ARBUSCULAR MYCORRHIZAL COLONIZATION IN SOME VEGETABLE AND
OILSEED CROPS IN DIST. SOLAPUR

MINOR RESEARCH PROJECT
REPORT

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INTRODUCTION
The word *mycorrhiza* was first used by German researcher A.B. Frank in 1885, and originates from the Greek *mycos*, meaning 'fungus' and *rhiza*, meaning 'root'. The term mycorrhiza describes symbiotic associations between plants and fungi. These associations are assumed to play an important role in the land colonization by plants due to the ability of the symbiotic organisms in acquiring nutrients unavailable to non-mycorrhizal individuals (Simon et al. 1993; Smith and Read 2008). The plants in return supply the fungal partner with carbohydrates. Two main types of mycorrhizae may be found, depending on whether the fungus penetrates into the root cells or not: ectomycorrhizae and endomycorrhizae or arbuscular mycorrhizae (AM).

The AM is formed between an enormously wide variety of herbaceous plants and obligatory biotrophic fungi (Smith and Read 2008). In detail plants which are able to form arbuscular mycorrhizas are taxonomically very diverse and belong to almost all phyla. All groups of Gymnospermaes, the majority of the Angiospermae families as well as all Bryophytes and almost all groups of Pteridophytes are capable to establish the symbiosis with AM fungi (Smith and Read 2008). The AM symbiosis is characterized by a bidirectional nutrient transfer, the plant supplies the fungus with carbon and in return the plant gets nutrients in particular phosphorus (P) from the fungal symbiont. The fungus efficiently acquires P, which is required in large amounts for the biosynthesis of primary and secondary compounds, since P has essential functions in the energy metabolism of the cells and as constituent of nucleic acids and phospholipids (Marschner 2002). The AM consists of three important components: the plant root itself, the fungal structures within and between the root cells and the huge extraradical mycelium in the soil. The name ‘arbuscular’ is derived from highly branched, tree-like structures formed in rootcortical cells, the so-called arbuscules. Further intraradical structures are vesicles, thickwalled, mainly lipid-filled storage organs, inter- and intracellular hyphae, moreover very rarely intracellular coils are formed. It is assumed that arbuscules are the main structures where the carbohydrate transfer between the plant and the fungus takes place, but a carbon transfer may also occur from the intracellular hyphae (Smith and Read 2008). The formation of arbuscules and vesicles is not obligatory even though almost all fungi develop arbuscules and approximately 80% of the AM fungi form vesicles the latter depending on the fungal genera (Smith and Read 2008).

VAM fungi have been proved to increase the productivity of several cereals, pulses, oilseed crops, vegetable crops, medicinal plants and also ornamental plants. The VAM
fungi are obligate symbionts and not host specific (Bonfonte-Fasolo, 1987). A better understanding of the mycorrhizae of agronomic crops is necessary because of their potential involvement in the sustainable agriculture systems (Khalil et al., 1992). The abundance and distribution of VAM fungi in various plants have been studied in various parts of the world. The present work was undertaken to study the mycorrhizal association in some vegetable and oil yielding crops cultivated in Solapur district on the light of distribution and abundance.
AIMS

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OBJECTIVES
The present research project has the following objectives.

1. Survey of some vegetable and oil yielding crops growing in various localities of district Solapur.
2. Assessment of arbuscular mycorrhizal fungal colonization in root of selected vegetable and oil yielding crops.
3. Isolation of native arbuscular mycorrhizal fungal spores from the rhizosphere soil of selected plants.
4. Quantification of AMF spores from rhizosphere of different localities of district Solapur.
5. To give first-hand information about occurrence of AM fungal spores in native soil.
REVIEW
OF
LITERATURE
Mycorrhizal colonization is common in tomato plants and well documented as a mycotrophic plant (Kubota et al., 2005). Satya Van et al., (2014) reported that the number of AM fungal propagules in brinjal crop of different localities varied from 635 to 1325 spores per 100 gm soil. Due to the widespread nature of AM fungi, they occurred in almost all the soil samples but with a variation both in number and type of spores and sporocarps. They isolated 20 AM fungal species belonging to the genera of Glomus, Acaulospora, Gigaspora, Sclerocystis and Entrophospora and concluded that Glomus was predominant followed by Acaulospora in the rhizosphere soils of brinjal.

Fifteen arbuscular mycorrhizal (AM) fungi are reported in the rhizosphere soil of three Solanaceous members namely, tomato, chilli and brinjal collected from five different locations (Reddy et al., 2006). The genus Glomus is the most dominant fungus followed by Acaulospora, Sclerocystis, Gigaspora and Entrophospora. Bhuvaneshwari and Sadhana (2014) studied diversity of AM fungi from the rhizosphere soil of selective plants such as amaranthus, chilli, brinjal, tomato and bhendi. They observed the AM spores belong to the Genus Acaulospora, Entrophospora, Glomus and Sclerocystis from the rhizosphere soil. Among these Glomus and Acaulospora are predominant in all rhizosphere soil. The hundred percent of AM fungal colonization was observed in Amaranthus roots and other plant root tissues showed 85-95% AM fungal colonization.

Aher (2015) carried out an experiment on Arachis hypogea L. to study the association of VAM and its influence on vegetative parameters. He concluded that among various oil seed crops Arachis hypogea, L. is an important crop. Seedling growth and vigour of various oil seed crops raised in pots were evaluated after inoculating nursery soil with four cultures of vesicular arbuscular mycorrhizal fungi. He tested four VA-mycorrhizal fungi for their ability to increase the growth, biomass by colonization of roots. Among the four VA-mycorrhizal fungi (Glomus fasciculatum, Glomus geosporum, Scutellospora nigra and Scutellospora sp.) Glomus fasciculatum was most effective in increasing the shoot and root growth, dryweight, % infection over control. VA-mycorrhizal fungi inoculated seedlings grew faster and healthier than uninoculated seedlings. Eighteen types of AMF spores belonging to four genera, namely Acaulospora, Glomus, Gigaspora, and Scutellospora. Out of these Glomus and Scutellospora were commonly found in rhizosphere soil.

Prasad et al., (2006) observed distribution of AM fungi in soybean. The treatment of Glomus fasciculatum showed good effect on shoots and root lengths, compared to the control. This is because of the ability of mycorrhizal plants to utilize the available nutrients efficiently than the non-mycorrhizal plants and mycorrhizal fungi are known to control the root
topology in response to soil conditions (Hetrick et al., 1988). The extensive colonization of AM fungi in soybean roots has been found in soils with high phosphorus (Khalil et al., 1992). Sharma et al. (2009) reported seven Acaulospora species (Acaulospora laevis, A. lacunose, A. rehmii, A. foveata, A. gerdemannii, A. bireticulata, A. scrobiculata) in sunflower rhizosphere.
MATERIALS AND METHODS
The plant materials used for the present study were collected from three regions i.e., Sangola region (Dongargaon, Nazara, Gheradi), Pandharpur region (Karakamb, Tarapu, Chale) and Mohol region (Patkul, Angar, Wadwal) of district Solapur. Following Vegetable and oil yielding plants were selected for present study- Lycopersicon esculentum Mill (Tomata), Solanum melongena L. (Brinjal), Raphanu ssativus L. (Radish), Cucumis sativus L. (Cucumber), Momordica charantia L. (Bitter gourd), Luffa acutangula (L.) Roxb. (Ridged gourd), Trigonella foenum-graecum (Fenugreek, Methi), Helianthus annuus (Sunflower), Carthamus tinctorius L. (Safflower), Arachis hypogea L. (Pea nut) etc. Plant material were collected in two seasons i.e., Kharip (June-September) and Rabi (October- January).

**Sampling of rhizosphere soils and root**

The rhizosphere soil samples of selected plants were collected randomly by lifting up gently a block of soil with the plant roots intact. Later the root system along with the sticky soil around was carefully removed and placed in a polythene bag. These samples were quickly transported to the laboratory and moisture content was determined immediately. Soils for further analysis were wrapped up in separate polythene bags and stored at 4°C. They were air dried and analysed for total nitrogen, phosphorus and potassium.

**Estimation of Total Nitrogen**

Total nitrogen content in rhizosphere soil was estimated following the method given by Hawk et al., (1948). Oven dried soil (0.5g) was taken in Kjeldahl’s flask with a pinch of microsalt (200 g K₂SO₄ + 5 g CuSO₄ dehydrated) and to it 5ml H₂SO₄ (1:1) were added. Few glass beads were added to avoid bumping and the material was digested on low flame. After complete digestion, a faint yellow solution was obtained which was cooled to room temperature, transferred to volumetric flask and diluted to 100 ml with distilled water. Then it was filtered through Whatman No. 1 filter paper and used for the estimation of nitrogen.

In a set of Nessler’s tubes, 2 ml of plant extract and different concentrations of standard ammonium sulfate solution (0.236g of oven dried ammonium sulfate dissolved in distilled water and few drops of conc. H₂SO₄ were added. The volume was made 1000 ml with distilled water. This solution contains 0.05mg of nitrogen per ml) were taken. To each of these tubes one drop of 8 % KHSO₄ was added and volume was made 35 ml with distilled water. To this 15 ml of freshly prepared Nessler’s reagent were added (Reagent A : 7g KI +
10 g HgI₂ in 40 ml distilled water, Reagent B : 10 NaOH in 50 ml water. A and B are to be mixed in proportion of 4:5 at the time of estimation).

The reaction between sample and the reagent gives the product NH₄Hg₂I₃ which has orange brown colour. The intensity of this colour was measured after 15 minutes at 520 nm on a double beam spectrophotometer (Shimadzu UV 190).

**Phosphorus**

The phosphorus content was estimated according to the method of Sekine et al., (1965). Here phosphorus reacts with molybdate vanadate reagent to give yellow colour complex. By estimating colorimetrically the intensity of the colour developed and by comparing it with the colour intensity of the known standards, phosphorus content was estimated.

Two ml of acid digest was taken in the test tubes and to this equal amount of 2 N HNO₃ followed by one ml of freshly prepared molybdate vanadate reagent (A - 25 g Ammonium molybdate in 500 ml of distilled water. B - 1.25 g Ammonium vanadate in 500 ml 1 N HNO₃, A and B were mixed equally at the time of using) were added. Then final volume in each test tube was adjusted to 10 ml with distilled water. The ingredients were mixed well and allowed to react for 20 minutes. After 20 minutes colour intensity was measured at 420 nm using a reaction blank containing no phosphorus.

Calibration curve of standard phosphorus was prepared from standard phosphorus solution (0.110 g KH₂PO₄ per liter = 0.025 mg P⁵⁺ ml⁻¹) taking different concentrations, other steps being essentially similar to the one described above. Amount of phosphorus in the plant material was expressed in mg 100 g⁻¹ on dry weight basis with the help of standard curve.

**Potassium**

The level of potassium was estimated using Atomic Absorption Spectrophotometer in acid digest sample. In case needed, appropriate dilution of plant extract was made with distilled water.

**Isolation and quantitative estimation of AM fungal spores**

VAM colonization was assessed following the method of Phillips and Hayman (1970). Roots of selected plants were washed in water, cleared with 10% KOH, acidified with
1 N HCl, and stained in 0.05% trypan blue in lactoglycerol. Quantification of root colonization of the AM fungi was carried out using the slide method (Giovannetti and Mosse, 1980). For every sampling, 100 root pieces (10 pieces of young branches from each seedling) measuring 1 cm. each were assessed for VAM colonization and the mean value was expressed in percent colonization. Spores of the AM fungi were extracted from rhizospheric soil using the modified wet-sieving and decanting method (Gerdemann and Nicolson, 1963). Quantification of the AM fungal spore density was carried out using the method given by Gaur and Adholeya (1994). Spore count was made on 100g dry soil and recorded as number of spores 100g\(^{-1}\) soil. VAM fungal species were identified by means of morphological characteristics of the spores using the relevant literature (Hall, 1984; Schenck and Perez, 1990). Each treatment was replicated three times.

**Root colonization:**

Per cent of AMF colonization was estimated by microscopically examination at 10X magnification. The mycorrhizal colonization was determined by using following formula.

\[
\text{Per cent of mycorrhizal colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \times 100
\]
RESULTS
AND
DISCUSSION
The data of physico-chemical properties of the rhizosphere soil samples of ten vegetable and oil yielding plants collected from three different places in relation to number of propagules is presented in Table 1. All the soils investigated in the present study were of red sandy loam type. The soils had a pH range between 6.5 and 8.5. The soils with pH neutral to slightly alkaline harboured more number of propagules. Moisture content of the samples ranged from 23-30%. Our results revealed range of nitrogen is 69 -79.5 mg 100 g⁻¹. The level of phosphorous is 20.5-32.5 mg100 g⁻¹ while range of potassium is 29.5-41.3 mg100 g⁻¹. Maximum number of spores was observed in rabbi season due to high moisture level as compared to kharip season (Table 1-5). Light textured sandy loam soil with neutral to slightly alkaline pH, low moisture percentage favoured extensive mycorrhizal root association (Sreeramulu and Bhagyaraj, 1986). In our study, the soil pH ranged from 6.5 to 8.5, which was closer to neutral to slightly alkaline and has more number of AM fungal propagules. The importance of soil moisture on the AM fungal distribution was reported by Saif and Khan (1977).

Studied vegetable and oil yielding crop plants of three sites of Solapur district were found to have spore densities in their rhizosphere soils ranging from 54-225 per 100g air dried soil in both kharip and rabbi season (Table 3-5). In rhizosphere soils we found more spore density in rabbi season while maximum root colonization in kharip season. Various types of VAM spores with colours like dark, reddish, brown and yellowish with various shapes such as oval, spherical and irregular were found in the rhizosphere soils of studied vegetable and oil yielding plants (Plate I). But specific identification for fungal species by spore characteristics was not attempted in this study. The spore-size ranged from 46 to 243 μm. Variations occurred in spore density among the plant species in different sites including the replications. We observed fungal structures like appresoria, arbuscules, hyphae, and vesicles in both season in selected vegetable and oil yielding crops (Plate II).

Association of VAM were studied and isolated from the rhizosphere soils of 10 vegetable and oil yielding plants (Table 3-5). The rhizosphere soils of all these plants were supported by a good number of VAM fungal colonisation. A maximum level of colonisation of VAM was recorded in the samples of Trigonella foenum–graceum followed by Helianthus annus and the minimum of colonisation were in the soil samples of Raphanus sativus in kharip season.

AM fungal species isolated from the rhizosphere soils of 10 vegetable and oil yielding crops in that Glomus was predominant followed by Acaulospora (Table 6). The results are very much supportive of the earlier studies (Singh et al., 2000). Earlier reports also revealed
the predominance of the above AM fungal genera in the rhizosphere soils of different plant cultivars (Gerdemann and Trappe, 1974; Hall and Abbott, 1984; Hindumathi and Reddy, 2011). In the present study it was observed that in many cases more than one appressorium is located at an entry point. In most cases, adjacent appressoria probably results from the branching of single external hyphae before or after contact with the root, which is accordance with Brundrett et al., (1985) described characteristic branching of the patterns of the internal hyphae of *Glomus* species. Occasionally numerous branches were produced by intercellular hyphae.
CONCLUSION
In the present study, *Glomus* sp. dominated the rhizosphere soils of all the selected plants. This was similar to earlier reports of VAM association on other vegetable crops (Subha, 2001). Similar reference was observed in the case of chilli (Bagyaraj and Manjunath, 1980; Bagyaraj and Sreeramulu, 1982; Manoharachary and Sulochana, 1989; Janakirani, 1991; Vani, 1993; Hindumathi, 1999; Subha, 2001) in studies of the AM fungal association in onion, chili, okra, sesame, safflower, castor, sorghum and sweet potato. Also, Karaarslan and Uyanoz (2010; 2011) reported that *Glomus* dominated the rhizosphere soils of natural plants. The distribution of VAM fungal species appears to be more closely related to host plant, soil structure and environmental conditions than to the competition by other VAM fungal species (Koske, 1981).
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PUBLICATION
Status of vesicular arbuscular mycorrhizae (VAM) in vegetable plants of Solapur district

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ABSTRACT

The present study is aimed to assess the association of vesicular arbuscular mycorrhizal fungi in some vegetable crops along with arbuscular mycorrhial (AM) fungal population density in the rhizosphere soil, investigate for qualitative composition of AM fungal species and per cent root colonization. The results showed that the number of AM fungal propagules in vegetable crop collected from different localities varied from 55.18-225 spores per 100gm soil. Due to the widespread nature of AM fungi, they occurred in almost all the soil samples but with a variation both in number and type of spores. Altogether, 20 AM fungal species were isolated belonging to the genera of Gigaspora, Acarospora, G. gaeumannii and Entrophospora. Gigaspora was observed to be predominant followed by Acarospora in the rhizosphere soils of all vegetable crops. The spore distribution, density and the composition of AM fungi was observed to be influenced by environmental and physico-chemical condition. The AM spore population, percentage of root colonization and distribution varied by the seasonal fluctuations in moisture, pH and soil mineral nutrient status such as N,P,K etc. The data showed that nitrogen deficient soils appeared to have more number of AM fungal propagules. The soils having high levels of phosphorus and potassium content harboured least number of AM fungal spore population while, low levels involved more spore density.

Keywords: Mycorrhizal, VAM, Fungi, Vegetables.

INTRODUCTION

Mycorrhizal fungi are key components of soil microbiota and obviously interact with other microorganisms in rhizosphere. Mycorrhizal association changes several aspects of plant physiology, nutritional and physical properties of the rhizosphere soil. Different types of mycorrhizal association have been observed in a wide range of land plants. AM fungi differ widely in the level of colonization they produce in a root system and in their impact on nutrient uptake and plant growth. The abundance and distribution of VAM fungi in various plants have been studied in various parts of the world. The present work was undertaken to study mycorrhizal association in some vegetable crops cultivated in Solapur district on the light of distribution and abundance.

MATERIALS AND METHODS

The plant materials used for the present study were collected from three regions i.e., Sangola region (Dongargaon, Nazara, Ghendi), Pandharpur region (Karmankh, Tampi, Chhae) and Mohol region (Petalk, Aaugar, Wadwad) of district Solapur. Following Vegetable plants were selected for present study- Lycopersicon esculentum Mill (Tomato), Solanum melongena L. (Eggplant), Raphanus sativus L. (Radish), Cucumis sativus L. (Cucumber), Moringa oleifera L. (Bitter gourd), Luffa acutangula (L.) Roem. (Bottle gourd), Trogneus foliopurpureus (Fenugreek, Methi) etc. Plant material were collected in two seasons i.e., Kharif (June-September) and Rabi (October-January).

Samples with roots of respective plant species were collected and placed in plastic bags. These samples were quickly transported to the laboratory and moisture content was determined immediately. The soil pH was determined by an electric pH meter using the suspension with soil water ratio of 1:2.5 (w/v). Even dried soil used for element analysis. Available nitrogen by micro-Kjeldahl method, available phosphorus (P) and potassium by Jackson (1978) method was analysed.

Mycorrhizal spores were separated from soil by wet sieving and decanning method of Gentzelman and Nichison (1965) and...