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Short Communication

ANTAGONISTIC ACTIVITY OF RHIZOSPHERE FUNGI AGAINST EARLY BLIGHT OF TOMATO CAUSED BY *ALTERNARIA ALTERNATA*

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ABSTRACT

Eight fungi were isolated from rhizosphere soil of tomato plants and their antagonistic properties were studied *in vitro* against early blight pathogen, *Alternaria alternata* f. sp. *Lycopersici* (AAL). Different grades of colony interaction were observed in dual culture between AAL and antagonistic fungi inhibiting fungal growth. *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus*, *Curvularia* sp, *Fusarium solani, Penicillium*sp and *Trichoderma harzianum* showed inhibitory effect against AAL, in which maximum inhibition was observed by *Trichoderma harzianum* (63%) and significantly least effect was seen by *Fusarium solani* (34%). Further investigation on their bio efficacy, plant growth promotion activity under field condition and mechanism of action against the pathogens can be carried out which helps in complete understanding and usage of rhizosphere microorganism as effective bioagents in plant disease management.

Keywords: Tomato, *Alternaria alternata,* Biocontrol, *Trichoderma harzianum*

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most popular and important commercial solanaceous vegetable crop grown all over the world. It is an excellent source of various micronutrients and antioxidants [1]. Tomato contains 95% of water, 0.07% calcium all of which have great importance in the metabolic activities of humans. It provides a balance of vitamin A, C, E and D needed to maintain good health [2]. It is native to South America and is widely cultivated in 140 countries of the world. In India, the major tomato producing states are Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and West Bengal [3]. And it has wider coverage in comparison to other vegetables. It is estimated that it has an annual production of 18,732 mt with 774 thousand ha area [4]. More than 800 million people in developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases [5]. Plant disease causes the greatest impact with regard to crop production losses either foliage or post harvested losses of fruits and vegetables through the decay resulted from fungal plant pathogen [6].

Tomato is found to suffer from a variety of diseases caused by fungi, bacteria, viruses and nematodes. The important diseases are damping off, early blight, late blight, *Fusarium* wilt, *Verticillium* wilt, bacterial wilt and tomato mosaic virus. Among the diseases early blight caused by *Alternaria* specie is one of the most yield limiting factors in India [7]. The survival of the pathogen mainly in the soil and penetrates into the plant through the root system. The causal organism is airborne and soil inhabiting and is responsible for late blight, seedling collar rot and fruit rot of tomato [8]. This disease causes direct loss by the infection of fruits and indirect loss by reducing plant vigour. The early blight was the most catastrophic disease incurring loss at pre- and post-harvest stages causing 35-37% reduction in yield [9].

The effective management of the disease could be through cultural practices, chemicals, biological control and use of resistant variety. Application of fungicides and botanicals against early blight has been reported in India by various workers. Unplanned and wide use of fungicides often leads to serious environmental problems besides affecting the health of the users and consumers. So, it is necessary to minimize the use of chemical for controlling the disease [10].

Antagonism refers to the action of any organism that suppresses or interfere the normal growth and activity of a plant pathogen, such as the main parts of bacteria or fungi. These organisms can be used as fast control and are referred to as biological control agents. Many soil microorganisms are antagonistic. They secrete a potent

enzyme which destroys other cell by digesting their cell walls and degrade the cellular material as well as released protoplasmic material serves as a nutrient for the inhibitor organism, for example *Aspergillus* has an antagonistic effect on *Penicillium* and *Cladosporium*. *Trichoderma* has an effect on actinomycetes. *Pseudomonas* shows antagonism on *Cladosporium* [11]. The aim of the present work is to assess the antagonistic activity of fungal organisms against early blight of tomato caused by *Alternaria alternata* f. sp. *Lycopersici* (AAL).

2. MATERIALS AND METHODS

2.1. Collection and isolation of phytopathogenic fungi

Infected tomato leaf materials were collected in sterile polythene bags from the field of Madahalli village, Nanjangud Taluk, Mysore District, during December 2018. The collected infected parts were cut into small pieces, and then treated with 70% ethanol, rinsed 3-5 times with sterile distilled water. All the glass wares used for the experiment were properly washed, dried and sterilized in the oven at 110℃ for one hour. The entire working surface was also disinfected with ethanol to reduce contamination. Potato Dextrose Agar (PDA) was prepared and poured aseptically into petri dishes. The sterilized infected parts were transferred to sterilize petri plates using sterile forceps and incubated for 5-7 days at 27°C for the growth of fungi with sporulation [12].

2.2. Identification of plant pathogen

The colonies that developed were sub cultured repeatedly on PDA to obtain a pure culture. Thin smear of the mycelia was made on a glass slide with sterile inoculating needle and stained with a drop of lactophenol cotton blue solution and covered with clean cover slip then viewed microscopically. The fungal pathogen was identified on the basis of morphological features like colony formation, mycelial colour, shape and size of the spores or conidia etc. Fungal identification was confirmed with the aid of books and manuals [13, 14].

2.3. Isolation of rhizosphere fungi

The soil sample was collected from the 15cm depths with the help of cork borer. Soil was emptied into sterilized polyester bags. Rhizosphere fungi were collected using serial dilution method. In this method, 1 gm of soil sample was mixed to 10 ml sterile distilled water, shaken vigorously, it is known as stock solution. From the stock solution 1 ml solution is transferred to 9 ml Sterile distilled water aseptically, shaken vigorously, this is a solution of 10^{-1} dilution. This step is repeated 9 more times to make the dilution till 10^{-10}. 0.1 ml of each solution is transferred aseptically to each plate containing potato dextrose agar and spread with spreader on the surface of the media. The plates are incubated for 5-7 days at room temperature [15].

2.4. Identification of the Soil Fungi

Identification of the fungal species was based on morphological characteristics of the colony and microscopic examinations. The colony growth, which includes the length and width of the colony, the presence or absence of aerial mycelium, and colour, wrinkles furrows and any other pigment production, basis of shape and size of conidiophores, conidial/spore arrangement and sporulation are identified with the help of identification keys and standard monographs [16].

2.5. Antagonistic activity of fungal organisms

In vitro biological activity of antagonists on *Alternaria alternata* was investigated on the PDA using Dual Culture Method [17]. In this method, a colony of test fungus was placed on one end of the Petri plates and antagonist's colony at other end parallel to each other in the experimental plate. A mycelium plugs from an actively growing *Alternaria alternate* on PDA was transferred to experimental plates using cork borer.The plates were incubated at 27°C till the test pathogens attains a maximum radial growth in the control plate.Radial growth of *Alternaria alternata* was recorded and percent inhibition was calculated using the formula [18].

Percentage inhibition (I) = [Control (C)–Treatment (T)/Control (C)] × 100

3. RESULTS AND DISCUSSION

The present investigation revealed that, the tomato plant was infected with early blight disease caused by *Alternaria alternata* f. sp. *Lycopersici* (AAL). Early blight disease in tomato is found to be infected in various parts of the country. *Alternaria alternata* was isolated from tomato plant which caused blight disease in Satara district, Maharashtra [19]. It is evident that the Pathogen *Alternaria alternata* infection triggered H_2O_2 production and ethylene evolution from tomato leaves within 6h of treatment, followed by blight symptoms. During disease development the pathogen secretes a toxin (AAL toxin) which exhibits high host specificity, plays a major role in pathogenesis and is responsible for early blight [20].

Rhizosphere fungi isolated using Serial dilution method revealed eight different species, namely *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus*, *Curvularia* sp*, Fusarium solani, Penicillium* sp and *Trichoderma harzianum*. These microorganisms are used as potential biocontrol agents because of their ability to reduce the incidence disease caused by plant pathogenic fungi [21].

In vitro antagonistic activity of isolated eight fungal organisms was treated against the pathogenic fungi *Alternaria alternate* using dual culture method. The inhibitory effect of these eight fungi towards plant pathogen *Alternaria alternata* was found to be maximum. On studying interaction between pathogen and antagonist (P×A) significantly high growth suppression was achieved by *Trichoderma harzianum* (63%) followed by *Aspergillus niger* (60%), *Penicillium* sp (60%), *Aspergillus terreus* (58%), *Aspergillus fumigatus* (56%), *Aspergillus flavus* (54%), *Curvularia* sp (41%) whereas minimum and significantly less inhibition was seen by *Fusarium solani* (34%) (Fig 1 and Table 1). *Trichoderma harzianum* is an effective biocontrol agent that is commercially produced to prevent development of several soil and foliar pathogenic fungi. *T. harzianum* was reported to be an effective biocontrol agent against *Alternaria alternata* isolated from *Lycopersicon esculentum* as their suppression range was around 63%.Similar results were obtained from *T. harzianum* which showed 67.07% of inhibition against *Alternaria alternate* [22].

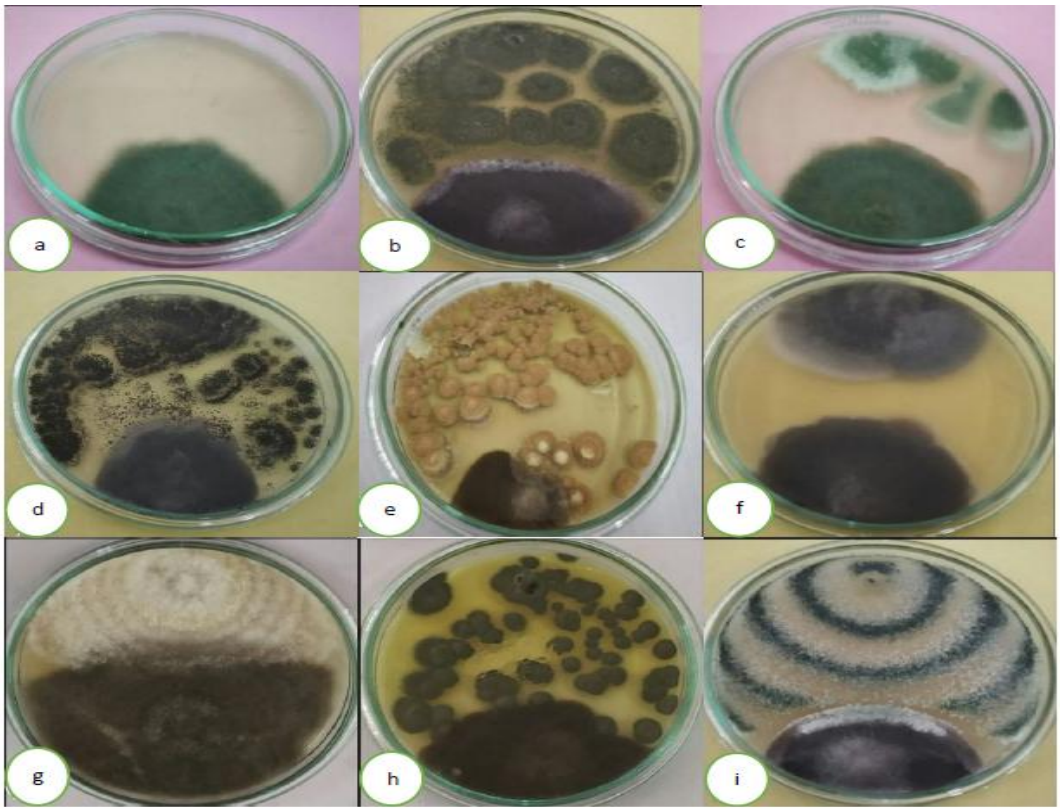

Fig 1: Antagonistic activity of different fungal organisms against *Alternaria alternata* plant pathogenic fungi: (a) Control (b) *Aspergillus flavus* (c) *Aspergillus fumigatus* (d) *Aspergillus niger* (e) *Aspergillus terreus* (f) *Curvularia* sp (g) *Fusarium solani* (h) *Penicillium* sp (i) *Trichoderma harzianum*

One of the key elements of sustainable agriculture is the ecological approach to solving the problems with plant pathogens, by the application of biocontrol agents. The genus *Trichoderma* is most important in achieving that and, at the same time, sustaining a favourable environment, instead of using chemicals. Fungi of the genus *Trichoderma* have long been recognized for their ability to act as biocontrol agents against plant pathogens. During this time, research has described their mechanisms of action and how they might be used for various purposes [23]. Hence it is suggested that *Trichoderma* species were capable enough to inhibit the growth of *Alternaria* species to a significant level.

Table 1: Efficacy of fungal antagonists against growth of *Alternaria alternata*

Antagonist	Mycelial growth of the pathogen under treatment (cm)	Percentage growth of inhibition over control
Aspergillus flavus	2.5	54
Aspergillus fumigatus	2.4	56
Aspergillus niger	2.2	60
Aspergillus terries	2.3	58
Curvularia sp	3.2	41
Fusarium solani	3.6	34
Penicillium sp	2.2	60
Trichoderma harzianum	2	63

4. CONCLUSION

Plant diseases caused by pathogenic fungi constrain the yields. In agriculture, farmers still depend on the use of chemical fungicides to control diseases. However, misuse of these synthetic chemicals causes hazardous to both environment and health. The alternative method for replacement of chemical fungicides had led to the use of these biological control agents. Biocontrol of plant pathogens can be met by the introduction of microorganisms.

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