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Antifungal activity of selected plant extracts against *Trichothecium roseum* (Pers.) Link (1809) (Sordariomycetes: Hypocreales), causal organism of fungal rot of *Solanum melongena* L. (Solanales: Solanaceae) in Kashmir, India

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Abstract. Egg plant *Solanum melongena* L. (Solanales: Solanaceae) is an important vegetable grown in Kashmir. It is attacked by number of fungal pathogens in storage and in the field. These fungi caused several fungal rot diseases resulting heavy losses to the growers. Therefore, present study was carried out to study the incidence and management of fungal rot of egg plant using some selected plant extracts. It was revealed from the study that *Trichothecium roseum* (Pers.) Link (1809) (Sordariomycetes: Hypocreales) causing decaying of egg plant under storage. Study was also undertaken to evaluate the efficacy of some plant extract against *Trichothecium roseum* on inhibition of spore germination and mycelial growth under *in vitro* conditions. It was observed from the results that amongst the plant extracts, plant extract of *Ajuga bracteosa* at highest concentration was found most effective against *Trichothecium roseum* and cause highest inhibition in the mycelial growth and spore germination followed by plant extract of *Taraxicum officinale*, *Mentha arvensis* and *Iris kashmiriana* at the same concentrations. Other concentrations of plant extracts also bought about significant reduction in mycelial growth and spore germination of the test fungus but to a lesser extent as compared to control.

Keywords: Pink rot; Botanical fungicides; Inhibition; Mycelia growth; Spore germination; Brinjal.

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Introduction

Vegetables are important constituents of human diet. They are important sources of carbohydrates, minerals and vitamins. They are attacked by many pathogens like fungi and bacteria that result in loss of fresh produce (Mitcham and Mitchell, 2002; Wani, 2011). Losses from the post-harvest diseases in fresh produce fall into two categories i.e., loss in quantity and quality. Loss in quantity occurs where deep penetration of decay occurs in the infected vegetables. This is often as a result of infection of the vegetables in the field before harvest. Loss in quality occurs when the diseases affects only the surface of the vegetables. It may cause skin blemishes that can lower the value of the commercial crop. Rot diseases cause greatest losses to the vegetables in storage as well as in fields (Snowdon, 2003; Bashar et al., 2012).

Egg plant *Solanum melongena* L. belongs to Family Solanaceae and is indigenous vegetable crop of India including Kashmir. It contributes 9% of the total vegetable production of the country. China is the largest producer of brinjal followed by India. It is grown in India over an area of 0.4 million hectors with an annual production of 7.8 million tonnes (Datar, 1999). Post harvest decays of fruits and vegetables account for significant levels of post harvest losses. It is estimated that about 20%-25% of the harvested fruits and vegetables are decayed by pathogens during post-harvest handling even in developed countries (El-Ghaouth et al., 2004; Droby et al., 2009; Abano and San-Amaoh, 2012). In developing countries, post harvest losses are often more severe (more than 30%) due to inadequate post harvest handling, packaging, transportation and storage which may result in decay and production by micro-organisms which become activated because of the changing physiological state of the fruit (Tripathi and Dubey, 2004; Korsten, 2006; Singh and Sharma, 2007).

It has been reported that on an average, the oblong-fruited eggplant cultivars are rich in total soluble sugars, whereas the long-fruited cultivars contain a higher content of free reducing sugars, anthocyanin, phenols, glycoalkaloids (such as solasodine), dry matter, and amide proteins (Bajaj et al., 1979). The glycoalkaloid contents in the Indian commercial cultivars vary from 0.37 mg/100 g fresh weight to 4.83 mg) (Bajaj et al., 1981). Brinjal is known to have ayurvedic medicinal properties and is good for diabetic patients. It has also been recommended as an excellent remedy for those suffering from liver complaints (Shukla and Naik, 1993). To the best of our knowledge, this is the first report of *T. roseum* causing pink rot on Brinjal in Kashmir region of Jammu and Kashmir, India. Various chemical fungicides have been used to control these fungal rot diseases, but these fungicides cause hazardous effect on humans and environment. Hence strong regulatory actions have been imposed on their use. So there is a strong need to control these diseases in an ecofriendly way.

Various biocontrol fungi and extracts obtained from many medicinal plants have gained much popularity and scientific interest for their antifungal and

antibacterial activities (Parveen et al., 2016; Koka et al., 2017). They are believed to be less hazardous than chemical fungicides and can therefore be used as an alternative to control fungal rot diseases (Jobling, 2000). The use of these plant extracts for inhibition of fungal diseases is an important step towards the assessment of the degree of variability among the diverse natural flora (Khandare and Vasait, 2017). The antifungal activities of these plant extracts are attributed to different chemical compounds like phenols, flavonoids, isoflavonoids, coumarins, pyrones, alkaloids, etc. present in these plants which effect the growth of pathogenic fungi (Jantasorn et al., 2016). Hence these plant extracts may have potential as a new natural fungicide for management of fungal rot pathogens. So in an approach towards ecofriendly management strategy, an attempt was made to study the efficacy of some plant extracts against *Trichothecium roseum* causal organism of fungal rot of brinjal in Kashmir.

The present study was carried out to evaluate the effect of different concentrations of plant extracts on mycelia growth and spore germination of *Trichothecium roseum* causing pink rot of brinjal.

Materials and methods

To investigate the fungi which cause the rotting of brinjal fruits in Kashmir Valley, diseased brinjal fruits were collected in separate polythene bags from different fields, markets, godowns and storage houses of Kashmir valley. These samples were either used immediately or stored at 10 °C in the laboratory for different pathological studies.

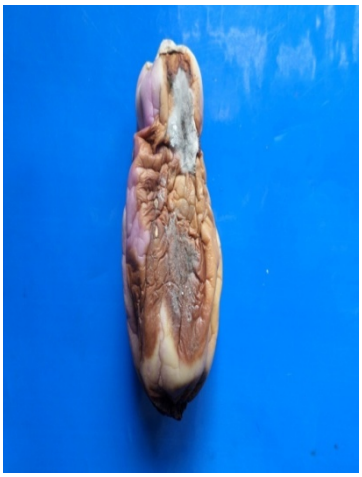

Figure 1. Infected brinjal fruit.

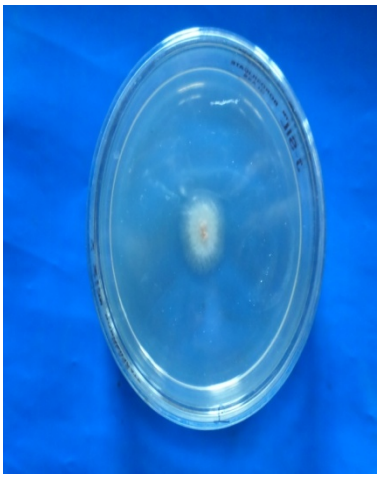

Figure 2. Culture of *T. roseum* on PDA (*Trichothecium* possess pinkish colored colonies).

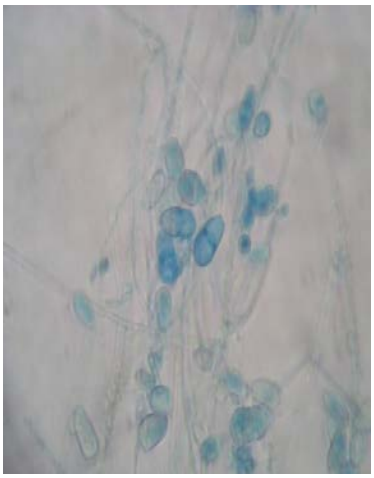

Figure 3. Conidiophore with conidia of *T. roseum* (100x) (Conidiophore was simple hyphae, septate in their lower half and bear clusters of conidia at the tip. Conidia of *T. roseum* were smooth and clavate).

Small portions of rotted tissues were cut out aseptically from the diseased brinjal fruits and transferred to Potato Dextrose Agar medium (PDA). The casual pathogen was identified on the basis of symptoms caused by the fungus on brinjal fruits, cultural and microscopic characteristics. The fungus

on Potato Dextrose Agar medium (PDA) after 48 h of inoculation at 24 °C ± 2 °C, produced white colonies and then due to conidial production the colonies turn light pink in colour (Figure 1, 2, and 3).

Pure colony cultures were obtained by sub-culturing the fungal growth in separate Petri plates containing the same medium. A pink rot fungus was identified by their morphological, reproductive and cultural characteristics (Ellis, 1971; Barnett and Hunter, 1972; Watanabe, 2002; Gilman, 2008).

For pathogenicity, pathogens were re-inoculated after isolation onto the healthy brinjal fruits (Tomkin and Trout, 1931). Then all the five brinjal fruits were kept in clean polythene bags and incubated at 25 °C ± 2 °C for ten days. These pathogenicity tests were used for the identification of plant pathogens and to confirm the detection of a particular disease. Identification of the disease and the pathogen was done following Koch's postulates. Parameters such as symptoms caused by these fungi on the healthy brinjal fruits, cultural characteristics of the pathogens and microscopic features of the pathogens were studied.

Preparation and evaluation of different concentrations of plant extracts

Different concentrations (i.e., S, S/2, S/5 and S/10) of aqueous extracts of leaves of *Ajuga bracteosa* Wall *ex* Benth, *Taraxacum officinale* Weber *ex* Wiggers, *Mentha arvensis* L. and *Iris kashmiriana* Baker were evaluated for their effect on the inhibition in mycelial growth and in spore germination of *Trichothecium roseum* isolated from decayed brinjal fruits.

To prepare various concentrations of plant extracts leaves were collected, cleaned and cut into small pieces before being dried under shade at room temperature. The dried material was ground to fine powder using a mechanical blender. Dry leaf powder (200 g) was packed in Soxhlet apparatus and extracted with distilled water at 80 °C-85 °C. The extracts were filtered through Whatmann filter paper No. 1 and the solvent was removed under reduced pressure at 35 °C-45 °C using rotavapor.

The dried extract (5 mg/5 mL) was considered as standard solutions (S) and stored at 4 °C in storage vials for experimental use (Kaul, 1997). Then other concentrations such as S/2, S/5 and S/10 were obtained by adding appropriate amount of sterilized distilled water to the standard concentration. These concentrations were evaluated for their effect on the mycelial growth by food poisoning technique (Adams and Wong, 1991). 1 mL from each concentration of the plant extract was mixed with nine ml of autoclaved and cooled PDA just before pouring into Petri plates. The medium was then dispensed uniformly into 90 mm sterile Petri plates and then inoculated with 5 mm mycelial disc of the pathogen from 10 day old fungal culture. Three replicates were maintained for each concentration including the control without any treatment. The Petri plates were incubated at 25 °C ± 2 °C and observations of the mycelial growth of test fungus were recorded after seven days of incubation.

The percent inhibition in fungal growth due to various treatments at different concentrations was computed as follows:

$$\textbf{Mycelial growth inhibition (\%)} = \{(dc - dt) / dc\} \times 100$$

Where:
dc = average diameter of fungal colony in control, and
dt = average diameter of fungal colony in treatment group.

The plant extracts of tested plants were also evaluated for their effect on the spore germination of *Trichothecium roseum*. Spore suspension was prepared from 10 days old fungal culture. A drop about 0.1 mL of spore suspension was then placed in a cavity glass slide containing a drop (about 0.1 mL) of different concentration of plant extract and then incubated at 25 °C ± 2 °C for 24 h in a moist chamber created in 100 mm Petri plates by covering both sides of the Petri plates with moist filter paper to maintain enough humidity. Three replicates were maintained for each treatment including the control. The slides were examined after 24 h by hand tally methods at different microscopic fields. Percent spore germination for each was recorded using formula given by Kiraly et al. (1974).

Percent spore germination = (No. of spores germinated/Total no. of spores examined) × 100

Statistical analysis

Statistical analysis was carried out using SPSS statistical software (version 16.0). Data was analyzed by one way analysis of variance (ANOVA) and comparison of the means was done by Duncan multiple comparison tests at $P \leq 0.05$.

Results

Effect of different concentrations of plant extracts on the mycelial growth of *T. roseum*

It was revealed from the results (Table 1) that different concentration of plant extracts caused significant inhibition in the mycelial growth of *Trichothecium roseum* as compared to control. However, the maximum inhibition in mycelial growth was found at highest concentration 'S' followed by lower concentrations S/2, S/5 and S/10 of the plant extracts. Among the plant extracts used *Ajuga bracteosa* at highest concentration 'S' was found most effective against *Trichothecium roseum* and cause highest inhibition in the mycelial growth (42.52%) followed by *Taraxacum officinale* (34.03%), *Mentha arvensis* (29.75%) and *Iris kashmiriana* (25.54%) at the same concentration.

Other concentrations of plant extracts also bought about significant reduction in the mycelial growth but to a lesser extent. In different concentrations of *Ajuga bractosea*, the inhibition in mycelial growth ranges from 42.52% to 23.37% and in *T. officinale*, the inhibition in mycelial growth ranges from 34.03% to 4.21%, respectively. Likewise, the inhibition in mycelial growth in different concentrations of *Mentha arvensis* ranges from 29.75% to 14.87% and in *Iris kashmiriana* the inhibition ranges from 25.54% to 19.15%, respectively.

Table 1. Effect of different concentrations of plant extracts on the mycelial growth of *Trichothecium roseum*.

	Mycelial growth (mm)				
	S	**S/2**	**S/5**	**S/10**	**Control**
Ajuga bracteosa	9.00 ±1.00^{c} (45.52%)	10.00±1.0^{c} (36.14%)	11.66±0.57^{b} (25.54%)	12.00±1.00^{b} (23.37%)	15.66±0.57^{a}
Taraxicum officinale	10.33±0.57^{b} (34.03%)	11.33±1.15^{b} (27.65%)	14.33±0.57^{a} (8.49 %)	15.00±1.00^{a} (4.21 %)	15.66± 0.57^{a}
Mentha arvensis	11.00±1.00 ^{c} (29.75%)	12.00±1.00$^{b\ bc}$ (23.37%)	12.66±1.15^{bc} (19.15%)	13.33±0.57^{b} (14.87 %)	15.66± 0.57^{a}
Iris kashmiriana.	11.66±0.57^{b} (25.54%)	12.00±1.00^{b} (23.37%)	12.33±2.08^{b} (21.26%)	12.66±1.52^{b} (19.15%)	15.66± 0.57^{a}

*Each value is mean of 3 replicates ± SD. Figures in parenthesis is the mycelial growth inhibition (%)

Effect of different concentrations of plant extracts on the spore germination of *T. roseum*

It was observed from the results (Table 2) that different concentrations (S, S/2, S/5 and S/10) of plant extracts caused significant reduction in spore germination of *T. roseum* compared to control. Among the plant extracts used, *A. bractosea* at highest concentration (S) was found most effective and caused highest reduction in spore germination followed by *T. officinale*, *M. arvensis L.* and *Iris kashmiriana*, respectively, at the same concentration. Other concentrations of plant extracts also bought about significant reduction in spore germination but to a lesser extent. In *A. bracteosa*, the inhibition in spore germination varies from 23.40% to 18.28% in different concentrations. In different concentrations of *Taraxicum officinale* the inhibition varies from 27.42% to 23.23% and in *Mentha arvensis* the inhibition varies from 33.48% to 26.49%, respectively. Likewise, the inhibition in the spore germination varies from 37.09% to 29.99% in different concentrations of *Iris kashmiriana*, respectively.

Table 2. Effect of different concentrations of plant extracts on the spore germination of *Trichothecium roseum*.

	Spore germination (%)				
	S	**S/2**	**S/5**	**S/10**	**Control**
Ajuga *bracteosa*	18.28±1.67^{c}	18.78±1.05^{c}	22.40±2.50^{b}	23.40±1.43^{b}	36.05±2.57^{a}
Taraxicum officinale	23.23±2.88^{b}	23.66±1.52^{b}	26.02±3.57^{b}	27.42±2.50^{b}	36.05±2.57^{a}
Mentha arvensis	26.49±1.29^{b}	28.33±2.88^{b}	33.01±2.86^{a}	33.48±2.80^{a}	36.05±2.57^{a}
Iris kashmiriana	29.99±3.33^{d}	32.91±1.84^{cd}	35.32±0.81^{bc}	37.09±0.88^{ab}	39.41±0.83^{a}

*Each value represents the mean spore germination %age of 3 replicates ± SD.

Discussion

It was clear from the results that brinjal fruits in storage are attacked by Pink rot fungi and caused Pink rot of brinjal. Such studies on fungal rot of brinjal have been carried out for the first time in Kashmir Valley. However, some earlier studies have been carried out on the fungal rot of brinjal in India and all

over the world. In the present study some plant extracts were evaluated for their antifungal activity against the fungus causing Pink rot of brinjal. *T. roseum* is Isolated from apples (Zabka et al., 2006) and eggplants (Pandey, 2010). Mycotoxins (roseotoxin B and trichothecin) production was carried from *T. roseum* by Engstrom et al. (1975) and Ghosal et al. (1982). Shamim Shamsi (2008) reported the association of this fungus with chickpea *Cicer arietinum* L.

Antifungal activities of these plant extracts are attributed to different chemical compounds like phenols, flavonoids, isoflavonoids, coumarins, pyrones, alkaloids, etc. present in these plants which effect the growth of pathogenic fungi (Jantasorn et al., 2016). Hence these plant extracts may have potential as a new natural fungicide for management of fungal rot pathogens.

Presently, Mancozeb proved effective in reducing the losses caused by *Trichothecium roseum.* Parveen et al. (2013) observed the effect of fungicides and plant extracts on fruit rot pathogens, viz. *Alternaria alternata* and *Mucor piriformis.* Likewise, some other studies also revealed the antimycotic activity of some plant extracts against fungi causing fungal rot of vegetables (Oyelana et al., 2011; Raji and Raveendran, 2013; Pawar, 2013).

Dal Bello (2008) reported that *Trichothecium roseum* causing postharvest fruit rot on tomato fruit for the first time in Argentina. The fungus has been cited causing fruit rot on many crops including tomato (Welch et al. 1975; Farr et al., 2007). It is also clear from the above study that the plant extracts of all the tested plants proved effective against *Trichothecium* rot pathogen, *Trichothecium roseum*. Gupta et al. (1996) reported that extracts of *Calotropis gigantea* and *Azadirachta indica* were most effective against *Fusarium oxysporum* Schlecht inhibiting the mycelial growth by 78.5% and 73.2%, respectively. Antifungal properties of *Azadirachta indica* and *Allium sativum* extracts against *F. oxysporum* have also been reported by Thakur et al. (1995). The variation in inhibition among test extracts could be due to variation in the components of antifungal chemicals in different plant species (Gautam et al., 2003).

Sengul et al. (2009) studied that the methanol extract of *Inula aucherana*, *Fumaria officinalis*, *Crocus sativus*, *Vicum album*, *Tribulus terestris*, *Polygonatum multiflorum*, *Alkanna tinctoria* and *Taraxacum officinale* were proved to possess considerable antimicrobial potentiality against a number of microorganisms. The methanolic extract had shown better antimicrobial activity compared to aqueous extract. Balkan et al. (2017) screened the fifty plant species for their antifungal effects against *T. roseum*. *Anthemis arvensis*, *Origanum vulgare*, *Sambucus ebulus* and *Thymus longicaulis* powders totally inhibited the mycelia growth of *T. roseum* at 10% (w/v). *Chelidonium majus* and *Clinopodium vulgare* powders were effective to *T. roseum*, with a percentage of inhibition of mycelia growth higher than 70%.

Conclusion

It is concluded from the study that these plant extracts showed antifungal activity and can possibly be exploited in the management of pathogenic fungi to prevent biodeterioration in an eco-friendly way but after further investigation.

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Conflicts of interest

Authors declare that they have no conflict of interests.

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