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Antimycotic activity of plant extracts on the spore germination of some pathogenic fungi

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Abstract

In an approach towards development of eco-friendly antifungal control strategy, plant extracts of three plants, onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and mint (*Mentha arvensis* L.) were tested for their antifungal activity on some pathogenic fungi. Different concentrations of extracts obtained from leaves of mint, and bulbs of onion and garlic were evaluated for their efficacy on the spore germination of *Alternaria alternata* and *Rhizopus stolonifer*. Results revealed that all the concentrations of plant extracts brought about significant inhibition in the spore germination of *A. alternata* and *R. stolonife*r. However, the highest concentrations caused maximum inhibition in the spore germination followed by lower concentrations of plant extracts of onion, garlic and mint respectively. The extract of *A. sativum* at highest concentration proved highly effective in reducing the spore germination of *A. alternata* followed by extract of *A. cepa* and *M. arvensis* respectively. Other concentrations of leaf and bulb extracts of tested plants also caused inhibition in spore germination but to a lesser extent than highest concentrations. Similar results were also observed on the spore germination of *Rhizopus stolonifer*. The highest inhibition in spore germination was observed at highest concentration of leaf and bulb extract of mint onion and garlic. It was followed by lower concentration concentrations of plant extract respectively.

Key words: Antimycotic activity, *Alternaria alternata*, bulb extract of garlic, leaf extract of mint, onion, *Rhizopus stolonifer, spore germination.*

Introduction

The fungal rots are world-wide in occurrence and have been reported almost in all parts of the world (Soki, 1994; Snowndon, 1990; Ali *et al*., 2005; Fontema *et al.*, 1996; Mitsovek *et al*., 2007), resulting in huge economic losses to the plants (Jones *et al*., 2001). Several management strategies such as cultural, physical, chemical, biological and regulatory methods have been used for the control of pathogenic fungi but each of these have one or other limitations. The continuous use of fungicides develop resistance in these fungi and are toxic to the environment. Therefore, there is need for developing novel plant protectant that interfere with the fungal pathogenicity factors. Use of natural plant products for the control of fungal disease is considered as alternative to synthetic fungicides due to their slower negative effect on the environment. The plant extracts being harmless and non-phytotoxic proved effective on the germination and viability of fungal spores. Several plants and their products have been used for the control of plant disease and have proved to be harmless and non-toxic unlike that of chemical fungicides (Khalil, 2001; Abu-Jawdah *et al*., 2002; Bovers and Locke, 2002; Sharma and Kumar, 2009; Satish *et al*., 2009). Keeping in view the non- phytotoxic effect of plant extracts, three plants, garlic (*Allium sativum* L.*)*, onion (*A. cepa* L.*)* and mint (*Mintha arvensis)* were evaluated for their efficacy on the spore germination of *Alternaria alternata* and *Rhizopus stolonifer* responsible for post harvest decay of vegetables.

Materials and Methods

Different concentrations of aqueous extracts of leaves of mint and bulbs of onion and garlic. were evaluated for their effect on the spore germination of *Alternaria alternata* and *Rhizopus stolonifer* causing rotting in vegetables. For the preparation of different concentrations of plant extracts, 200g each of leaves of mint and bulbs of onion and garlicwere washed with sterilized distilled water, grinded in Mortor and pestle using 200ml amount of sterilized distilled water (Bhat and Sivaprakasan, 1994). The material was homogenized for 5 minutes and filtered through double layered muslin cloth followed by Whattman's filter paper No. 1. The filtrate was

then centrifuged at 5000 rpm for 10 min. and was considered as standard solutions (S). Then other concentrations such as S/2, S/10, and S/100 were obtained by adding appropriate amount of sterilized distilled water to standard concentration. These concentrations were used to study the spore germination of various rot causing fungi.

Spore suspension of each isolate of fungi containing at least 20-30 spores per microscopic field was prepared from 10 days old fungal culture. One drop about 0.1ml of spore suspension was put in a cavity glass slide containing a drop (about 0.1 mL) of different concentration of plant extract. These slides were kept in moist chamber prepared by putting two folds of filter paper in both sides of Petri-plates. These Petri plates were incubated at 24±2 °C for 24 hours. Each treatment was replicated five times.The data were analysed for critical difference (Pansey and Sukhatme, 1978).

The percent spore germination was recorded using formula given by Kiraly *et al.* (1974) as:

$$\text{Percent spore germination} = \frac{\text{No. of spores germinated}}{\text{Total no. of spores examined}} \times 100$$

Results and Discussion

Effect of plant extracts on the spore germination of *Alternaria alternata*

It was revealed from the results (Table 1, Fig. 1) that different concentrations of plant extracts caused significant inhibition in the spore germination. However, the maximum inhibition in the spore germination was found at highest concentration 'S'. It was followed by S/2, S/10, and S/100 concentrations of plant extracts as compared to control which showed least inhibition in spore germination. The extract of *Allium sativum* at highest concentration ('S' concentration) was found most effective in reducing the spore germination followed by highest concentration (S) of extract of *Allium cepa* and *Mentha arvensis* respectively. The inhibition in spore germination varies from 43.94% to 9.7% in different concentrations of *Allium sativum.* In different concentrations of extract of *Allium cepa*, the inhibition in spore germination ranges from 60.51% to 17.58% whereas inhibition of spore germination ranges from 69.39% to 20.44% in different concentration of extract of *Mentha arvensis* respectively as compared to untreated control which showed least inhibition in spore germination.

Effect of plant extracts on the spore germination of *Rhizopus stolonifer*

It was revealed from the study (Table 2, Fig. 2) that plant extracts of garlic, onion and mint caused inhibition in the spore germination. However, the inhibition in spore germination increased with the increase in the concentration of plant extracts. The maximum inhibition in the spore germination was found at highest concentration 'S'. It was followed by S/2, S/10, and S/100 concentrations of plant extracts. The extract of *Allium sativum* at highest concentration ('S' concentration) was found most effective in reducing the spore germination followed by standard concentration 'S' of *Allium cepa* and *Mentha arvensis*, respectively. The inhibition in spore germination varies from 42.62% to 9.14% in different concentrations of *Allium sativum*. The decrease in spore germination in different concentration of extracts of *Allium cepa* ranges from 45.27% to 21.29% respectively, whereas in *Mentha arvensis* the inhibition in spore germination ranges from 55.34% to 22.57% in different concentrations of its extracts respectively as compared to control which showed least inhibition in spore germination.

An attempt was made in the present study to observe the efficacy of different concentrations of plant extracts of garlic (*Allium sativum*), onion (*Allium cepa*) and mint (*Mentha arvensis*) on the spore germination of fungi such as *Alternaria alternata, Rhizopus stolonifer*, causing fungal rot diseases on vegetables. It was clear from the results, that different concentration of leaf and bulb extract of mint, garlic and onion caused significant inhibition in the spore germination as compared to control. And highest concentration (S) of plant extracts proved highly effective followed by lower concentration of extract i.e. S/2, S/10 and S/100 respectively. The result further indicates that the extract of *Allium sativum was* highly effective as compared to extracts of *Allium cepa* and *Mentha arvensis* respectively. The effect of different concentrations of plant extract on the inhibition of spore germination may be due to fungicidal effect of extract on the spore germination and such study have been carried out for the first time in Kashmir. Similar studies have been carried out by Misra and Dixit (1976) on the antifungal activity of *Allium sativum* against eighteen different fungi including *Fusarium* spp. and they reported that crude leaf extract of *A. sativum* completely checked the mycelia growth of all the test fungi. Jacob and Sivaprakasan (1994) and Arya *et al.* (1995) studied the antifungal activity of the extracts of various plant species

against *Fusarium pallidoroseum* and reported inhibitory effect of extracts of garlic bulbs and Bignonia leaves on the mycelial growth of *Fusarium pallidoroseum*. Karade and Sawant (1999), Datar (1999), and Anwar and Khan (2001) observed the same results with the plant extracts of other plants. Our findings are also in agreement with those of Bashir (2001) and Bhat (2002). Bowers and Locke (2000) indicates that the maximum inhibition in spore germination of *Fusarium solani* f. sp. *melongenae* was exhibited by the extract of *Allium sativum* followed by *Datura stramonium*, *Artemisia* spp. *Mentha spicata* and *Juglans regia*. Thirumala and Sitaramaiah (2000), Lolpuri, (2002) and Teqida Menesens *et al.* (2002) reported antifungal activity of some wild plants against *Penicillium* sp. Various plant extracts of neem, mint, mehendi, safeda and garlic were used for their effect on the inhibition control of spore germination of *Alternaria brassicae* causing *Alternaria* blight in rapeseed and mustard. All the plant extracts showed inhibitory effect but garlic proved most effective in inhibiting spore germination (Khurana *et al.*, 2005). Several studies with plant extract of onion, ginger and other plants also indicates their inhibitory effect on the spore germination and mycelial growth of several pathogenic fungi (Moubasher *et al*., 1970; Singh *et al.*, 1990; Hassan *et al*., 2005; Tagoe *et al*., 2011).

Table 1: Effect of plant extracts on the spore germination of *Alternaria alternata*.

Concentration / Treatment	Spore germination (%)				
	Control	S/100	S/10	S/2	S
Allium sativum	90.50 (72.06)	43.94 (41.52)	32.60 (34.82)	21.29 (27.48)	9.7 (18.20
Allium cepa	91.90 (73.47)**	60.51 (51.07)	51.93 (46.26)	41.26 (39.97)	17.58 (24.79)
Mentha arvensis	92.57 (74.19)	69.39 (56.41)	58.61 (49.96)	39.26 (38.80)	20.44 (26.88)

	SE. diff.	C.D (P = 0.05)	C.D (P = 0.01)
Fungicides	1.80	1.61	2.09
Concentration	0.93	1.86	2.42
Fungicide x conc.	1.61	3.22	4.19

Mean of five replicates; ** Figures in parentheses are arc Sin√%age transformed value and are statistically identical.

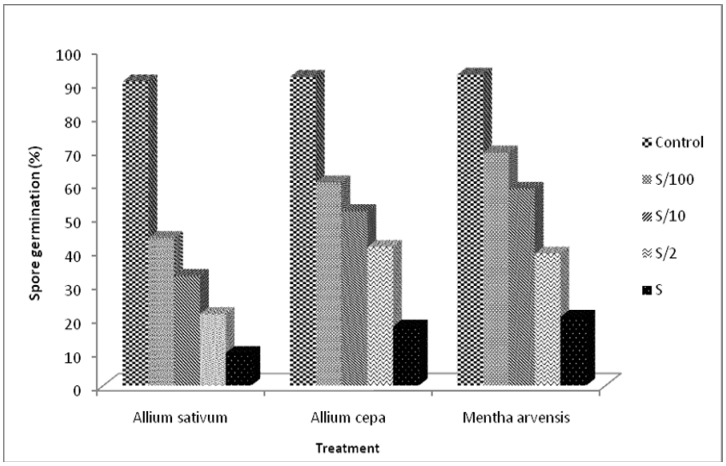

Fig. 1: Effect of plant extracts on the spore germination of *Alternaria alternata*.

Table 2: Effect of plant extracts on the spore germination of Rhizopus stolonifer.

Concentration / Treatment	Spore germination (%)				
	Control	S/100	S/10	S/2	S
Allium sativum	87.78 (69.54)	42.60 (40.75)	25.21 (30.14)	17.17 (24.48)	9.14 (17.60)
Allium cepa	89.28 (70.89)	45.27 (42.29)	36.58 (37.22)	28.52 (32.28)	21.29 (27.48
Mentha arvensis	91.11 (72.66)	55.34 (48.07)	47.95 (43.83)	35.24 (36.42)	22.57 (28.37)

	SE. diff.	C.D (P = 0.05)	C.D (P = 0.01)
Fungicides	0.80	1.58	2.09
Concentration	0.92	1.83	2.41
Fungicide x conc.	1.60	3.17	4.18

Mean of five replicates; ** Figures in parentheses are arc Sin√%age transformed value and are statistically identical.

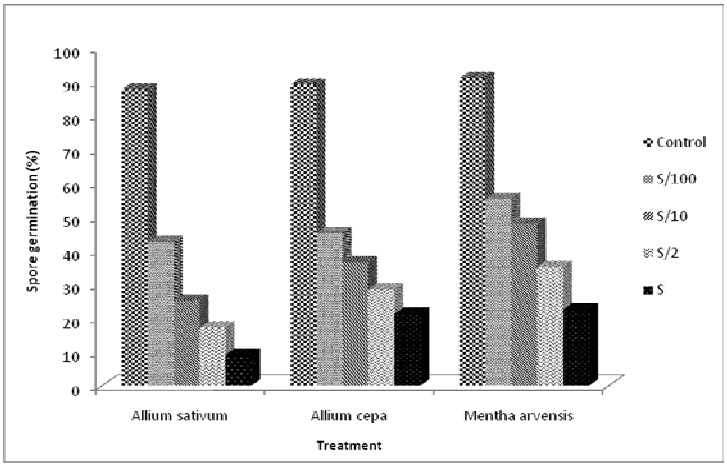

Fig. 2: Effect of plant extracts on the spore germination of *Rhizopus stolonifer*.

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