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Management of the Green Mould of Milky Mushroom (*Calocybe indica*) by Fungicides and Botanicals

Kundan Kumar, Manoj Kumar*, Jaywant Kumar Singh, S.P. Goyal and Surjeet Singh

Department of Plant Pathology, CCS Haryana Agricultural University,
Hisar- 125 004, Haryana, India
**Corresponding author*

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A B S T R A C T

The toxicants (fungicide and botanicals) showing maximum efficacy against the pathogen and minimum efficacy against milky mushroom (*Calocybe indica*) mycelia as per results of *in vitro* studies were further evaluated against *T. harzianum* under *in vivo* condition in mushroom house. Among fungicides only Bavistin (@150 ppm) and out of three botanicals only Nimbicidin (@4 per cent) were selected for management of the green mould disease in milky mushroom cultivation in bags. The different yield parameters and yield were recorded and found that days for spawn run (DFSR), days for pinhead formation (DFPF) and days for first harvest (DFFH) were significantly reduced in case Bavistin and Nimbicidin treatment.

Introduction

Commercial production of edible mushrooms represents unique exploitation of the microbial technology for the bio-conversion of the agricultural, industrial, forestry and household waste into nutritious food (mushrooms). Integrating mushroom cultivation in the existing farming systems not only supplements the income of the farmers but also promotes proper recycling of agro-residues thereby improving soil health and promoting organic agriculture. Therefore, there is need to have mushroom which can grow during summer months being longer shelf-life. Milky mushroom (*Calocybe indica*) fits well under these conditions because of its ability to grow at temperature above 30°C, white sporophore, excellent shelf life, high biological efficiency (80-90%) and easier in post-harvest handling.

The green mould due to *Trichoderma harzianum* is of common occurrence in milky mushroom cultivation and therefore also called as *Trichoderma* spot, *Trichoderma* blotch, *Trichoderma* mildew etc. and causing losses from 63-65% in cultivated mushrooms (Bhatt and Singh, 2000). It is a ubiquitous fungus found in air, soil, plant materials and other substrates. Disease control in mushroom farms by treatment of casing soil with disinfectants and fungicides. Gae (2008) also reported that benomyl and thiabendazole were

effective in controlling green mould disease during mushroom cultivation. Shah and Nasreen (2013) evaluated antifungal potential of different botanicals.

Materials and Methods

The present research work was carried out at Mushroom Technology Laboratory, Department of Plant Pathology, College of Agriculture, CCS Haryana Agricultural University Hisar Haryana (India) during 2014-2015. The culture of *C. indica* (P & C) was obtained from Directorate of Mushroom Research, Chambaghat, Solan. The culture of *C. indica* (P & C) used during present study was maintained on PDA medium. The culture of *C. indica* (P & C) after inoculation was incubated in a B.O.D. incubator at 30±1˚C. The pieces of cleaned mushroom pilus were placed on potato dextrose slants and amended with streptocycline to avoid bacterial contamination and incubated at 25±1˚C. The identification of fungus was based on visual observation of culture and microscopic studies.

Sensitivity of *C. indica* against fungicides and botanicals

The Poison Food Technique was adopted for testing the sensitivity *C. indica* against different fungicides and botanicals under *in vitro* condition (Nene and Thapliyal, 2000). Three different fungicides namely Dithane M-45, Bavistin and Topsin-M each at 100, 150, and 200 ppm concentrations were evaluated in present study. The inhibitory effect of some botanicals *viz*, Bakain seed extract (*Melia azedarach*), Turmeric powder and Nimbicidin were tested against *C. indica.* Observations for radial growth were taken after 7 days of incubation at 30±1˚C, and per cent inhibition of *C. indica* mycelial growth over control was calculated by using formula suggested by Vincent (1947).

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

Sensitivity of *T. harzianum* against fungitoxicants and botanicals

Those fungicides and botanicals were evaluated against *T. harzianum* which exhibited least toxic effect to *C. indica* (as per previous experiment's result). Further Poison Food Technique was used and radial growth was observed, finally per cent inhibition of *C. indica* mycelial growth over control was calculated.

Results and Discussion

To manage the green mould disease of milky mushroom, different fungitoxicants were used and these not only inhibit the growth of the pathogen but also affect the mushroom growth. Per cent growth inhibition by fungicides were calculated which are presented in Table 1. It is evident from the table 1 that maximum growth inhibition of *C. indica* was observed in case of Dithane M-45 at all tested concentrations followed by Topsin-M and least in case of Bavistin. Diathane M-45 and Topsin-M completely checked the growth of *C. indica* when used at their highest concentration taken in the present work, whereas Bavistin inhibited only 67 % growth at the highest concentration (200 ppm).

Sensitivity of *C. indica* against to Botanicals

Different botanicals *viz.*, Bakain Seed Extract (BSE), Nimbicidin (Neem product) and Turmeric powder were taken to evaluate their effect on *C. indica* under *in vitro* conditions. Using Poison Food Technique radial growth of *C. indica* was recorded, data analyzed thereafter per cent inhibition was calculated which are depicted in Table 2. Among the

botanicals evaluated BSE (15 %) showed 11 per cent inhibition followed by Nimbicidin (4 %) which inhibited growth upto 10 per cent and least in case of Turmeric powder (1 %) where only 9 per cent growth of *C. indica* was checked. Regarding inhibition range by botanical to *C. indica* is concerned it was 1.3 to 11 per cent which is very less in comparison to fungitoxicants evaluated in the previous experiment.

Sensitivity of *T. harzianum* against botanicals and fungitoxicants

The fungitoxicant and botanicals initially evaluated for their influence on *C. indica* growth and the one which had least inhibitory effect on the milky mushroom mycelium was selected for the present experiment. Radial growth of *T. harzianum* were measured after incubation at 30±1˚C temperature, then data analyzed and finally percent inhibition was calculated, depicted in Table 3. From the result it is clear that Bavistin at 150 ppm concentration completely inhibited the *T. harzianum* growth. Among botanicals Nimbicidin exhibited maximum inhibition (90.68 per cent) followed by Turmeric powder (35.45 per cent) and least in case of Bakain Seed Extract (33.63 per cent) at their respective highest concentration.

Management of green mould by botanicals and fungitoxicants

For this experiment fungicide (Bavistin @150 ppm) and botanical (Nimbicidin @ 4 %) were selected on the basis of *in vitro* studies result for management of green mould disease. Yield parameters *viz.*, days for spawn run (DFSR), days for pinhead formation (DFPF), days for first harvest (DFFH), number of fruiting body (NOFB), and weight of fruiting body (WOFB) besides Yield were taken for observation as shown in Table 4. The result from the table 4 evident that the average numbers of DFSR of *C. indica* was significantly less in both Bavistin (13.5 days) and Nimbicidin (15.6 days) treated bags when compared with control-I (18.6 days) and control-II (17.8 days). But the average numbers of the DFSR in case of control-I (mushroom bags without pathogen and toxicants) and control-II (mushroom bags with pathogen but without toxicants) was found statistically identical with each other. Similarly DFPF was observed minimum in Bavistin treatment (11.6 days) followed by Nimbicidin (13.4 days), control-II (14.8 days) and maximum in case of control-I (16.6 days) which significantly differ with each other and same trend was observed in case of DFFH. As far as average number of fruiting bodies is concerned it was found maximum in case of control-I (9.4/bags) followed by Bavistin treatment (8/bag) and Nimbicidin treated bags (6.8/bag) but least in case of control-II (2.8/bags). The yield of bags treated with Nimbicidin (326.8 g/bag) was statistically at par with respect to yield in case of the bags treated with Bavistin (351.4 g/bags) but in both cases yield was significantly higher than as that of in case of pathogen infested bags *i.e* in case of control-II (107 g/bags).

As per the result, growth inhibition of *C. indica* was maximum in case of Dithane M-45 at all assigned concentrations in the present work followed by Topsin-M and least being when Bavistin was used. Dithane M-45 (150 ppm) and Topsin-M (200 ppm) completely inhibited *C. indica* growth, while Bavistin at the highest concentration used (200 ppm) inhibited only 67 % mycelial growth of mushroom that is in accordance with the findings of Bhardwaj (2003). Gandy (2005) observed that Bavistin fungicide was less toxic to the fungi of basidiomycetes class which include *C. indica*. Thus it may be inferred that to minimize damage to *C. indica* extra care should be taken in the selection of fungicides application to manage the green mould disease. So based on the result of present experiment Bavistin was selected for

further experimentation to evaluate the inhibitory against *T. harzianum* which incites green mould disease during milky mushroom cultivation.

Table.1 Effect of different fungitoxicants on mycelial growth of *C. indica*

Sr. No	Treatments	Concentration (ppm)	*Radial growth of *C. indica* (cm)	Per cent inhibition
1	Dithane M-45	100	0.65	85
		150	0.00	100
		200	0.00	100
2	Bavistin	100	2.20	50
		150	1.72	60
		200	1.42	67
3	Topsin-M	100	1.30	70
		150	0.70	84
		200	0.00	100
4	Control	-	4.40	0.0
C.D at 5%			**0.22**	-

* Denotes all values of radial growth of average of three replications

Table.2 Effect of different botanicals on mycelial growth of *C. indica*

Sr. No	Treatments	Concentration (Per cent)	*Radial growth (cm)	Per cent inhibition
1	Turmeric powder	0.25	4.35	1.3
		0.50	4.23	4
		1.0	4.00	9
2	Nimbicidin	1.0	4.33	2
		2.0	4.14	6
		4.0	3.96	10
3	Bakain Seed Extract	5.0	4.30	2
		10.0	4.18	5
		15.0	3.90	11
4	Control	-	4.40	0
C.D at 5%			0.19	

* Denotes all values of radial growth are average of three replications

Table.3 Effect of botanicals and fungitoxicant on mycelial growth *T. harzianum*

Sr. No	Treatments	Concentration (per cent)	*Radial growth (cm)	Per cent inhibition
1	Bakain (*Melia azedarach*) seed extract	5.0	3.93	10.68
		10	3.43	22.04
		15	2.92	33.63
2	Nimbicidin	1.0	2.41	45.22
		2.0	1.40	68.18
		4.0	0.41	90.68
3	Turmeric solution	0.25	3.83	12.95
		0.50	3.16	28.18
		1.0	2.84	35.45
4	Bavistin	0.010	0.50	88.63
		0.015	0	100
		0.020	0	100
5	Control	-	4.40	0
C.D at 5%			**0.76**	-

* Denotes all values of radial growth are average of three replications

Table.4 Effect of botanical and fungitoxicant on yield and yield parameters

Sr. No	Treatments	*Yield and other important parameters of milky mushroom					
		DFSR	DFPF	DFFH	NOFB/Bag	WOFB(g)	Y(g/bag)
1	Control-I	18.6	16.6	10.6	9.4	37.4	416.0
2	Control-II	17.8	14.8	9.4	2.8	39.4	107.0
3	Bavistin (150 ppm)	13.5	11.6	6.2	6.2	34.2	351.4
4	Nimbicidin (4 per cent)	15.6	13.4	8.4	8.4	36.6	326.8
C.D at 5%		**1.4**	**1.8**	**1.5**	**2.0**	**NS**	**32.3**

Control-I= without pathogen and toxicants, Control-II= with pathogen without toxicant
* Denotes all value of radial growth are average of five replications
DFSR=Days for spawn run, DFPF=Days for pinhead formation
DFFH=Days for first harvest, NOFB= No. of fruiting body,
WOFB=Weight of fruiting body and Y=Yield
NS- Non significant

Three botanicals namely Bakain (*Melia azedarach*) Seed Extract, Nimbicidin and Turmeric powder were evaluated in the present experiment for their sensitivity to *C. indica*. Unlike fungitoxicants, botanicals exhibited less inhibitory effect on *C. indica.* Among the botanicals maximum inhibition to *C. indica* (11 per cent) was recorded in case of Bakain Seed Extract (15 %) followed by 10 percent inhibition in case of Nimbicidin (4 %) and least (9 per cent inhibition) in case of Turmeric powder (1 %). Present studies support the findings of Shah and Nasreen (2013) who evaluated antifungal potential of eight botanicals *viz., Azadiracta indica, Artemesia indica, Allium sativum, Urtica dioeca, Licopercicon esculantum, Dathura strimonia, Mentha* and *Juglans regia* against *T. harzianum* and mushroom under *in-vitro* using Poison Food Technique as well as *in vivo* studies. In the present studies, inhibition range of botanicals to *C. indica* varied from 1.3 to 11 per cent which is very less in comparison to fungitoxicants. So from the present experiment all botanicals were selected for evaluating their inhibitory effect on *T. harzianum*.

As fungicides being environment pollutant also has residual toxicity which directly harm to human beings and gradually pathogen developing resistance against fungicides, botanicals for managements of green mould disease in case of milky mushroom is also an alternative option. In this experiment those fungicides and botanicals were evaluated against *T. harzianum* which exhibited least toxic to *C. indica* (as per previous experiment's result). Among three fungicides only Bavistin at 100, 150, and 200 ppm concentration was selected as it showed very less inhibitory effect on mushroom mycelia than Mancozeb and Topsin- M. The result is evident that Bavistin at 150 ppm completely inhibited the *T. harzianum* growth which is in agreement with the findings of Bhardwaj (2003).

Similarly all botanicals that exhibited very less inhibitory effect on *C. indica* were evaluated against the pathogen (*T. harzianum*) under *in vitro*. Among botanicals Nimbicidin showed maximum inhibition (90.68 per cent) followed by Turmeric powder (35.45 per cent) and least in case of Bakain seed extract (33.63 per cent) at their respective highest concentration used in the present studies. Similarly Sharma and Jandaik (1994) who observed that *A. indica,* Eucalyptus, *T. erecta and* garlic extract inhibited the growth of various fungi affecting the yield during mushroom cultivation.

Management of green mould by Fungitoxicants and Botanicals

Mushroom like any other crop are subjected to attack by various fungal, bacterial and viral

pathogens which cause symptoms predominantly on the sporophores and consequently results in loss of marketable mushrooms. During present studies green mould which competes for food and space causing thereby reduction in yield, considering the threat posed by this (*T. harzianum*) in present experiment, fungicide (Bavistin @ 150 ppm) and botanical *viz.*, Nimbicidin (@ 4 per cent) selected on the basis of raised under natural conditions in bags. The results thus obtained indicate that the average numbers of the day for spawn run (DFSR) of *C. indica* was significantly less in both Bavistin (13.5 days) and Nimbicidin (15.6 days) treated bags when compared with control-I (18.6 days) and control-II (17.8 days). But the average numbers of the DFSR in case of control-I (mushroom bags without pathogen and toxicants) and control-II (mushroom bags with pathogen but without toxicants) was found statistically identical with each other. Similarly days for pinhead formation (DFPF) was observed minimum in Bavistin treatment (11.6 days) followed by Nimbicidin (13.4 days), control-II (14.8 days) and maximum in case of control-I (16.6 days) which significantly differ with each other and same trend was observed in case of days for first harvest (DFFH).

As far as average number of fruiting bodies is concerned it was found maximum in case of control-I (9.4/bags) followed by Bavistin treatment (8/bag) and Nimbicidin treated bags (6.8/bag) but least in case of control-II (2.8/bags). The mushroom yield was significantly higher when Bavistin and Nimbicidin were used for the management of the disease which is in agreement with findings of Shah and Nasreen (2013). Danesh and Goltapeh (2007) evaluated two fungicides namely benomyl and carbendazim for control of *Trichoderma* green mould of white button and reported that yield of mushroom was significantly higher over control (devoid of fungicides), which also support the present studies.

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