ISOLATION IDENTIFICATION OF MYCORRHIZAL SPORES FROM THE RHIZOPSPHERIC SOIL OF MAIZE IN PATNA DISTRICT.

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ABSTRACT

Mycorrhiza is symbiotic mutualism relation between special soil and fungi and fine plant root. They form a fundamental link between biotic and abiotic components of the soil system. The present study aims at isolation and identification of some mycorrhizal fungal spores from the maize field of Vaishali District of Bihar during 2009-2011. Random collection of rhizospheric soil samples of maize field from Patna, Mithapur, Danapur, Maner and Didarganj regions of Patna District was done. Soil samples were collected before and on cultivation of maize plant. Wet sieving and decanting method were employed for the isolation of mycorrhizal spores. Collected rhizospheric soil was suspended in water and passed through sieves of different sizes of 500 μm, 250 μm, 100 μm and 38 μm. Spores concentration of different size, shape, colour and hyphal attachment were examined under Stereo microscope. The spore density seemed to be dominated mainly by species of Glomus. However, Gigaspora, Acaulospora, Scutellospora too were identified at comparatively lesser percentage.

INTRODUCTION: -

One of the most successful strategies that terrestrial plants have evolved to withstand different edaphic condition is the capacity if their root system to establish close relationships with a variety of fungi living in their vicinity. The AM fungal symbiosis is certainly one of the most prevalent symbiotic association found in nature in a wide range of ecosystem. AM fungi are found to occur throughout the plant kingdom (Gerdemann, 1968). AM fungi form symbiotic relationship with over 80% of terrestrial plant species (Brundrett, 2002).

The roots are colonized by the AM fungus, which ramifies through the soil. Typically, fungal spores germinate, infect fine roots of the host plants and form characteristic structure, vesicles and arbuscules, inside the roots. Outside the root mycelia spread profusely in the soil. The AM fungus enhances the function of the plant's root hairs and acts as an extension of the root system allowing the mycorrhizal plants to explore and capture nutrients and water (Myer, 2007) from a larger volume of soil compared to non – mycorrhizal plants (Machovej, 2001; Joubert & Archer, 2004).

AMF present in the most natural and agricultural ecosystems, they are important for plant health, nutrient cycling, survival rate, and conservation of soil structure. AMF produce and transport phosphate and other nutrients from the soil to plant roots. On the other hand, the host plant provides fixed carbon to its fungal partner (Harrison, 1999). Furthermore, AMF

could facilitate the management of metal contamination in soil for a restoration and / or bioremediation program (Val Del, et al., 1999).

MATERIAL AND METHODS: -

Field survey was conducted during the year 2008, in the different regions of Patna district to determine the soil sample collection sites and status of Vesicular mycorrhizal fungal spores' concentration in the soil of those regions. Patna, Mithapur, Danapur, Maner and Didarganj regions of Patna district were selected for soil sample collection sites. Random collection of rhizosphere soil samples of Maize from Patna, Mithapur, Danapur, Maner and Didarganj region of Patna District was done during 2009-2011.

SOIL SAMPLING BEFORE CULTIVATION: -

Before sowing of maize, soil samples were randomly collected from the respected crop field of each region of Patna district. From each site of the collection, Soil was collected by a small showed from an area of 15 cm diameter of 10 cm depth after the elimination of organic debris and humus particles. For each sample 200 gm soil was collected in transparent polythene bags of 30 cm × 20 cm size and brought into the laboratory and stored at temperature of 4˚ C, for the estimation of mycorrhizal infection rates.

SOIL SAMPLING ON CULTIVATION: -

When above mentioned cereals plants reached at the flowering stage, soil samples were collected from rhizospheric regions of the plants. The collection of rhizosphere soil samples, a whole root system was dug out carefully so as not to lose the terminal and lateral fine feeder roots. A whole root system was shaken off gently and the compact excess mass of soil from the excavated root system was discarded and soil adhering to the root system was retained. 200 gm soil was collected from the soil mass still adhering to a root system in transparent polyethylene bags.

Wet sieving and decanting method are the commonly used method for spore isolation (Gerdemann and Nicolson, 1963; Walker et. al., 1982). Wet sieving and decanting are a simple method which used sieve of various size to separate spores and other similar size particles from sand and clay (Daniels and Skipper, 1982). The various steps involved in wet sieving and decanting method were as follows: -

1. First 10 g soil sample was taken and dissolved in 100 ml distilled water in conical flask.
2. Then conical flask was shaken for 30 min.
3. After that the conical flask was kept in undistributed condition for 30 min.

4. The heavier particles were allowed to settle down.
5. Suspension was decanted through a 500 μm sieve to remove organic matter and roots.
6. This suspension was decanted through 250 μm, 100μm and 38 μm sieve consequently.
7. The entire residue was collected on 38 μm sieve.
8. After settlement residue was dissolved in distilled water and filtered through filter paper.
9. This paper was spread in Petridis and a residue present in filter paper was taken and mounted on a slide and was examined.

CHARACTERIZATION OF MYCORRHIZAL FUNGAL SPORES: -

Extracted spores were mounted using polyvinyl Alcohol Lactic Acid Glycerol (PVLG) and then morphologically characterized with the help of Manuals (Schneck and Prez, 1990) under Compound Microscope (40 X – 100 X). Major and minor details regarding Shape, Color, Hyphal attachment, for identification unto generic level. The characteristics used for identification include Spore Color, Shape, Size, Wall structure, Ornamental hyphal attachment and Occultation.

PREPARATION OF POLYVINYL ALCOHOL LACTIC ACID GLYCEROL (PVLG): -

Poly vinyl alcohol lactic acid glycerol used in study of vesicular – arbuscular mycorrhizal fungi consists of the ingredients listed below: -

INGREDIENT	QUANTITY
Distilled Water	100 ml
Lactic Acid	100 ml
Glycerol	10 ml
Polyvinyl Alcohol	16.6 g

PROCEDURE: -

Polyvinyl Alcohol Lactic Acid Glycerol (PVLG) is used to prepare permanent slides with unbroken and crushed spores, as well as with fragments of Mycorrhizal roots. Its viscosity enables to manipulate the position of the specimen examined and hence accurately determine their properties.

ROOT STAINING TECHNIQUE: -

Root colonization was observed by rapid clearing and staining technique (Philip & Hayman, 1970). For AM colonization assessment root samples were cleared with 10% KOH, acidified with 1N Hcl and stained with Lacto Glycerol Trypan Blue (.05%).

The stained roots were mounted on Microscopic slides and the segment were examined by light Microscope (40 X – 100 X).

ESTIMATION OF VAM ROOT COLONIZATION

Vam root colonization of host plant was studied after processing the roots according to Kaski and Gemma (1989). The total percentage of root colonization was determined by using the formula

$$\textbf{Root Colonization} = \frac{\textbf{No. of root segment colonized}}{\textbf{Total No. of root segments observed}} \times 100$$

COLLECTION AND STORAGE OF ROOT SAMPLES

- Roots were taken from the regions between 50 cm and 100 cm of root material for each plant species.
- Care was taken to collect as many of the fine lateral roots as possible along with the main system.
- Roots were not collected if they were enlarged with the roots of other species in order to avoid incorrect assessment.

  Root samples were placed into labelled vials containing distilled water in order to wash the sand from them. They were generally processed the following day. However, if processing was to be delayed, they were transferred to vials containing 50% ethanol. Ethanol was chosen as the fixative over FAA (Formalin Acetic Acid Alcohol) due to the caustic nature of the latter.

OBSERVATION

Periodical survey of various places such as Patna, Mithapur, Danapur, Maner and Didarganj region of Patna district was undertaken to collect and identify different AM fungi Genera and Species association with maize Plant. Rhizosphere soil sample collected from various localities revealed presence of several species of different genera on the basis of resemblances the AM fungi as *Glomus spp., Acaulospora spp.* shown in plate.

Figure showing survey of wheat field.

Figure showing survey of maize field

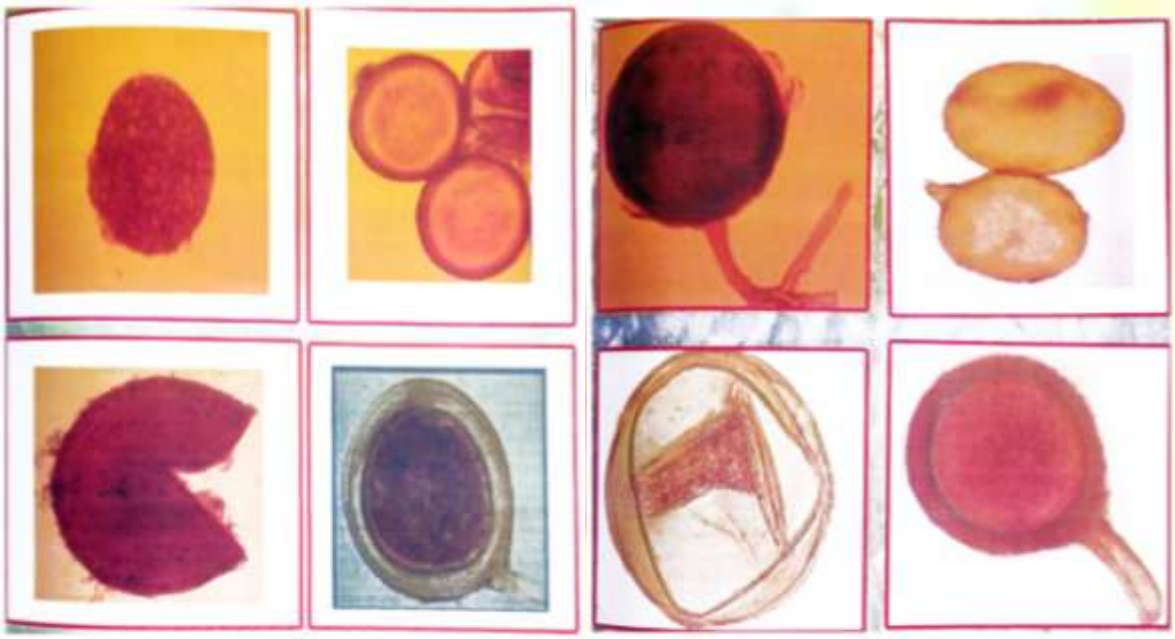

Figure showing different types of *Acaulospora spp.* spore collected from rhizospheric soil samples.

Figure showing different types of *Glomus spp.* from collected soil samples.

Figure showing spore of *Scutellospora spp.*

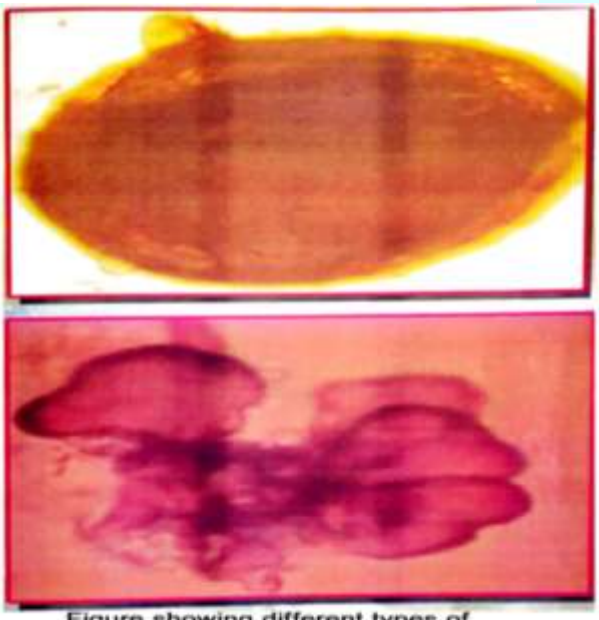

Figure showing different types of *Gigaspora spp.* spore collected from rhizospheric soil samples.

The number of AM fungal Spores isolated from different sites of Maize crop were given in table. The number of AM fungal spores ranged from 80 – 140 per 100 gm of soil. This study describes the distribution of AM fungi in the rhizosphere soil of Maize plant. Both plant and rhizosphere soils were collected during three-year period (2009 – 2011) at different site and during different seasons.

TABLE: - AVERAGE NUMBER OF SPORES AREA WISE AND YEAR WISE

YEAR	PATNA	MITHAPUR	DANAPUR	MANER	DIDARGANJ	AVERAGE SPORE YEAR WISE
2009	110	110	118	105	108	110.2
2010	122	90	141	120	98	114.2
2011	116	112	134	112	110	118
AVERAGE SPORES AREA WISE	116	106	131	112.33	105.3	

The average number of spores isolated from Maize plant collectively from year 2009 to 2011 from diverse has been show in tabular form table.

Root Infection Assessment: -

In order to find out the potential of AM fungi infection in roots of Maize, the root samples collected from were clean, chopped into small pieces and then subjected to Fixation, Cleaning, Rinsing and Bleaching in KOH solution following standard techniques for Microscopic observations.

In case of infected roots, the presence of AM fungi infection was observed on the basis of root cuts (1 cm size), infected and uninfected, the degree of root infectivity was worked out in term of percentage.

TABLE: - R.C. VALUE OF MAIZE

YEAR	MAIZE PLANT R.C. VALUE
2009	16
2010	28
2011	26

RESULT AND DISCUSSION

In the present study *Glomus* were the most common Genera and dominant in shifting system. My finding corroborates with the finding of Morton (1988) that the genus *Glomus* is predominantly distributed genus in the soil all over the world. Glomus were common and made up for more than 75% of total isolates followed by *Acaulospora* and *Gigaspora*. Dominancy of *Glomus* in the present study is in the agreement with the finding of (Panawar and Tarafdar, 2006; Pande and Tarafdar, 2004; Burni and Illahi 2004; Mirtha and Dhar, 2007; Sharma et al, 2009; Burni et al., 2009).In Indian context my finding is corroborate with the finding of Rani and Manoharachary (1994) that the most frequently identified VAM fungi were *Glomus spp* (7 species).Singh and Adholeya (2002) also observed that the genus glomus was ubiquitous. The predominance of *Glomus* species under varying soil conditions might be due to the fact that they were widely adaptable to the varied soil conditions and survive in acidic as well as in alkaline soils (Pande and Tarafdar, 2004). In the present study the maximum spores were observed from undisturbed natural vegetation of Danapur of Patna district. The potential reason for maximum number of spore's availability in undisturbed natural vegetation is that spores keep multiplying in association with plant whereas, in cultivated habitat the top soil is disturbed each time as some fresh crop was sown. Previously several researchers like Gaur and Kaushik 2011, also reported that quantitative spore population differed in cultivated and uncultivated soil. Mycorrhizas are an essential below-ground component in the establishment and sustainability of plant communities, but thorough knowledge is required to achieve maximum benefits from these microorganisms and their associations

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