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In vitro Evaluation of *Trichoderma harzianum* and Botanicals on the Radial Growth of *Colletotrichum dematium* Causing Anthracnose Disease of Groundnut (*Arachis hypogaea* L.)

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A B S T R A C T

A study was conducted *in vitro* to control *Colletotrichum dematium* causing anthracnose disease of groundnut with *Trichoderma harzianum* and botanicals. Five botanicals *viz.*, Datura leaf extract, Tulsi leaf extract, Garlic bulb extract, Neem oil and *Eucalyptus* oil at the rate of 5% were evaluated for their efficacy against the radial colony growth of *C. dematium.* The complete inhibition was obtained in *Eucalyptus* oil (100%) followed by *T. harzianum* (71.01%), datura leaf extract (64.78%), tulsi leaf extract (63.63%), neem oil (49.14%) and garlic bulb extract (43.35%). In the present study different culture media *viz.*, malt extract agar, Czapek dox agar, corn meal agar, Martin's rose Bengal agar and oat meal agar were used for the study of different cultural characters of *Colletotrichum dematium.*

Introduction

The peanut, groundnut pea, or groundnut (*Arachis hypogaea* L.) is a native of South America but was early carried to the old world tropics by the Portuguese explorers. Groundnut is the one of the world's important oilseed crops. Groundnut is called as the 'King' of oilseeds. It is one of the most important food and cash crops in India. While being a valuable source of all the nutrients, it is a low-priced commodity. In groundnut several diseases like tikka, rust, peanut bud necrosis, collar rot, and anthracnose are constraints the yield and productivity. Anthracnose of groundnut caused by *Colletotrichum dematium* was first reported by Subrahmanyam *et al.*, (2012). The term 'Anthracnose' literally means 'like coral' and first used by Fabre and Dunal to describe a disease of grapes in which blackening of tissues was characteristic feature black lesions, usually sunken caused by certain imperfect fungi that produce conidia in acervuli those

are hyaline, one-celled, that is *Colletotrichum* (Jha *et al.,* 2012). *Colletotrichum dematium* until recently was a relatively poorly known species in urgent need of epitypification. It was originally collected from a stem of *Eryngium* in France as well as solanaceous hosts and has been more recently recorded from numerous hosts such as a pathogen of chilli (Than *et al.,* 2008). It has been also recorded as a pathogen of *Polygonatum falcatum* (Tomioka *et al.,* 2008) and an endophyte of *Pteromiscum* sp. (Ren *et al.,* 2008). Disease symptoms are reported to range from fruit rot to shoot, leaf, and flower blight, e.g., Sutton reported that in herb. It was represented that 216 collections from 37 countries on 118 different host genera.

Colletotrichum dematium is difficult to recognize based on morphological characteristics, mainly because different researchers have described conidia width differently. Colonies of putative *C. dematium* strains have been reported by Sutton (1992) to be very variable with white to pale mouse-grey or grey-vinaceous patches with abundant setae and black, conical sclerotia. Conidia are formed in olive-grey to light vinaceous-salmon masses, and are 18–26 × 2–3 μm, falcate, fusiform, and gradually tapered to each end (Sutton, 1992). Appressoria are medium brown, clavate, ovate to irregular, margin entire or slightly irregularly lobed (Sutton, 1992).

Keeping in view the economic importance of anthracnose disease, the present study has therefore been undertaken with the objective to isolate and identify the pathogen *Colletotrichum dematium,* to observe the effect of *Trichoderma harzianum* and certain botanicals on the radial growth of *Colletotrichum dematium and* to study the cultural characters of *Colletotrichum dematium* on different culture media.

Materials and Methods

An experiment was conducted to evaluate effect *Trichoderma harzianum* and botanicals on the radial growth of *Colletotrichum dematium* causing anthracnose of groundnut *in vitro*. The experiment was conducted in the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.).

Isolation and identification of pathogen

Diseased leaves (anthracnose) of groundnut collected from research field of University were isolated by using standard procedure of Aneja (2004).

The pathogen was identified based on its cultural and morphological characters. Following single hyphal-tip technique, the fungus was transformed/subcultured aseptically onto the PDA slant in test tubes. Through frequent sub-culturing, the fungus was purified and pure culture was maintained on agar slants in test tubes stored in refrigerator for further studies.

In vitro evaluation of biological agent

Trichoderma harzianum was evaluated *in-vitro* on radial growth *C. dematium* applying Dual culture Technique (Dennis and Webster, 1971) and using Potato Dextrose Agar (PDA) as basal culture media.

In vitro evaluation of botanicals

A total of five botanicals viz. Datura leaf extract, Tulsi leaf extract, Garlic bulb extract, Neem oil and *Eucalyptus* oil at 5% concentration were evaluated *in vitro* on radial growth of *C. dematium* applying Poison Food Technique (Nene and Thapliyal, 1993) and using Potato Dextrose Agar (PDA) as basal culture media.

Cultural characters of *Colletotrichum dematium*

Different culture media *viz.*, Malt extract agar, Czapek dox agar, Corn meal agar, Martin's rose Bengal agar and Oat meal agar were used for assessing the cultural characters such as colony diameter, growth rate and different phenotypic characters such as colony shape, colony margin, colony color and substrate color of *Colletotrichum dematium*.

Three replications were maintained for each media and were incubated at room temperature and observation recorded. The different colony characters were recorded in each medium by visual observation after 7 days of incubation.

Colony diameter of every culture was recorded daily for 7 days. Growth rate was calculated as the 7-day average of mean daily growth (mm per day).

Collection and analysis of data

After 7 days of incubation, radial growth (mm) of *Colletotrichum dematium* in petridishes was recorded. The radial growth (mm) of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony.
Percentage inhibition of growth was calculated using the following formula:

Per cent growth inhibition (I) =

$$\frac{C - T}{C} \times 100$$

Where,
C = Growth of test fungus (mm) in control plate
T = Growth of test fungus (mm) in treatment plate

Results and Discussion

Identification of *Colletotrichum dematium*

Colony of putative *C. dematium* was very variable with white to pale mouse-grey or grey-vinaceous patches with abundant setae and black, conical sclerotia. The conidia are borne on conidiophores, each conidia was one celled hyaline, typically long, falcate, fusiform, and gradually tapered to each end the acervuli are main distinct features of this genus that are blackish to dark brown with pointed caps, the seta are hyaline and yellowish. The morphological observations of fungus were recorded by adapting slide culture technique. The fungus under study was identified as *Colletotrichum dematium* and its identification results were similar to the different fungal characters given by Sutton (1992) (Fig. 1).

In vitro evaluation of bioagent and botanicals

Different treatments tested in the present study gave appreciable inhibition in radial growth of *C. Dematium* as shown in the Table 1. Minimum radial growth of 0.0 mm was observed in T_6 (*Eucalyptus* oil @ 5%) which is statistically significant followed by T_1 (*Trichoderma harzianum*) 17 mm, T_2 (Datura leaf extract @ 5%) 20.66 mm, T_3 (Tulsi leaf extract @ 5%) 21.33 mm, T_5 (Neem oil @ 5%) 29.83 mm and T_4 (Garlic bulb extract @ 5%) 33.13 mm as compared to control (58.66 mm).Maximum per cent growth inhibition of *Colletotrichum dematium*100% was obtained by T_6 (*Eucalyptus* oil @ 5%) followed by T_1 (*Trichoderma harzianum*) 71.01%, T_2 (Datura leaf extract @ 5%) 64.78%, T_3 (Tulsi leaf extract @ 5%) 63.63%, T_5 (Neem oil @ 5%) 49.14% and T_4 (Garlic bulb extract @ 5%) 43.35% as compared to control (Table 1; Fig. 2 and 3).

Table.1 Effect of bioagent and botanicals on the radial growth and per cent growth inhibition of *Colletotrichum dematium*

Treatments		Radial growth (mm)	Growth inhibition (%)
T_0	Control	58.66	0.0
T_1	*Trichoderma harzianum*	17	71.01
T_2	Datura leaf extract	20.66	64.78
T_3	Tulsi leaf extract	21.33	63.63
T_4	Garlic bulb extract	33.13	43.35
T_5	Neem oil	29.83	49.14
T_6	***Eucalyptus* oil**	**0**	**100**
C. D. (P=0.05)		**6.897**	**-**
S.Ed. (±)		**3.213**	**-**

Table.2 Mean colony diameter and growth rate of *Colletotrichum dematium* on different culture media

Sr.No.	Media	Mean colony diameter (mm)	Growth rate (mm/day)
1	Malt extract agar	68.33	9.76
2	**Czapek dox agar**	**89**	**12.71**
3	Corn meal agar	63.50	9.07
4	Martin's rose Bengal agar	48.00	6.85
5	Oat meal agar	73.16	10.45
C. D. (P=0.05)		**6.068**	**-**
S.Ed. (±)		**2.720**	**-**

Fig.1 Effect of bioagent (*Trichoderma harzianum*) on radial growth of *Colletotrichum dematium*

Fig.2 Effect of botanicals on radial growth of *Colletotrichum dematium*. Where, A- Control, B- Datura leaf extract (5%), C- Tulsi leaf extract (5%), D- Garlic bulb extract (5%), E- Neem oil (5%), F- Eucalyptus oil (5%)

Fig.3 Growth of *Colletotrichum dematium* on different culture media

The minimum radial growth was observed in T_6(*Eucalyptus* oil @ 5%) whereas the maximum radial growth was observed in T_0 (Control). The probable reason for such findings may be that the mycelial growth of the test pathogen (*Colletotrichum dematium*) was checked due to the fungicidal properties of essential oil used during the experiment. Similar findings have been reported by Ramezani *et al.*, (2002).

Cultural characters of *Colletotrichum dematium* on different culture media

There was significant difference among different culture media with respect to colony diameter which ranged from 48 to 89 mm. The maximum mean colony diameter as observed in Czapek dox agar (89 mm) followed by Oat meal agar (73.16 mm), Malt extract agar (68.33 mm), Corn meal agar

(63.50 mm) and Martin's rose Bengal agar (48 mm). *Colletotrichum dematium* growth rate ranges from 6.85 to 12.71 mm/day. The fastest growth was recorded 12.71 mm/day on Czapek dox agar followed by Oat meal agar (10.45 mm/day), Malt extract agar (9.76 mm/day), Corn meal agar (9.07 mm/day) and Martin's rose Bengal agar (6.85 mm/day) (Table 2). The cultural characters and growth of *Colletotrichum dematium* varied on different media. This might be due to the variation in the nutritional requirement of the fungus. There was a wide variation in the colony shape, margin and colour of *Colletotrichum dematium* on different culture media. Similar observations were made by Denobys and Baudry (1995), Kuramae *et al.*, (1997) and Manjunath (2009).

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