



Effect of Neem Leaf Powder Supplementation in Casing Mixture on the Growth and Yield Parameters of white Button Mushroom [*Agaricus bisporus* (Lange) Imbach]

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Green mould caused by *Trichoderma harzianum* is a severe threat of white button mushroom [*Agaricus bisporus* (Lange) Imbach] cultivation causing heavy crop losses. The present experiment was conducted to manage 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% concentration showed maximum efficacy against pathogens. Further, an *In vivo* experiment was conducted to evaluate

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neem leaf powder at different concentrations @0.5%, 1.5%, 2.5%, 3.5% and 4.5% supplemented in the casing mixture combination viz., Farm yard manure (FYM) + Cocopeat + Sawdust (1:1:1) 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 (470.09 g) and biological efficiency (27.11%).

Keywords: *Agaricus bisporus*; casing mixture; green mould; neem leaf; *Trichoderma harzianum*.

1. 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 with a total production of 2,80,000 MT of mushrooms accounting for 0.58% of the total world mushroom production [1].

China, Japan, the USA and India are driving worldwide mushroom production. China is the world's biggest grower of edible mushrooms providing 45,428,000 MT, or 93.99 % of worldwide contribution [1].

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 [2].

In addition to their high protein content, mushrooms also 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 [3].

Among these moulds, *Trichoderma harzianum* 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 [3].

2. MATERIALS AND METHODS

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

2.1 Isolation and Preservation of *Trichoderma* sp

White button mushroom bags were naturally infected with typical green 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 ± 1° C for 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 until further use.

2.2 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, considered a standard solution.



Plate 1. Bag infected by green mould

2.3 *In vitro* Evaluation

The botanicals were evaluated *in vitro* through the poison food technique [4]. Sterilized media (20 ml) with botanical extract was poured into 90 mm Petri plates under aseptic conditions in laminar airflow. After solidification of the media, 5mm disc of 7 day old subculture of *Trichoderma harzianum* was placed in the center of the Petri plates and one control plate which had only the PDA medium inoculated with culture disc and used as check. Three replications were maintained for all the treatments. Per cent inhibition of mycelial growth of green mould fungus over control was calculated using the following formula of Vincent [5]:

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

2.4 *In vivo* evaluation Procurement of Spawn

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

2.5 Composting

The basic materials for compost, wheat straw, 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 outlet. The compost was prepared by long composting method [6]. Wetted wheat straw was spread thinly over an entire floor of the composting yard and then gradually wetted 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 per cent. 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 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 for the loss of water due to evaporation. Profenofos insecticide was mixed at 7 turning for prevention of insect pests. A total of eight turnings were done and each turning at four days interval. The compost was then ready for spawning *i.e.* it was dark brown and without any smell of ammonia and had sufficient moisture content (68-70%) when pressed between palms

2.6 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 contained 7 replications 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 7.5 g/kg compost [7] and pressed moderately. The sterilized newspaper was placed on the top of the compost bag to preserve moisture, kept folded on top, and transferred into the dark room for the spawn run.

2.7 Preparation of Casing Mixtures

The selected 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.

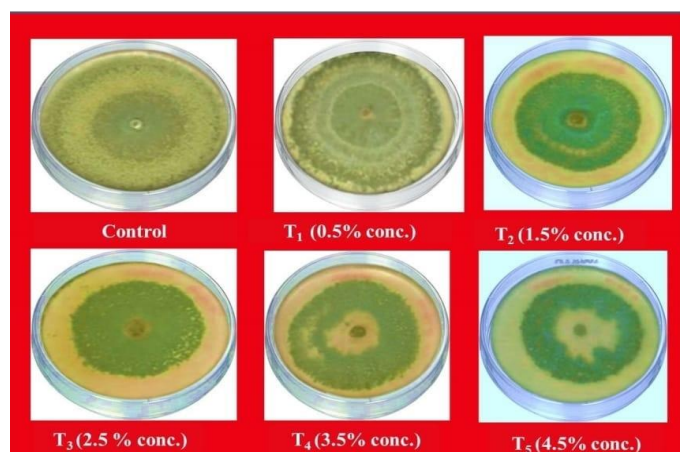


Plate 2. Growth of *Trichoderma harzianum* in Petri plate after 96 hours of inoculation

2.8 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.

2.9 Application of Treatment (Casing Material)

The different casing materials viz., FYM, Cocopeat, and Sawdust were used alone and different casing 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, 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 thereby making the casing layer free from contaminants. Each casing material was applied to seven uniform bags (replicates) containing spawned compost. After 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 case run) [8].

2.10 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 6-8hrs, and relative humidity 90-95% was maintained by spraying 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. Pinheads appeared between 13-16 days after casing, and they became ready for harvesting within the next week.

2.11 Biological Efficiency

The biological efficiency (%) of all the tested casing substrates was calculated further by adopting the following formula [9]:

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

2.12 Statistical Analysis

In this experiment, a Complete Randomized Design (CRD) was followed. The analysis of variance (ANOVA) technique was applied 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.

3. RESULTS

3.1 *In vitro* Effect of Different Concentrations of Neem Leaf Extract on Radial Growth (mm) of *Trichoderma harzianum*

Neem leaf extracts significantly inhibited the mycelial growth of *Trichoderma harzianum* at all the tested concentrations. The maximum mycelial inhibition percentage and least mycelial

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
	CV (%)		1.88	
	CD (0.05)		1.96	

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 (g)	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 = Farm yard manure, CP = Cocopeat, SD = Sawdust, NLP = Neem leaf powder

growth of *T. harzianum* (64.59%, 31.85 mm) were observed in T₅ (neem leaf extract@ 4.5%) followed by (57.22%, 37.80 mm) in T₄ (Neem leaf extract@3.5%) (Table 1).

3.2 *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), 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 (470.09g) with (27.11%) biological efficiency was recorded in T₂ [FYM + Sawdust + Cocopeat (1:1:1) + Neem leaf powder @ 1.5%].

4. 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 [3]. 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

fungi to die [10] 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. [11]. Similar findings have been reported by Singh et al. [12], Kakraliya et al. [13] and Kakraliya and Paswal [14].

5. CONCLUSION

From 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 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 mushrooms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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