Brazilian Journal of Biological Sciences, 2019, Vol. 6, No. 12, p. 133-139.
ISSN 2358-2731
https://doi.org/10.21472/bjbs.061212

Effect of culture filtrates of pathogenic and antagonistic fungi on seed germination of some economically important vegetables

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Abstract. The subject of present study was to check whether the pathogenic fungi that were associated with different rot diseases of fruits and vegetables and the antagonistic fungal species produce extracellular growth regulating substances. For this present study healthy seeds of four economically important crop plants, viz. *Solanum lycopersicum, Brassica rapa, Raphanus sativus* and *Trigonella melongena* were selected. The results showed that all the pathogenic fungi except *Fusarium solani* decrease the germination percentage of the all seeds. *Solanum lycopersicum* seed germination was completely inhibited by the culture filtrate of *Trichothecium roseum* and *Alternaria alternata*. Likewise, the culture filtrate of *Penicillium expansum* caused complete inhibition of the germination of *Brassica rapa* seeds. The culture filtrate of *Fusarium solani* was found to increase the germination percentage of all the seeds tested during the present study. Amongst the three *Trichoderma* spp., *T. asperellum* and *T. harzianum* culture filtrate effectively increases the seed germination percentage of all the seeds tested while the culture filtrate of *T. viride* have negative effect on the germination percentage of *Solanum lycopersicum, Brassica rapa,* and *Raphanus sativus* seeds. This stimulatory or inhibitory effect of the culture filtrates can be attributed to the presence of certain metabolites/substances that the test fungi have released in the medium. To identify the substances present and the nature of these substances further studies will be carried out.

Keywords: Antagonistic fungi; Culture filtrate; Pathogenic fungi; Seed germination.

Received
January 18, 2019

Accepted
February 13, 2019

Released
April 30, 2019

Full Text Article

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Introduction

Fungal rot of fruits and vegetables is the predominant postharvest disease (Janisiewicz and Korsten 2002) and cause huge losses to the growers in terms of yield (Bhale 2011). Certain fungal pathogens also synthesize certain mycotoxins or metabolites on their hosts which are harmful to the consumers and restrict their growth (Roy et al., 1972; Alwakeel, 2013). These fungal metabolites are substances that are discharged by the fungal species during their metabolic processes. The metabolites can be aminoacids, phenols, terpenoids, plant growth regulators (Madhosing, 1995).

Certain fungal genera like *Aspergillus*, *Penicillium*, *Alternaria*, *Rhizoctonia* produce mycotoxins and degrade the seed quality and their viability (Caster and Frederiksen, 1980). These toxins are known to suppress the germination and sprouting of several seeds (Roy et al., 1972; Kunwar et al., 1987). Strains of *Trichoderma* spp. used as biological agents are capable of increase in plant growth even in absence of pathogen. The application of *Trichoderma* causes increase in the germination, plant height, leaf area and dry weight (Lindsey and Baker, 1967; Inbar et al., 1996).

So the aim of the present study was to check the effect of extracellular metabolites produced by the pathogenic fungi and the antagonistic fungi on seed germination of some economically important vegetable plants.

Materials and methods

Fungal isolates

The fungal pathogens and the antagonistic fungi used during the present study were obtained from Section of Mycology and Plant Pathology, Department of Botany, University of Kashmir, Srinagar. The fungal pathogens, *Trichothecium roseum*, *Aspergillus niger*, *Cladosporium herbarum*, *Alternaria alternata*, *Penicillium chrysogenum*, *Penicillium expansum* and *Fusarium solani* were isolated from decayed fruits and were found to be the common postharvest rot causing fungal pathogens of fruits and vegetables (Parveen et al., 2013, 2014; Parveen and Wani, 2015). Three different *Trichoderma* spp., viz. *T. harzianum*, *T. viride* and *T. asperellum* were isolated from healthy fruits and were further identified from IARI, New Delhi.

Preparation of culture filtrate

The fungal organisms both pathogenic as well as antagonistic used in this study were grown in Richard's solution. 100 mL of Richard's solution was transferred to 250 ml conical flasks and inoculated with fungal inoculum taken from the edge of growing hyphae of 7 days old culture grown on PDA medium. The flasks were incubated at 25 °C ± 2 °C for 20 days. The mycelial mat was then removed by filtering through whatman's filter paper. The filtrate was then centrifuged to obtain a cell free culture filtrate. The filtrate was collected in presterilized conical flasks and used for further experiments.

Effect of culture filtrate on seed germination of some vegetables

The effect of culture filtrates of the fruit rot causing fungal pathogens and the antagonists was determined by their effect on seed germination. Healthy seeds of crop plants, viz. *Solanum lycopersicum, Brassica rapa, Raphanus sativus* and *Trigonella melongena* were selected and surface sterilized by 0.1% mercuric chloride and then by distilled water. Sterilized seeds were soaked in the culture filtrate for 24 h. These were them removed from the culture filtrate and placed on moist filter paper in presterilized Petriplates. The petriplates were then sealed with parafilm and kept at room temperature. Seeds soaked in uninoculated liquid medium and distilled water served as control. 10 replicates were taken for each treatment. Percent germination of seeds was observed and recorded as under:

$$\textbf{Percent seed germination} = \frac{\textbf{Number of seeds germinated}}{\textbf{Total number of seeds}} \textbf{ x 100}$$

Results

The toxicity of the culture filtrates prepared from the isolated rot causing fungal pathogens and the biological control agents was tested against the seed germination of *Solanum lycopersicum, Brassica rapa, Raphanus sativus* and *Trigonella melongena* to check the effect of extracellular metabolites released by the fungus in the liquid culture medium. Amongst the fruit rot pathogenic fungi, the highest toxicity or least germination was found by the culture filtrate obtained from *Trichothecium roseum*, *Alternaria alternata* and *Penicillium expansum* (Table 1, Figure 1). This inhibition in germination can be attributed to certain metabolites which are produced by the fungal pathogens to restrict the germination of the seeds. *Solanum lycopersicum* seed germination was completely inhibited by the culture filtrate of *Trichothecium roseum* and *Alternaria alternata*. Likewise, the culture filtrate of *Penicillium expansum* caused complete inhibition of the germination of *Brassica rapa* seeds. Amongst the fruit rot fungal pathogens *Fusarium solani* was found to accelerate the germination of all the tested seeds.

Table 1. Effect of culture filtrates of isolated fungi on seed germination of different crop plants.

Culture filtrate	Seed germination (%)			
	Solanum lycopersicum	***Brassica rapa***	***Raphanus sativus***	***Trigonella melongena***
Trichothecium roseum	0	10	20	50
Aspergillus niger	10	10	50	60
Cladosporium herbarum	10	10	40	70
Alternaria alternata	0	0	30	60
Penicillium chrysogenum	20	20	60	60
Penicillium expansum	20	0	40	50
Fusarium solani	50	70	100	100
Liquid culture	30	60	80	100
Control (H_2O)	50	50	70	80

The culture filtrate of the three antagonistic fungi that are used for the biological control studies, *Trichoderma harzianum, Trichoderma viride* and *Trichoderma asperellum* were also evaluated for the effect on the seed germination (Table 2, Figure 2). Amongst the three species, *T. asperellum* and *T. harzianum* culture filtrate were found to have stimulatory effect on seed germination and was found to cause increase in the seed germination percentage. Culture filtrate of *T. viride* cause inhibition in seed germination of *B. rapa* and *R. sativus*. Culture filtrate of all *Trichoderma* spp. were found to increase the seed germination percentage of *Solanum lycopersicum* and *Trigonella melongena,* whereas a negative effect was found on the germination percentage of *B. rapa* and *R. sativus* seeds by culture filtrate of *T. viride*.

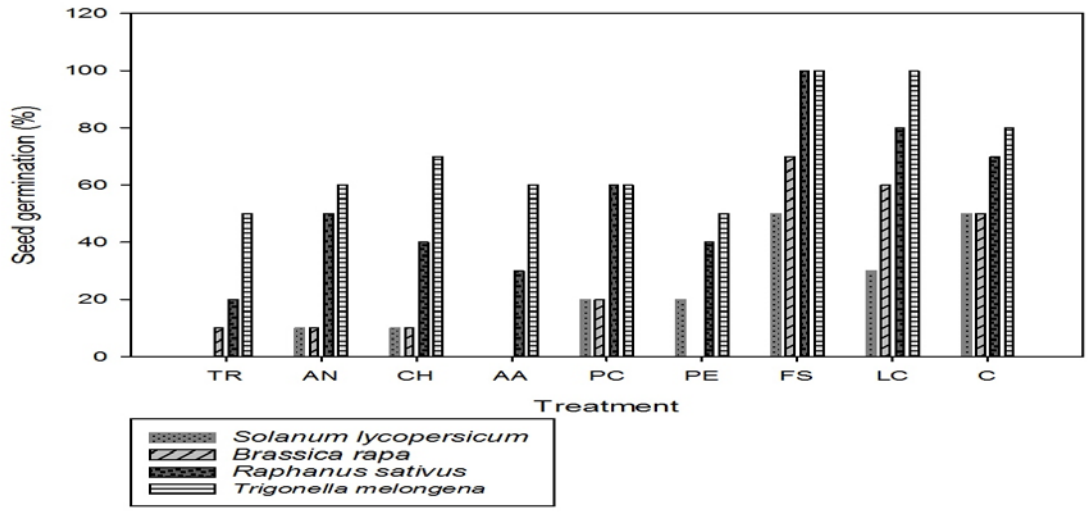

Figure 1. Germination percentage of different seed by treatment with different culture filtrates of pathogenic fungi. TR - *Trichothecium roseum*, AN - *Aspergillus niger*, CH - *Cladosporium herbarum*, AA - *Alternaria alternata*, PE - *Penicillium chrysogenum*, PC - *Penicillium expansum*, FS - *Fusarium solani*, LC - liquid culture control, C - Control (distilled H_2O).

Table 2. Effect of culture filtrates of antagonistic fungi on seed germination of different crop plants

Culture filtrate	Seed germination (%)			
	Solanum lycopersicum	***Brassica rapa***	***Raphanus sativus***	***Trigonella melongena***
Trichoderma harzianum	50	60	80	90
Trichoderma viride	40	30	50	100
Trichoderma asperellum	60	40	90	100
Liquid culture	30	60	80	100
Control (H_2O)	50	50	70	80

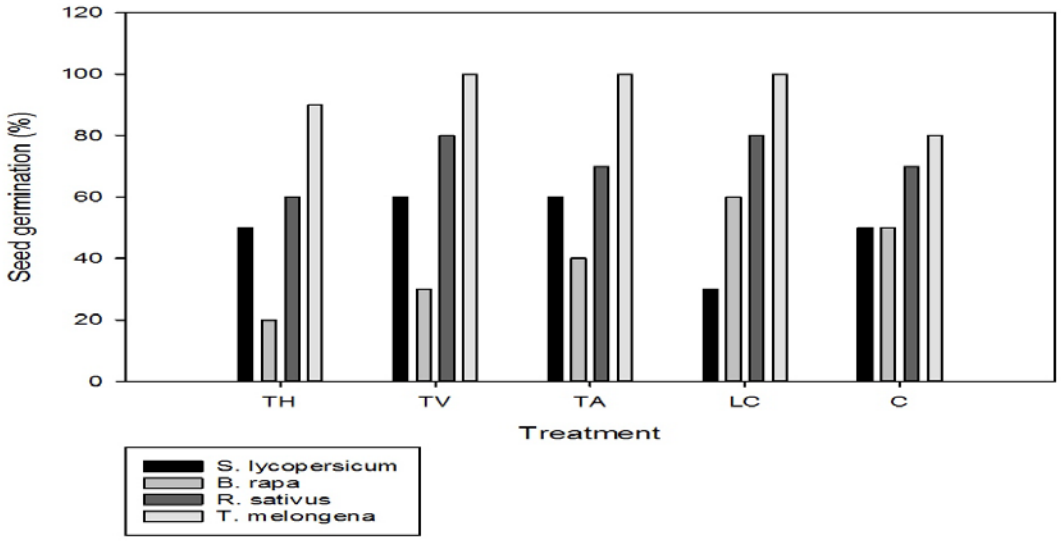

Figure 2. Germination percentage of different seed treated with culture filtrate of antagonistic fungi. TH - *Trichoderma harzianum*, TV - *Trichoderma viride*, TA - *Trichoderma asperellum*, LC - Liquid culture control, C- Control (H_2O).

Discussion

During the present study, it was reported that the culture filtrates of the pathogenic fungi caused inhibition in germination of all the tested seeds, viz. *Solanum lycopersicum, Brassica rapa, Raphanus sativus* and *Trigonella melongena*. Similar results were obtained by Haikal (2008), Jalander and Gachande (2012), Garuba et al. (2014). Kunwar and Mehrotra (1988) studied the effect of culture filtrate of storage fungi on germination and sprouting of wheat grains and reported that culture filtrate of all the fungal species screened inhibited germination and sprouting of grains, with complete inhibition by culture filtrate of *Aspergillus clavatus* and *Penicillium urticae.* Umechuruba and Nwachukwu (1997) reported that *Aspergillus niger*, *Aspergillus flavus*, *Fusarium moniliforme* and *Penicillium* sp. produce various metabolites that are known to reduce the seed germination percentage and seedling development of bean seeds. Jalander and Gachande (2012) reported that the culture filtrate of *Aspergillus niger* inhibit seed germination of several pulses and cereals. Haikal (2008) reported that the culture filtrates of *Aspergillus niger*, *Fusarium culmorium*, *Penicillium* sp. and *Rhizoctonia solani* inhibited seed germination and seedling development of soyabean, representing that these fungi produced some toxic substances in the culture media in which they were grown, inhibiting or reducing the germination percentage of seeds. Inhibition or stimulation of seed germination may be related to the certain substances that these fungi produce extracellularly, that can regulate the activity of hydrolytic enzymes (Negi et al., 1983).

Present study also depicted that the culture filtrate of antagonistic fungi like *Trichoderma harzianum, Trichoderma viride* and *Trichoderma asperellum* have a pronounced effect on the seed germination of *Solanum lycopersicum, Brassica rapa, Raphanus sativus* and *Trigonella melongena*. *T. harzianum* and *T. asperellum* effectively increased the seed germination percentage of all the seeds tested. Similar results were reported by Gupta and Sharma (1995), Celar and Valic (2005). Cole and Zvenyika (1988) reported that seeds of few plants like tobacco grew better when *T. harzianum* was added to the soil. Gupta and Sharma (1995) reported that the culture filtrate of *Trichoderma* spp. increased the seed germination of black gram.

Reduction in the seed germination by the culture filtrate can be attributed to the presence of certain mycotoxins, enzymes released by the respective fungal species (Karman and Malavuly, 2005). Antagonistic fungi used may directly or in directly influence the growth and development of higher plants. In previous studies, it was reported that the antagonistic fungi have stimulating effect on plants (Chang et al., 1986) with very little negative effects reported only when large amount of inoculum of antagonistic fungi is used (Kohl and Schlosser, 1989).

Conflicts of interest

Authors declare that they have no conflict of interests.

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