Physiological changes in groundnut (*Arachis hypogaea* L.) Plantsinoculated with *Sclerotium rolfsii* and*Trichoderma* species

Saraswathi Maddu and Jaya Madhuri Ravuri

Department of Applied Microbiology

Sri Padmavati Mahila Visvavidyalayam

Tirupati-517502

Abstract: Groundnut contributes immensely to food security, income generation and maintenance of the environment for millions of small-scale farmers in India. Stem rot of groundnut caused by *Sclerotium rolfsii* is a Stem rot of groundnut caused by *Sclerotium rolfsii* is found throughout groundnut producing areas of the world and causes the severe damage during any stage of the crop growth with greatest yield losses up to 80% in severe conditions. In this study, the effect of Trichoderma species on resistance induction in groundnut plants was studied in vivo in pot culture experiments by maintaining three treatments i.e., Tt1- plants treated with neither pathogen nor antagonist, Tt2- plants inoculated with S. rolfsii only and Tt3- Plants inoculated with *S. rolfsii* + *Trichoderma*. Results obtained showed that *Trichoderma* species have significantly reduced the disease incidence and induced the growth of the plant.

Keywords: Groundnut, Induced systemic resistance, *Sclerotium rolfsii,* stem rot, *Trichoderma*

1. Introduction

Among the soil-borne fungal diseases of groundnut, stem rot or southern blight caused by *S.rolfsii* (Doidge and Bottomly, 1931) is a potential threat to groundnut production and is of considerable economic significance for groundnut grown under irrigated conditions [5, 10]. *S.rolfsii* causes severe damage during any stage of crop growth [2, 5] and attacks all parts of the plant but stem infection is the most common and serious and yield losses over 25% have been reported [8]. The fungi from the genus *Trichoderma* as biocontrol agents is due to their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanism. Different mechanisms have been suggested as being responsible for their biocontrol which include mycoparasitism, antibiosis, competition for nutrients and space. Besides, the fungus can colonize plant roots leading to induce growth and nutrient adsorption [6]. Moreover, *Trichoderma* spp. was used not only for control plant disease, but also stimulated nutritional adsorption [6].

Materials and Methods

2.1 Isolation of pathogen:

Cultured *S. rolfsii* strain was isolated on PDA from the plants showing stem rot or southern blight symptoms of groundnut from the area of Sadhanavaripalem, Chittoor District in Kharif season on PDA. The pure culture of the fungus was obtained and maintained on PDA for further study. The stock culture was maintained on PDA slants in a refrigerator and subcultured every two months.

2.2 Preparation of *S. rolfsii*: Oatmeal-sand culture of the test fungus was used for inoculation. This oatmeal-sand culture of *S.rolfsii* was thoroughly mixed with sterilized soil at 7 level (w/w- 93 g sterilized soil + 7 g inoculum). This inoculum-soil mixture was then distributed in 12" diameter earthenware pots and left undistributed for two days. After this period, one week old seedlings grown in seed pans were lifted carefully without causing much damage to the root system and transplanted into the pots. They were watered on alternate days and kept in an open atmosphere.

2.3 Isolation of *Trichoderma* Species

Trichoderma species was isolated from the rhizospheric soil of groundnut fields from area of Chittoor District by serial dilution plate technique using modified *Trichoderma* specific medium. The isolate was maintained on PDA slants at 40C for further use.

2.4 Preparation of *Trichoderma* culture: Oatmeal-sand culture of *Trichoderma* was used for inoculation. This oatmeal-sand culture of *Trichoderama* was thoroughly mixed with sterilized soil at 5% level.

2.5 Plant Material: *S. rolfsii* susceptible TMV-2 variety groundnut seeds were selected and surface disinfected by 0.5% mercuric chloride for 3 min, followed by extensive rinsing in sterile distilled water. Then the seeds were sown in earthenware pots filled with previously sterilized soil. Ten old seedlings were used for inoculation purposes.

2.6 Treatment of groundnut plants with *Trichoderma* and *S. rolfsii*:

In this essay, four treatments were performed. In the first one, antagonists were added to the soil one week before the addition of pathogen, in the second, antagonists and the pathogen were applied at the same time, in the third treatment, antagonists were added to the soil one week after addition of the pathogen and in the fourth treatment plants were treated neither with antagonist nor with pathogen. Three sets were maintained for each treatment with 10 seedlings per pot. All the treatments were incubated at identical conditions and observed for the disease incidence and physiological changes in the above four treatments. The disease incidence was calculated by following the formula:

$$PDI = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \times 100$$

3 Results and Discussion

Trichoderma species have significantly reduced the disease incidence especially when they were applied one week before inoculation with the pathogen (Tab 1). Plants treated with antagonist one week before inoculation with the pathogen, appeared healthy and with no wilting symptoms (Fig. 1). Likewise, observations of the root length and shoot length in four treatments showed that the in T1 and T2 treatments root length and shoot length had a better development compared to the T3 and T4 treatments (Fig 2).

Table 1: Effect of four treatments on disease incidence, root and shoot length of groundnut plant

S.No	Treatments	% of disease incidence	Shoot length(cm)	Root length(cm)
1	T1	0	34.8	15.6
2	T2	10	30.5	13.8
3	T3	70	11.4	6.3
4	T4	0	18.7	7.5

Fig 1: Comparison between *Trichoderma* treated and untreated groundnut plants

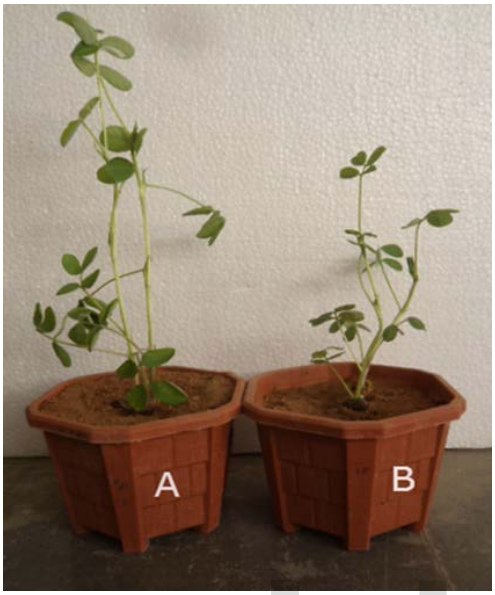

Fig 3: Comparison between shoot and root length of groundnut plants treated with *Trichoderma* and *S. rolfsii*

Simultaneous application of antagonist and pathogen to groundnut plants has also significantly reduced disease incidence. However, applying antagonist one week after the inoculation with the pathogen has low effect on disease incidence and there is non-significant difference between treated and untreated groundnut plants. Besides the use of potential microbial antagonists [4] and beneficial microorganisms such as plant growth-promoting rhizobacteria [7, 9] to control pathogen populations, the possibility of stimulating a plant's immune system by using non-pathogenic organisms has opened novel areas for plant disease management.

The numerous benefits of these fungi on plant growth and resistance must be reflected in changes in the physiology of plants [6]. *Trichoderma* spp. has many effects on plant physiology, making it challenging to isolate the interactions associated with triggering plant resistance. *Trichoderma* species have been shown to reduce disease incidence of several pathogens by stimulating vegetative growth and root development of the treated plants (1, 11). Reducing disease incidence by antagonists was the object of several studies. Reduction of disease incidence was more important when the antagonist was added to the nutrient solution 10 days before inoculation with the pathogen.

References:

1. Ayed, F., Daami-Remadi, M., Jabnoun- Khiareddine, H., and El Mahjoub, M. 2006. Potato Vascular Fusarium wilt in Tunisia: Incidence and biocontrol by *Trichoderma* spp. Plant Pathology Journal 5: 92-98.

2. Cilliers, A.J., Herselman, L. and Pretorius, Z.A.2000. Genetic viability within and among mycelial compat-

ibility groups of *Sclerotium rolfsii* in South Africa. *Phytopathology* 90 (9): 1026-1031.

3. Doidge, E.M. and Bottomly, A.M. 1931. A revised list of plant diseases occurring in South Africa. *Bot. Surv. S. Afr. Mem*.11.

4. *Duijff, B. J., Pouhair, D., Olivan, C., Alabouvette, C., and Lemanceau, P. 1998. Implication of systemic induced resistance in suppression of Fusarium wilt of tomato by Pseudomonas fluorescens WC417r and non -pathogenic Fusarium oxysporum Fo47. European Journal of Plant Pathology 104: 903- 910.*

5. Ganesan, S., Ganesh, K.R. and Sekar, R. 2007. Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.*)* using *Rhizobium* and *Trichoderma harzianum* (ITCC-4572). *Turk. J. Agric. For* 31: 103-108.

6. Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Larito, M. 2004. *Trichoderma* species- Opportunistic, avirulent plant symbionts. *Nature Reviews* 2: 43-56.

7. *Liu, L., Kloepper, J. W., and Tuzun, S. 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth promoting rhizobacteria. Phytopathology 85: 695-698.*

8. Mayee, C.D. and Datar, V.V. 1988. Diseases of groundnut in the tropics. *Review of Tropical Plant Pathology* 5: 169-198.

9. Pieterse, C. M. J., Van Pelt, J. A., Van Wees, S. C. M., Ton, J., Léon-Kloosterziel, K. M., Keurentjes, J. J. B., Verhagen, B. W. M., Knoester, M., Van der Sluis, I., Bakker, P. A. H. M., and Van Loon, L. C. 2001. Rhizobacteria- mediated induced systemic resistance: triggering, signaling and expression. European Journal of Plant Pathology 107: 51-61.

10. Tiwari, R.K.S., Chandravanshi, S.S. and Ojha, B.M. 2004. Efficacy of extracts of medicinal plants species on growth of *Sclerotium rolfsii* root rot in tomato. *Journal of Mycology and Plant Pathology* 34(2): 461-464.

11. Yedidia, I., Benhamou, N., and Chet, I. 1999. Induction of defense responses in cucumber plants (Cucumis sativus L.) by biocontrol agent Trichoderma harzianum . Applied and Environmental Microbiology 65: 1061-1070.