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In vitro evaluation of fungal antagonists against purple leaf blotch of onion (*Alternaria porri)*

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Abstract
Purple leaf blotch of onion *(Alternaria porri*) is most devastating disease of onion in India and a challenge for producer is to find the effective means of control for this disease. As Fungal bio agents are also playing an important role in controlling the incidence of purple leaf blotch disease. An experiment was carried out in the Department of Plant Pathology, College of Horticulture, Anantharajupeta to isolate the causative agents of purple leaf blotch to study the effect of different fungal antagonists to control the disease in *in vitro*. The efficacy of 5 fungal antagonists *viz*, *Trichoderma harzianum, Tichoderma viride*, TCT4, TCT10 and *Penicillium chrysogenum* on growth of *A. porri* was studied *in vitro* by dual culture technique. The results revealed that the fungal antagonists significantly reduced the growth of the pathogen either by antibiosis (exhibiting inhibition zones) or competition (over growing). It was noticed that maximum reduction in colony growth of *A. porri* was observed in *T. harzianum* (54.84%) and significantly superior over all other bioagents tested and followed by *T. viride* (44.72%) and the next best was TCT4 (42.19%). Least inhibition was noticed in P*enicillium chrysogenum* (27.42%).

Keywords: onion, fungal antagonists, *Alternaria porri, Trichoderma harzianum*

Introduction
Onion (*Allium cepa* L.) is extremely important vegetable crop not only for internal consumption but also as highest foreign exchange earner among the fruits and vegetables. Onion belongs to the family Alliaceae. According to Vavilov (1951) [12] the primary center of origin is Central Asia and the near East and Mediterranean are the secondary centers of origin. Onion was introduced from Palestine to India. Onion has manifold uses as spice, vegetable, salad dressing *etc*, hence it is known as "queen of kitchen". It is also used as condiments for flavouring a number of food and medicines. The reason for very low productivity may be attributed to occurrence of diseases *viz*., purple blotch, *stemphyilum* blight, downy mildew, basal rot and storage rots *etc*. (Priya *et al*., 2016, Singh *et al*. 2017a; Singh *et al*. 2017b; Singh *et al*. 2017c; Singh *et al*. 2018; Tiwari *et al*. 2018; Tiwari *et al*. 2019a; Tiwari *et al*. 2019b; Kour *et al*. 2019; Singh *et al*. 2019) [9, 14, 15, 16, 17, 18, 19, 20, 21] and non-availability of varieties resistant to biotic and abiotic stresses. Among the foliar diseases, purple blotch is one of the most destructive diseases, commonly prevailing in almost all onion growing pockets of the world, which causes heavy loss in onions under field conditions, ranging from 30 to 100%.

2. Materials and methods
2.1 General laboratory procedures
2.1.1 Glassware
Different types of glassware used in the present study were Petri plates (90 mm diameter), conical flasks (250, 500, 1000 ml), measuring cylinder (25, 250 and 500 ml), test tubes *etc*.

2.1.2 Glassware cleaning
For all the laboratory experimental studies, Borosil glasswares were used. The glasswares were kept overnight in the cleaning solution prepared by dissolving 60 g of potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated Sulphuric acid (H_2SO_4) in one liter of distilled water. Then, they were washed with detergent powder followed by rinsed 3-4 times in running tap water, air dried and sterilized before use.

2.1.3 Equipments
Different types of laboratory equipments were used for the present investigation. Compound microscope (10x, 40x, 100x magnifications Olympus) was used to identify the pathogen. Hot air oven was used for the sterilization of glassware. Autoclave was used for the sterilization of the media. Incubator was used for the incubating test materials at different temperatures. Refrigerators were used for storage of cultures. Electronic balance was used for the measuring the chemicals. Other types of tools used in the present investigation for various purposes include surgical knife, camel brush, inoculation needle, corkborer, scalpel, forceps *etc*.

2.1.4 Sterilization of glassware and media
Petri plates were sterilized in hot air oven at 110 ºC for 90 minutes. Work benches were sterilized with 70 per cent ethyl alcohol. Cork borer, scalpel and inoculation loop were sterilized over flame. Media and water used in the study were sterilized at 15 lb psi (121 ^{0}C) for 15 minutes in an autoclave.

2.1.5 Culture media used
The following culture media was used for isolation, culturing and maintenance of pathogen in the laboratory. Potato Dextrose Agar (PDA) medium is most commonly used for the isolation and maintenance of the fungi.

2.1.6 Preparation of PDA
Materials required
Peeled potato slices: 200 g
Dextrose: 20 g
Agar agar: 20 g
Distilled water: 1000 ml

Isolation and identification of the pathogen
Onion leaves showing purple blotch symptoms were collected from my research plot. These leaves were put in sterilized polythene bags and brought to the laboratory for isolation and identification of the organism involved. Isolation of the pathogen was made under aseptic conditions by tissue segment method (Aneja, 2003) [1] from the onion leaf samples showing typical purple blotch symptoms and the culture was further purified by single spore isolation (Tuite, 1969) [11].

Isolation of pathogen
Onion leaves showing purple leaf blotch symptoms of the disease were selected and washed with sterile water. Small bits of diseased tissue along with some healthy tissue were cut with the help of a sterile scalpel and the surface was sterilized with 1 per cent sodium hypochlorite solution for 30 seconds. The bits were washed thrice with sterile distilled water to remove traces of sodium hypochlorite and blotted dry on clean, sterile paper towels. These leaf tissue pieces were aseptically transferred in to PDA containing Petri plates and incubated at 25±1 ºC for 2 to 3 days. Fungal growth emerging from diseased leaf tissue was directly transferred to the PDA plates.

3.1 Evaluation of fungal antagonists against *A. porri*
The efficacy of bioagents was tested against *A. porri* on PDA media using dual culture technique under *in vitro* condition. The promising bioagent was mass multiplied and included in organic management *in vivo*.

3.1.1 List of bioagents used against *A. porri* are mentioned below

S. No	Sets	Bioagents	Effective against	Source
1	Set:1	*Trichoderma* spp. (TCT_4, TCT_{10})	*Fusarium* dry root rot in sweet orange	Gopal *et al.*(2014) [5]
2	Set:2	*Trichoderma harzianum*	Purple leaf blotch of onion	Nagalakshmi (2018) [8]
3	Set:3	*Penicilium chrysogenum Trichoderma viride*	Turmeric rhizome rot	Nandini (2017)

3.1.2 Dual culture test
Fungal bioagents were evaluated for their efficacy through dual culture technique. Twenty ml of sterilized PDA medium melted and cooled to 45 ^{0}C was poured aseptically into sterilized Petri dishes of 9 cm diameter. Mycelial discs of 5 mm diameter cut from the edge of actively growing seven days old culture of pathogen and mycelial discs (5 mm) of *Trichoderma* spp. cut from actively growing colony of the respective fungal species with the help of a sterilized cork borer, these were placed on the periphery about one cm from the edge of the petri dish at opposite sides. All the treatments were replicated and incubated at room temperature (27±1 ^{0}C). After incubation when the growth of the pathogen was completed in the control, the colony diameter of fungal antagonists was measured in each treatment and the per cent inhibition of the pathogen over control was calculated by using the formula given by Vincent (1947) [13]. Later data were analyzed statistically after suitable transformation.

$$I = \frac{C - T}{C} X100$$

Where, I= Per cent inhibition
C= Radial growth in control
T= Radial growth in treatment

Result and discussion
4.1 *In vitro* study on efficacy of plant extracts against *A. porri*
Results (Table 1) revealed that efficacy of 5 antagonists *viz*, *Trichoderma harzianum*, *Tichoderma viride*, TCT4, TCT10 and *Penicillium chrysogenum* on growth of *A. porri* was studied *in vitro* by dual culture technique as explained under "material and methods" and presented in Table 1 and plate 1.
The results revealed that the fungal antagonists significantly reduced the growth of the pathogen either by antibiosis (exhibiting inhibition zones) or competition (over growing). It was noticed that maximum reduction in colony growth of *A. porri* was observed in *T. harzianum* (54.84%) and significantly superior over all other bioagents tested. Which was followed by *T. viride* (44.72%) and the next best was TCT4 (42.19%). Least inhibition was noticed in P*enicillium*

chrysogenum (27.42%). (Table 1 and plate 1 and Fig. 1)

Biological control through the use of antagonistic microorganisms is a potential, non-chemical means of controlling plant disease by reducing inoculum levels of pathogens. Such a management would help in preventing the pollution and also health hazards. The inhibitory effect of these bioagents was probably The inhibitory effect of these bioagents was probably due to competition and/or antibiosis.

Table 1: *In vitro* evaluation of fungal antagonists against *A. porri*

S. No	Bioagents	Linear mycelia growth of pathogen in (mm)	Percent inhibition over control
1	*T. harzianum*	36.60	54.84 (47.78)*
2	*T. viride*	43.60	44.72 (41.97)
3	TCT4	45.60	42.19 (40.51)
4	TCT10	50.60	35.86 (36.79)
5	*P. chrysogenum*	57.30	27.42 (31.58)
6	Control	79.00	-
	SE m±	0.133	0.582
	C.D (*P* 0.05)	0.426	1.857

*Figures in the parenthesis are angular transformed values

In the present investigation, the antagonistic effect of different bio-agents was assessed against *A. porri* and by dual culture technique. Maximum reduction in colony growth of *A. porri* was observed in *T. harzianum* which was significantly superior over all the other bioagents tested folloed by *T. viride* and next best was TCT4 and least inhibition was noticed in *Penicillium chrysogenum.* In general, species of *Trichoderma, viz., T. harzianum*, *T. viride*, TCT4 *and* TCT10 showed more mycelial inhibition of *A. porri* compared to other antagonist. This could be obviously attributed to several possibilities of existence of mechanisms and microbial interactions such as higher competitive ability, stimulation and antibiosis by these *Trichoderma* isolates over test pathogen. This has been enumerated by many workers (Mishra and Gupta, 2012; Gopal *et al.*, 2014; Brahmane *et al*. 2015; Arunakumara *et al.*, 2016) [7, 5, 3, 2]. The antagonism of *Trichoderma* sps. Against many fungi is mainly due to production of acetaldehyde, a carbonyl compound (Robinson and Park, 1966; Dennies and Webster, 1971) [10, 4]. This may also be the reason for its antagonistic effect on *A. porri.*

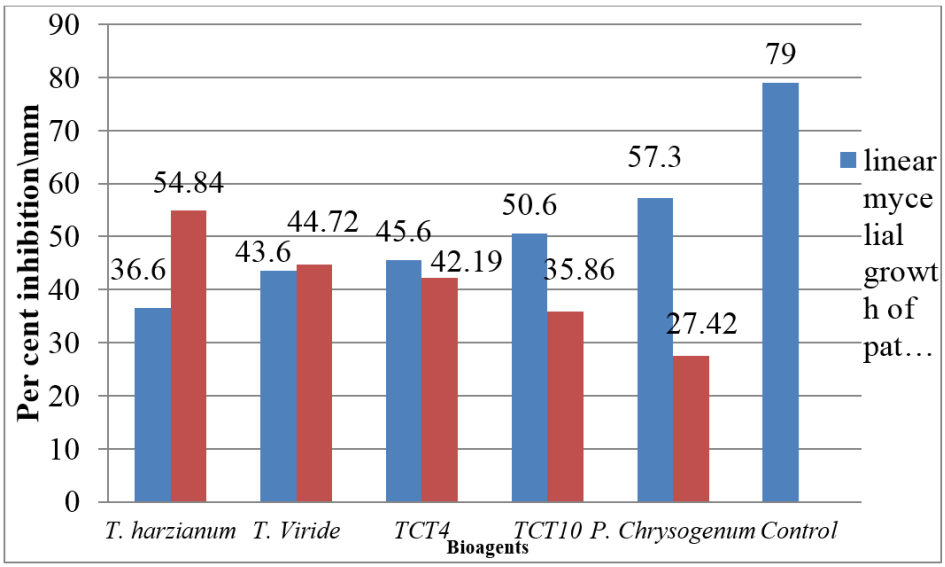

Fig 1: *In vitro* evaluation of fungal antagonists against *Alternaria porri*

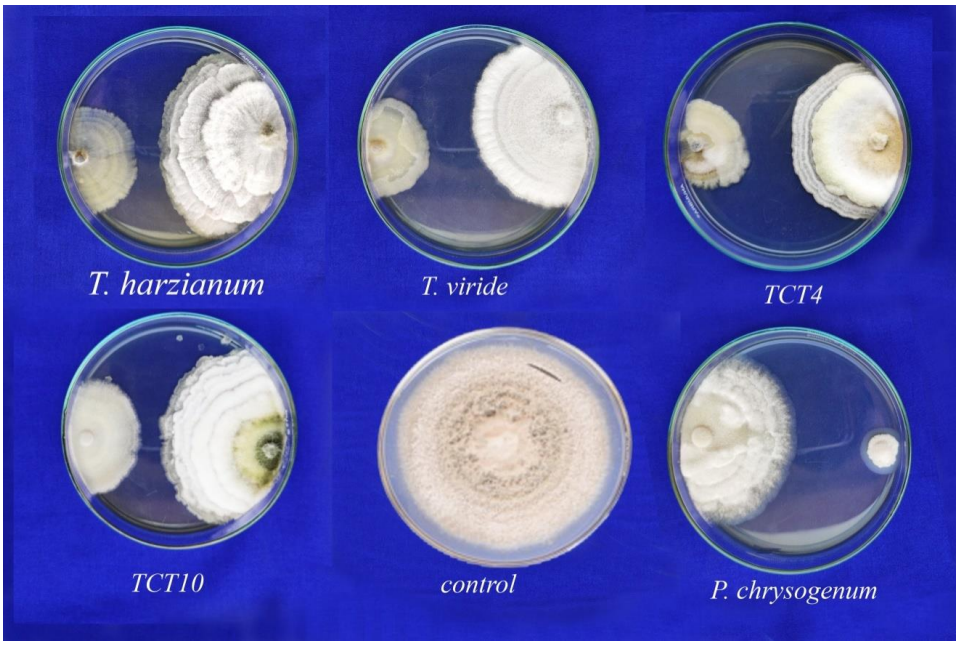

Plate 1: *In vitro* evaluation of fungal antagonists against *A. porri*

Conclusion

A close analysis of the present investigation revealed that among the five antagonists tested against *A. porri* under laboratory condition in dual culture, *T. harzianum* recorded highest inhibition of radial growth. The antagonists *T. viride*, TCT4, TCT10 and *Penicillium chrysogenum* were next in order. Among all the treatments *pencillium chrysogenum* proved to be least effective.

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