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# EFFECT OF FUNGAL AND BACTERIAL BIOAGENTS AGAINST *ALTERNARIA ALTERNATA* (FR.) KEISSLER *IN VITRO* CONDITION

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**ABSTRACT**
Investigation on leaf spot disease (*Alternaria alternata* (FR.) Keissler) of Brinjal (*Solanum melongena* L.) under south Gujarat condition was carried out to find out suitable management strategies. Due to hazardous effect of chemical fungicides, search for safer alternative to control the pathogen is better choice. This led to trials on the use of bioagents to control the pathogen. The eight known bioagents were evaluated by dual culture, pathogen at periphery and pathogen at the centre technique to monitor antagonistic effect. The results revealed that out of all the eight bioagents used, three bioagents *viz., Trichoderma viride* (IARI isolate) (74.77%, 69.04% and 79.45%), *Trichoderma viride* (Navsari isolate) (74.14%, 66.08%, and 76.99%) maximum growth inhibition in dual culture, pathogen at periphery and pathogen at the centre methods respectively), *T. harzianum* (Junagadh isolate) (71.25%, 59.96% and 74.78%) maximum growth inhibition in dual culture, pathogen at periphery and pathogen at the centre methods respectively, showed strong antagonistic effect to inhibit the mycelia growth of the pathogen significantly.

## INTRODUCTION

Brinjal or egg plant (*Solanum melongena* L.) belongs to the kingdom: Plantae, division: Magnoliophyta, class: Magnoliopsida, order: Solanales, family: Solanaceae. Egg plant is the second major vegetable crop next to potato in India. It is considered as a "King of vegetables".Total production of brinjal is about 32 million tonnes in the world wherein India is world's second largest producer after China. It contains about 1.4 g proteins, 4.0g carbohydrates, 0.3g fat, 18 mg calcium, 2.0mg potassium and 0.9mg iron per 100 g of edible portion. It also provides vitamins like A, B and C. (Choudhary and Gaur, 2009). In India, the crop is grown about 5, 66, 000 hectares and produces 95, 96, 000 metric tons of fruits (Anon, 2008). West Bengal, Orissa, Bihar, Karnataka Maharashtra and Gujarat are the major brinjal growing states of the country. Brinjal crop is attacked by number of fungal, bacterial, viral and phytoplasmal diseases, during various growth stages which reduce its yield and quality of fruits. Diseases like *Alternaria* Leaf spot (*Alternaria alternata* (Fr.) Keissler), Damping off (*Pythium aphanidermatum* (Eds.) fitz.; *Phytophthora* spp.; *Rhizoctonia* spp.), *Phomopsis* blight (*Phomopsis vexans* Sacc. and Syd.) Harter., *Cercospora* Leaf spot (*Cercospora solani-melongenae* Chupp., *C.solani*.), *Verticillium* wilt (*Verticillium dahliae* Kleb), *fusarium* wilt (*Fusarium solani* (Mart.) App and Wollenw, Bacterial wilt (*Ralstonia solanacearum* Smith), Little leaf (*Phytoplasma.),* Mosaic virus, Root-knot nematodes (*Meloidogyne javanica* (Treub) Chitwood).Among all the fungal diseases, *Alternaria* leaf spot, *Alternaria* leaf blight and fruit rot diseases are of regular occurrence in moderate to severe proportion in India and causes extensive damage to the quality of fruits (Pandey and Vishwakarma 1999). Infected seeds caused reduction in seed germination and yield loss up to 30-50 Per cent. (Thippeswamy *et al.,* 2005). *Alternaria* leaf spot caused by *Alternaria alternata, Alternaria solani* and *Alternaria melongenae,* heavily damage the plants. Cool and humid weather, coupled with cloudiness which favors the occurrence and spread of the disease. When humid conditions prevailing at ground level, lower leaves are first attacked and infection spreads to the upper leaves and fruits. The disease causes characteristics leaf spot with concentric rings. The spots are mostly irregular and collapse to cover a large leaf area. Considering the seriousness of the problem, the present investigation was carried out. The hazardous effects of chemicals used in plant disease management have diverted plant pathologists to find out the alternative techniques of plant disease control which may cause little or no adverse effect on environment. Notable success of disease management through the use of antagonistic bioagents in the laboratory, glass house and field has been achieved during past several years. On the basis of this information, there is possibility of development of biological control for plant diseases. Now a day, the commercial formulation of some of the biocontrol agents has already become available in the market. In the present study, attempts have been made to identify antagonistic bioagents against *Alternaria alternata in vitro* condition.

## MATERIALS AND METHODS

Eight known fungal and bacterial bioagents (antagonists) viz.,

**Table 1: Antagonistic effect of different microorganisms against *Alternaria alternata* under dual culture method**

| Sr. No. | Test organism | Average colony diameter of pathogen (mm) | Per cent growth inhibition |
|---|---|---|---|
| 1 | *Trichoderma viride* (IARI isolate ) | 13.50 | 74.77 |
| 2 | *Trichoderma viride* (Navsari isolate ) | 13.83 | 74.14 |
| 3 | *Trichoderma harzianum* (Junagadh isolate) | 15.38 | 71.25 |
| 4 | *Bacillus subtilis* (Navsari isolate) | 16.19 | 69.73 |
| 5 | *Gliocladium virens* (Junagadh isolate) | 17.53 | 67.23 |
| 6 | *Aspergillus niger* (IARI isolate ) | 18.96 | 64.55 |
| 7 | *Pseudomonas fluorescens* (Navsari isolate) | 23.16 | 56.70 |
| 8 | *Chaetomium globosum* (Navsari isolate) | 25.16 | 52.96 |
| 9 | Control | 53.50 | |
| | S.Em ± | 0.56 | |
| | C.D. at 5% | 1.68 | |
| | C.V. % | 4.49 | |

**Table 2: Antagonistic effect of different microorganisms against *Alternaria alternata* under pathogen at periphery method**

| Sr. No. | Test organism | Average colony diameter of pathogen (mm) | Per cent growth inhibition |
|---|---|---|---|
| 1 | *Trichoderma viride* (IARI isolate) | 16.36 | 69.04 |
| 2 | *Trichoderma viride* (Navsari isolate) | 17.93 | 66.08 |
| 3 | *Trichoderma harzianum* (Junagadh isolate) | 21.16 | 59.96 |
| 4 | *Bacillus subtilis* (Navsari isolate) | 22.03 | 58.32 |
| 5 | *Gliocladium virens* (Junagadh isolate) | 22.53 | 57.38 |
| 6 | *Aspergillus niger* (IARI isolate) | 23.50 | 55.55 |
| 7 | *Pseudomonas fluorescens* (Navsari isolate) | 26.16 | 50.50 |
| 8 | *Chaetomium globosum* (Navsari isolate) | 27.46 | 48.05 |
| 9 | Control | 52.86 | |
| | S.Em ± | 0.61 | |
| | C.D. at 5% | 1.83 | |
| | C.V. % | 4.19 | |

*Trichoderma viride* Pers Ex. Grey. (IARI isolate), *Trichoderma viride* (Navsari isolate), *T. harzianum* Rifai (Junagadh isolate), *Bacillus subtilis* Ell. (Navsari isolate), *Pseudomonas fluorescens* Migula. (Navsari isolate), *Aspergillus niger* Link. (IARI isolate), *Gliocladium virens* Miller. (Junagadh isolate) and *Chaetomium globosum* Kunze. (Navsari isolate) were tested *in vitro* against *Alternaria alternata*. The culture discs measuring 5mm of test organism and pathogen were cut aseptically from the colony of pure culture grown on PDA medium and kept at different positions according to different techniques employed in the present investigation. In dual culture technique (Dennis and Webster, 1971), culture discs of test organisms and the pathogen were placed opposite to each other at 4cm apart in the Petri plate containing 20mL PDA aseptically and real antagonistic properties of the test bioagents were exhibited .In Pathogen at the periphery technique (Asalmol and Awasthi, 1990), the culture disc of the pathogen placed aseptically 4cm away radially at four corners keeping one disc of test organism at centre in the plate containing 20mL PDA aseptically. In Pathogen at the centre the culture disc of the pathogen was placed in the center and four similar discs of the test organisms were placed 4cm away from the pathogen at the periphery in the Petri plate containing 20mL PDA aseptically. The culture discs of the pathogens were kept at respective places of pathogen in each technique without bioagent served as control. All the treatments were incubated at room temperature (27 ± 2ºC) and after 6 days the radial growth of the test organism and pathogen was measured. CRD design with three repetitions of each treatment was employed in the present experiment. The per cent growth inhibition (PGI) was calculated by using formula given as below:

$$PGI = \frac{100\ (DC-DT)}{DC}$$

Where,

PGI = Per cent growth inhibition

DC = Average diameter of mycelial colony of control set (mm)

DT = Average diameter of mycelial colony of treated set (mm)

## RESULTS

All the antagonists under test were significantly superior over control in all the techniques against *A. alternata* however in Dual culture technique, Out of eight antagonists tested, *T. viride* (IARI isolate) (74.77%) and *T. viride* (Navsari isolate) (74.14%) showed maximum growth inhibition of the pathogen and appeared to be the most superior over all the antagonists tested. Next best in order of merit was *T. harzianum* (Junagadh isolate) (71.25%) followed by *B. subtillis* (Navsari isolate) (69.73%), *G. virens* (Junagadh isolate) (67.23%), *A. niger* (IARI isolate) (64.55%) and rest of the antagonists showed comparatively least growth inhibition (Table 1). In Pathogen at the periphery techniques, *T. viride* (IARI isolate) gave maximum growth inhibition (69.04%) and appeared to be the most superior antagonists against *A. alternata* over all the antagonists tested. Next best in order of merit was *T. viride* (Navsari isolate) (66.08%) which was statistically at par with *T. harzianum* (Junagadh isolate) (59.96%) and *Bacillus subtilis* (Navsari isolate) (58.32%) followed by *G. virens* (Junagadh isolate) (57.38%), *A. niger* (IARI isolate) (55.55%) while rest of the antagonists showed comparatively least growth inhibition (Table 2). In Pathogen at the center technique, maximum inhibition was found in *T. viride* (IARI isolate) (79.45 %). Which was statistically at par with *T. viride* (Navsari isolate) (76.99 %) next best in order of merit was *T. harzianum* (Junagadh isolate (74.78%) and *B. subtillis* (Navsari isolate) (73.14%). The rest of the antagonists showed comparatively least growth inhibition (Table 3).

## DISCUSSION

It appears from the results that all the antagonists tested by

**Table 3: Antagonistic effect of different microorganisms against *Alternaria alternata* under pathogen at centre method**

| Sr.No. | Test organism (Antagonists) | Average colony diameter of pathogen (mm) | Per cent growth inhibition |
|---|---|---|---|
| 1 | *Trichoderma viride* (IARI isolate ) | 10.86 | 79.45 |
| 2 | *Trichoderma viride* (Navsari isolate) | 12.16 | 76.99 |
| 3 | *Trichoderma harzianum* (Junagadh isolate) | 13.33 | 74.78 |
| 4 | *Bacillus subtilis* (Navsari isolate) | 14.20 | 73.14 |
| 5 | *Pseudomonas fluorescens* (Navsari isolate) | 14.36 | 72.82 |
| 6 | *Aspergillus niger* (IARI isolate) | 16.36 | 69.04 |
| 7 | *Gliocladium virens* (Junagadh isolate) | 18.23 | 65.51 |
| 8 | *Chaetomium globosum* (Navsari isolate) | 21.56 | 59.21 |
| 9 | Control | 52.86 | — |
| | S.Em ± | 0.57 | |
| | C.D. at 5% | 1.70 | |
| | C.V. % | 5.15 | |

three different methods were effective against *A.alternata* and useful as potential biological control agents. Among them, *T. viride* (IARI isolate), *T. viride* (Navsari isolate) and *harzianum* (Junagadh isolate) proved to be effective antagonist against *A. alternata*. This may be due to undeniably its mode of action like competition, antibiosis and mycoparasitim and it possess some important secondary metabolites and antibiotics like viridin, harzianiol and so many. These are in harmony with earlier workers *viz*., Ghosh *et al*. (2002) and Akbari and Parakhia (2007), who observed strong antagonism of *T. viride* on *A. alternata*. Patel (1991) and Sempere *et al*. (2007) observed strong antagonism on *A. alternata* with *T. harzianum and T viride*. Among the bacterial antagonist Akbari and Parakhia (2007) observed good antagonism of *Bacillus subtilis* with *A. alternata*. The present findings in complete agreement with the findings of above workers. Hence it can be recommended after rigorous testing in the pot and field condition against the pathogen for management of Brinjal leaf spot disease.

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