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RESEARCH ARTICLE

Effect of various nitrogen sources and antagonists on the growth of *Colletotrichum capsici* (Syd.) Butler and Bisby causing anthracnose of yam (*Dioscorea alata* L.)

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ABSTRACT

The yam crop was found infected with anthracnose disease caused by *Colletotrichum capsici*. In present investigation out of seven different nitrogenous sources tried, potassium nitrate proved to be the best for the growth (345.33 mg) and sporulation (380.3 spores/LPM) of the pathogen. Ammonium sulphate, Sodium nitrate and Ammonium nitrate also showed stimulation of growth and sporulation of *C. capsici* as compared to other sources tried. Also the result from the *in vitro* study revealed that nine antagonists *viz*., *Trichoderma viride* Pers. ex Grey, *Trichoderma harzianum* Rifai, *Trichoderma longibrachyatum* Rifai, *Gliocladium virens* Miller., *Chaetomium globosum* Kunze., *Pseudomonas fluorescens* Migula, *Aspergillus niger* Link, *A. flavus* and *Bacillus subtilis* Ell. were tested against *C.capsici.* by dual culture technique. *In vitro* studies on interaction of antagonists revealed strong antagonism of *T. viride* Pers., *P.fluorescens* Migula and *Aspergillus flavus* Link.

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INTRODUCTION

The yam is an important tuber crop originated from the Indo-Burmese region of South East Asia. The yam is a common name for some species in the genus *Dioscorea* (Family: Dioscoreaceae). The major yam producing states in India includes Gujarat, Maharashtra, Orissa, Rajasthan, Kerala, West Bengal, Bihar and Assam. Two Asiatic yams, *viz*., *Dioscorea alata* Linn. (greater yam) and *Dioscorea esculenta*. (Lour.) Burkill (lesser yam) are the major food of the Indians. The yams exploited for pharmaceutical purposes are non-edible. (Thamburaj and Singh, 2005). The 100 g edible portion of yams contains calcium 38mg, phosphorous 28 mg, iron 1.1mg, vitamin A 5 mg, thiamine 0.10 mg, riboflavin 0.04 mg, niacin 0.5 mg and ascorbic acid 6 mg (Tindall,1983).

In the year 2007, in Horticulture farm of Navsari Agricultural University, Navsari, the yam crop was found to be severely affected by anthracnose disease resulting in severe losses.

MATERIALS AND METHODS

Effect of various nitrogen sources on growth and sporulation of *Colletotrichum capsici* :

The present *in vitro* study was conducted in the Plant Pathology Laboratory of ASPEE College of Horticulture and Forestry, Navsari, by using Completely Randomized Block Design having 4 repetitions. *C.capsici* was repeatedly isolated from naturally infected yam (*D.alata*) leaves on Potato dextrose agar medium in laboratory. The culture was further purified by frequent sub culturing and maintained on Potato dextrose agar (PDA) slants for further investigation.

Fifty ml of sterilized liquid Richard's medium was poured in to 150 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 121^0C (15 psi pressure) for 20 minutes. Potassium nitrate in the basal medium was replaced by various

inorganic and organic sources of nitrogen *viz.*, ammonium sulphate, ammonium nitrate, sodium nitrate, calcium nitrate, ammonium chloride, urea and potassium nitrate. Nitrogen sources were added singly to furnish 1.38 g of nitrogen per litre of basal medium and treatment without nitrogen source served as control. Each treatment was replicated four times. The flasks were inoculated under aseptic conditions with 5 mm diameter culture block cut from 10 days old actively growing pure culture of *C. capsici* and transferred to each flask. Inoculated flasks were incubated at room temperature (27 ± 2°C). Mycelial mats were collected from three repetitions in each case after 15 days of incubation on previously weighed Whatman's filter paper no. 42 and dried in an oven at 60°C for 3 consecutive days (uptill constant weight). The average dry weights of the mats obtained from three replications of each treatments were used as quantitative measure for comparing the growth under different treatments.The dry weight of mycelium was expressed in milligrams.

The sporulation of the fungus was recorded from the fourth repetition of each treatment .The data obtained were analysed statistically using standard procedure.

Effect of antagonists on the growth of *Colletotrichum capsici*:

The test organism and the pathogen were grown on PDA and from 10 days old culture, a 5 mm disc of the test organism (antagonist) was cut aseptically from the periphery of the colony and placed at one end of the Petri plate containing 20 ml solidified PDA. In the opposite place and approximately 70 mm away from the first, a similar disc of the pathogen was aseptically placed. Three repetitions of each were kept and the plates with only pathogen served as control. The plates were incubated at room temperature (27 ± 2°C) and the radial growth of the test organism and pathogen was measured after 6 days. The per cent growth inhibition (PGI) was calculated as per Sundar *et al.* (1995) :

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

where,

X= Growth of pathogen in control plate (mm)

Y= Growth of pathogen in treated plate (mm)

RESULTS AND DISCUSSION

The results obtained from the present investigation (Table 1 and Fig.1) revealed that, among seven different nitrogenous sources tested for their effects on the growth and sporulation of *C.capsici,* significantly superior growth of the pathogen was recorded in potassium nitrate (345.33 mg). The next best in order of merit were ammonium sulphate (318.33 mg), sodium nitrate (295.00mg) which was statistically at par with ammonium nitrate (283.00 mg). The rest of the nitrogen sources supported moderate mycelial growth. Similarly, the sporulation was significantly superior in potassium nitrate (380.33 spores/LPM). The next best in order of merit were ammonium sulphate (318.33 spores/LPM) which

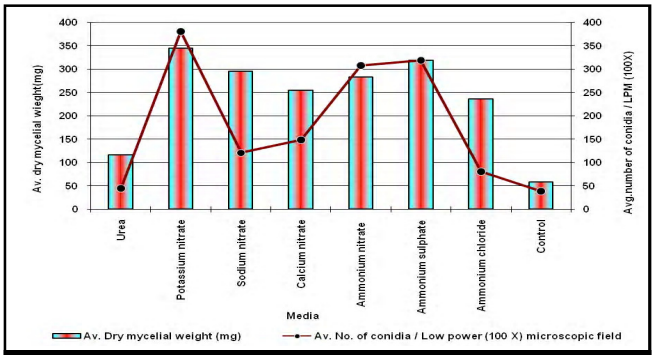

Fig. 1: Effect of various nitrogen sources on growth and sporulation of *Colletotrichum capsici in vitro*

Table 1: Effect of various nitrogen sources on growth and sporulation of *C. capsici in vitro*

Sr.No.	Nitrogen source	Liquid medium (after 15 days)			
		Average dry weight of mycelium (mg)		No. of conidia/low power microfield (100x)	
1.	Urea	2.07*	(116.00) **	1.66 *	(44.67)**
2.	Potassium nitrate	2.54	(345.33)	2.58	(380.33)
3.	Sodium nitrate	2.47	(295.00)	2.09	(120.67)
4.	Calcium nitrate	2.41	(254.67)	2.17	(148.00)
5.	Ammonium nitrate	2.45	(283.00)	2.49	(307.33)
6.	Ammonium sulphate	2.50	(318.33)	2.50	(318.33)
7.	Ammonium chloride	2.37	(235.67)	1.91	(80.67)
8.	Control	1.78	(58.33)	1.59	(38.33)
	S.E. ±	0.005		0.002	
	C.D.at 5%	0.015		0.006	
	C.V.%	0.37		0.15	

* Figures indicate logarithmically transformed values, ** Figures indicate original values

Table 2: Testing of antagonists against *C. capsici* under dual culture method

Sr. No.	Test organism	Average colony diameter (mm)	Per cent growth inhibition
1.	*Trichoderma viride*	6.33	86.24
2.	*Trichoderma harzianum*	20.33	55.80
3.	*Trichoderma longibrachyatum*	21.67	52.89
4.	*Aspergillus niger*	24.67	46.37
5.	*Gliocladium virens*	8.33	81.89
6.	*Pseudomonas fluorescens*	6.67	85.50
7.	*Aspergillus flavus*	7.67	83.33
8.	*Bacillus subtilis*	26.33	42.76
9.	*Chaetomium globosum*	25.00	45.65
10.	Control	46.00	-
	S.E. $\pm$	0.54	-
	C.D. at 5%	1.59	-
	C.V. %	4.82	-

was statistically at par with ammonium nitrate (307.33 spores/ LPM). These results are in harmony with the findings of Solanki *et al*. (1974), Palarpawar and Ghurde (1997) and Kumar and Yadav (2005).

The results presented in Table 2 revealed that all the antagonists screened against *C. capsici* were significantly superior over the control. Out of these, *Trichoderma viride* Pers. (6.33 mm) significantly reduced growth of the pathogen which was statistically at par with *Pseudomonas fluorescens* Migula. (6.67 mm) and *Aspergillus flavus* Link. (7.67) which was statistically at par with. *Gliocladium virens* Miller. (8.33 mm) followed by *Trichoderma harzianum* Rifai (20.33 mm) and *Trichoderma longibrachyatum* Rifai (21.67 mm) and rest of the antagonists inhibited comparatively least growth of *C.capsici*.

Trichoderma viride Pers. (86.24%) showed maximum growth inhibition and appeared to be the most superior over all other antagonists tested which was at par with *Pseudomonas fluorescens* Migula (85.50 %) and *Aspergillus flavus* Link. (83.33 %) which was statistically at par with *Gliocladium virens* Miller (81.89 %) followed by *Trichoderma harzianum* Rifai (55.80 %) and *Trichoderma longibrachyatum* Rifai (52.89 %) and rest of the antagonists showed comparatively least growth inhibition.These results are in harmony with the findings of earlier workers *viz.,* Hegde *et al*. (2002), Chirame and Padule (2005), Kaur *et al.* (2006) and Anand and Bhaskaran (2009).

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