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Evaluation of some plant extracts in management of dry bubble (*Verticillium fungicola*) disease of white button mushroom [*Agaricus bisporus* (Lange) Imbach]

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Abstract: The study was undertaken to determine antifungal potentials of some plant extracts against dry bubble (*Verticillium fungicola*) disease of white button mushroom (*Agaricus bisporus*). Twelve botanicals namely, *Allium cepa, A. sativum, Saraca asoca, Aloe vera, Azadirachta indica, Lantana camara, Ocimum sanctum, Solanum lycopersicum* (*Lycopersicon esculentum*), *Tagetes erecta, Psidium guajava, Catharanthus roseus* and *Aparagus racemosus* were evaluated *in-vitro* and *in-vivo* for their efficacy against both *A. bisporus* and *V. fungicola,* causing dry bubble disease of mushroom. The efficacy of botanicals was examined by poison food technique in *in-vitro*. The percent inhibition produced by botanicals against *V. Fungicola* recorded *in-vitro* was; *A. cepa* (25.87%), *A. sativum* (24.70%), *S. asoca* (12.35%), *A. vera* (22.35%), *A. indica* (35.11%), *L. camara* (28.48%), *O. sanctum* (20.59%), *S. lycopersicum* (20.34%), *T. erecta* (14.11%), *P. guajava* (15.11%), *C. roseus* (18.11%) and *A. racemosus* (13.52%). Among these plant extracts, *A. indica* was found best treatment followed by *L. Camara* and *A. Cepa.* Plant extracts showing maximum efficacy against *V. fungicola* and minimum inhibition against mushroom were further evaluated against *V. fungicola* infection in mushroom crop room (*in-vivo* test). In *in-vivo* test, the polybags which receive *A. indica* show maximum mean increase in yield (43.46%) over control and exhibited minimum mean disease incidence (27.7%).

Keywords: *Agaricus bisporus,* Dry bubble disease, Plant extracts, *Verticillium fungicola*

INTRODUCTION

The cultivation of edible mushrooms is regarded as a biotechnological process for conservation of various lignocellulosic, agricultural, industrial, forestry and horticultural wastes or their by-products into proteins, especially in developing countries. Mushroom cultivation is a viable alternative venture for minimizing the ever increasing protein malnutrition gap and multitude of allied problems in these countries (Eswaran and Ramabadran, 2000).

Edible mushrooms are the important component of many countries diet (Gbolagade *et al.*, 2006). Mushrooms such as *A. bisporus* contained high amounts of protein, minerals, B vitamins group, D and K vitamin and sometimes A and C vitamins. Against, fat amount, calorie, sodium and cholesterol are low (Saiqa *et al.*, 2008). *A. bisporus* is one of the most important mushrooms that cultivated in the world (Toker *et al.*, 2007).

V. fungicola var. *fungicola* (Preuss) Hassebrauk, *Mycogone perniciosa* (Magnus) Delacroix, and *Cladobotryum* spp. (Cooke) – the causal agents of dry bubble, wet bubble, and cobweb disease – are important fungal pathogens of the button mushroom, *A. bisporus* (Lange) Imbach (Grogan and Gaze, 2000; Umar *et al.*, 2000; Gea *et al.*, 2003). Symptoms of dry bubble, caused by *V. fungicola* var. *fungicola*, vary depending on the time of infection. Infection at an early stage in mushroom development results in the production of undifferentiated masses of mushrooms. If maturing mushrooms are infected, then spotting symptoms develop (Grogan *et al.*, 2000; Potocnik *et al.*, 2008). Control of myco-pathogens is based on the use of chemicals, cultural practices, and sanitation. Some workers have recommended fungicides for management. But growers hardly use the fungicides for the treatment of this disease. They often found fungicidal treatment as non-economical (Shah and Nasreen, 2011). Accordingly, the objectives of this study were to evaluate antifungal activity of some plant extracts against *V. fungicola* both *in vitro* and *in-vivo*; and also to develop economically viable and eco-friendly management of this disease by using plant extracts.

MATERIALS AND METHODS

The study was conducted during August-September, 2014 at the laboratory of the Department of Plant Pathology, SHIATS-Deemed University, Allahabad (UP). The cultures of *V. fungicola* (ITCC No. 4909) and *A. bisporus* (ITCC No. 1927) were procured from Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi.

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***In-vitro* evaluation:** In this experiment, ethanol extract of 12 botanicals *viz. A. cepa* (onion), *A. sativum* (garlic), *S. asoca* (ashoka), *A. vera* (aloevera), *A. indica* (neem), *L. camara* (lantana), *O. sanctum* (tulsi), *S. lycopersicum* (tomato), *T. erecta* (marigold), *P. guajava* (guava), *C. roseus* (sadabahar) and *A. racemosus* (satawar) were evaluated in the laboratory for their efficacy against both *A. bisporus* and *V. fungicola.* The plant extracts were evaluated *in-vitro* through poison food technique (Nene and Thapliyal, 2000). Five per cent and ten per cent test concentrations were obtained by adding appropriate amount of sterile distilled water to the standard solution (100%). Two ml of each extract (5% and 10%) was dispensed in petriplates (90mm) and then 20 ml of molten PDA was poured gently in petriplates containing extract solution. After solidification, inoculations were done with 5 mm dia mycelial cut from 6 days old cultures of both *A. bisporus* and *V. fungicola* separately. The media without the plant extract served as control. The plates were incubated at 27±1°C till the complete growth was observed in control plates. Percent inhibition in growth was calculated in relation to growth in control using the following formula of Vincent (1947):

$$\text{Mycelial inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

***In-vivo* evaluation :** In this study, the botanicals which showed least adverse effects on the growth of *A. bisporus* were evaluated against *V. fungicola* in *in-vivo* condition during October-February, 2014 in Mushroom Crop Room, Department of Plant Pathology, SHIATS-Deemed University, Allahabad (UP). The spawn of *A. bisporus* was procured from Directorate of Mushroom Research, Solan (HP) and the strain of *A. bisporus* spawn was DMR-3. Wheat straw was used as substrate for cultivation of white button mushroom (*A. bisporus*). Compost was made using long method (28 days). The dried powder of selected plant materials was incorporated separately in the compost @ 1, 2 and 3% (w/w) and filled in polythene bags @ 500 g of compost. The untreated bags (devoid of botanicals) were kept as control. All the treatments including control were replicated six times. Spawn of *A. bisporus* was added @ 7.5g/kg of compost (Kapoor, 2004). Then, the bags were incubated inside the Mushroom Crop Room, where temperature (20±2^{0}C) and humidity (80-85%) was maintained. Room having spawn running bags was kept in dark for 10-15 days till complete colonization of the compost with fungal mycelium (El-Kattan and El-Hadded, 1998).

After complete colonization on compost with mycelium of *A. bisporus*, the bags were inoculated with 3 mL spore suspension of *V. fungicola* with a spore load of 1×10^3 spores mL^{-1} in the middle of bag, with the help of syringe. The untreated bags (devoid of botanicals) with the same inoculums load were kept as control (Shah *et al.*, 2012).

While carrying the above experiment *in-vivo*, the observations on days for complete spawn run, days for pin head initiation, per cent increase in yield over control and disease incidence were recorded. Per cent increase in yield over control was calculated by using following formula:

$$\text{Per cent increase in yield over control} = \frac{\text{Yield treatment} - \text{Yield control}}{\text{Yield treatment}} \times 100$$

Disease incidence (%) was recorded on a 0-5 scale with 0 = disease free, 1 = 1-20% area covered by disease, 2 = 21-40% area covered by disease, 3 = 41-60% area covered by disease, 4 = 61-80% area covered by disease and 5 = 81-100% area covered by disease

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Sum of numerical values}}{\text{Total No. of observations} \times \text{Maximum grades}} \times 100$$

(Bhardawaj, 1992). Per cent disease incidence (PDI) was calculated as follows

Statistical analysis: In the *in-vitro* experiments, complete randomized design was applied. In *in-vivo* trial, factorial design (RBD) was applied. All the experiments were analyzed statistically by Analysis of Variance (ANOVA). The calculated value was compared with tabulated value at 0.05% level of probability for the appropriate degree of freedom.

RESULTS AND DISCUSSION

The results obtained on inhibition of mycelia growth of *V. fungicola* by poison food plate technique were presented in table 1. All twelve plant extracts more or less significantly inhibited mycelial growth of *V. fungicola* at both concentrations (5% and 10%). The results revealed that out of twelve selected plant extracts, maximum inhibition of mycelia growth of *V. fungicola* was observed in the *A. indica* (35.11%), followed by *L. camara* (28.48%) and *A. cepa* (25.87%). The least inhibition was exhibited by *S. asoca* (12.35%) and *T. erecta* (14.11%). As maximum inhibition of pathogen was recorded in *A. indica*, it was found that this plant extract also showed least toxicity to *A. bisporus*, inhibiting the mycelial growth of mushroom by (10.58%) (Table 2).

It was further observed that the effect of two concentrations (5 and 10%) on mycelial inhibition of *V. fungicola* varied significantly i.e., with the increase in concentration from 5% to 10%, there was an increase in the inhibition of mycelial growth of *V. fungicola*, except in the treatments which contain *T. erecta* leaf extract inhibiting pathogen mycelium by 15.29% at the rate of 5% but showing the inhibition of 12.94% at the rate of 10%. However, *A. bisporus* also showed the same results.

In this experiment, the plant extracts which displayed the maximum efficacy against *V. fungicola* and least adverse effects on the growth of *A. bisporus* were fur-

Table 1. In-vitro efficacy of ethanol extract of selected botanicals on inhibition of mycelial growth (in cm) of V. fungicola.

Treatments	*Percent inhibition over control		
	Concentration (%)		
	5	10	Mean
Allium cepa	24.70	27.05	25.87
Allium sativum	22.35	27.05	24.70
Saraca asoca	10.58	14.12	12.35
Aloe vera	21.17	23.53	22.35
Azadirachta indica	30.95	39.28	35.11
Lantana camara	26.74	30.23	28.48
Ocimum sanctum	20.00	21.18	20.59
Solanum lycopersicum	18.60	22.09	20.34
Tagetes erecta	15.29	12.94	14.11
Psidium guajava	12.79	17.44	15.11
Catharanthus roseus	17.41	18.82	18.11
Aparagus racemosus	11.76	15.29	13.52
Mean	19.36	22.41	

*Mean of four replications

ther evaluated *in-vivo*. The botanicals selected for *in-vivo* trial were; *A. indica, L. Camara* and *A. cepa*. Out of twelve plant extracts selected, only three botanicals, *viz*., *A. indica, L. camara* and *A. cepa* were further evaluated against *V. fungicola* under *in-vivo* condition. The data pertaining to *in-vivo* evaluation of botanicals against *A. bisporus* and *V. fungicola* is presented under the following heads:

Days taken for complete mycelium run: It is evident from the table 3 that there was significant difference between the influences of plant extracts on time taken for complete mycelium run by *A. bisporus*. The maximum days required for complete mycelium run in *A. bisporus* was significantly less (15.03 days) in *A. indica*. It was followed by *L. camara* (15.13 days) and *A. cepa* (15.23 days) as compared to control (17.5 days), devoid of plant extracts.

Days taken for pin head formation: The data recorded to influence of selected plant extracts on time taken for pin head initiation by *A. bisporus* presented in table 4, revealed that there was no significant difference between the effect of botanicals and their concentrations on time taken for pinhead formation by *A. bisporus*. The maximum days required for pin head initiation in *A. bisporus* was significantly less (5.0 days) in *A. indica*. It was followed by *L. camara* (5.4 days) and *A. cepa* (5.5 days) as compared to control (6.5 days).

Per cent increase in yield over control: It was revealed that there was a significant difference between the influences of the plant extracts on the effect of total yield of *A. bisporus* (Table 5). Maximum increase in yield over control was recorded in treatment *A. indica* (43.46%) followed by *L. camara* (31.37%) and *A. cepa* (12.36%), which gives the least performance of increase in yield over control. It was further observed that the mushroom yield increased on increasing the concentrations (1, 2 and 3%) of plant extracts. Maximum increase in yield over control was recorded in treatment *A. indica* (48.55%) at 3% concentration, 42.00% at 2% concentration and 39.83% at 1% concentration, followed by *L. Camara* (36.04%) at 3% concentration, 31.73% at 2% concentration and 26.35% at 1% concentration. Minimum increase in yield over control was obtained in *A. cepa* (6.33%) at 1% concentration, 12.35% at 2% and 18.40% at 3% concentration.

Per cent disease incidence: It is clear from the table 6 that all the three botanicals at all concentrations of 1, 2 and 3% were more or less significantly effective in reducing the incidence of *V. fungicola* of *A. bisporus* as compared to the control. *A. indica* was most effective treatment where the incidence was reduced to 27.7%, followed by *L. camara* (43.3%) and *A. cepa*

Table 2. *In-vitro* efficacy of ethanol extract of selected botanicals on inhibition of mycelial growth (in cm) of *A. bisporus.*

Treatments	*Percent inhibition over control		
	Concentration (%)		
	5	10	Mean
Allium cepa	12.94	14.11	13.52
Allium sativum	13.95	16.28	15.11
Saraca asoca	32.14	34.52	33.33
Aloe vera	10.71	11.90	11.30
Azadirachta indica	9.41	11.76	10.58
Lantana camara	12.20	15.12	13.66
Ocimum sanctum	11.62	13.95	12.78
Solanum lycopersicum	22.35	24.70	23.52
Tagetes erecta	27.05	29.41	28.23
Psidium guajava	35.29	37.64	36.46
Catharanthus roseus	29.41	31.76	30.58
Aparagus racemosus	31.39	32.55	31.97
Mean	20.70	22.80	

*Mean of four replications

Table 3. Influence of selected botanicals on time taken for complete mycelium run of A. bisporus (in vivo).

Treatments	*Time taken for complete mycelium run (in days)			
	Concentration (%)			
	1	2	3	Mean
Azadirachta indica	14.50	15.30	15.30	15.03
Lantana camara	15.10	15.00	15.30	15.13
Allium cepa	15.10	15.30	15.30	15.23

Control- 17.5 days *Mean of six replications

Table 4. Influence of selected botanicals on time taken for pin head initiation of *A. bisporus* (*in vivo*).

Treatments	*Time taken for pin head initiation (in days)			
	Concentration (%)			
	1	2	3	Mean
Azadirachta indica	4.6	5.1	5.3	5.0
Lantana camara	5.5	5.5	5.3	5.4
Allium cepa	5.5	5.6	5.6	5.5

Control- 6.5 days, *Mean of six replications

Table 5. Influence of selected botanicals on yield of *A. bisporus.*

Treatments	Conc. (%)	*Yield (g)			
		Control	Treatment	Per cent increase in yield over control	Mean of per cent increase
Azadirachta indica	1	59.16	98.33	39.83	43.46
	2		102.00	42.00	
	3		115.00	48.55	
Lantana camara	1		80.33	26.35	31.37
	2		86.66	31.73	
	3		92.50	36.04	
Allium cepa	1		63.16	6.33	12.36
	2		67.50	12.35	
	3		72.50	18.40	

*Mean of six replications

(74.4%). Maximum disease incidence was recorded in control (86.6%). Furthermore, it was found with increase in concentrations of plant extracts, the disease incidence was reduced. Minimum disease incidence (23.3%) was recorded in *A. indica* at 3% concentration, 26.6% at 2 concentration and 33.3% at 1% concentration. Maximum disease incidence (80.0%) was recorded in *A. cepa* at 1% concentration, 73.3% at 2% and 70.0% at 3% concentrations (Table 6).

Evaluation of plant extracts against both *V. fungicola* and *A. bisporus* under *in-vitro* condition, revealed that all the plant extracts more or less suppress the growth of *V. fungicola*. Out of twelve selected plant extracts, *A. indica* (neem) expressed the strongest antifungal activity against *V. fungicola*. It was followed by *A. cepa, L. camara, A. sativum, A. vera, O. sanctum, S. lycopersicum, C. roseus, P. guajava, T. erecta, A. racemosus* and *S. asoca.* These plant species may contain chemical compounds having antifungal properties. Sharma and Jarial (2000) evaluated neem leaves against False Truffle (*Diehliomyces microsporus*) disease of *Agaricus* spp. and recorded good results in controlling this disease *in vitro* which supports the present investigation. Sharma and Rajesh (2005) observed that 10% neem leaf extract was effective in inhibiting the growth of *Sepedonium chrysospermum,*

Table 6. Influence of selected botanicals on disease incidence of *V. fungicola* in white button mushoom (*A. bisporus*) cultivation.

Treatments	Conc. (%)	Disease incidence (%)	Mean
Azadirachta indica	1	33.3	27.7
	2	26.6	
	3	23.3	
Lantana camara	1	46.6	43.3
	2	43.3	
	3	40.0	
Allium cepa	1	80.0	74.4
	2	73.3	
	3	70.0	
Control (no botanicals)		86.6	

responsible for causing yellow mold disease in white button mushroom which support the present investigation. Similar findings have also been reported by Shah *et al.* (2012), who observed that neem (*A. indica*) leaf extract at both 5% and 10% was found effective in inhibiting the growth of *Trichoderma harzianum.* Mishra (2009) also reported similar results with the use of neem leaf extract, neem cake solution and neem saw dust against *Trichoderma viride.*

In-vivo evaluation of selected plant extracts, *viz., A. indica, L. camara* and *A. cepa* against *V. fungicola* was carried out in mushroom crop room. *A. indica* was found best treatment among the selected botanicals for *in-vivo* evaluation in all parameters, *viz.,* time taken for complete mycelium run and pin head initiation, yield and per cent disease incidence. Antifungal activity of the product of *L. camara* and *A. indica* plant have very low toxic to mammals (Kleeberg, 1992) and are relatively safe to non-target organisms (Schmutterer, 1995). Similar findings have been reported by Grewal and Grewal (1988), Sharma and Jandiak (1994), Sharma and Jarial (2000), Sharma and Rajesh (2005) and Inam-ul-Haq *et al.* (2010) in that the neem was the best treatment among all used botanicals. Findings of mentioned authors support the present investigation that neem increases the yield of *A. bisporus* and suppresses the infection by *V. fungicola.*

Conclusion

This study has found two most promising botanicals, *A. indica* and *L. camara* that were able to inhibit the infection of dry bubble disease (*V. fungicola*) of white button mushroom (*A. bisporus*) under both *in-vitro* and *in-vivo* conditions. In *in-vitro* study, *A. indica* and *L. camara* have been best treatments among all botanicals as they inhibit the growth of *V. fungicola* (35.11% and 28.48% respectively). In *in-vivo* study, incorporation of these botanicals in the compost reduces the disease incidence (27.7% and 43.3% respectively) and enhances the yield (mean of per cent increase- 43.46 g and 31.37 g respectively) when compared to control (without botanicals). However, there are still some further studies to be carried out to validate these findings.

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