-culture cycles, spores were isolated by following the technique of wet sieving and decanting then counted. The spores were grouped based on morphology (colour, size, and shape). Rhizospheric soil from each trap culture was examined for AM fungal spores. Once the data were obtained, the following were calculated for AM fungal diversity analysis (1) Spore density (Total number of spores in 100g of soil sample). (2) AM fungal species richness (the total number of AM fungal species in each sites) (3) relative abundance (the ratio between the number of spores of particular fungal species to the total number of AM spores) (4) Shannon–Weiner index (H') calculated for each sites using Eq. (1), where $Pi = ni/N$, ni is the number of individuals of species i, and N is the total number individuals in all species).

$$H' = - \sum (Pi) \ln (Pi) \qquad (1)$$

S-W index is expressed as $e^{H'}$

Results and Discussion

Spores of AMF collected from trap cultures set up from soils of organically managed agricultural field were classified into 20 spore morphotypes/isolates, 13 of which represented the Genus *Rhizophagus* and *Funneliformis* 7 represented *Gigaspora* and *Scutellospora* genera. Spores produced in these trap cultures were appeared white to yellowish brown in reflected light and were globose to subglobose, 60–300 µm in diameter. Spores of *Gigaspora* and *Scutellospora* species were recorded in these trap culture were larger (150–300 µm) and more abundant ($P<0.001$, Fig.2). Spores size class 180-240µm significantly more were recorded in these trap culture than trap culture originated from conventional practices sites ($F= 26.6$, $P<0.00$, Fig.2). When the total number of AMF species contained in the four replicate trap culture for each treatments were considered, the species richness decrease in order of ORG>ZTL>RBP>CML (Fig.1A). While AM fungal species diversity as expressed by the H'decrease in the order of ORG> RBP≥ ZTL>CML (Fig.1B). Diversity index H′ value was greater (5.83± 0.1809) in trap culture originated from ORG field and lowest (H'= 1.988 ± 0.1349) in trap culture originated from conventional managed land (CML). A hierarchical cluster analysis showed that the highest similarity of AMF species composition between the three treatments (ORG,RBP & ZTL) however AM specis composition in chemically managed soil were distinct (Fig.1C). Present studied indicated that absence or lower abundance of species belongs to *Scutellospora* and *Gigaspora* were recorded in both field of chemically managed soil samples and during trap culture cycle condition. This supporting the earlier hypothesis by Jansa et al. (2003), who

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observed that lower number of *Gigasporacaea sp*. in chemically managed land. Moreover, present invest indicates that significant difference in abundance of *Glomeraceae* spores between trap cultures set up from conventional tillage and zero tillage field. Recent investigation by Mirás-Avalos et al. (2011) based on Denaturing gradient gel electrophoresis (DGGE) sequencing found that increased presence of *Glomus* fungi in agricultural soil under conventional tillage practices. This may be due to spores of genera *Glomeraceae* were more susceptible to disturbance than compared with *Gigasporaceae* because species of *Glomeraceae* initially colonize with host roots by hypha which are easily damage by tillage (Hart and Reader 2004). Similar finding by Hijri et al. (2006) observed that AMF species diversity, low in agricultural field, also found that low-input agriculture land including crop rotation practices induce relatively high AMF species richness.

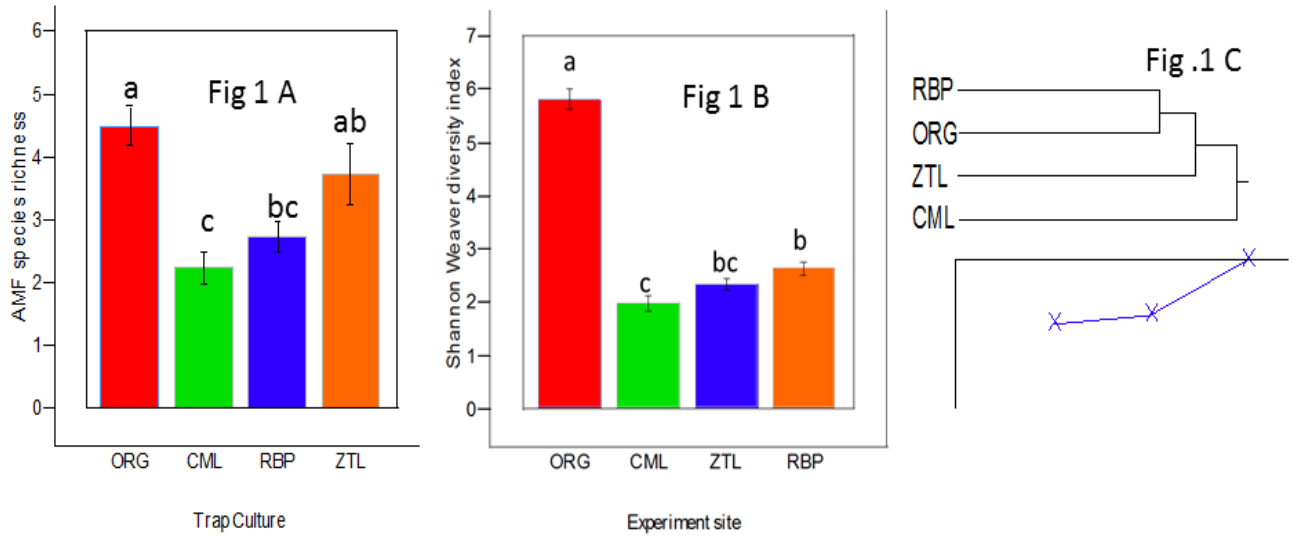

Figure 1 (A-C): A. Species richness (total number of species) of AMF in trap cultures initiated from samples, 100 g each, of rhizosphere soil set up from ORG, CML, RBP & ZTL. Data are reported means and standard errors of means are shown for four replicate plots per site; B. AMF diversity (Shannon-Weiner index) in different trap cultures originated from ORG, CML, RBP & ZTL. Diversities are reported means and standard errors of means are shown for five replicate plots per site. Different letters were used when the difference were significant ($p \leq 0.05$) for one-way Tukey HSD test. Bars indicate ±S.E as calculated from one way ANOVA; C. A hierarchical cluster analysis showed that the highest similarity of AMF species composition between the three treatments (ORG, RBP and ZTL) on the other hand conventional treatments under chemically fertilized separated into different subcluster (CML) showing in Fig.(1C).

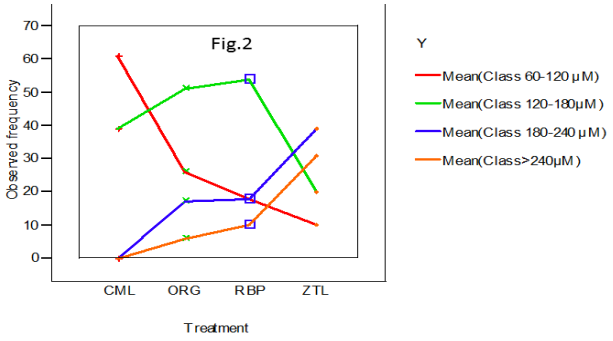

Figure 2: Size of spores in trap soil affected by ORG, CML, RBP & ZTL land. 100 AMF spores of size class (180-240µm) were sampled for size distribution measurement. Means and standard errors of means are shown for five replicate plots per site. Non-significant differences between sites are indicated by identical letters above the bar. Error bars are ±S.E

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Conclusions and outlook

The present study showed that both abundance and diversity of AMF is favoured by low-input agriculture incorporating planting on raised beds and management practices such as zero tillage that do not disturb the physical properties of soil. This investigation also explored the extent to which soils under different farming systems in the tropical zone differ in terms of the composition and species richness of AMF. Present study revealed that conventional agricultural practices causes loss of AM fungal biodiversity and also selectively favours smaller spores (*Rhizophagus* sp.). We also suggested that strong selection pressure favors sporulation of certain group of AM fungi especially species belonging to genera *Gigasporaceae*. Present investigation also suggested that biodiversity study of AM fungi in complex habitat would be integration of molecular, morphological and biochemical approach will be future interest of research. These integrated systematic and ecological researches look forward to the development of powerful and cost effective techniques for AM ecotype selection from complex environmental soil.

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