DOI: https://doi.org/10.36036/MR.34.1.2025.151814
Mushroom Research 34 (1) : 45-53, 2025

Management of green mould (*Trichoderma viride)* in button mushroom (*Agaricus bisporus)*

Akshay Pathania, Rajender Singh Jarial, Kumud Jarial and Riya*

Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Himachal Pradesh, India
**Corresponding author,* E-mail: dhimanriya38@gmail.com

ABSTRACT

Green mould is a highly destructive affliction that plagues button mushroom cultivation, resulting in substantial annual losses. This study focused on evaluating the effectiveness of diverse fungicides and botanicals for its mitigation. Seven fungicides and eight botanicals were subjected to rigorous testing against *Trichoderma viride* and *Agaricus bisporus* mycelium. Result revealed that under *in vitro* conditions, Bavistin, Tilt and Contaf fungicides showed maximum inhibition (100%) against *T. viride* Whereas, among tested botanicals, *Allium sativum* exhibited a maximum (72.51%) inhibition of *T. viride* mycelium followed by *Curcuma longa* (56.33%) displayed significant efficacy against *T. viride*. Further assessments under natural hut conditions included *in vivo* trials with four botanicals (*Tagetes* sp., *Allium sativum, Azadirachta indica, Justicia adhatoda*) and one fungicide (Bavistin) alongside positive and negative controls concluded that *Tagetes* sp. and Bavistin significantly increased mushroom yield compared to the negative control infected with the green mould pathogen. In a commercial production setting, the application of *Justicia adhatoda* leaf extract on bags yielded minimal loss at 6.43%, compared to the positive control.

Keywords: *Agaricus bisporus, Trichoderma viride*, green mould, botanicals, fungicides

Mushroom cultivation is a significant microbial technique with economic importance, utilizing agro-waste efficiently under controlled conditions. This method helps alleviate pressure on arable land. Mushrooms, as saprobic macrofungi, thrive on decomposed organic matter. They have captured the interest of scientists and the public alike due to their critical roles in forest ecology, food and pharmaceutical industries, bio-degradation, and remarkable health benefits (Stojchev *et al*., 1998; Altaf *et al*., 2022). *Agaricus bisporus*, commonly known as the white button mushroom or European mushroom, holds a prominent position among commercially cultivated mushrooms worldwide. Its production constitutes a substantial portion, ranging from 35 to 45 percent of the global total (Sharma *et al*., 2017). In India, the current output stands at approximately 60,733 metric tonnes, with the majority being white button mushrooms (Anonymous, 2018). Himachal Pradesh, specifically, contributes around 9,150 metric tonnes annually, with a significant portion stemming from small farms (Sharma *et al*., 2017).

Furthermore, various bacterial, fungal, and viral pathogens infect the crop, leading to either partial or complete crop failure or a decrease in product quality. Among the significant fungal diseases such as wet bubble, cobweb, dry bubble, and competitor molds,

Recieved on 21-05-2024; Accepted on 06-01-2025
https://epubs.icar.org.in/index.php/MR/article/view/151814

green mold disease caused by *Trichoderma* spp. has been identified as a major culprit (Bhatt and Singh, 2000) resulting in significant damage and production losses of around 63–65% in mushroom farming (Castle *et al.*, 1998). Despite the presence of numerous *Trichoderma* species (including *T. crassum* Bissett, *T. koningii* Oudem., *T. citrinoviride* Bissett, *T. spirale* Bissett, *T. longibrachiatum* Rifai, *T. hamatum* (Bonord.) Bainier) in mushroom compost, initial outbreaks of green mold in *A. bisporus* cultivation were attributed to *T. harzianum* (Forer *et al*., 1974; Allaga *et al*., 2021). However, with various diseases and competitive molds, resulting in notable yield losses. Among these challenges, the green mold caused by *Trichoderma* spp. emerges as a particularly severe threat to white button mushroom production in the sub-tropical regions of Himachal Pradesh.

Consequently, extensive studies on green mold management were conducted, encompassing both in vitro and in vivo assessments conducted in natural bamboo huts. Seven distinct systemic and non-systemic fungicides, along with eight botanicals, were scrutinized in vitro across four different concentrations for their efficacy against *T. viride* (NCFT ID No. 9774.20) and their impact on *A. bisporus* growth using the poisoned food technique pioneered by Falck in 1907. The most effective fungicides and botanicals, demonstrating inhibition of the test pathogen while supporting *A. bisporus* growth, were then selected for in vivo evaluation under real-world hut conditions in a commercial mushroom production trial.

MATERIALS AND METHODS

In vitro evaluation of different chemicals against test pathogen and their effect on the growth of *A. bisporus*

Seven different fungicides, including both systemic and non-systemic types, were assessed *in vitro*. Chlorothalonil®, Dithane M-45®, and Captra® (non-systemic) were tested at concentrations of 500, 1000, 1500, and 2000 ppm. Meanwhile, systemic fungicides like CabrioTop®, Bavistin®, Tilt® and Contaf® were evaluated at 125, 250, 375 and 500 ppm. The poisoned food technique by Falck in 1907 was employed to gauge their effectiveness against the target pathogen and their impact on *A. bisporus* growth. Petri plates with the respective chemical concentrations in CDA were inoculated with pathogen and *A. bisporus* culture bits (5mm dia.) and kept at ± 25°C until the untreated control plates showed complete mycelial growth of the fungus. Data were recorded in terms of diametric growth of the fungus (mm) and growth inhibition (%) in relation to untreated control was further calculated as follows:

$$\text{Inhibition (\%)} = \frac{C-T}{C} \text{ X } 100$$

Where:
C = Diametric growth (mm) in control
T = Diametric growth (mm) in treatment

In vitro evaluation of different botanicals against test pathogen and their effect on the growth of *A. bisporus*

Eight botanicals, including *Allium sativum* (garlic), *Azadirachta indica* (neem), *Lantana camara* (lantana), *Murraya koenigii* (curry patta), *Tagetes* sp. (marigold), *Justicia adhatoda* (basuti), *Melia azedarach* (darek), and *Curcuma longa* (turmeric) were assessed in vitro for their effectiveness against the test pathogen and their impact *on A. bisporus* growth. The poisoned food technique (Falck, 1907) was used with concentrations of 5, 10, 15, and 20 percent for each botanical extract. Plates with these concentrations were inoculated with pathogen and *A. bisporus* culture bits and incubated until untreated control plates showed complete mycelial growth. Data on diametric growth (mm) and growth inhibition (%) were recorded and calculated in relation to the untreated control.

In vivo evaluation of selected chemicals and botanicals under natural bamboo hut conditions

The most effective fungicides and botanicals, which suppressed the test pathogen while promoting *A. bisporus* growth, were chosen for *in vivo* assessment in a commercial mushroom production trial conducted in natural hut conditions. Commercial 10 kg compost bags were acquired, spawned with button mushroom spawn and cased. These bags were then artificially inoculated with a spore suspension of the pathogen (10^4 spores/ml) and treated with the selected fungicides (at field concentration of @100 ppm) and botanicals (at 10%). The treatments were applied three times at 15-day intervals from casing. Positive and negative control groups were maintained for comparison, with all treatments replicated thrice. Data were recorded in terms of cumulative mushroom yield and yield loss (%) w.r.t. positive control and yield gain (%) w.r.t. negative control were further calculated as follows:

$$\text{Yield loss (\%)} = \frac{\text{Yield in positive control - Yield in treatment}}{\text{Yield in positive control}} \times 100$$

$$\text{Yield gain (\%)} = \frac{\text{Yield in treatment - Yield in negetive control}}{\text{Yield in negetive control}} \times 100$$

Statistical analysis

The experiments followed a Completely Randomized Design (CRD) with three replications per treatment. Data were analyzed using Panse and Sukhatme's (2000) statistical methods.

RESULTS AND DISCUSSION

In vitro evaluation of different fungicides against _T. viride_

The findings as shown in Table 1 and Fig 1, unequivocally demonstrate the inhibitory effects of all assessed fungicides on *T. viride* growth compared to the untreated control. Among the seven fungicides, Tilt, Contaf, and Bavistin exhibited complete suppression of the test fungus across all concentrations. CabrioTop, at 500 ppm, 375 ppm and 250 ppm resulted in growth inhibition 91.30, 87.04, and 82.59 per cent respectively, while Dithane M-45 at 500 ppm showed only 16.30 per cent inhibition. The remaining fungicides across various concentrations supported intermediate levels of diametric growth corresponding to respective levels of growth inhibition.

In vitro evaluation of different botanicals against _T. viride_

As depicted in Table 2, *Allium sativum* employed at a 20 percent concentration, exhibited the lowest diametric growth (18.77 mm) and achieved a substantial 79.14 per cent inhibition of the test pathogen. This performance was statistically comparable to the treatment with *A. sativum* at a 15 percent concentration which resulted in a diametric growth of 19.87 mm and 77.92 percent inhibition of the pathogen as shown in Fig. 2. *Curcuma longa* at a 20 percent concentration led to a diametric growth of 21.80 mm and 75.78 percent inhibition. Conversely, *Lantana camara* at a 5 percent concentration failed to impede *T. viride* growth resulting in no inhibition. The remaining botanicals, at various concentrations, demonstrated intermediate levels of growth inhibition. Among these, *A. sativum* emerged as the most effective plant extract against *T. viride* recording a mean inhibition of 72.51 percent compared to the control. Following closely were *Curcuma longa, Tagetes sp., Justicia adhatoda* and *Azadirachta indica.*

In vivo evaluation of selected fungicides and botanicals against green mould

In an *in vivo* evaluation against green mould under natural hut conditions, four botanicals -

Table 1. *In vitro* evaluation of different fungicides against *Trichoderma viride*

Treatment	Diametric growth (mm), at concentrations (ppm)				Overall mean	Inhibition (%) in diametric growth at concentrations (ppm)				Overall mean
	125	250	375	500		125	250	375	500	
CabrioTop*	19.23 (25.99)	15.67 (23.31)	11.67 (19.96)	7.83 (16.23)	13.60 (21.37)	78.63	82.59	87.04	91.30	84.89
Chlorothalonil**	30.53 (33.53)	26.13 (30.73)	22.90 (28.57)	19.68 (26.30)	24.81 (29.78)	66.07	70.96	74.56	78.15	72.44
Dithane M-45**	75.33 (60.22)	68.33 (55.74)	50.33 (45.17)	43.33 (41.15)	59.33 (50.57)	16.30	24.07	44.07	51.85	34.07
Captra**	60.13 (50.83)	40.33 (39.41)	36.37 (37.07)	33.43 (35.31)	42.57 (40.66)	33.19	55.19	59.59	62.85	52.70
Tilt*	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100.00	100.00	100.00	100.00	100.00
Contaf*	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100.00	100.00	100.00	100.00	100.00
Bavistin*	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100.00	100.00	100.00	100.00	100.00
Control	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)					
Mean	34.59 (31.78)	30.25 (29.11)	26.60 (26.81)	24.47 (25.34)						
		$CD_{Pe''0.05}$	**SE(d)**							
Fungicide		0.58	0.29							
Concentration		0.41	0.20							
Fungicide X Concentration		1.15	0.58							

**Concentrations were 125, 250, 375 and 500 ppm for systemic fungicides; **Concentrations were 500, 1000, 1500 and 2000 ppm for non-systemic fungicides; Figures in parentheses represent angular transformed values*

Azadirachta indica, Allium sativum, Tagetes sp. and *Justicia adhatoda* along with the chemical fungicide Bavistin were assessed as shown in Table 3 and Figure 3. Additionally, a negative control (artificially inoculated with green mould) and a positive control (without green mould inoculation) were included. Table 3 highlights a notable increase in mushroom yield across all treatments compared to the negative control, except for *Tagetes* sp. The highest yield (1906.67 g/ 10 kg compost) was achieved when casing soil was sprayed with a 10 per cent extract of *J. adhatoda* statistically on par with the yield from *A. sativum* at 10 per cent concentration (1876.33 g/10 kg compost). This represented a 26.80 and 24.78 per cent gain in yield, respectively, compared to the negative control (1503.676 g/10 kg compost). Conversely, the lowest yield (1515.67 g/10 kg compost) was recorded in bags treated with *Tagetes* sp. extract, statistically similar to the yield in the negative control.

In terms of yield loss due to disease, the negative control exhibited the highest loss at 26.21%, followed by bags treated with *Tagetes* sp. extract (25.61%), carbendazim (20.94%) and *A. indica* leaf extract (17.26%). The lowest yield loss was observed in bags sprayed with *J. adhatoda* leaf extract (6.43%) followed by *A. sativum* leaf extract (7.91%).

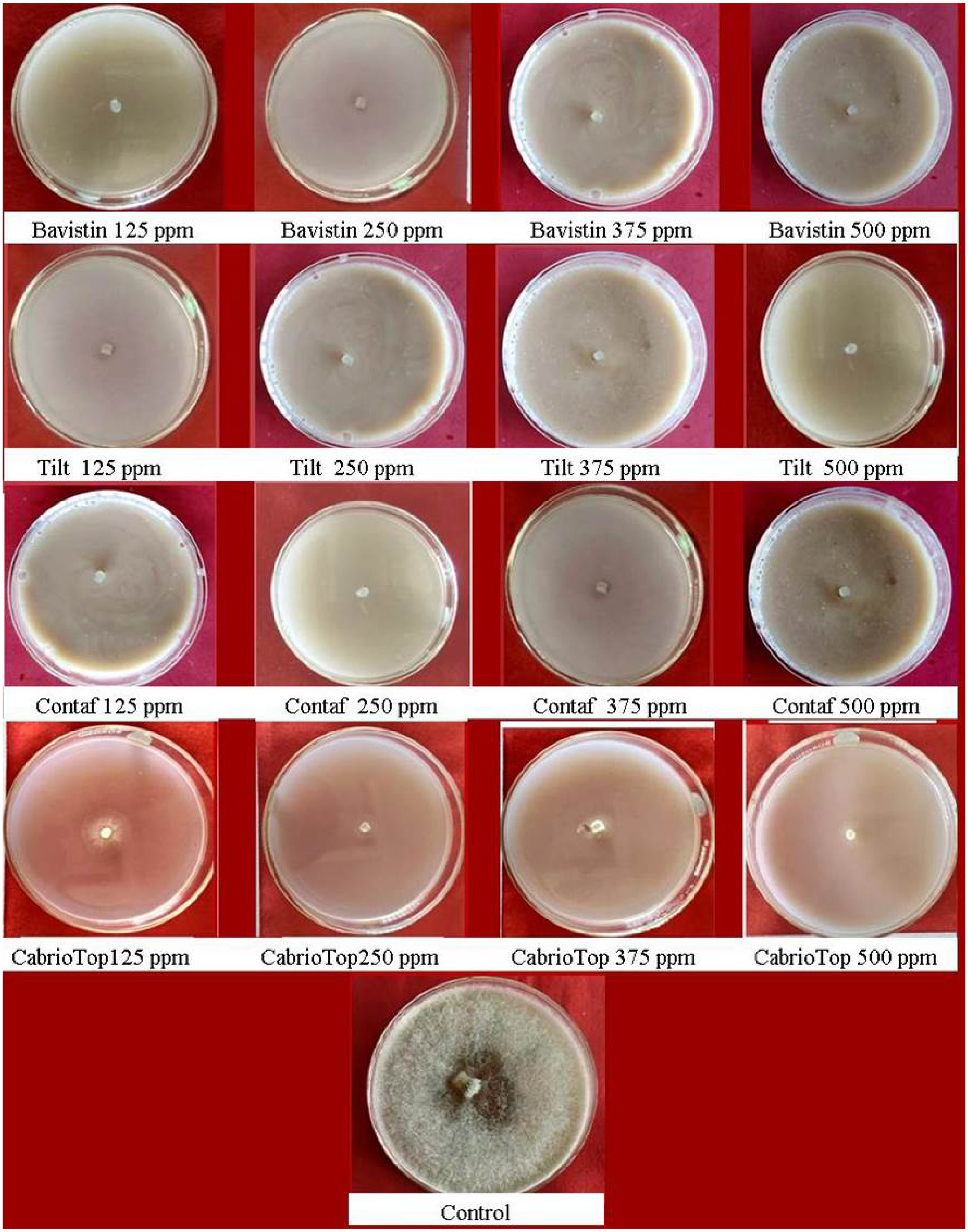

Fig. 1. *In vitro* evaluation of chemicals against *Trichoderma viride*

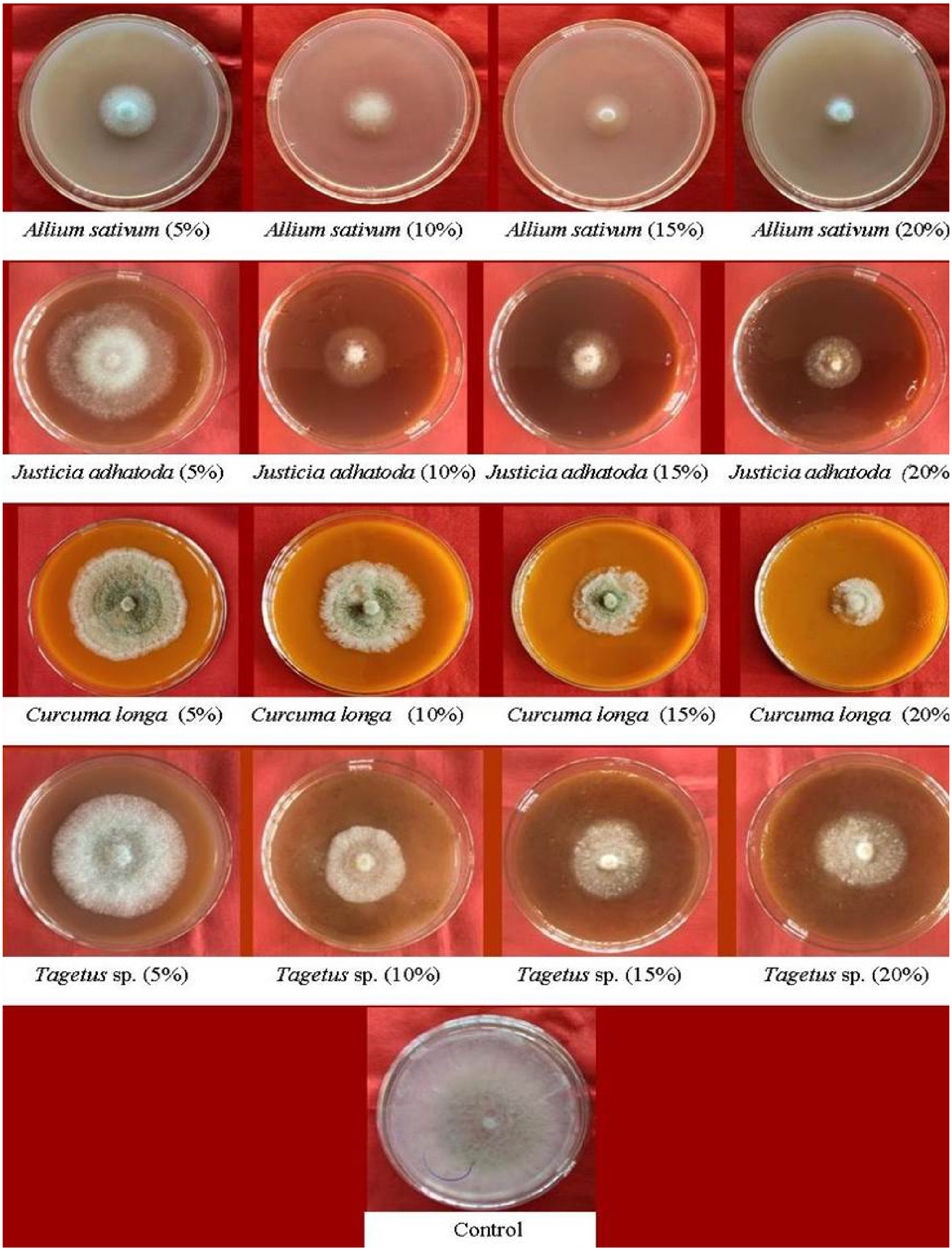

Fig. 2. *In vitro* evaluation of botanical extracts against *Trichoderma viride*

Table 2. *In vitro* evaluation of different plant extracts against *Trichoderma viride*

Treatment	Diametric growth (mm) at concentrations (%)				Overall mean	Inhibition (%) in diametric growth at concentrations (%)				Overall mean
	5	10	15	20		5	10	15	20	
Lantana camara	90.00	75.87	70.00	65.00	75.26	0	15.70	22.22	27.78	16.42
Murraya koengii	80.00	79.63	66.43	61.23	71.82	11.11	11.52	28.41	31.96	20.75
Melia azedarach	77.30	70.60	70.00	69.67	71.89	14.11	21.56	22.22	22.59	20.11
Azadirachta indica	70.30	64.80	40.10	31.90	51.78	21.89	28.00	55.44	64.56	42.48
Justicia adhatoda	68.30	38.87	33.13	30.20	42.63	24.11	56.81	63.19	66.44	52.63
Tagetes sp.	47.77	38.67	37.67	35.90	40.00	47.03	56.81	58.14	60.11	55.56
Allium sativum	34.13	26.20	19.87	18.77	24.74	62.08	70.89	77.92	79.14	72.51
Curcuma longa	56.10	49.10	30.23	21.80	39.31	37.67	45.44	66.41	75.78	56.33
Control	90.00	90.00	90.00	90.00	90.00					
Overall Mean	68.17	59.34	50.83	47.16						
		$CD_{Pe”0.05}$	**SE(d)**							
Treatment		1.26	0.63							
Concentration		0.84	0.42							
Treatment X Concentration		2.52	1.26							

Table 3. *In vivo* evaluation of botanicals and fungicides in terms of yield of *Agaricus bisporus* infected with green mould disease.

Treatment	Mushroom yield (g/10kg compost)	Yield loss (%) w.r.t. +ve control	Yield gain (%) w.r.t. -ve control
Azadirachta indica	1,686.00	17.26	12.12
Allium sativum	1,876.33	7.91	24.78
Tagetes spp.	1,515.67	25.61	0.01
Justicia adhatoda	1,906.67	6.43	26.80
Bavistin	1,611.00	20.94	0.71
Control (-)	1,503.67	26.21	
Control (+)	2,037.67		35.51
$CD_{Pe”0.05}$	77.66		
$SE_{(d)}$	35.86		

These results indicate that all plant extracts, except *Tagetes* sp. and Bavistin, significantly increased mushroom yield compared to the negative control infected with the green mould pathogen. Noticeable yield gains were observed in bags treated with *J. adhatoda, A. sativum* and *A. indica* extracts in descending order.

The findings align with previous studies by Gupta *et al*. (1995), Kamal *et al*. (1997), Domondon and Poppe (2000) and Shah *et al*. (2013) affirming the effectiveness of carbendazim against *Trichoderma* spp. Narzari *et al*. (2007) reported that *A. sativum* extract completely inhibited the mycelium of *T. harzianum*. Shah *et al*. (2011) and Verma and

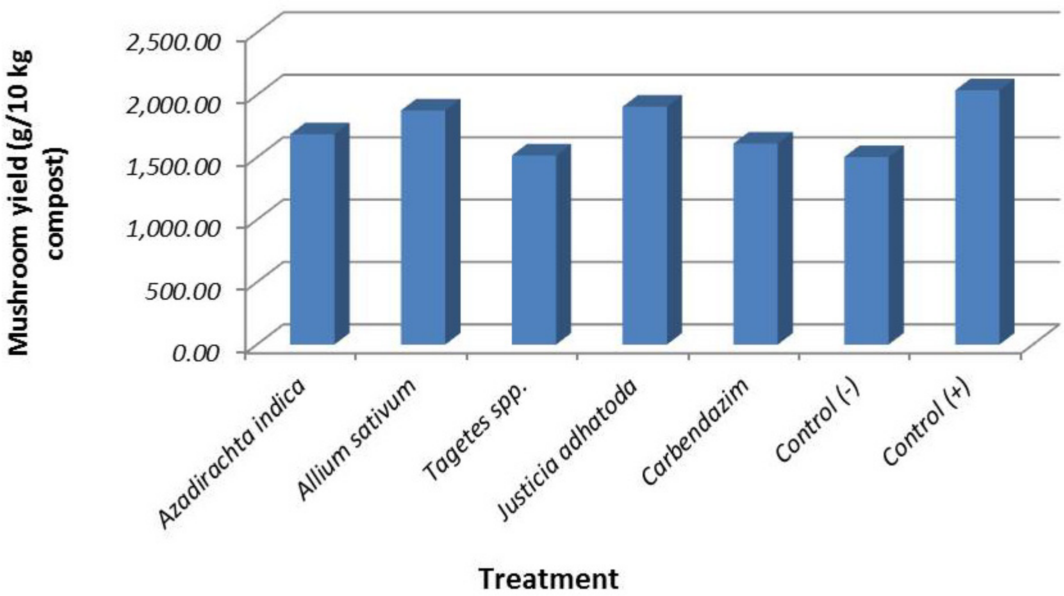

Fig. 3. Yield of *Agaricus bisporus* as affected by botanicals and fungicides evaluated for green mould management

Ratnoo (2012) also supported these results, indicating inhibitory effects of *A. indica* and *A. sativum* extracts against *T. harzianum* without affecting *A. bisporus* growth.

CONCLUSION

The current research assessing both non-systemic, systemic fungicides and botanical has demonstrated promising effects in reducing green mold infection. It was concluded that an among tested fungicides and botanicals, Bavistin and *Allium sativum* showed good results extract against green mould. Under in vivo conditions, *Tagetes* sp and Bavistin treatments helped in significant increase in mushroom yield in comparison to negative control infected with pathogen. Use of fungicides as well as botanicals showed significant result in controlling green mould in *Agaricus bisporus*.

ACKNOWLEDGEMENT

I would like to give my special thanks for the encouragement and help provided by Dr. R.S. Jarial, Dr. Kumud Jarial and Riya. I would also like to acknowledge the help provided by the Department of Plant pathology, COHF, Neri, Dr. Y.S. Parmar University of Horticulture and Forestry for providing the laboratory facilities for research.

REFERENCES

1. Allaga, H., A. Zhumakayev, R. Büchner, S. Kocsubé, A. Szûcs, C. Vágvölgyi, L. Kredics and L. Hatvani. 2021. Members of the *Trichoderma harzianum* Species Complex with Mushroom Pathogenic Potential. *Agronomy* **11:** 2434.

2. Altaf, S., S.K. Jan, S.A. Ahanger, U. Basu, R.A. Rather, O.A. Wani, F. Rasool , M. Mushtaq, M.T. Yassin, A.A. Mostafa, A.M. Elgorban, E. El-Haroun, A.M. El-Sabrout, R. Casini and H.O. Elansary. 2022. Management of Green Mold Disease in White Button Mushroom (*Agaricus bisporus*) and Its Yield Improvement. *Journal of Fungi* **8(6):** 554.

3. Annonymous 2018. Mushrooms and truffles. *FAO STAT*, Food and Agricultural Organisation. https://data.un.org/Data.aspx?d=FAO&f=itemCode%3A449

4. Bhatt, J.C. and R. Singh. 2000. Influence of the *Trichoderma* exudates on the growth and yield of mushroom cultivation in north plain of India. *Indian Phytopathology* **68:**178–188.

5. Castle, A., D. Speranzini, N. Rghei, G. Alm, D. Rinker and J. Bissett.1998. Morphological and molecular identification of Trichoderma isolates on North American mushroom farms. *Applied and Environental Microbiology* **64**:133–137.

6. Domondon, D. and J Poppe. 2000. *Mededelingen van de Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen*. Volume 65. Universiteit Gent; Gent, Belgium: Prevention of yield loss as influenced by *Trichoderma* in mushroom cultivation; pp. 771–781.

7. Falck, R. 1907. Wachutumgesetze, wachstum Laktorehnund temperature wertder holzersrenden. *Myceture* **32:** 38-39.

8. Forer, L.B., P. J. Wuest and V.R. Wanger. 1975. Occurrence and economic impact of fungal diseases of mushrooms in Pennsylvania. *Plant Disease Report* **58:** 987–991.

9. Gupta, A. and P.A. Kerni. 1995. *In vitro* evaluation of different chemicals against. *T. viride* isolated from button mushroom. *Research and Develpoment Report* **12:** 44-47.

10. Kamal, S., A.K. Sinha and R.N. Verma. 1997. *In vitro* studies of fungicidal control of pathogens and competitors of white button mushrooms prevalent in Haryana (India). *Indian Mushroom Conference*. 55p.

11. Panse, V.G. and P.V. Sukhatme. 2000. *Statistical Methods for Agricultural Workers* (2nd edition). Indian Council of Agricultural Research, New Delhi. 381 p.

12. Shah, S., S. Nasreen and N.A. Munshi. 2011. Evaluation of some botanicals in controlling green mould (*Trichoderma harzianum*) disease in oyster mushroom cultivation. *International Journal of Botany* **7:** 209-215.

13. Sharma, V.P., S.K. Annepu, Y. Gautam, M. Singh and S. Kamal. 2017. Status of mushroom production in India. *Mushroom Research* **26 (2):** 111-120.

14. Stojchev, G., A. Asan A and F. Giicin. 1998. Some macrofungi species of European part of Turkey. *Turkish Journal of Botany* **24:** 341–348.

15. Verma, R. and R.S. Ratnoo. 2012. Mangaement of green mould (*Trichoderma harzianum*) with fungicide and botanical. *Journal of Plant Disease Sciences* **7(1):** 103-110.