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Symbiotic effectiveness of arbuscular mycorrhizal technology and *Azotobacterization* in citrus nursery production under soil disinfestation and moisture conservation practices

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ABSTRACT

The present study was carried out with the objective to exploit indigenous arbuscular mycorrhizal (AM) fungal symbiosis and *Azotobacterization* on citrus seedlings inoculated under solarized, chemical sterilized and natural soil conditions along with moisture conservation mulch practices. Two potent indigenous species of AM fungi namely, *Glomus fasciculatum* (Thaxter sensu Gerdemann) (AM_1) and *G. macrocarpum* Tul. & Tul. (AM_2), and two strains of *Azotobacter chroococcum* viz., *A. chroococcum* strain-I (AZ_1) and *A. chroococcum* strain-II (AZ_2) were inoculated at nursery stage under four different moisture conservation mulch practices viz., black plastic mulch (BPM), grass mulch (GM), cover crops (CC), green manuring + clean cultivation (Gm + Cc). The effects of AM fungi and *A. chroococcum* strains on mycorrhizal spore population, *Azotobacter* bacterial count, vegetative growth characteristics and soil microbial densities (bacteria, fungi and actinobacteria) were studied. Different AM fungi showed the difference in the effectiveness of inter-species in citrus seedlings. The observations on the individual effect of soil disinfestations and mulch practices on microbial density, percent root colonization, vegetative growth characteristics (plant height, stem diameter, leaf area and total root length) and nutrient content of leaf of the seedlings were also recorded. Inoculation with *G. fasciculatum* and AZ_1 showed greater positive effects in the seedlings compared to *G. macrocarpum* and AZ_2 strain recording highest AM colonization, plant height, stem diameter, leaf area, root length and had the density of soil borne bacteria, fungus and actinobacteria reduced drastically in the solarized soil plots. Solarization increased the maximum daily temperature to 48.5 °C and the average minimum daily soil temperature to 28.8 °C at 5–25 cm depth. It is also noticed that the plants inoculated with *G. fasciculatum* and AZ_1 had improved vegetative growth characteristics, microbial consortium of the rhizosphere soil and nutrient content of leaf N, P, K and Zn under plots of solarization and black plastic mulching compared to chemical sterilized and natural plots under different mulch types. The present findings inferred that the combining soil solarization and inoculation of rhizosphere soil to AM fungi and *A. chroococcum* using black plastic mulching as moisture conservation practice could be considered as an efficient and feasible approach for excellent citrus nursery management under mid-hill rain-fed agro-climatic conditions of Himachal Pradesh.

1. Introduction

Arbuscular mycorrhizal (AM) symbiosis are unique associations formed among more than 80% of all terrestrial plants to assist the host plants with the acquisition of mineral nutrients due to the fine exploration of the rhizosphere by the external hyphae (Harrison, 2005). They are responsible for the most part of water and mineral uptake from natural soils, enhanced tolerance of adverse conditions, and the creation, maintenance and restoration of good soil structure. Besides, another important beneficial microorganism is *Azotobacter chroococcum*, a non-symbiotic, free-living, heterotrophic and aerobic bacterium found worldwide, not predominantly in Indian soils, capable of fixing on an average of 20 kg N ha^{-1} $year^{-1}$ and enhances growth and productivity of fruit crops (Kizilkaya, 2009).

Soil solarization is a hydrothermal, non-pesticidal, ecofriendly and low cost method using high temperatures produced by capturing radiant energy from the sun, achieved by covering (mulching) soil with transparent polyethylene sheets to trap solar radiation to heat for 4–6 weeks during a hot period of the year when the

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soil will receive the most direct sunlight. Solarization also initiates the changes in the physico-chemical and biological properties of soil that improve plant growth, development, quality and yield (Freeman and Katan, 1988). Plants often grow faster and produce yields of increased quantity and quality (size and appearance) when grown in solarized compared to non-treated soils. A partial explanation may be found in a combination of mechanisms. First, because major pathogens and pests are controlled, it is likely that minor pathogens and pests are also controlled. Second, the mode of action of solarization practice liberates vapor, liquid N compounds and increases the concentration of reduced N (NH_4–N) which then nitrify (convert to NO_3–N) to provide NO_3 for increased crop growth response.

According to FAO, 140 countries have produced citrus fruits in the world. In India, citrus is grown in more than 6, 18,000 ha area and ranks sixth in the production in the world. Healthy nursery is pre-requisite for establishing a good orchard with potential of high productivity. The occurrence and distribution of AM fungi in citrus was reported for the first time in 1933 (Reed and Fremount, 1935). There is increasing evidence that AM fungi affect citrus root growth independent of phosphorus nutrition (Peng et al., 1993). The presence of root hairs has been found in many citrus species, but root hairs of citrus are relatively shorter than those of other tree species. Citrus depends on AM fungi which can improve the supply of water and nutrients to the host plant (Wu and Zou, 2009). The inoculation of seedlings at nursery stage helps plant to establish at orchard site as it carries the AM fungal colonization to the site of plantation. Moreover, the feasibility of inoculation of AM fungal and *Azotobacter* is much greater for citrus nursery grown in field conditions. Due to the economic importance of citrus industry in the world and relatively its higher dependence on mycorrhizal fungi, the nursery inoculation has received great attention. Much of the research has documented the effect of dual inoculation of AM fungi and *A. chroococcum* to enhance plant growth particularly in fruit crops (Sharma and Kumar, 2008; Sharma et al., 2011a). The aims of this work were: (1) isolation and identification of indigenous AM fungal species and *A. chroococcum* strains associated with local citrus orchards being grown in the Shiwalik hill ranges of north-western Himalayan region of India, (2) evaluation of the comparative potential of indigenous AM fungal symbiosis and *Azotobacterization* on plant growth, soil biological properties and leaf nutrient content of citrus seedlings under different soil disinfestations and moisture conservation mulch practices in order to ascertain the eventual usefulness of such technique for sustainable citrus nursery production.

2. Materials and methods

2.1. Site selection and sampling

The study was carried out in the Department of Fruit Science and Experimental Research Stations of the Dr. Y S Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India. Forty-five citrus orchards of uniform age group (15 years) were selected purposely for the isolation, characterization and identification of indigenous AM fungal species and *A. chroococcum* strains associated with local citrus orchards. These places experienced warm weather during April–June with average temperature ranges between 41–21 °C and 27–8 °C during the winters (November–February). The monsoon arrives in the beginning of July up to month of September. In each orchard, 10 uniform and healthy trees were selected for the observations using standard procedures. Soil samples were collected between July and August to quantify the indigenous soil microbial community. Composite samples at 30 cm depth, weighing up to 1 kg were collected, taken to the laboratory in polythene bags and stored in refrigerator at 4 °C. The samples were prepared and processed according to standard procedures for further microbial assessment.

2.2. Layout of experiment

The experimental unit comprised 4 rows 0.5 m apart and 8 m long with 30 cm interval. The soil was clay loam in texture having 23% sand, 32.6% silt and 40.4% clay content with near neutral pH (6.7) and free from salinity (0.09 dS m^{-1}). The soil contained organic carbon (0.38%), available N (1.39 g kg^{-1}), available P ($NaHCO_3$ extractable – 68 mg kg^{-1}), available K (116 mg kg^{-1}) and DTPA extractable available Zn (4.4 mg kg^{-1}). The experimental soil was collected from the field that was kept under fallow to avoid any indigenous AM fungal propagules in the plots.

2.3. Isolation, characterization and identification of AM fungi and A. chroococcum

AM fungal spores were isolated from rhizosphere soils using wet sieving and decanting method according to Gerdemann and Nicolson (1963). 100 g of soil was mixed with 1000 ml of water and wet sieved through 450, 250, 150, 100, and 45 μm mesh sieves. The sieved soil on each mesh was centrifuged at 2000 rpm for 5 min and the floating particles removed. Spores were recovered on filter paper and the quantification of spores was carried out under microscope. Diagnostic slides with spores were prepared using polyvinyl alcohol lactoglycerol as mountant. Spores were examined and identified up to species level according to spore morphology and wall characteristics (Hall and Fish, 1979; Trappe, 1982). Twelve AM fungal species namely, *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe, *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *G. macrocarpum* Tul. & Tul., *G. epigeaum* Daniels & Trappe, *G. magnicaule* Hall, *G. sinuosum* (Gerdemann & Bakshi) Almeida & Schenck, *Gigaspora albida* Schenck & Smith, *G. heterogamma* Gerdemann & Trappe, *G. margarita* Trappe, *Entrophospora spp.* Ames & Schneider, *Scutellospora pellucida* (Ferr. & Herr.) Walker & Scander and *S. calospora* (Nicol. & Gerd.) Walker & Scander were isolated, identified and characterized. In most of the orchards species like *G. fasciculatum, G. mosseae* and *G. macrocarpum* were dominant. Similarly, two strains of *A. chroococcum* viz., *Azotobacter* strain-I (AZ_1) and *Azotobacter* strain-II (AZ_2) were isolated by serial dilution technique from the rhizosphere soil. Out of the each sample, 10 g of soil was drawn and serial dilutions were made up to 10^{-6} in sterile saline. One ml of 10^{-6} dilution was taken on sterilized Jenson's media (sucrose, 20 g; dipotassium phosphate, 1 g; magnesium sulphate, 0.5 g; NaCl, 0.5 g; ferrous sulphate, 0.1 g; sodium molybdate, 0.005 g; $CaCO_3$, 2 g; agar, 15 g and distilled water, 1000 ml) for isolation of pure and viable bacterial culture. Taxonomic identification of the strains was done according to Bergey's Manual of Systematic Bacteriology (Tchan, 1984).

2.4. Preparation of inocula

Two potent indigenous AM fungal species viz., *Glomus fasciculatum* (Thaxter sensu Gerdemann) and *Glomus macrocarpum* Tul. & Tul. were selected as inocula for field experimentation. AM fungal culture was multiplied in the autoclaved sterilized soil for 6 months in earthen pots on green gram (*Vigna radiata* L. (Wilczek)) as host plant. The host plants were uprooted and the roots were chopped into pieces. The inocula contained spores, colonized chopped root segments and mycelia in the pot cultured soil. Similarly, the charcoal based culture slurry of *A. chroococcum* strains (AZ_1 and AZ_2) was also prepared. The culture carrier for each strain was prepared