

Original Research Article

Effect of neem leaf powder supplementation in casing mixture on the growth and yield parameters of white button mushroom [*Agaricus bisporus* (Lange) Imbach]

ABSTRACT

Green mould caused by *Trichoderma harzianum* is a serious threat in severe threat of white button mushroom [*Agaricus bisporus* (Lange) Imbach] cultivation causing heavy crop losses. The present experiment was conducted to manage green mould the green mould fungus *Trichoderma harzianum* using neem leaf extracts. *In vitro* results showed maximum mycelial growth inhibition of 64.59 % in *T. harzianum* using neem leaf extract at 4.5% concentration. Neem leaf extracts at 4.5% concentrations showed maximum efficacy against pathogen pathogens. Further, an *in vivo* experiment was conducted to evaluate neem leaf powder at different concentration concentrations ranging from @0.5% to @4.5% supplemented in the casing mixture combination viz., Farm yard manure (FYM) + Cocopeat + Sawdust (1:1:1) with an objective to monitor the growth and yield aspects of white button mushroom. Among all treatments used in the study, the results revealed that minimum average time taken for completion of spawn run (15.14 days) and pinhead initiation (18.28 days) as well as maximum number of fruiting bodies (14.71), fruiting bodies weight (22.54g), stalk length (2.81 cm) stalk diameter (1.85 cm) and pileus diameter (5.64 cm), and were recorded in T₂ [FYM + Cocopeat + Sawdust (1:1:1) + neem leaf powder @ 1.5%]. Results have shown that T₂ [FYM + Cocopeat + Sawdust (1:1:1) + neem leaf powder @ 1.5%] had the highest yield (470.09g) and biological efficiency (27.11 %).

Key words: *Agaricus bisporus*, Casing mixture, Green mould mould, Neem leaf, *Trichoderma harzianum*.

INTRODUCTION

Mushrooms are a prominent fruiting body of a macrofungus that belongs to the phylum Basidiomycota and possesses the ability to produce spores and can be found as epigeous or hypogeous. White button mushroom [*Agaricus bisporus* (Lange) Imbach] is the most

popular cultivated edible mushroom, fetching high prices and still dominating the Indian and International markets. Mushrooms are one such component that not only impart diversification but also help in addressing the problems of health, environment quality and food-related

issues. Its first cultivation was started in China. Though mushroom production is of recent origin in India, it ranked 4th in the world ~~mushroom production~~ with a total production of 2,80,000 MT of mushrooms accounting for 0.58% of the total world mushroom production (FAO, 2022).

China, Japan, ~~the~~ USA and India are driving ~~in~~ worldwide mushroom production. China is the world's biggest grower of ~~eatable-edible~~ mushrooms providing 45,428,000 MT, or 93.99 % of worldwide contribution (FAO, 2022).

Agaricus bisporus requires two different substrates to form the fruiting bodies, i.e. the compost for nutrition on which it grows vegetatively and the casing soil in which the suitable physicochemical/biological conditions stimulate the initiation process of pin head formation for fruit body production (Kaure *et al.*, 2017).

In addition to ~~having a their~~ high protein content, mushrooms also ~~have contained~~ high levels of several vitamins, including vitamins B, C, and D, riboflavin, thiamine, and five nicotinic acids. In addition to folic acid, mushrooms are a good source of potassium, phosphorus and iron. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Kumare *et al.*, 2023).

Among these moulds, *Trichoderma harzianum* ~~inducee-induces~~ significant quantitative and qualitative losses in the yield of *Agaricus bisporus*, *Pleurotus* spp., *Auricularia*, *Calocybe indica* and *Lentinula edodes* reported 5-46.87 per cent and 6.25-50.0 per cent yield losses due to *T. viride* and *T. harzianum*, respectively, under artificial inoculation conditions. (Kumar *et al.*, 2023).

MATERIALS AND METHODS

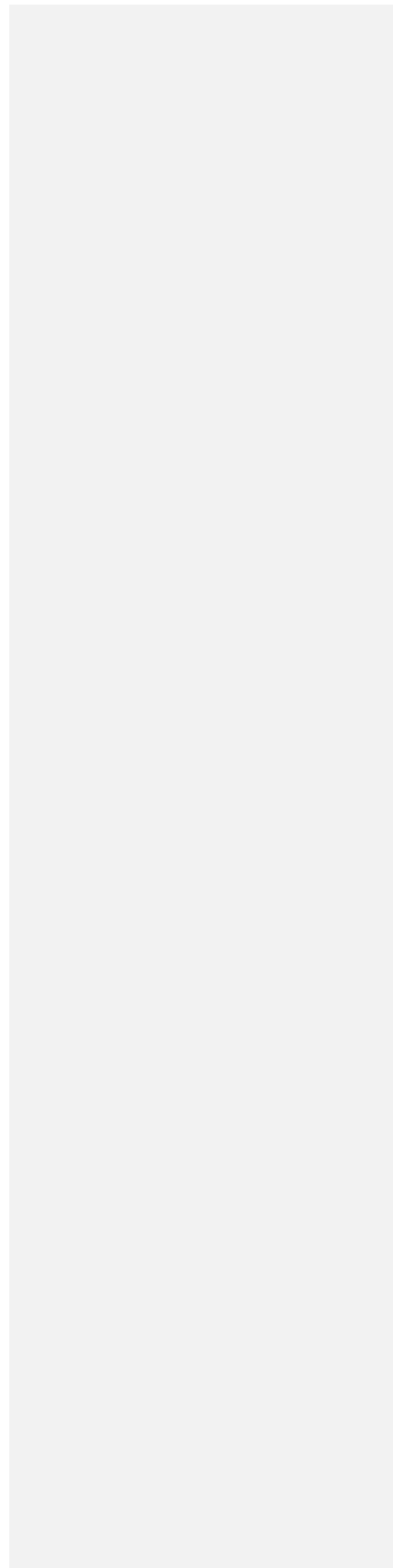
The present experiment was conducted ~~during the Rabi season 2023 at Mushroom crop room, Department of Plant Pathology, SHUATS, Prayagraj, Uttar Pradesh during the season October 2023— March 2024~~ from October 2023 to March 2024 during the Rabi season 2023 at the Mushroom crop room, Department of Plant Pathology, SHUATS, Prayagraj, Uttar Pradesh.

Isolation and preservation of *Trichoderma* sp

White button mushroom bags were naturally infected with typical green ~~mould used to isolate mould~~ used to isolate the causal organism. The isolated fungi were identified using microscopy. Casing material from ~~an~~ infected bag was taken from sterile forceps. Serially diluted using

with sterile distilled water, inoculated on the Potato Dextrose Agar plates and incubated at $25 \pm 1^\circ\text{C}$ for

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96 hours. Fungal culture was purified by single spore isolation and periodic sub-culture. All the pure cultures were kept in refrigerator at 4°C ~~for till~~ until further use.

Preparation of neem leaf extract:

For the preparation of neem leaf extract, 100 g of fresh neem leaf was collected, washed in distilled water, air dried and homogenized with an equal amount of distilled water (100 ml) by crushing them with an electric grinder machine. The extract was filtered through double-layered muslin cloth and centrifuged at 4000 rpm, for 10 minutes. The supernatant was collected and filtered through Whatman No.1 filter paper ~~which was considered as~~ considered a standard solution.



Plate 1: Bag infected by green mould

***In vitro* evaluation:**

The botanicals were evaluated *in vitro* through the poison food technique (Nene and Thapliyal, 2000). Sterilized media (20ml) with botanical extract was poured into 90 mm Petri plates under aseptic conditions in laminar ~~air flow~~ airflow. After solidification of the media, a 5mm disc of 7 days old subculture of *Trichoderma harzianum* ~~were~~ was placed in the centre of the ~~petri~~ Petri plates and one control plate which ~~has had~~ only the PDA medium inoculated with culture disc and used as check. Three replications were maintained for all the treatments. ~~Per cent~~ Per cent inhibition of mycelial growth of green ~~mold~~ mould fungus over control was calculated using the following formula of Vincent (1947):

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

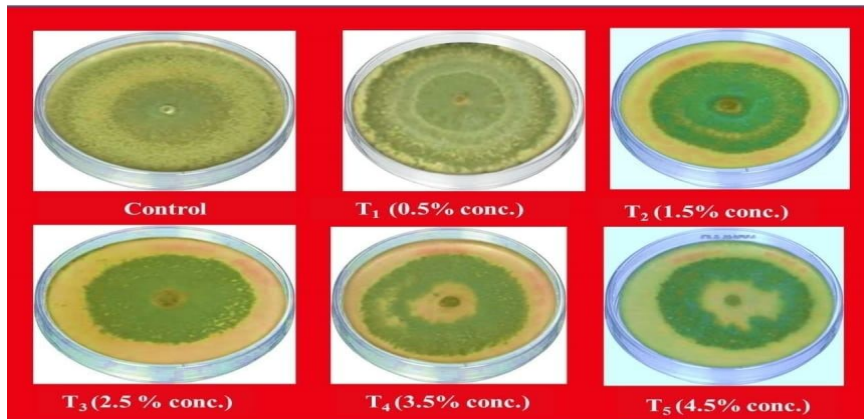


Plate 2: Growth of *Trichoderma harzianum* in Petri plate after 96 hours of inoculation

In vivo

evaluation Procurement of Spaw

n:

The spawn strain – *Agaricus bisporus* (DMR NBS-5), was procured from Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh.

Composting:

The basic materials for compost, wheat straw ~~was, were~~ taken from Agro farm. Other ingredients like wheat bran, urea, potassium (Murate of Potash), phosphorus (Single Super Phosphate), gypsum and lindane were obtained from commercial ~~out let~~ outlet. The compost was prepared by long ~~method of composting~~ composting method (Mantel *et al.*, 1972). Watted wheat straw ~~had spread thinly over entire floor of the composting yard and then gradually watted by sprinkling water, till the straw was taken~~ was spread thinly over an entire floor of the composting yard and then gradually watted by sprinkling water, an entire floor of

the composting yard and then gradually wetted by sprinkling water till the straw took no more water. The straw was then turned for even wetting at the stage, and water content was maintained at 75 percent. The moist straw was mixed with wheat bran and fertilizer uniformly scattered over the straw. A heap was made after each turning but not compressed tightly ~~so as to~~ maintain the aerobic condition in the compost heap. Gypsum was mixed at the third turning ~~and at each turning water was sprayed to make up,~~ and at each turning, water was sprayed to make up for the loss of water due to evaporation. Profenofos insecticide was mixed at 7 turning for prevention of ~~insects~~ insect pests. ~~Total~~ A total of eight ~~turning~~ turnings was done and each turning at four days interval. The compost was then ready for spawning *i.e.* it was dark brown ~~in colour~~ and without any smell of ammonia and had sufficient moisture content (68-70%) when pressed between palms.

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Spawning:

A unit of 5 kg mildly wet compost was used for each bag in a perforated polypropylene bag, which was equally distributed in 6 treatments including control which ~~contain~~ contained 7 replication each. The moisture content of the compost at the time of spawning was kept around 25-30%. The spawn ~~was~~ thoroughly mixed with the compost at ~~the rate of~~ 7.5 g/kg compost (Kapoor, 2004) and pressed moderately. The sterilized newspaper was placed on the top of ~~he~~ compost bag to preserve moisture ~~and kept, kept~~ folded on top, and transferred ~~in to~~ into the dark room for ~~the~~ spawn run.

Preparation of casing mixtures:

The selected ~~basic~~ primary material for ~~the~~ preparation of casing soil, such as Farm yard manure was obtained from ~~an~~ Agro-farm, SHUATS and other casing materials, cocopeat and sawdust were obtained from commercial outlet Prayagraj.

Preparation of botanicals (Neem leaf powder):

Fresh neem leaves were collected, well dried in sunlight, and then made into a fine powder with the help of a mortar pestle and mixer. The dried powder of the selected botanical was mixed with the casing mixtures in different concentrations (0.5%, 1.5%, 2.5%, 3.5% and 4.5%) for 5 treatments, and the untreated check bags were kept as control.

Application of treatment (casing material):

The different casing materials viz., FYM, Cocopeat, ~~and~~ Sawdust ~~was~~ were used alone and different casing ~~mixture~~ mixtures with neem leaf powder. [FYM + Cocopeat + Sawdust (1:1:1) + neem leaf powder ranging from @ 0.5-4.5%]. First, ~~the~~ cocopeat was soaked in water for 2 hours. Initially, individual casing materials, FYM, Sawdust, ~~Cocopeat sterilization was done by using~~ and Cocopeat sterilization were done using a 2% formalin solution. Before applying the casing layer to the compost, it was kept under a polythene film so that evaporation occurs ~~and~~ thereby making the casing layer free from contaminants. Each of ~~the casing materials~~ casing material was applied to seven uniform bags (replicates) containing spawned compost. After ~~casing was done the temperature of the room was again maintained at 23±2°C and relative humidity of 85-90% for another 8-10 days (till the~~

casing was done, the temperature of the room was again maintained at $23\pm 2^{\circ}\text{C}$ and relative humidity of 85-90% for another 8-10 days (till the casing run) (Singh *et al.* 2016).

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Fruiting and Harvesting

The mycelium emerged on casing soil after 10 days, the environmental conditions were changed in [the](#) cropping room by providing fresh air through ventilation and light for 6-8 hrs, [and](#) [relative humidity 90-95%](#) [were](#) [was](#) maintained by spraying of the water thrice a day. The temperature of [the](#) cropping room was maintained at $16 \pm 2^\circ\text{C}$. Low CO_2 concentration (0.08-0.15%) is favorable for reproductive growth at this stage. ~~Pin heads were appeared between 13-16 days after casing and they became ready for harvesting within next one~~ Pinheads appeared between 13-16 days after casing, and they became ready for harvesting within the next week.

Biological efficiency:

~~Biological~~ The biological efficiency (%) of all the tested casing substrates was calculated further by adopting [the](#) following formula (Change *et al.*, 1981):

$$\text{Biological efficiency (\%)} = \frac{\text{Total weight of fresh mushroom}}{\text{Total dry weight of compost}} \times 100$$

Statistical analysis:

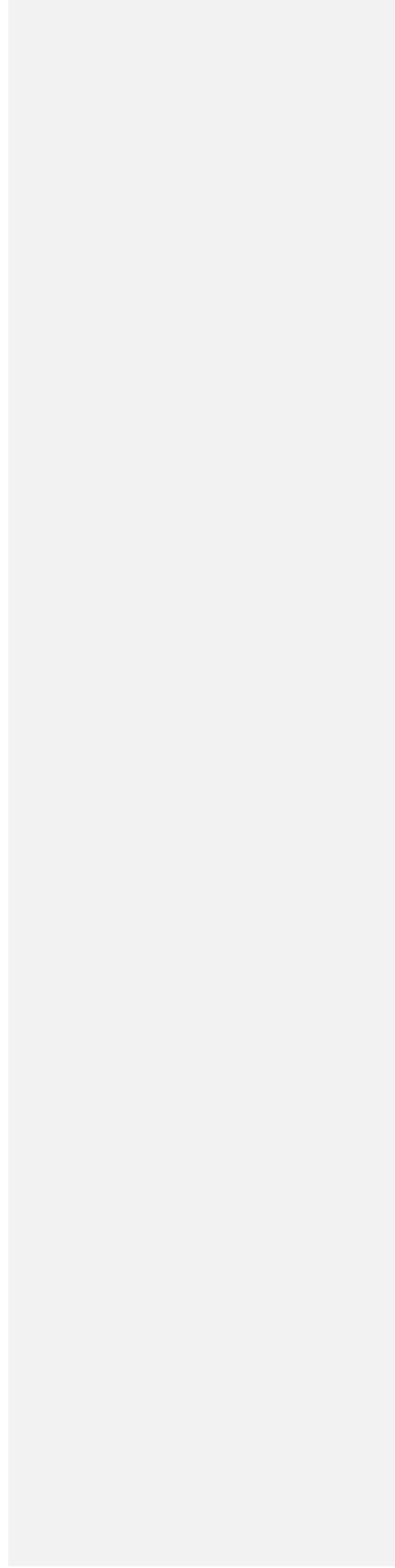
In this experiment, [a](#) Complete Randomized Design (CRD) was followed. The analysis of variance (ANOVA) technique was applied ~~for drawing to draw~~ conclusion from [the](#) data. The calculated values were compared ~~to~~ the tabulated values at [a](#) 5% level of probability for the appropriate degree of freedom.

RESULTS AND DISCUSSION

In vitro effect of different concentrations of neem leaf extract on radial growth (mm) of *Trichoderma harzianum*

Neem leaf extract ~~more or less significantly~~ [significantly](#) inhibited the mycelial growth of *Trichoderma harzianum* at all the tested concentrations. The maximum mycelial inhibition percentage and least mycelial growth of *T. harzianum* (64.59%, 31.85 mm) were observed in T_5 (neem leaf extract @ 4.5%) followed by (57.22%, 37.80 mm) in T_4 (Neem leaf extract @ 3.5%) (Table 1).

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In vivo* effect of neem leaf powder supplementation in casing mixture on the growth and yield parameters of *Agaricus bisporus

It is evident from (Table 2) that the minimum days taken for the mycelium run and pin head initiation in the casing layer were observed in T₂ [FYM + Sawdust + Cocopeat (1:1:1) + Neem leaf powder @ 1.5% (15.14, 18.28)]. It was further observed that the maximum stalk length (2.81 cm), stalk diameter (1.85 cm), pileus diameter (5.64 cm), and maximum number of fruiting bodies (14.71), weight of fruiting bodies (22.54 g) were recorded in T₂ [FYM + Sawdust + Cocopeat (1:1:1) + Neem leaf powder @ 1.5%].

A significant difference was recorded in the effect of neem leaf powder supplemented in the casing mixture on the yield of *Agaricus bisporus* (Table 2). The maximum yield (470.09 g) with (27.11%) biological efficiency was recorded in T₂ [FYM + Sawdust + Cocopeat (1:1:1) + Neem leaf powder @ 1.5%].

Table 1: *In vitro* effect of different concentrations of neem leaf extract on radial growth (mm) of *Trichoderma harzianum*

Treatment number	Treatment name	Concentration (%)	Mean colony diameter (mm)	Percent inhibition (%)
T ₀	Control		90.00	00.00
T ₁	Neem leaf extract	0.5%	85.11	5.96
T ₂	Neem leaf extract	1.5%	62.04	30.96
T ₃	Neem leaf extract	2.5%	45.51	49.41
T ₄	Neem leaf extract	3.5%	37.80	57.22
T ₅	Neem leaf extract	4.5%	31.85	64.59
	C.V.		1.88	

	CD(0.05)		1.96	
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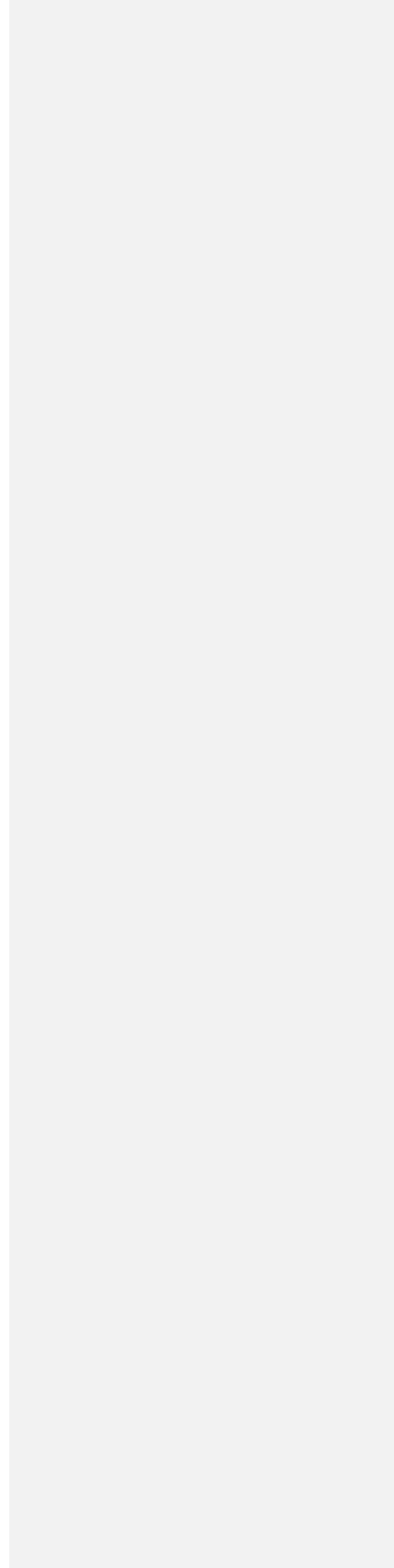


Table 2: Effect of neem leaf powder supplementation in casing mixture on the growth and yield parameters of white button mushroom [*Agaricus bisporus* (Lange) Imbach

Tr no.	Treatments	Case run (days)	Pinhead initiation (days)	Number of fruiting bodies	Weight of fruiting bodies	Stalk length (cm)	Stalk diameter (cm)	Pileus diameter (cm)	Yield (g)	Biological Efficiency (%)
T ₀	FYM+SD+CP	23.14	25.57	6.42	13.65	1.26	0.97	1.33	283.18	19.51
T ₁	FYM+SD+CP NLP@0.5%	16.42	19.42	13.57	21.40 ^{cd}	1.85 ^d	1.53 ^d	4.20	385.99	24.62
T ₂	FYM+SD+CP NLP@1.5%	15.14	18.28	14.71	22.54 ^d	2.81	1.85	5.64	470.09	27.11
T ₃	FYM+SD+CP NLP@2.5%	17.14 ^c	20.28 ^c	11.14 ^c	20.30 ^{bc}	1.78 ^{cd}	1.46 ^{cd}	3.84	342.47	23.80
T ₄	FYM+SD+CP NLP@3.5%	17.28 ^c	20.42 ^c	10.28 ^{bc}	19.50 ^b	1.67 ^{bc}	1.40 ^{bc}	2.73 ^d	329.64	23.08
T ₅	FYM+SD+CP NLP@4.5%	18.00	21.28	9.57 ^b	16.30	1.60 ^b	1.33 ^b	2.43 ^d	301.76	21.04
	CD (0.05)	0.61	0.55	0.97	1.33	0.18	0.10	0.32	7.29	0.31

FYM=Farmyardmanure, CP=Cocopeat, SD =Sawdust, NLP =Neemleaf powder

DISCUSSION

The probable reason for such a result may be due to the inhibitory effect of neem on the mycelium growth of *Trichoderma harzianum* due to which maximum inhibition per cent at 4.5% concentration of neem leaf extract (Kumar *et al.*, 2023). This may be due to the presence of active biological compounds such as quercetin, sitosterol, polyphenolic flavonoid, and many other important compounds cause the pathogen's cell to rupture, restrict the activity of certain enzymes that are found in proliferating fungi and ultimately cause the fungus to die (Jha *et al.*, 2023). Neem leaf powder at 1.5% concentration promoted faster mycelial run and thus resulted in requiring least days for mycelium run and pinhead initiation and provided the sufficient level of nutrients for *Agaricus bisporus* Sidhu *et al.* (2021). Similar findings have been reported by Singh *et al.* (2017), Kakraliya *et al.* (2022) and Kakraliya and paswal (2024).

CONCLUSION

From present study, it was concluded that the tested botanical it was found that the neem leaf more or less inhibited the growth of *Trichoderma harzianum*, the probable reason might be the presence of anti-microbial properties in neem leaf which inhibited the growth green mould disease caused the present study, it was concluded that the tested botanical it was found that neem leaf more or less inhibited the growth of *Trichoderma harzianum*; the probable reason might be the presence of anti-microbial properties in neem leaf, which inhibited the growth of green mould disease caused by *Trichoderma harzianum*. The casing had a positive effects a positive effect on the yield, size, and quality of button mushrooms. The mixture of T₂ [FYM+Cocopeat+Sawdust + Neem leaf powder@ 1.5%] performed best in terms of yield and growth parameters and biological efficiency of white button mushroom mushrooms.

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REFERENCES

Chang, S. T., Lau, O. W. and Cho, K. Y. (1981). The cultivation and nutritional value of *Pleurotus sajor- caju*. *European Journal of Applied Microbiology and Biotechnology*, 12: 58-62.

Food and Agriculture Organization (FAO), 2023. (www.fao.org)

Jha, K., A., Choudhary, S., J., Shinde, R. and Singh K., A. (2023). Management of green mould infestation in oyster mushroom crop [*Pleurotus ostreatus* (Jacq.) Kumm.] using plant extracts. *Mushroom Research*, 32 (1): 67-73.

Kakraliya, S. S., Gupta, S., Choudhary, S., Diskit, S., Paswal, S., Pandit, D. and Khushboo, S. S. (2022). Evaluation of some botanical extract in controlling dry bubble (*Verticillium fungicola*) disease of button mushroom (*Agaricus bisporus*) under the conditions of subtropics of Jammu. *The Pharma Innovation Journal*, 11(1): 1456-1459.

Kakraliya, S. S. and Paswal, S. (2024). Sustainable management of wet bubble disease (*Mycogone Perniciosa*) in button mushroom (*Agaricus Bisporus*) using

botanicals agents under temperate conditions. *Indian Journal of Ecology*, 51(1): 225-230.

Kaur, A., Johl, P., P. and Sodhi, H., S. (2017). Evaluation of casing variables for cultivation of *Agaricus bisporus* strain U3 in Punjab, India. *Journal of Applied and Natural Science*, 9(1): 305 – 310.

Kapoor, J. N. (2004). Mushroom cultivation. Department of Mycology and Plant Pathology, IARI, New Delhi. PP: 14-6.

Kumar, P., Singh, G., Singh, R., Khilari, K., Singh, D. V. and Prakash, S. (2023). Evaluation of different spices and medicinal plant leaf powder on production potential of milk mushroom (*Calocybe indica*). *The Pharma Innovation Journal*, 12 (9): 128– 133.

Nene, Y. L. and Thapliyal, P. N. (2000). Fungicides in Plant Disease Control. *Oxford and IBH Publishing Company*, PP: 531-533.

Mantel, E. F. K., Agrawal, R. K., and Seth, P. K. (1972). A guide to mushroom cultivation. Ministry of Agriculture, Farm Information Unit, Directorate of Extension, New Delhi, Farm Bull. No.2.

Sidhu, K. G., Joshi, N. and Sharma, S. (2021). Natural bio-agents in casing mixture for yield improvement of *Agaricus bisporus*. *Mushroom Research*, 30(2): 161-168.

Singh, S., Lal, A. A. and Simon, S. (2017). Efficacy of some botanicals in controlling dry bubble (*Verticillium fungicola*) disease in white button mushroom (*Agaricus bisporus*) cultivation. *The Bioscan*, 11(4): 2969-2973.

Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159: 850-850.

Singh, S., Lal, A. A., Singh, A., Yaduman, R. and Murmu, R. (2016). Evaluation of some plant extracts in management of dry bubble (*Verticillium fungicola*) disease of white button mushroom [*Agaricus bisporus* (Lange) Imbach]. *Journal of Applied and Natural Sciences*, 8(3): 1205- 1209.