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Compatibility of Fungal Antagonists Filtrates Against Germination of Powdery Mildew Spores, *Leveillula taurica* (Lev.) Arn. of Bell Pepper

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Abstract: Interaction between powdery mildew spores of *Leveillula taurica* (Lev.) Arn. and the culture filtrates of biological control agents *Trichoderma harizianum* and *T. viride* against conidial spore germination was studied *in vitro*. For each biological control agent two media were tested viz., Potato Dextrose Broth (PDB) and *Trichoderma* specific medium (TSM). Maximum of 1.25 and 2% conidial spore germination of *L. taurica* was observed against culture filtrates of *T. harizianum* and 2.25 and 3% in *T. viride* on PDB and TSM, respectively. However, a maximum spore germination of 68.5 and 65.5% was observed in un inoculated PDB and TSM, respectively. Single distilled water considered as control recording 30.5% powdery mildew spore germination.

Key words: Conidial spores, *Leveillula taurica*, powdery mildew, culture filtrates, *Trichoderma harizianum*, *T. viride*, percent germination

INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is important vegetable crop next to potato and tomato. In India, bell pepper is grown in 4,783 hectares and production is 42,230 tones (Madhavi, 2003). The increasing trend in the more area of cultivation under this crop is due to the large demand of vegetables, not only for table purpose but also because of their medicinal values (http://www.calantilles.com/cateframes/capsicum_peppers.htm). *Capsicum* is prone to many air borne fungal diseases of which the powdery mildew, *Leveillula taurica* (Lev.) Arn. takes heavy toll under greenhouse and field every year all over the world (Palti, 1988). Since several strains of the biological control agent i.e., genus *Trichoderma* are being tested as alternatives to chemical fungicides (Harman and Kubicek, 1998) and also are eco-friendly and were more effective in controlling powdery mildew (Brand *et al.*, 2002). Hence, in the present study the plant antagonists, *Trichoderma harizianum* Rifai and *Trichoderma viride* Pers.:Fr. culture filtrates against conidial spore germination of *L. taurica* were considered.

MATERIALS AND METHODS

The fungal antagonists *Trichoderma harizianum* and *Trichoderma viride* used in horticultural crops as an effective biological control agents were considered for the study to determine the influence of the fungal antagonists filtrates on the germination of the *Leveillula taurica*,

conidial spores. The study was aimed to determine percent conidial spore germination on different media, temperatures, humidity and also per cent suppression of spore germination against different fungal antagonists filtrates at different concentrations. The experiments were carried out at G.K.V.K, UAS, Bangalore, India during 2006.

Preparation of fungal antagonists filtrates: *Trichoderma harizianum* and *T. viride*, were cultured in Petri plates (9 cm width) on Potato Dextrose Agar (PDA) (HIMEDIA Laboratory Ltd., Mumbai, India) at 27±1°C, 65% RH and photoperiod of 16:8 L:D for a period of 7 days in BOD incubator (S.K. Enterprises, Bangalore, India). From the above plates a 6 mm circular cut disc of fungal antagonists transferred on to the Potato Dextrose Broth (PDB) and *Trichoderma* Specific Medium (TSM) (Elad *et al.*, 1981) separately. The inoculated PDB and TSM was kept in shaker at 60 rpm, 27°C for two weeks; later the full-grown culture of PDB and TSM was filtered through whatman paper No. 1 to avoid *Trichoderma* spores and mycelial fragments. The filtrates of *T. harizianum* and *T. viride* devoid of its spores was taken freshly for the experiment.

Preparation of powdery mildew spore solution: The lower leaves of bell pepper (*Capsicum annuum* L.) infected by powdery mildew, *Leveillula taurica* was freshly collected from the unsprayed control plots of experimental field at G.K.V.K, UAS, Bangalore. Later the lower sporulated surface of leaves were washed with 1 mL of sterile water and average number of spores per milliliter was calculated.

Freshly prepared powdery mildew spore solution was tested for spore germination against 1) media viz., Glucose 5%, Glucose 2% Sucrose 5%, Sucrose 2%, sterile water, distilled water and tap water at room temperature. 2) temperatures viz., 5, 10, 15, 20, 25, 30 and 35°C on 5% sucrose solution at 50% RH. 3) RH viz., 20, 30, 40, 50, 60, 70, 80 and 90% on 5% sucrose solution at 20°C. 4) Filtrates of *Trichoderma harizianum* grown on PDA and TSM at room temperature. 5) Filtrates of *T. viride* grown on PDA and TSM at room temperature. Single distilled water served as control. All the treatments were replicated five times and the experiment was repeated to ensure the reproducibility. The conidial spore germination from the each treatment was recorded using improved Neubauer double ruled haemocytometer and phase contrast microscope at a magnification of 600x after one hour of incubation at 25°C and 65% RH. The results were expressed in per cent conidial spores germination to determine the overall effect. Compatibility was decided finally based on the percent germination of conidial spores.

The experiment was conducted in a Completely Randomized Design (CRD). Squire root transformation was used to analyze the differences in germination of spores. The data was expressed in mean percent germination of conidial spores. The resultant data was subjected to analysis of variance (ANOVA) SAS (1996). The treatment means were compared using Critical Difference (CD) at p = 0.05.

RESULTS

Among the antagonist filtrates tested, *in vitro*, filtrates of both the species of *Trichoderma* viz., *T. harizianum* and *T. viride* inhibited the maximum germination of asexual spores of *Leveillula taurica*. The results found significant at p = 0.05 (Table 1).

Table 1: Influence of PDB and TSM alone and culture filtrates of *T. harizianum* and *T. viride* against spore germination of *Leveillula taurica*

Treatments	Mean (%) germination**	Treatments	Mean (%) germination**
SDW: PDB (µL)*		SDW: <i>T. h</i> filtrate on TSM (µL)*	
95:05	32.5 (5.74)	95:05	2.00 (1.56)
90:10	33.0 (5.79)	90:10	1.25 (1.31)
80:20	37.5 (6.16)	80:20	0.25 (0.84)
70:30	40.0 (6.36)	70:30	0.00 (0.71)
60:40	40.5 (6.40)	60:40	0.00 (0.71)
50:50	42.8 (6.58)	50:50	0.00 (0.71)
40:60	48.0 (6.96)	40:60	0.00 (0.71)

Table 1: Continued

Treatments	Mean (%) germination**	Treatments	Mean (%) germination**
30:70	51.3 (7.19)	30:70	0.00 (0.71)
20:80	53.5 (7.35)	20:80	0.00 (0.71)
10:90	58.0 (7.65)	10:90	0.00 (0.71)
05:95	60.8 (7.83)	05:95	0.00 (0.71)
00:100	65.5 (8.12)	00:100	0.00 (0.71)
SDW: TSM (µL)*		SDW: <i>T. v</i> filtrate on PDB (µL)*	
95:05	32.3 (5.72)	95:05	2.25 (1.65)
90:10	34.3 (5.89)	90:10	1.00 (1.22)
80:20	38.5 (6.24)	80:20	0.00 (0.71)
70:30	40.8 (6.42)	70:30	0.00 (0.71)
60:40	41.3 (6.46)	60:40	0.00 (0.71)
50:50	42.8 (6.57)	50:50	0.00 (0.71)
40:60	48.0 (6.96)	40:60	0.00 (0.71)
30:70	52.3 (7.26)	30:70	0.00 (0.71)
20:80	55.5 (7.48)	20:80	0.00 (0.71)
10:90	58.5 (7.68)	10:90	0.00 (0.71)
05:95	62.0 (7.90)	05:95	0.00 (0.71)
00:100	68.5 (8.31)	00:100	0.00 (0.71)
SDW: <i>T. h</i> filtrate on PDB (µL)*		SDW: <i>T. v</i> filtrate on TSM (µL)*	
95:05	1.25 (1.31)	95:05	3.00 (1.86)
90:10	1.00 (1.22)	90:10	0.75 (1.10)
80:20	0.25 (0.84)	80:20	0.00 (0.71)
70:30	0.00 (0.71)	70:30	0.00 (0.71)
60:40	0.00 (0.71)	60:40	0.00 (0.71)
50:50	0.00 (0.71)	50:50	0.00 (0.71)
40:60	0.00 (0.71)	40:60	0.00 (0.71)
30:70	0.00 (0.71)	30:70	0.00 (0.71)
20:80	0.00 (0.71)	20:80	0.00 (0.71)
10:90	0.00 (0.71)	10:90	0.00 (0.71)
05:95	0.00 (0.71)	05:95	0.00 (0.71)
00:100	0.00 (0.71)	00:100	0.00 (0.71)
		Control (SDW)	30.5 (5.57)

CD (p = 0.05%): 0.137
SEM±0.049

* SDW = Single Distilled water; PDB = Potato Dextrose Broth; TSM = *Trichoderma* Specific Medium; *T. h* = *Trichoderma harizianum*; *T. v* = *T. viride*. ** Mean of five replications. Figures in parentheses are squire root transformed values

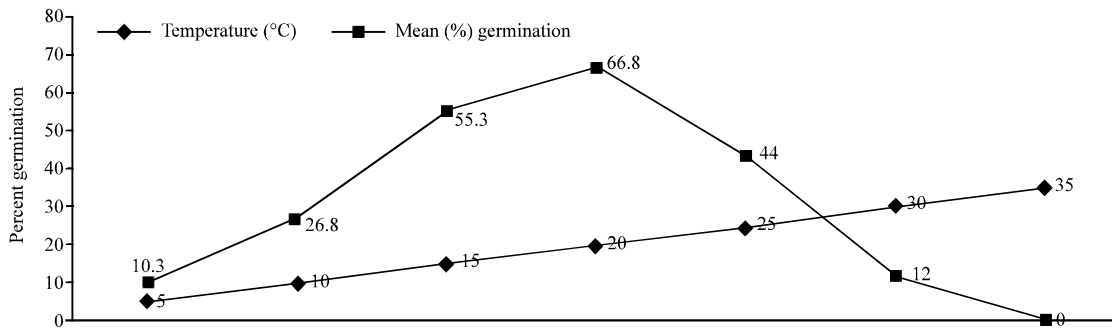


Fig. 1: Percent germination of powdery mildew spores against different temperatures (°C)

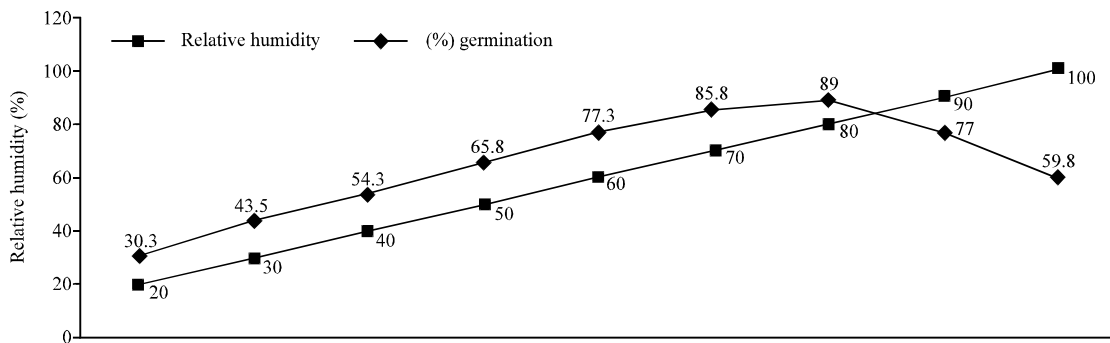


Fig. 2: Percent germination of powdery mildew spores against different relative humidity (%)

Influence of PDB and TSM against spore germination of *Leveillula taurica*: When powdery mildew spores were allowed to germinate over PDB, there was highly significant difference was observed between concentrations. Form the Table 1 it is clear, as the concentration of PDB increases the mean percent germination increased gradually and the maximum conidial spore germination recorded was 65.5% in undiluted PDB as against 30.5% in control which is single distilled water alone. Similar trend was observed in the TSM, recording maximum conidial spore germination of 68.5% when the medium was taken without any dilution.

***Trichoderma harzianum* filtrate on PDB and TSM, against powdery mildew spore germination:** Deprived germination of powdery mildew spores was observed against *T. harzianum* culture filtrates. The maximum spore germination of 1.25 and 2% was recorded in 95:5 μ L (SDW: *T. harzianum* filtrate) *T. h* filtrates of PDB and TSM, respectively. A consistent percent germination of conidia was recorded in control, throughout the experiment i.e., 30.5%. However, no conidial spore germination was observed in any of the *T. harzianum* filtrate concentrations above 70:30 μ L (SDW:

T. harzianum filtrate) indicating complete inhibition of conidial germination even after the extended time of incubation (Table 1).

***Trichoderma viride* filtrate on PDB and TSM, against powdery mildew spore germination:** A similar trend as in *T. harzianum*, was observed in *Trichoderma viride* filtrates on PDB and TSM (Table 1), against spore germination was recorded a maximum of 2.25 and 3% mean, respectively, in 95:05 μ L (SDW: *T. viride* filtrate) *T. viride* filtrates of PDB and TSM. Complete conidial spore inhibition was recorded form the concentrations above 90:10 μ L (SDW: *T. harzianum* filtrate) of *T. viride* culture filtrate.

However, among the cultures filtrates *T. harzianum* on both the media i.e., PDB and TSM performed better in inhibiting germination of powdery mildew spores and could able to limit the percent germination 1.25 and 2 in PDB and TSM, respectively. In contrast, maximum of 2.25 and 3% germination was observed in *T. viride*, respectively in PDB and TSM.

In the other set of study significant result obtained, when powdery mildew conidial spores allowed to germinate over different media (Table 2). A maximum

Table 2: Percent germination of powdery mildew spores against different media

Solutions	Mean (%) germination*
Glucose 5 %	54.5 (7.38) ^e
Glucose 2 %	50.5 (7.11) ^d
Sucrose 5 %	66.5 (8.15) ^a
Sucrose 2 %	61.5 (7.84) ^b
Sterile water	32.0 (5.66) ^g
Distilled water	35.5 (5.96) ^f
Tap water	39.5 (6.28) ^f

CD (p = 0.05%): 1.18, * Mean of four replications, Figures in parentheses are square root transformed values, Similar alphabet letters do not show significant difference

percent germination of 66.5 was observed on 5% sucrose solution followed by 2% sucrose recording 61.5% against 32, 35.5 and 39.5% in sterile water, distilled water and tap water, respectively.

A comprehensive study revealed among different temperatures (°C) tested against spore germination at 50% RH, in 5% sucrose solution; the maximum conidial spore germination of 66.8% at 20°C was recorded as a peak in the graph. As the temperature increased the percent germination was also increased upto 20°C and it drastically declined to no-percent germination at 35°C (Fig. 1).

It is clear from the curve (Fig. 2) of percent conidial spore germination against relative humidity (%) that percent germination gradually increased when the relative humidity (%) increased keeping the temperature constant (20°C) upto 80% RH and gradually declined. A peak of 89% of conidial spore germination was observed at 80% RH followed by 85.8% in 70% RH.

DISCUSSION

Spore germination implies a change from an inactive to active growing condition. This is accomplished in most fungi by the formation of germ tube (that is germinability); In one set of the experiment, where powdery mildew spores were tested for its better germinability against different media. Among that, sucrose found to be the best at its 5 and 2% concentrations. Similarly, Biju (2000) observed maximum spore germination of *Erysiphe polygoni* DC. in 5% sucrose solution. Based on this results, germinability at different temperatures and humidity were carried out in 5% sucrose solution in which 80% RH reported to be best at 20°C. Carroll and Willcox (2003) data suggested that germination frequency of *Uncinula nector* conidia *in vitro* (grape seedlings) feel sharply at RH levels above a mean of 87%.

Based on the present investigations, in both the mediums viz., PDB and TSM, there is more than two fold

increase in mean per cent germination of powdery mildew spores when compared with control. When PDB and TSM were inoculated with *T. harzianum*, later powdery mildew spores were allowed for germination in its filtrates resulted in significantly poor spore germination at very low concentrations (90:10 SDW:*T.h/T.v* filtrate). The fungal antagonist filtrate completely inhibited conidial spore germination above 80:20 to 0:100 (SDW:*T.h/T.v* filtrate) concentrations. The volatile metabolites produced by *Trichoderma* sp. could inhibit the conidial spore germination, Mannina *et al.* (1997) isolated new tetracyclic direrpene (C₂OH₂8O₂) from culture filtrates of *T. viride* was found as a strong inhibitor of fungal growth and spore germination. However, there was no significant difference found between the two *Trichoderma* sp. in inhibiting the powdery mildew spore germination. Over all, the antagonist fungal filtrates were found significant in inhibiting germination of *Leveillula taurica* conidial spores. Rajakumar *et al.* (2005) observed similar results when they conducted bioassay with culture filtrates of *T. viride*, *Chaetomium globosum* and *Acremonium implicatum* resulted in significant inhibition of Pycnidiospore germination of *Ascochyta rabiei*. The present study would form an important component in our future research programmes that aims to control powdery mildews of different crops of horticultural importance.

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