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# Influence of inoculation of Efficient AM fungi (*Glomus fasciculatum*) against wilt of tomato caused by *Sclerotium rolfsii*

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**Abstract**
Mycorrhizae are highly evolved symbiotic association between roots of most vascular plants and certain specialized soil fungi that colonize the cortical tissues of roots during periods of active plant growth both in natural environment and in cultivation. The present study is aimed to assess the association of arbuscular mycorrhizal fungi in tomato crop in terms of nutrition, growth and suppression of root infecting pathogens, along with arbuscular mycorrhizal (AM) fungal population density in the rhizosphere soils. Sclerotial wilt is one of the major causes for yield loss in tomato crop. Hence, a survey was conducted in different villages in the sclerotial wilt on standing crop during Nov- 2016 to Feb 2017. Later, per cent disease incidence was calculated to finalize the villages for demonstration trials. The efficient AM fungal inocula were mass produced as axenic cultures in a glasshouse and have been kept ready for their use for field trials in farmers fields during Kharif 2017-18. The field trials were conducted in farmers fields at Yadvada & Narendra (Dharwad dist) and Gundagatti and Chanahalli (Haveri Dist) in tomato crop to demonstrate the importance of efficient AM fungi on plant growth, yield, nutrient status (Major and minor) in soil & plant in addition to disease incidence. The present investigate for inoculation of AM fungi in tomato significantly increased spore count and per cent root colonization as compared to uninoculated nursery bed at 30 DAS and 45 DAS and in field at 60 DAT. At harvest stage, the AM spore counts were 188 and 186 in Yaadavada and Gundagatti villages per 25 g of soil while the per cent root colonization was 93 both in Narendra and Gundagatti respectively in the inoculated tomato fields. The number of plants wilted at vegetative stage ranged from 1 to 2 plant wilt in the inoculated field as compared to uninoculated field which was 9 to 27 in tomato. The number of pickings and the fruit yield increased in the inoculated fields of tomato (0.5 to 1.2 kg/plant) as compared to uninoculated control in all the villages.

**Keywords:** Influence, *Glomus fasciculatum*, tomato caused, *Sclerotium rolfsii*

## Introduction

Mycorrhizae are highly evolved and have non-pathogenic symbiotic association between roots of most vascular plants and certain specialized soil fungi that colonize the cortical tissues of roots during periods of active plant growth both in natural environment and in cultivation. Arbuscular Mycorrhizal (AM) fungi are an important component of the terrestrial communities. They are active living components of the soil and have some properties like those of roots and some like those of microorganisms. Mycorrhizal associations may influence both biodiversity (Bever *et al.*, 2002) [4] and biogeochemistry (Hoffland *et al.*, 2004) [7]. The mycorrhizal fungi obtain organic nutrition (carbohydrates, vitamins, amino acids and plant growth substances) from plants and also perfect ecological niche that is necessary for fungal growth and development including the completion of the sexual cycle. They benefit the host by improving soil fertility and by producing enzymes for absorption, translocation and assimilation of major mineral ions like phosphates and inorganic nitrogen, and many number of genes required for symbiosis. Phosphorous (P) is one of the most essential mineral nutrients for plant growth and development as it plays crucial role related to metabolism, energy transfer and various other regulatory functions in plants (Schachtman *et al.*, 1998) [15]. Alternatively, plants associates in symbiotic relationship with beneficial soil microorganisms, predominantly preferring Arbuscular Mycorrhizal (AM) fungi as a mutualistic partner (Karandashov and Bucher, 2005) [10]. It is very well established that AM fungi help plants to withstand different stresses and to ward off soil borne root infecting pathogens. Thus AM fungi safeguard plant health and in turn promote crop yield (Bethlenfalvay and Linderman, 1992, Barea and Jeffries, 1995) [5, 3]. Though many scientists carried out research on this aspect, a little is known on underlying mechanisms (Carling *et. al.,* 1989) [6]. All these studies however clearly revealed that AM fungi can alleviate or mitigate the severity of the disease caused by root infecting pathogen (Bagyaraj, 1984: Sreenivasa, 2008) [2]. Pre colonization of AM fungi was found to be effective in reducing damage caused by pathogen in citrus (Schenck *et. al.,* 1977) [13]. Similar observations have been made on *Rhizoctonia* (Iqbal *et. al.,* 1977) [8] and *Sclerotium rolfsii* (Sreenivasa, 1992) [14].

Vegetables are considered as essential for well balanced diets since they supply vitamins, minerals, dietary fiber and phytochemicals. In the daily diet, vegetables have been strongly associated with improvement of gastro-intestinal health, good vision and reduced risk of heart disease, stroke, chronic diseases such as diabetes and some forms of cancer. Among the vegetables, tomato is the second most consumed and widely grown vegetable in the world after potato. Tomato is popular in fresh and in many processed forms (e.g., ketchup, canned whole or in pieces, puree, sauce, soup and juice). The ripe fruits are good In Karnataka, tomato occupies 63.73 thousand ha with a production of 2.14 million tonnes having productivity of 33.55 tonnes per hectare (Anon., 2017) [1]. Tomato requires a relatively cool, dry climate for high yield and premium quality. Eventhough tomato is a perennial plant, it is grown as a warm-season annual crop due to its sensitiveness to frost. Tomato can be grown both in open field and protected culture. The latter is used when the outdoor environmental conditions are not favourable for its growth. However, tomato is adapted to a wide range of climatic conditions from temperate to hot and humid tropic (Nicola *et al*., 2009) [12]. Among the pathogens that affect the tomato crop, soil-borne fungal pathogens, including species belonging to *Sclerotium*, *Fusarium*, *Pythium*, *Rhizoctonia*, and *Verticillium* genera causing the root rot or damping- off and wilt which affect the quality with yield reduction. Many scientists have reported the extent of losses caused by different soil-borne pathogens. The losses range from 10 to 90 per cent depending upon the stage of occurence and other factors (Mandal *et al*., 2017 and Kumar and Sharma, 2015) [11, 9]. Some of these pathogens are particularly challenging because they often survive in soil for many years through different survival mechanisms and structures (Steven, 2003) [16]. To manage such diseases, farmers presently use different fungicides with application schedules utilizing two or more different fungicide groups or fungicide formulations containing two different chemical groups atleast for 8-10 times in one growing season which has resulted in several undesirable effects like pesticide pollution, fungicide resistance, elimination of beneficial flora and fauna, environmental pollution and human health hazards (Kumar and Sharma, 2015) [9]. So integrated disease management where biological control is one practice is becoming key consideration for soil- borne diseases. Use of AM fungi as biocontrol agent may open up new area of research in plant protection in the recent period under various agro-climatic situations of India in general and Karnataka in particular.

## Material and Method

Survey was conducted following villages in Dharwad, Haveri and Belgaum districts of Karnataka to record the per cent disease incidence (PDI) in Tomato caused by *Sclerotium rolfsii*. GPS (Ground Positioning System) was used for survey work. The incidence of Sclerotial wilt in chilli and tomato were recorded in farmers fields in the above listed villages and it was calculated using the following formula

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plant observed}} \times 100$$

## Mass production of efficient Arbuscular Mycorrhizal (AM) fungi

The efficient AM fungi for chilli and tomato crops developed by the Principal Investigator and released by UAS Dharwad, Tomato (*Glomus fasciculatum*).

Both these efficient AM fungal inocula were mass produced as axenic cultures in a glasshouse and have been kept ready for their use in field trials in farmers fields during Kharif 2017-18.

The field trials were conducted in farmers fields at Yadvada & Narendra (Dharwad dist) and Gundagatti and Chanahalli (Haveri Dist) in both chilli and tomato crops to demonstrate the importance of efficient AM fungi on plant growth, yield, nutrient status (Major and minor) in soil & plant in addition to disease incidence.

## Results and Discussion

A survey was conducted in different villages in the sclerotial wilt on standing crop during 2016- 2017. The per cent disease incidence ranged between 6.32 to 9.47 in tomato while. Since this is a soil borne polyphagous pathogen, it will spread and cause severe yield losses at later stages of the crop growth (Table 1).

**Table 1:** Disease incidence of Tomato wilt in Dharwad and Haveri districts

| District | Village | Altitude | Latitude | Per cent disease incidence |
|---|---|---|---|---|
| Haveri | Hullathi | N $14^0$ 39.169 | E $075^0$ 38.432 | 8.19 |
| | Midur | N $14^0$ 41.464 | E $075^0$ 40.142 | 7.00 |
| | Kudrihal | N $14^0$ 30.586 | E $075^0$ 32.605 | 7.95 |
| | Itagi | N $14^0$ 33.111 | E $075^0$ 40.975 | 8.88 |
| | Chalageri | N $14^0$ 55.732 | E $075^0$ 71.530 | 8.97 |
| | Kamadod | N $14^0$ 57.049 | E $075^0$ 67.395 | 6.32 |
| | Heremoraba | N $14^0$ 34.730 | E $075^0$ 42.680 | 8.88 |
| Dharwad | Hangarki | N $15^0$ 35.385 | E $074^0$ 55.059 | 9.18 |
| | Kotabagi | N $15^0$ 36.663 | E $074^0$ 58.279 | 8.64 |
| | Kardigudda | N $15^0$ 34.082 | E $075^0$ 02.034 | 6.32 |
| | Yadvada | N $15^0$ 33.446 | E $074^0$ 57.054 | 8.16 |
| | Kumbapur | N $15^0$ 49.184 | E $074^0$ 96.572 | 7.95 |
| | Chikkamalingawad | N $15^0$ 48.603 | E $074^0$ 95.434 | 9.47 |
| | Garag | N $15^0$ 34.456 | E $074^0$ 56.908 | 7.21 |
| | Narendra | N $15^0$ 31.231 | E $074^0$ 58.857 | 6.81 |

### Symptoms

*Sclerotium rolfsii* primarily attacks host stems, although it may infect any part of a plant under favorable environmental conditions including roots, fruits, petioles, leaves, and flowers. The preliminary signs of infection, though usually undetectable, are dark-brown lesions on the stem at or just beneath the soil level; the first visible symptoms are progressive yellowing and wilting of the leaves (drooping). Following this, the fungus produces abundant white, fluffy mycelium on the infected tissues and the soil. *Sclerotia* of relative uniform size are produced on the mycelium, roundish and white when immature, then becoming dark brown to black. Mature sclerotia resemble mustard seed. The fungus occasionally produces basidiospores (the sexual stage of reproduction) at the margins of lesions and under humid conditions, though this form is not common (Fig 1).

The seedlings are very susceptible and die quickly once they become infected. The older plants that have formed woody tissue are gradually girdled by lesions and eventually die. The invaded tissues are pale brown and soft, but not watery.

### Effect of AM inoculation on spore count, per cent root colonization and wilting at different stages and yield in tomato

In general, the mycorrhizal parameters were significantly highest in the inoculated plots as compared to the uninoculated plots in both the crops in all 4 villages. The highest spore count and per cent root colonization was observed in the fields inoculated with *Glomus fasciculatum*. Among four villages, the higher spore count and per cent root colonization was observed in the inoculated fields of Yaadavada followed by Narendra in Dharwad district and Gundagatti followed by Channahalli in Haveri district. The inoculation of AM fungi in tomato significantly increased spore count and per cent root colonization as compared to uninoculated nursery bed at 30 DAS (days after sowing) and 45 DAS and in field at 60 DAT (days after transplanting) (Table 2).

The number of plants wilted at vegetative stage ranged from 1 to 2 plant wilt in the inoculated field as compared to uninoculated field which was 9 to 27 in tomato plants in uninoculated fields. (Table 3).

At harvest stage, the AM spore counts were 188 and 186 in Yaadavada and Gundagatti villages per 25 g of soil while the per cent root colonization was 92 and 93 respectively in the inoculated tomato fields The number of pickings and the fruit yield increased in the inoculated fields in tomato (0.5 to 1.2 kg/plant) as compared to uninoculated control in all the villages (Table 4).

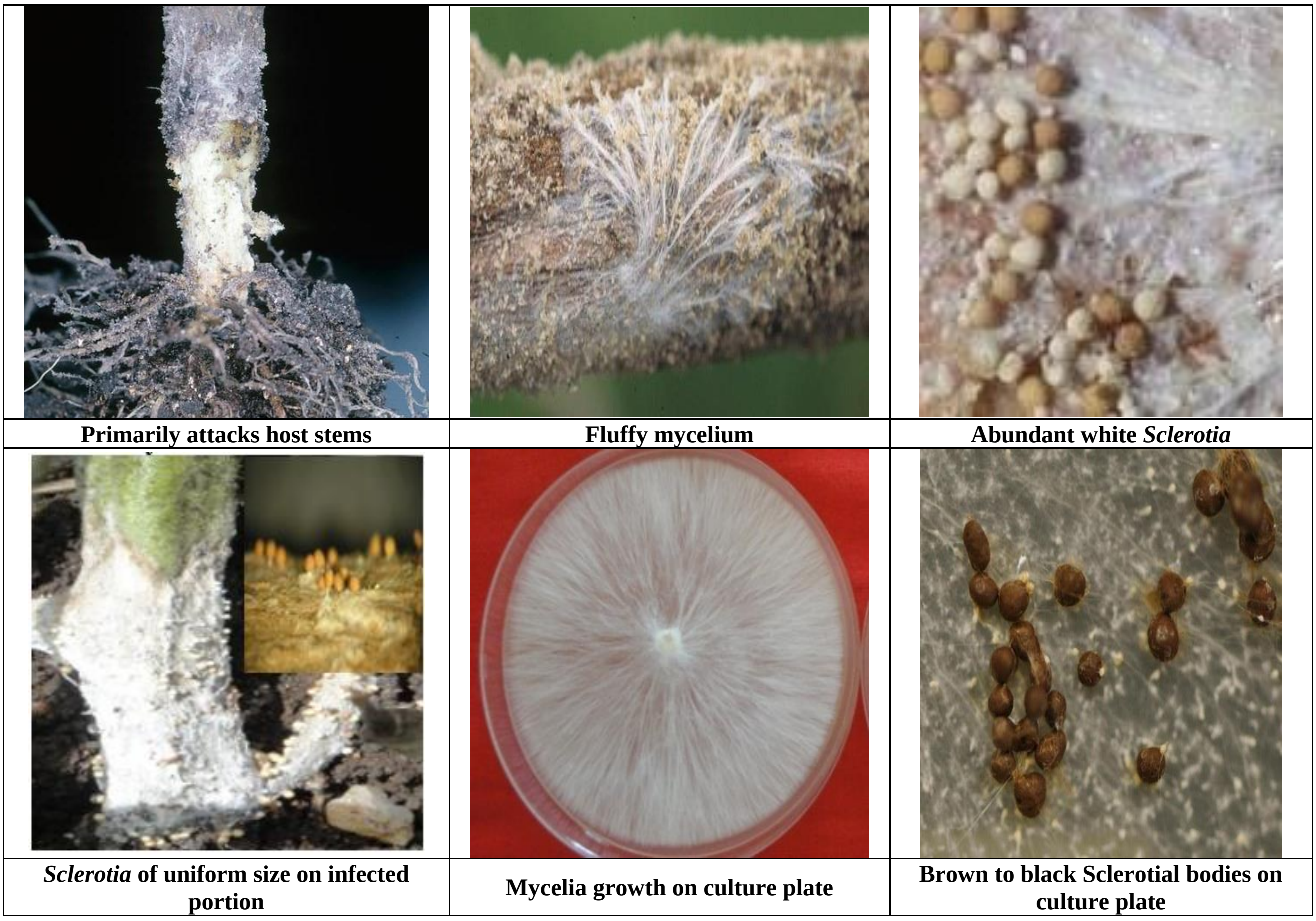


**Fig 1:** Symptoms of *Sclerotium rolfsii*

**Table 2:** Mycorrhizal spore count and per cent root colonization in tomato in different villages at 30, 45 DAS and 60 DAT

| District | Villages | AM spore count at 30 DAS (Nursery Bed) per 25 g of soil | | Per cent root colonization at 30 DAS (Nursery Bed) | | AM spore count at 45 DAS (Nursery Bed) per 25 g of soil | | Per cent root colonization 45 DAS (Nursery Bed) | | AM spore count at 60 DAT per 25 g of soil | | Per cent root colonization at 60 DAT | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Uninoculated | Inoculated | Uninoculated | Inoculated | Uninoculated | Inoculated | Uninoculated | Inoculated | Uninoculated | Inoculated | Uninoc-ulated | Inoc-ulated |
| Dharwad | Yaadavada | 101 | 161 | 22 | 51 | 131 | 199 | 34 | 66 | 48 | 63 | 43 | 72 |
| | Narendra | 86 | 138 | 21 | 48 | 76 | 140 | 25 | 54 | 42 | 57 | 33 | 69 |
| Haveri | Gundagatti | 62 | 94 | 19 | 52 | 135 | 188 | 30 | 65 | 58 | 73 | 32 | 73 |
| | Channahalli | 58 | 91 | 21 | 40 | 87 | 112 | 28 | 52 | 48 | 59 | 30 | 60 |

DAS: Days after showing DAT: Days after transplanting

**Table 3:** Per cent wilt in tomato at peak vegetative and 3rd harvest stage

| District | Villages | Per cent wilt at vegetative Stage (50 DAT) | | Per cent wilt at 3rd harvest stage (130 DAT) | |
|---|---|---|---|---|---|
| | | Uninoculated | Inoculated | Uninoculated | Inoculated |
| Dharwad | Yaadavada | 12 | 2 | 15 | 3 |
| | Narendra | 21 | 2 | 35 | 4 |
| Haveri | Gundagatti | 9 | 1 | 14 | 3 |
| | Channahalli | 27 | 2 | 32 | 5 |

DAT: Days after transplanting

**Table 4:** Mycorrhizal spore count, per cent root colonization and fruit yield in tomato at final harvest

| District | Villages | AM spore count at final harvest per 25 g of soil | | Per cent root colonization at final harvest | | Yield per plant (kg) | |
|---|---|---|---|---|---|---|---|
| | | Uninoculated | Inoculated | Uninoculated | Inoculated | Uninoculated | Inoculated |
| Dharwad | Yaadavada | 96 | 188 | 58 | 92 | 3.22 | 4.45 |
| | Narendra | 73 | 166 | 40 | 93 | 3.10 | 4.32 |
| Haveri | Gundagatti | 94 | 186 | 50 | 93 | 3.53 | 4.05 |
| | Channahalli | 79 | 158 | 43 | 84 | 3.19 | 3.65 |

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