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Assessment of different phyto extracts in controlling Green Mould Disease pathogen-*Trichoderma harzianum* Rifai in cultivation of *Pleurotus florida* (Mont.) Singer

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Green mould disease caused by *Trichoderma harzianum* is considered as the major constraint in mushroom production. The present investigation was attempted with an aim to determine the botanicals that can be ecofriendly and cause minimum residual side effects on mushrooms. Antifungal potential of ten different botanicals *viz*. *Ageratinaa denophora, Artemisia annua, Ocimum sanctum, Juglans regia, Acorus calamus,Castanea sativa, Cymbopogon flexuosus, Lantana camara, Zingiber officinale, Mentha spicata* against *Trichoderma harzianum* and *Pleurotus florida* were evaluated by poison food technique. Among all tested botanicals *L. camara* showed the maximum inhibitory effect (41.21, 51.82 and 64.14%) against *T. harzianum* and the minimum inhibitory effect was shown by *Mentha spicata* (14.10, 20.19 and 28.00%) at 1, 2 and 3 % concentration respectively. The result indicated that *L. camara* was found compatible with *P. florida*. On the other hand, maximum inhibition of *P. florida* was shown by *J. regia*- 59.27%, 64.84% and 69.93% and it was found most incompatible and significantly inferior. *Z.officinale* was found to have minimum inhibition of *P. florida* (6.72, 12.26 and 15.18 %) at 1, 2 and 3 %.

Keywords: Botanicals, Greenmould, *Lantana, Pleurotus florida*, percent inhibition, residual effect, *Trichoderma harzianum*

INTRODUCTION

Oyster mushroom, *Pleurotus* species is also known as “Wood fungus”. In India, it is commonly known as “Dhingri” (Bahl, 2005). *Pleurotus florida* belonging to family Pleurotaceae is an edible mushroom having desirable attributes like saprophytic colonization, simple cultivation techniques have made it popular among mushroom growers. Maintaining proper humidity 80 to 95% and temperature (25 ± 5ºC) are two vital factors for getting a good crop.

Major medicinal properties of oyster mushrooms include anticancer, anti-inflammatory antiviral activities, immune- modulator effect and blood lipid lowering effects.They also synthesize a polycyclic aromatic compound “Pleurotin” which has antibiotic properties. Hundred gram of fresh *P. florida* contain 2.5 - 2.75 g of proteins, 0.5 5.0 - 5.6 g of carbohydrates (Lavi *et al*. 2010).

The genus *Trichoderma*, commonly called as weed mould in mushroom industry is responsible for causing Green mould disease of mushrooms. The mould fungi colonizes the substrates and start releasing enzymes (lytic enzyme) that degrade the cell structures of the host fungi. Some of the secondary metabolites released by the *Trichoderma* include trichorovins and trichodecenins. A dense pure white mycelium of *Trichoderma harzianum* may resembles to mushroom mycelium which later turns to green. Green sporulation areas were observed in growing substrate during spawn run resulting in large unproductive area which occurred in severely infected bags. Botanical control offers an important alternative to synthetic chemicals and has been shown to be economical and environmentally friendly (Shah *et al*. 2013).

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MATERIALS AND METHODS

Sample was collected from the infected substrate, showing characteristic symptoms of Green mould disease in Mushroom Research Unit, College of Horticulture, VCSH UUHF, Bharsar (Pauri Garhwal) Uttarakhand. Sample was transferred into PDA plates using sterilized inoculating needle under *in vitro* conditions and incubated at 27±2ºC for 96 hours. Pure culture was stored in refrigerator at 4°C for futureuse. Microscopic study was undertaken to confirm the identity of the isolated pathogen (Jayalal and Adikaram, 2007). The powdered material obtained from selected plants were placed in thimble chamber of the soxhlet apparatus. The extract was collected separately in a round bottom flask containing ethanol and filtered through Whatman's No.1 filter and then concentrated in oven at 60ºC till the extract remained (about 4 mL) (Redfern *et al*. 2014). The standard plant extracts were added to PDA medium in conical flasks, so as to get final desired concentration (1%, 2% and 3%). After solidification of the medium a small mycelial disc (5 mm) was cut from 6 days old culture of *T. harzianum* with the help of sterilized cork borer and transferred aseptically in centre of the Petri dish. The media without botanical extract served as control.

Preparation of botanicals

The powdered material obtained from tested plants were placed in a thimble made from a strong filter paper and then placed in thimble chamber of the soxhlet apparatus. The round boiling flask was filled with ethanol. The extracts were filtered through Whatman's No.1 filter and concentrated in oven at 60ºC till the yield of extracted plant material remained (about 4 mL) in the glass bottom flask. (Redfern *et al*. 2014).

In vitro evaluation

The poisoned food technique was adopted for *in vitro* testing of botanicals (Nene and Thapliyal, 1993). The standard plant extracts of ten botanicals were added to 60 mL of PDA medium in conical flasks, so as to get final desired concentration (1%, 2% and 3%) of each separately.The flasks were shaken gently to ensure the proper mixing of botanicals in media. 20 mL of mixture of botanical and PDA was poured in each Petri plate. After solidification of the media a small mycelial disc (5 mm) were cut from 6 days old culture of *T. harzianum* with the help of sterilized cork borer and transferred aseptically in centre of the Petri dish. The media without botanical extract served as control.

Per cent inhibition in growth was calculated in relation to growth in control using the following formula (Vincent 1947) :

$$\text{Per cent inhibition} = \frac{\text{Radial growth in control-Radial growth in treatement}}{\text{Radial growth in control}} \times 100$$

The data obtained were analyzed by using standard statistical procedure in the Completely Randomized Design (CRD) with help of OPSTAT.

RESULTS AND DISCUSSION

In the morphological study, the colony of *T. harzianum* was recorded as compact with smooth edge.The green globose to subglobose shaped conidia were small (2.2- 2.4 µm) and conidiophores were hyaline, branched and compact. The irregularly flask shaped phialides were about (3.5-3.8 ×1.8-2.2 µm) and chlamydospores were mostly 7 to 8 µm in diameter (Jayalal and Adikuram, 2007). The result obtained on inhibition of mycelial growth of *T. harzianum* in food poisoning technique are presented in Table 1.

Among the tested botanicals, *L. camara* (41.21%, 51.82% & 64.14%) showed the best inhibitory effect against *T. harzianum* and the minimum inhibitory effect was shown by *M. spicata* (14.10%, 20.19% & 28.00%) at 1, 2 and 3 % concentration respectively. Mousumi *et al.* (2017) also found that *L. camara* was able to inhibit the growth of *T. harzianum.* Similar result was recorded by Pervez *et al*. (2012). From a glance of data depicted in Table 2, it is clear that all the extracted botanicals suppressed the growth of *P. florida* at varying degree.

Table 1: Effect of botanicals on mycelial growth and percent inhibition of *Trichoderma harzianum* at different concentration at 96 hours after inoculation

Treatments	*Trichoderma harzianum*					
	Mycelial growth (mm)± S.F.(m)			Per cent mycelium growth inhibition		
	Concentrations (%)			Concentrations (%)		
	1%	2%	3%	1%	2%	3%
Control	90.00 ± 0.00	90.00 ± 0.00	90.00 ± 0.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ageratina adenophora	60.96 ± 0.10	52.01 ± 0.46	43.45 ± 0.53	32.26* (34.59)	42.20* (40.50)	51.71* (45.96)
Artemisia annua	67.00 ± 0.06	56.82 ± 0.37	47.70 ± 0.69	25.55* (30.35)	37.18* (37.55)	47.32* (43.45)
Ocimum sanctum	68.56 ± 0.33	57.62 ± 0.48	49.24 ± 0.47	23.45* (28.95)	35.97* (36.83)	45.28* (42.27)
Juglans regia	54.82 ± 0.23	48.29 ± 0.18	38.72 ± 0.78	39.08* (38.67)	45.64* (42.48)	56.96* (48.98)
Acorus calamus	55.89 ± 0.49	49.90 ± 0.06	39.91 ± 0.70	37.89* (37.97)	44.22* (41.66)	55.65* (48.98)
Castanea sativa	73.78 ± 0.24	67.71 ± 0.36	62.80 ± 0.54	18.01* (25.10)	24.75* (29.82)	30.66* (33.60)
Cymbopogon flexuosus	62.45 ± 0.43	55.02 ± 0.26	46.68 ± 0.32	30.60* (33.57)	38.85* (38.85)	48. 12* (43.90)
Lantana camara	52.90 ± 0.51	43.35 ± 0.30	32.26 ± 0.24	41.21* (39.92)	51.82* (46.02)	64. 14* (53.19)
Zingiber officinale	75.99 ± 0.05	68.93 ± 0.49	63.73 ± 0.36	15.21* (22.94)	23.40* (28.91)	29. 17* (32.68)
Mentha spicata	78.30 ± 0.29	71.82 ± 0.17	65.07 ± 0.61	14.10* (22.03)	20.19* (26.69)	28.00* (31.93)
S.E.(d)	0.43	0.46	0.74	0.56	0.57	0.72
C.D.(0.05%)	0.90	0.97	1.55	1.17	1.91	1.503

*=Significantat 5%level of significance as compared with control
Mean of three replications
Values in parenthesis indicate Angular transformed value

The maximum per cent inhibition of mycelium was shown by *J. regia* followed by *A. annua* so found least compatible for mycelium growth of *P. florida*. Similar observation was recorded by Shah *et al*. (2011). *J. regia* produce “Juglone” (5 hydroxy-alphanapthoquinone) that show strong antifungal and allelopathy effect. The study has found *Lantana camara* as the most promising botanical that was able to minimize the infection of green mould disease as it show less inhibition percentage reduction of *P. florida* (37.31%) and showed highest efficiency against *T. harzianum* (64.14%).

CONCLUSION

Generally, synthetic fungicides are used against green mould disease of mushroom. The continuous use of chemical fungicides in the management of plant disease has become a major threat to mankind which often imposes various undesirable side effects so screening the plant products for their effective antifungal activity against the *Trichoderma harzianum* as an alternative is essential to minimize residual effects. The study has found *Lantana camara* as the most promising botanicals, that was able to minimize the infection of green mould disease of *Pleurotus florida*.

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Table 2: Effect of botanicals on mycelia growth and per cent inhibition of *Pleurotus florida* at different concentration at 9 days after inoculation

Treatments	*Pleurotus florida*					
	Mycelial growth (mm) ± S.F.(m)			Per cent inhibition (%)		
	Concentrations (%)			Concentrations (%)		
	1%	2%	3%	1%	2%	3%
Control	90.00 ± 0.00	90.00 ± 0.00	90.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Ageratina adenophora	64.83* ± 0.16	58.36* ± 0.26	51.24* ± 0.36	27.96* (31.91)	35.15* (36.34)	43.05* (40.99)
Artemisia annua	55.25* ± 0.63	47.23* ± 0.31	37.92* ± 0.50	38.60* (38.39)	47.47* (43.53)	57.86* (49.50)
Ocimum sanctum	78.49* ± 0.27	67.25* ± 0.17	58.44* ± 0.29	12.78* (20.93)	25.27* (30.16)	35.06* (36.29)
Juglans regia	36.65* ± 0.32	31.14* ± 0.23	27.05* ± 0.56	59.27* (50.32)	64.84* (53.61)	69.93* (56.72)
Acorus calamus	66.16* ± 0.16	59.30* ± 0.15	52.22* ± 0.42	26.47* (30.95)	34.10* (35.71)	41.97* (40.36)
Castanea sativa	83.10* ± 0.09	77.53* ± 0.47	70.61* ± 0.40	07.66* (16.06)	13.85* (21.83)	21.53* (27.63)
Cymbopogon flexuosus	70.85* ± 0.33	63.84* ± 0.48	57.32* ± 0.52	21.26* (27.44)	29.06* (32.60)	36.30* (37.03)
Lantana camara	68.19* ± 0.40	62.36* ± 0.29	56.41* ± 0.28	24.22* (29.47)	30.70* (33.63)	37.3* (37.63)
Zingiber officinale	83.82* ± 0.15	78.96 * ± 0.49	76.13* ± 0.58	06.72* (15.02)	12.26* (20.47)	15.18* (22.91)
Mentha spicata	81.83* ± 0.44	75.25* ± 0.33	69.44* ± 0.26	09.06* (17.50)	16.37* (23.85)	22.84* (28.53)
S.E. (d)	0.45	0.46	0.58	0.50	0.51	0.67
C.D. (0.05%)	0.95	0.96	1.22	1.05	1.06	1.39

*=Significantat 5%level of significance as compared with control
Mean of three replications
Values in parenthesis indicate Angular transformed value

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