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Effect of Soil Factors on Arbuscular Mycorrhizal Fungi of Castor [*Ricinus communis* (L.)]

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Abstract: ***The rhizospheric soil samples of castor collected from ten (10) different localities of Mahabubnagar district were analyzed for physico-chemical parameters. The data on rhizosphere soil analysis of physico-chemical characteristics viz., soil texture varied from sandy clay, sandy to clayey type, pH was neutral (7.0) to moderately alkaline (8.0), Electricon conductivity (EC) ranged from normal (0.07 mmhos/cm) to satisfactory levels (0.86mmhos/cm). The soils were optimum in nutrient levels particularly with Organic Carbon ranged from low (<0.5%) to medium (0.5% - 0.72%) levels, moisture content from 16.5% to 21%, levels of nitrogen were moderate to high (68- 94 kgs/ Acre), phosphorus levels ranged from low to medium (12 - 27 kgs/Acre) and levels of potassium were moderate to high (119- 165 kgs/ Acre). Among the ten localities studied, highest spore count was noticed from (318-298/100g) Velgonda (S7) and Sharepally (S10). The lowest number of spores (112-230/100g soil) was recorded in the soil samples of Chinnadarpally (S3) and Molgara (S8). A total of fifteen AM fungal species were isolated from the rhizosphere soil of castor. Which belonging to 3 genera namely Acaulospora, Glomus, and Scutellospora, most of them supporting castor cultivars.***

Keywords: AM Fungi; Root Colonization; Castor; Physico- chemical characters

1. Introduction

Castor [*Ricinus communis* (L.)] is one of the medicinally important oil seed crop (24). Castor oil has a wide range of industrial applications like soaps, lubricants, hydraulic and brake fluids, paints, dyes, waxes, polishes nylon, pharmaceuticals and perfumes. Oil cake makes an excellent fertilizer (17). Antihistamine and anti-inflammatory properties were found in ethanolic extract of castor root bark. The oil has great promises in the field of biodiesel production (29). Castor is contributing a major portion to the Indian agricultural economy and reported to have a mycorrhizal association.

The arbuscular mycorrhizal fungi (AMF) are universal and ubiquitous, rhizosphere microflora forming symbiosis with plant roots and acting as biofertilizers, bioprotectants and biodegraders. Mycorrhizal fungi are one of the soil organisms that create a direct connection between the soil mass and plant root systems (3).

Many aspects of Arbuscular mycorrhizal (AM) interactions including growth effect, nutritional exchanges, biocontrol toward plant pathogens, tolerance to water stress and adverse environmental conditions were studied, but little is known about their potential effect on the quantitative and qualitative profile of the secondary metabolites (e.g., essential oils) in medicinal and aromatic plants (22), (8), (26). 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 (15), (32). The use of microorganisms with the aim of improving nutrient availability for plants is an important practice and necessing for agriculture (10).

However, population sizes and species composition are highly variable and influenced by physico-chemical characteristics of the soil viz., soil moisture, temperature, soil pH, texture, nutrient levels, heavy metal concentration, the presence of other microorganisms and soil salinity (7). The physico-chemical factors were studied in relation to AM fungal distribution and seasonal variations. The number of spores are also influenced by varying depths of soils and different altitudes and the type of vegetation (4). This review gives on overview on the role of mycorrhizae in nutrition.

2. Materials and Methods

2.1 Study Site

The present study was carried out with selected castor plants [*Ricinus communis* (L.)] naturally cultivated in the fields of mahabubnagar district located in Telangana., India. Mahabubnagar is the largest district in Telangana in terms of area (18432.00 sq. km) covered. The district was situated between 77° 15' and 79° 15'E, of the eastern longitudes and 15° 55' and 17° 20'N, of northern latitudes. An annual rainfall between 700-900 mm. Avg annual temperature 35.0 °C (95.0 °F), Avg. summer temperature 40.9 °C (105 °F). Rhizosphere soil of castor cultivars were collected from ten different locations i.e. Koilkonda, Rajapur, Chinnadarpally, Manganur, Velkicherla, Palamur university campus, Velgonda, Molgara, Goplapur, Sharpally. All these localities will come under Mahabubnagar is the largest district in Telangana, India. Three soil samples were collected randomly from each site in all the ten locations. The soil samples were collected with the help of a widger by lifting up gently a block of rhizosphere soil and placed in sterile polythene bags. The soil samples were transferred to the laboratory and processed for isolation of AM propagules.

2.2 Isolation and Estimation of Arbuscular mycorrhizal (AM) spore population

The rhizosphere soil samples were used to determine AM spore population from 50 grams portion of soil. The AM fungal spores/ propagules consisting of spores, sporocarps were isolated from composite soil samples by employing wet sieving and decanting technique (Gerdemann and

Nicolson, 1963) and were quantified by using the grid-line interesting method (2). 100 g soil have been oven dried at 80°C for 24 h so that the units for spore number can be expressed per 100 g dry soil. 100 g of composite rhizosphere soil samples from each plant/cultivar of castor from each field site was suspended in water (1L) and stirred thoroughly. The resulting soil suspension was passed through sieves of sizes 450ìm, 245µm, 105µm, 75µm and 53µm, which were kept one below the other in descending order. The process was repeated 4 to 5 times with an interval of 20 min, until the sand and stones are left in the beaker. The debris retained on the sieves was carefully collected into a beaker with the help of a level pipe separately through single synthetic fibred imported white cloth.

This cloth was kept in a Petri dish divided into compartments with the glass marker for easy counting of spores. The Petri dish was observed under a stereo-binocular microscope. Spores and sporocarps were counted by scanning each filter cloth sieved under 420µm, 250µm, 105µm, and 75µm sieves. The spores were picked up using narrow injecting needles and mounted on slides in a drop of lactophenol and stained in 0.05% trypan blue. Cover glasses were placed on the slides and sealed with DPX mountant. (41).

2.3 Estimation of Arbuscular Mycorrhizal Root Colonization

Roots of the test plant species after thorough washing with tap water were cut into approximately 1-cm pieces and processed by employing "rapid clearing and staining technique". 50-stained root bits were examined under microscope for mycorrhizal root colonization. The observations were recorded from each sample containing 50 root segments according to the method suggested by Bierman and Linderman. The relative abundance of mycelium, vesicles and arbuscules of mycorrhizal fungi in the host tissues were recorded as abundant, moderate and scanty. Per cent root colonization was quantified based on the number of root segments colonized by AM fungi using the formula:

$$\% \text{ of root colonization} = \frac{\text{Total number of infected roots}}{\text{Total Number of roots}} X\,100$$

2.4. Identification of AM fungal species

Intact spores and sporocarps were mounted in lactophenol and identified according to their spore morphology by using taxonomic key 9 (38), (27), (28). The qualitative estimation was expressed as percentage frequency occurrence of AM fungal species.

2.5. Physical and Chemical Properties of the Soil

Various physico-chemical characters viz. soil type, soil pH, soil moisture, N, P, K status, were determined following standard methods and techniques (5). The number of AM fungal propagules and percentage of AM infection were estimated by following the standard methods (18), (19), (20), (31), (13), (30), (42), (16). Analysis were done in the Mycology & Plant Pathology Lab, Department of Botany, Osmania University. For all the parameters, triplicate values were taken into consideration.

3. Results & Discussions

3.1. Isolation of Arbuscular Mycorrhizal (AM) spore population and Estimation of AM root colonization

It is clear from Table.1 that AM spore counts were higher in S7, and S10 sample sites whereas the least AM spore counts were observed in S3 and S8 sample sites. From the results (Table 1) we can say that S7 and S10 soil sample site collections are highly significant in all the parameters. The lowest percent root colonization in *Ricinus communis L.* (65–72%) at Koilkonda (S1) and Sharepally (S10). Whereas, the highest percent root colonization from (85-88%) was determined in the places of Chinnadarpally (S3) and Velgonda (S7). Later on, with increased colonization mycelium, vesicles and arbuscules were found to be very abundantly associated during the period of harvest, accordingly the roots were heavily colonized both qualitatively and quantitatively. Sporocarps were observed inside the root cortical cells. Similarly, highest spore count (318-298/100g) was noticed in sample sites of Velgonda (S7) and Sharepally (S10), the lowest number of spores (112-230/100g soil) was recorded in the soil samples of Chinnadarpally (S3) and Molgara (S8) shown in table 1. From this, it is concluded that there is no direct relation between the number of spores and number of species. It is a well-established fact that these two factors need not be directly proportional to one another. In the present study, *Acaulospora* dominated the soils supporting castor cultivars followed by *Glomus*, Our results are in agreement with the earlier observations (1), (40), (41). Difference in the response of AM fungi with in host suggests that under some conditions selection should occur to favor certain host-fungus combinations. Later on, with increased colonization mycelium, vesicles and arbuscules were found abundantly associated during the period of harvest.

Table 1: Arbuscular Mycorrhizal fungal spore number and percent of root colonization(%) in the rhizosphere soils of castor fields from different locations of Mahabubnagar District, Telangana, India.

S. No	Crop location	AM spore density/100 gm of soil	Percent root colonization (%)
1	Koilkonda	234	65
2	Rajapur	260	75
3	Chinnadarpally	112	85
4	Manganur	250	80
5	Velkicherla	278	75
6	Palamur University campus	246	82
7	Velgonda	318	88
8	Molgara	230	84
9	Goplapur	267	75
10	Sharepally	298	72

3.2 Soil analysis for Physico-chemical characteristics

The data of physico-chemical characteristics of the rhizosphere soil samples of castor in relation to the number of AM propagules is presented in Table 1 & 2. Soils with neutral pH and slightly alkaline pH i.e.,7 to 8 consisted of a

greater number of propagules. Though the pH varied greatly in different soils. Soil pH showed negative correlation with AMF spore count and AMF % colonization in castor plants. Soil moisture, in the present investigation showed negative correlation with AMF spore count and AMF colonization indicating that low soil moisture had increased the number of AMF spore population and colonization. Nitrogen showed positive correlation with AM spore count and AMF % colonization. Phosphorous in the present findings, shows negative correlation with AM spore count and AMF % colonization. The potassium content in the present investigation is positively correlated with both the AMF spore count and % of colonization. All the soils investigated in the present study are of sandy clay type receiving less fertilizer and water. Therefore, the above soils have been considered as good habitats for AM fungal association. The spore distribution, density and the composition of AM fungi were observed to be influenced by environmental and physico-chemical factors. The AM spore population, percentage of root colonization and distribution was affected by the seasonal fluctuations in moisture, temperature, pH and soil mineral nutrients such as N, P, K, Zn, Fe, etc. The results are in concurrence with the earlier findings (6), (23), (33), (34), (16).

Table 2: Physico-chemical factors of the rhizosphere soils of castor collected from different locations of Mahabubnagar District.

Different location sites	Texture	Moisture content (%)	pH	EC	OC	Nitrogen kgs/Ac	Phosphorous kgs/Ac	Potassium kgs/Ac
S1	S	19.0	7.0	0.54	L	68	20	119
S2	S	19.0	8.0	0.07	M	94	22	165
S3	SC	21.0	8.0	0.22	M	90	27	153
S4	SC	18.0	7.5	0.72	M	71	21	140
S5	SC	17.0	8.0	0.62	L	76	18	158
S6	SC	19.0	8.0	0.62	M	88	26	148
S7	S	16.5	7.0	0.32	M	81	17	132
S8	SC	20.0	7.5	0.27	M	79	15	149
S9	SC	18.0	7.0	0.54	L	82	14	136
S10	S	18.5	8.0	0.60	M	80	12	127

Texture: Sc-Sandy clay,S-Sandy,C-Clay; EC- Electron conductivity; OC Organic Carbon (L=low < 0.5%;M= medium 0.5% - 0.72%); Kgs Kilograms; Ac Acre. Soil samples: 1-Koilkonda, S2 Rajapur, S3 Chinnadarpally ,S4-Manganur,S5-Velkicherla,S6-Palamur University campus,S7-Velgonda,S8-Molgara,S9-Goplapur,S10-Sharpally.

3.3. Isolation and identification of AMF from the rhizosphere soil of castor

AM fungal species were isolated from rhizosphere soils of castor collected from Koilkonda, Rajapur, Chinnadarpally , Manganur, Velkicherla, Palamur university campus, Velgonda, Molgara, Goplapur, Sharpally in mahabubnagar. In the present study a total of Fifteen AM fungal species were isolated from the rhizosphere soil of castor by wet-sieving technique (11), and identified according to their spore morphology by using manual (38) and taxonomic key 9 (28). Which belonging to 3 genera namely *Acaulospora*, *Glomus*, and *Scutellospora* (Table 3). *Acaulospora* was represented by the highest number of species with 9 species identified, followed by Glomus 5 and *Scutellospora* 1, species. Majority of the AM fungal species belong to *Acaulospora* species. There was awide variation in spore number especially among *Acaulospora* species followed by *Glomus* species. However, the distribution of *Scutellospora* species was very less in all the localities. (9) reported has the *Glomus* was the most dominant isolated mycorrhizal genus with three dominant species *Glomus fasciculatum, G.macrocarpum and G. mosseae.* Earlier reports have also revealed the predominance of the AM fungal genera in the rhizosphere soils of different plant cultivars (12), (14), (25), (39), (43), (21), (16), (35), (36), (37).

Table 3: Presence of Arbuscular mycorrhizal fungi in the rhizosphere soil samples of castor

S no	AM fungi	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
1	*Acaulospora bireticulata,* Rothwell and Trappe	-	+	+	-	-	-	-	+	-	-
2	*Acaulospora delicata,* Walker, Pfeiffer and Bioss	-	+	-	+	+	-	-	-	+	-
3	*Acaulospora foveata, Trappe and Janos*	-	-	-	-	-	-	+	-	-	-
4	*Acaulospora koskei,* Blaszk	+	+	-	-	+	+	-	-	-	+
5	*Acaulospora lacunosa,* Morton	-	-	+	-	-	-	+	-	+	+
6	*Acaulospora laevis,* Gerdemann & Trappe	+	-	-	-	+	+	-	-	-	+
7	*Acaulospora mellea,* Spain & Schenck	+	+	+	-	+	+	+	-	+	-
8	*Acaulospora nicolsonii* Walker, Reed and Sanders	+	+	-	-	+	-	+	-	-	-
9	*Acaulospora spinosa,* Walker and Trappe	-	-	-	-	+	+	-	-	-	-
10	*Glomus aggregatum,* Schenck and Smith emend, Koske	+	+	-	-	+	-	-	-	-	-
11	*Glomus citricola,* Tang and zang	-	-	-	-	-	-	-	-	-	-
12	*Glomus fasciculatum,* Gardemann and Trappe	-	+	-	-	-	-	-	+	-	-
13	*Glomus halonatum* Rose and Trappe	-	+	+	-	-	-	-	+	-	-
14	*Glomus warcupii,* McGee	-	+	-	+	+	-	-	-	+	-
15	*scutellospora coralliodea,* Koske and Walker	-	-	-	-	-	-	+	-	-	-

Soil samples: S1-Koilkonda, S2- Rajapur, S3- Chinnadarpally ,S4-Manganur,S5-Velkicherla,S6-Palamur University campus,S7-Velgonda,S8-Molgara,S9-Goplapur,S10-Sharpally.

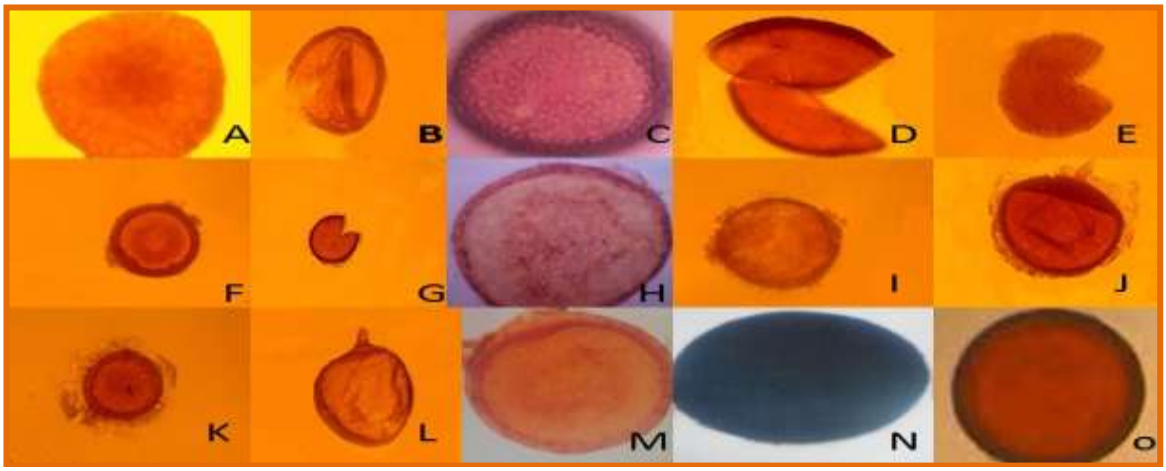

Figure 1: AM Fungi isolated from the rhizosphere samples of castor
A . *Acaulospora bireticulata,* **B.** *Acaulospora delicate,* **C.** *Acaulospora foveata,* **D.** *Acaulospora koskei,* **E.** *Acaulospora lacunose,* **F**. *Acaulospora laevis,* **G.** *Acaulospora mellea,* **H.** *Acaulospora nicolsonii,* **I.** *Acaulospora spinosa,* **J.** *Glomus aggregatum,* **K.** *Glomus citricola,* **L.** *Glomus fasciculatum,* **M.** *Glomus halonatum,* **N.** *Glomus warcupii,* **O.** *scutellospora coralliodea.*

4. Conclusion

Physico-chemical characteristics of the soil showed varied influence on AM fungal distribution and occurrence. Soils with slightly alkaline, medium organic matter and low 'P' content have influenced the AMF distribution, occurrence and quantitative account.

5. Future Scope

The isolates tested positive for supporting growth effects on castor cultivars and will be further exploed in for pot and field experiments to study plant growth, yield, and bio-control ability against (pathogen) on castor.

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