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EFFECT OF BIOAGENTS ON SPORE GERMINATION OF *ALTERNARIA BURNSII*

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ABSTRACT
The effect of cultural filtrate of *Trichoderma* and *Pseudomonas fluorescens* isolates on spore germination of *A. burnsii*. The cultural filtrate of *Trichoderma viz*. *Trichoderma* isolate-11 and *Trichoderma* isolate-19 were found most effective and spore germination inhibition were 63.98 per cent and 63.30 per cent against *A. burnsii*, respectively. Among culture filtrate of *P. fluorescens viz*. *P. Fluorescens* isolate-7 and *P. fluorescens* isolate-2 were highly effective in the reduction of spore germination of *A. burnsii*. Which were inhibited spore germination 59.32 per cent with *P. fluorescens* isolate-7 and 52.92 per cent with *P. fluorescens* isolate-2 against *A. burnsii*.

INTRODUCTION

Cumin (*Cuminumcyminum*L.) locally known as *Jeera* or *Jiru* and is an annual herb of the family *Apiaceae* (*Umbelliferae*). It is a cross pollinated crop, and bees helps for pollination. Moderate sub-tropical climate is ideal for cumin cultivation. Moderately cool and dry climate is best. Cumin crop does not stand under high humidity and heavy rainfall. It grows to about 30-50 cm tall. It has dissected leaves with white or rose-coloured flowers. Cumin seeds have yellowish brown, white or black colour. The seed content essential oil between 2.5 to 4.5% (Pruthi, 1996). Alternaria blight incited by *Alternaria burnsii* is an economically important andwidely distributed disease throughout the world. Cumin crop is affected mainly with three important diseases *viz*., blight (*Alternaria burnsii*), wilt (*Fusarium oxysporum*f.sp. *cumini)* and powdery mildew (*Erysiphe polygoni*) (Dange, 1995). Cumin is susceptible to blight after flowering. In initial stage of the disease, ash-coloured spots or lesions are observed on the leaf and branches. Under wet and warm conditions infection rapidly spreads to the stem and blossoms, and in severe conditions, the whole plant dries-up and become black in colour as if it burnt. In cases of very severe infection, there may not be any seed production. Even if seeds are produced, they are shriveled, dark-coloured, light in weight and usually non-viable (Gemawat and Prasad, 1972). In India, the major cumin growing states are Gujarat and Rajasthan, together accounts for more than 70 per cent of total country's production (Anonymous, 2010-11). The area under cumin cultivation in India is about 858900 ha with annual production of 513850 tonnes (Anonymous, 2013-14).Considering its domestic use and export potential, it is high time to reduce fungicide applications with partial or complete substitution with bio products to suit international standard of chemical residue and sale of quality product at domestic level.Use of bio agents have beensuggested by workers as alternative to syntheticchemicals in order to counter the potential hazardous effecton the environment associated with the use of syntheticchemicals (Ganie, *et al*., 2013, Heydari and Pessarakli, 2010). Natural plant products and bio agents areimportant sources of new agrochemicals for the control ofplant diseases (Kagale *et al*., 2004). Therefore, in the present investigation, inhibition spore germination of cumin blight fungus, *Alternaria blight,* exposed to different concentrations of cultural filtrate of *Trichoderma* and *Pseudomonas fluorescens* isolates were studied.

MATERIALS AND METHODS

Slide germination technique was employed with 30 *Trichoderma* and *Pseudomonas fluorescens* isolates cultural filtrates to study their efficacy on spore germination inhibition of *A. burnsii*. Double strength than required concentration for *Trichoderma* and *Pseudomonas fluorescens* filtrates were obtained by dilution technique in sterilized distilled water. Spore suspension was prepared in sterilized distilled water separately. Spore germination of *A. burnsii* was obtained by following procedure.

Preparation of spore suspension

Spores were collected from 10 day old culture of *A. burnsii* and respective suspensions prepared utilizing 10mL. of sterilized water. The suspension was then examined under the microscope (10x) and again adjusted to about 30 spores per one optical field.

Preparation of cultural filtrate of bioagents

The effect of cultural filtrate of 30 *Trichoderma* and 10 *Pseudomonas fluorescens* isolates on the growth of *A. burnsii* was studied. Potato dextrose broth and King's-B broth were prepared and inoculated with respective antagonist(s). Cultural filtrate of the respective antagonist(s) grown in potato dextrose broth and nutrient broth for 12 days were filtered using what man paper no. 41 in laminar air flow and recovered cultural filtrates.

Preparation of the slides

One drop of each *Trichoderma* and *Pseudomonas fluorescens* filtrate suspension was placed separately on a glass slide and one drop of spore suspension was placed exactly on this respective drop so that required concentration was obtained in each of the treatment. The experiments were replicated four times. These slides were kept in Petri plates lined with moist blotting paper and incubated at room temperature.

Observations of germinated spores were recorded at interval of after 24, 48 hrs and 72 hrs, per cent spore germination and spore germination inhibition were worked out as per following formula (Vincent, 1947).

$$PG = \frac{A}{B} X\ 100$$

Where,

PG = Per cent germination

A = Number of conidia germinated

B = Total number of conidia examined

$$I = \frac{C - T}{C} X\ 100$$

Where,

I = Per cent inhibition

C = Number of germinated spores, in control

T = Number of germinated spores, in treatment

RESULTS AND DISCUSSION

Effect of cultural filtrate of different *Trichoderma* isolates

Table1: Spore germination of *A. burnsii* under cultural filtrate of different *Trichoderma* isolates

Sr.No	*Trichoderma* isolates	Concent-ration(%)	Per cent spore germination after* 24 hrs	48 hrs	72 hrs	Mean (pooled)	Per cent inhibition over control
1	*Trichoderma* -1	40	43.44	44.89	46.11	44.81	44.90
2	*Trichoderma* -2	40	39.44	40.11	41.99	40.51	50.19
3	*Trichoderma* -3	40	33.55	34.22	35.89	34.55	57.51
4	*Trichoderma* -4	40	41.33	42.55	43.22	42.36	47.91
5	*Trichoderma* -5	40	37.44	38.55	39.11	38.36	52.83
6	*Trichoderma* -6	40	38.66	39.77	40.89	39.77	51.10
7	*Trichoderma* -7	40	32.99	33.55	34.44	33.99	58.20
8	*Trichoderma* -8	40	35.11	36.44	37.77	36.44	55.19
9	*Trichoderma* -9	40	38.77	39.44	40.66	39.62	51.28
10	*Trichoderma* -10	40	40.89	42.22	43.44	42.18	48.13
11	*Trichoderma*-11	40	28.11	29.00	30.78	29.29	63.98
12	*Trichoderma* -12	40	29.66	31.22	31.89	30.92	61.98
13	*Trichoderma* -13	40	35.22	36.66	37.88	35.58	56.25
14	*Trichoderma* -14	40	30.11	31.33	32.21	31.21	61.62
15	*Trichoderma* -15	40	44.22	45.11	46.11	45.14	44.49
16	*Trichoderma* -16	40	41.22	42.55	43.55	42.44	47.81
17	*Trichoderma* -17	40	33.22	34.66	35.89	34.59	57.46
18	*Trichoderma* -18	40	33.44	35.18	36.51	35.04	56.91
19	*Trichoderma* -19	40	28.77	29.55	31.22	29.84	63.30
20	*Trichoderma* -20	40	36.55	37.32	38.55	37.47	53.92
21	*Trichoderma* -21	40	36.44	37.77	39.00	37.73	53.60
22	*Trichoderma* -22	40	32.00	33.11	34.33	33.14	59.25
23	*Trichoderma* -23	40	39.77	40.89	41.55	40.73	49.92
24	*Trichoderma* -24	40	31.89	33.77	35.55	33.73	58.52
25	*Trichoderma* -25	40	33.77	35.27	36.55	35.19	56.73
26	*Trichoderma* -26	40	36.77	38.11	39.21	38.03	53.23
27	*Trichoderma* -27	40	39.66	40.33	41.11	40.36	50.37
28	*Trichoderma* -28	40	37.66	38.66	39.44	38.58	52.56
29	*Trichoderma* -29	40	41.66	42.66	44.00	42.77	47.41
30	*Trichoderma* -30	40	31.55	32.66	33.44	32.53	60.00
31	Control	-	74.00	82.00	88.00	81.33	
	S. Em. ±		0.73	0.78	0.75	0.10	-
	CD at 5%		2.06	2.19	2.11	2.80	-
	CV %		3.39	3.47	3.23	4.44	

* Mean of four replications

Table 2: Spore germination of *A. burnsii* under cultural filtrate of different *P. fluorescens* isolates

Sr.No	*Pseudomonas fluorescens* isolates	Concent-ration(%)	Per cent spore germination after*			Mean (pooled)	Per cent inhibition over control
			24 hrs	48 hrs	72 hrs		
1	*P. fluorescens*-1	40	38.29	40.81	42.18	40.42	49.47
2	*P. fluorescens*-2	40	36.77	37.44	38.77	37.66	52.92
3	*P. fluorescens*-3	40	45.77	47.55	49.22	47.51	40.61
4	*P. fluorescens*-4	40	40.63	42.63	43.63	42.29	47.13
5	*P. fluorescens*-5	40	47.63	49.11	50.11	48.95	38.81
6	*P. fluorescens*-6	40	41.96	43.63	45.29	43.62	45.47
7	*P. fluorescens*-7	40	30.44	32.77	34.41	32.54	59.32
8	*P. fluorescens*-8	40	51.44	53.55	54.77	53.25	33.43
9	*P. fluorescens*-9	40	41.44	43.55	45.07	43.36	45.80
10	*P. fluorescens*-10	40	49.33	51.11	53.89	51.44	35.70
11	Control	-	72.00	81.00	87.00	80.00	
	S. Em. ±		0.55	0.53	0.40	1.62	-
	CD at 5%		1.63	1.55	1.17	4.76	-
	CV%		2.13	1.92	1.40	5.94	

* Mean of four replications

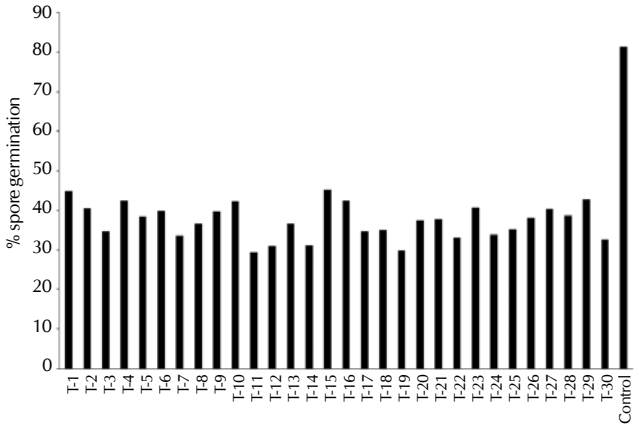

Figure 1: Spore germination of *A. burnsii* in cultural filtrate of *Trichoderma* isolates

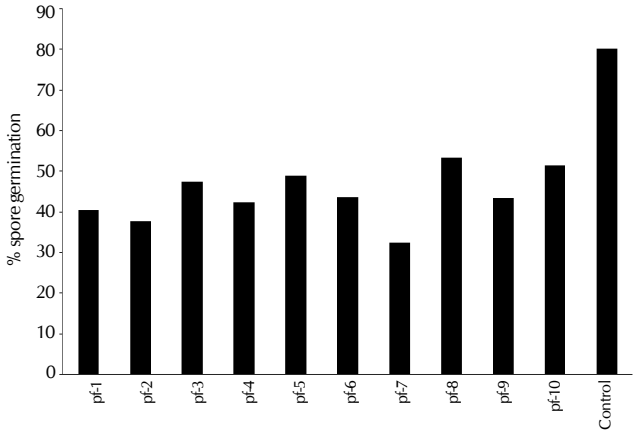

Figure 2: Spore germination of *A. burnsii* in cultural filtrates of *P. fluorescens* isolates

on spore germination of *A. burnsii*

An effect of cultural filtrate of thirty different *Trichoderma* isolates on the spore germination of *A. burnsii* was evaluated at 40 per cent concentrations by slide spore germination technique (Table. 1). All the isolates significantly reduced spore germination of test fungus with variation in their efficacy. Minimum spore germination (29.29 %) of *A. burnsii* was observed with the culture filtrate of *Trichoderma* isolate-11 which was closely followed by *Trichoderma* isolate-19 with 29.84 % and *Trichoderma* isolates-12 (30.92 %) as against 81.33 per cent in control. Overall the spore germination inhibition ranged between 44.49 to 63.98 per cent among all isolates. There was little increase in spore germination when kept for more hours (Fig. 1)

For detecting the antifungal properties of cultural filtrate of *Trichoderma* isolates when tested against the *A. burnsii* fungus to measure their inhibitory effect on spore germination, the*Trichoderma* isolates 11, 19 and 12 are proved highly effective. This findings are supported by results of Odebode (2006). He examined the antagonistic activity of cultural filtrates of*T. harzianum* and *T. pseudokoningii* strains completely inhibited germination of conidia/spores of rot pathogens*viz. Macrophomina phaseolina, Fusarium solani, Alternaria* sp. and *Aspergillus niger*. Among two spp. of *Trichoderma, T.viride* exhibited significantly higher inhibition of *A. solani* causing blight of tomato (Patel, *et al*., 2012). Among the three species of *Trichoderma* tested so far, *T. viride* was found to be the most effective with 61.62% inhibition followed by *T. harzianum* and *T. virens* with 60.80 and 59.49 per cent inhibition, respectively, over control after 96h of incubation period (Chakrabarty,*et al*., 2013).The effectiveness of*Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2 and *T. piluliferum* against *A. alternata*was reported by (Thakur and Harsh,2014).

Effect of cultural filtrate of different *P. fluorescens* isolates on spore germination of *A. burnsii*

An effect of ten different *Pseudomonas fluorescens* isolates on the spore germination of test fungus was evaluated at 40 per cent concentrations (Table.2.). Different isolates varied in their efficacy to inhibit the spore germination of the fungus under study. Minimum mean spore germination (32.54%) of *A. burnsii* was observed with the culture filtrate of *P. fluorescens* isolate-7, it was followed by *P. fluorescens* isolate-2with 37.66 per cent spore germination. Rest of the cultural filtrates of *P.*

fluorescens exhibited meanspore germination of *A. burnsii* with the range of 40.42 to 53.25 percent as compared to 80.00 per cent in control. (Fig. 2)

All the 10 isolates *Pseudomonas fluorescens* effectively reduced spore inhibition of *A. burnsii*. More than 50 per cent spore inhibition was observed in isolates 7 and isolate 2.This result are supported by the findings of Singh and Singh(2014) where they have reported antagonist potential of seventeen isolates of *Trichoderma harzianum* and ten isolates of *Pseudomonas fluorescens*was determined against *Exserohilum turcicum* under *in vitro* condition. They found that Th-39 and Psf- 82 gave maximum inhibition of mycelial growth of the pathogen by 77.11 and 56.00 percent respectively. Akbari and Parakhia (2007) where they have reported strong growth inhibition of *Alternaria alter nata* causing blight of sesame using *T. viride-I, T. harzianum-IV* and *V* and *Bacillus subtilis-G*.

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