Original Research Article

Effect of neem leaf powder supplementation in casing mixture on the growth and yieldparametersof whitebuttonmushroom[*Agaricusbisporus*(Lange)Imbach]

ABSTRACT

Greenmouldcausedby*Trichodermaharzianum*isaseriousthreatinwhitebuttonmushroom [*Agaricus bisporus*(Lange) Imbach] cultivation causing heavy crop losses. Thepresent experiment was conducted to manage green mold fungus *Trichoderma harzianum*usingneemleaf extracts.*Invitro*resultsshowedmaximummycelialgrowthinhibitionof 64.59 % in *T. harzianum*using neem leaf extract at 4.5% concentration. Neem leaf extracts at4.5%concentrationshowedmaximumefficacyagainstpathogen.Further,*invivo*experiment was conducted to evaluate neem leaf powder at different concentration rangingfrom@0.5% to @4.5% supplemented in the casing mixture combination *viz.*, Farm yardmanure (FYM) + Cocopeat + Sawdust (1:1:1) with an objective to monitor the growthand yield aspectsof white button mushroom. Among all treatments used in the study, theresults revealed that minimum average time taken for completion of spawn run (15.14 days)and pinhead initiation (18.28 days) as well asmaximum number of fruiting bodies (14.71),fruiting bodies weight (22.54g), stalk length (2.81 cm) stalk diameter(1.85 cm) andpileusdiameter(5.64 cm),andwere recorded in T_2 [FYM + Cocopeat+ Sawdust (1:1:1) + neemleaf powder @ 1.5%]. Results have shown that T_2 [FYM + Cocopeat + Sawdust (1:1:1) +neemleafpowder@1.5%]hadthehighestyield(470.09g)andbiologicalefficiency(27.11 %).

Key words: *Agaricus bisporus*, Casing mixture, Green mold, Neemleaf,*Trichodermaharzianum.*

INTRODUCTION

Mushrooms are a prominent fruiting body of a macrofungus that belongs to the phylumBasidiomycota and possesses the ability to produce spores and can be found as epigeous orhypogeous. White button mushroom [*Agaricus bisporus*(Lange) Imbach] is the most popularcultivatedediblemushroom,fetchinghighpriceandstilldominatingtheIndianandInternatio nal market. Mushrooms are one such component that not only impart diversificationbutalsohelpinaddressingtheproblemsofhealth,environmentqualityandfoodrelate d

issues. Its first cultivation was started in China. Though mushroom production is of recent originin India, it ranked 4th in the world mushroom production with a total production of 2,80,000 MT ofmushroomsaccountingfor0.58%ofthetotal world mushroomproduction **(FAO,2022).**

China, Japan, USA and India are driving in worldwide mushroom production. China is theworld's biggest growerof eatable mushrooms providing 45,428,000MT, or 93.99 % ofworldwidecontribution **(FAO,2022).**

*Agaricusbisporus*requirestwodifferentsubstratestoformthefruitingbodies*i.e*.thecompost for nutrition on which it grows vegetatively and the casing soil in which the suitablephysicochemical/biological conditions stimulate the initiation process of pin head formationforfruit bodyproduction(**Kaur*et al*., 2017**).

In addition to having a high protein content, mushrooms also have high levels of severalvitamins, including vitamins B, C, and D, riboflavin, thiamine, and five nicotinic acids. Inaddition to folic acid, the 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**(Kumar*et al*., 2023)**.

Among these moulds, *Trichoderma harzianum*induce significant quantitative and qualitativelosses 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).**

MATERIALSANDMETHODS

The present experiment was conducted during the *Rabi* season 2023 at Mushroom crop room,Department of Plant Pathology, SHUATS, Prayagraj, Uttar Pradesh during the seasonOctober2023 – March 2024.

Isolationandpreservationof*Trichoderma*sp

Whitebuttonmushroombagswerenaturallyinfectedwithtypicalgreenmoldusedtoisolates the causal organism. The isolated fungi were identified using microscopy. Casingmaterial from infected bag was taken from sterile forceps. Serially diluted using with steriledistilledwater,inoculatedonthePotatoDextroseAgarplatesandincubatedat25±1°Cfor

96 hours. Fungal culture was purified by single spore isolation and periodic sub-culture. Allthepurecultureswerekeptin refrigerator at 4°C fortill furtheruse.

Preparationof neemleafextract:

For preparation of neem leaf extract, 100 g fresh neem leaf was collected, washed in distilledwater, air dried and homogenized with equal amount of distilled water (100 ml) by crushingthem with electric grinder machine. The extract was filtered through double-layered musdincloth and centrifuged at 4000 rpm, for 10 minutes. The supernatant was collected and filteredthroughWhatman No.1filterpaperwhich wasconsidered asstandard solution.

Plate 1: Bag infected by green mould

***Invitro*evaluation:**

The botanicals were evaluated *in vitro* through poison food technique (**Nene and Thapliyal,2000**). Sterilized media (20ml) with botanical extract was poured into 90 mm Petri platesunder aseptic conditions in laminar air flow. After solidification of media 5mm disc of 7 daysoldsubcultureof*Trichodermaharzianum*wereplacedinthecentreofthepetriplatesandone control plate which has only the PDA medium inoculated with culture disc and used ascheck.Threereplicationsweremaintainedforallthetreatments.Per-centinhibitionofmycelialgrowthofgreenmoldfungusovercontrolwascalculatedusingthefollowingformulaof**Vincent(1947):**

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

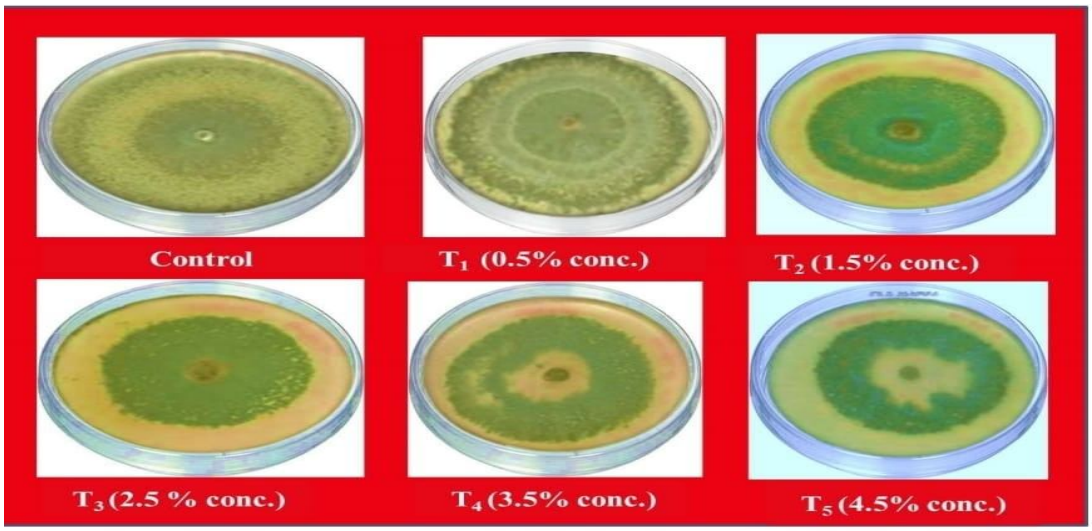

Plate 2: Growth of *Trichoderma harzianum* in Petri plate after 96 hours of inoculation

In vivo

evaluationProcurementofSpaw

n:

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

Composting:

The basic materials for compost, wheat straw was taken from Agro farm. Other ingredientslike wheat bran, urea, potassium (Murate of Potash), phosphorus (Single Super Phosphate),gypsumand lindane were obtained from commercial out let. The compost was prepared bylong method of composting (**Mantel *et al*., 1972**). Wetted wheat straw had spread thinly overentire floor of the composting yard and then gradually wetted by sprinkling water, till thestraw was taken no more water. The straw was then turned for even wetting at the stage andwatercontentwasmaintainedat75percent.The moiststrawwasmixedwithwheatbranandfertilizer uniformly scattered over the straw. A heap was made after each turning but notcompressed tightly so as to maintain the aerobic condition in the compost heap. Gypsum wasmixed at the third turning and at each turning

water was sprayed to make up the loss of waterdue to evaporation. Profenofos insecticide was mixed at 7 turning for prevention of insectspests. Total eight turning was done and each turning at four days interval. The compost wasthen ready for spawning *i.e.* it was dark brown in colour and without any smell of ammoniaandhad sufficientmoisturecontent(68-70%)whenpressedbetweenpalms.

Spawning:

A unit of 5 kg mildly wet compost was used for each bag in perforated polypropylene bag,which was equally distributed in 6 treatments including control which contain 7 replicationseach. The moisture content of the compost at the time of spawning was kept around 25-30%.The spawn thoroughly mixed with the compostat the rate of 7.5 g/kg compost**(Kapoor,2004)**andpressedmoderately.Thesterilizednewspaperwasplacedonthetopofc ompostbag to preserve moisture and kept folded on top and transferred in to the dark room forspawnrun.

Preparationofcasingmixtures:

The selected basic material for preparation of casing soil such as Farm yard manure wasobtained from Agro-farm, SHUATSand other casing materials, cocopeatand sawdustwereobtained from commercial outlet Prayagraj.

Preparation of botanicals(Neemleaf powder):

Fresh neem leaves were collected, welldried in sunlight and then made into fine powderwith the help of mortar pestle and mixer. The dried powder of the selected botanical wasmixedwiththecasingmixturesindifferentconcentrations(0.5%,1.5%,2.5%,3.5%and 4.5%)for5 treatmentsandtheuntreatedcheckbagswerekeptascontrol.

Applicationoftreatment(casing material):

The different casing material viz., FYM, Cocopeat, Sawdustwas used alone anddifferentcasingmixture with neem leaf powder. [FYM + Cocopeat + Sawdust (1:1:1) + neem leafpowder ranging from@0.5-4.5%].First cocopeatwassoakedinwaterfor2hours.Initiallyindividual casing materials, FYM, Sawdust, Cocopeat sterilization was done by using 2% formalinsolution. Before applying the casing layer to the compost, it was kept under a polythene filmso that evaporation occurs and thereby making the casing layer free from contaminants. Eachof the casing materials was applied to seven uniform bags (replicates) containing spawnedcompost. After casing was done the temperature of the room was again maintained at 23±2℃andrelativehumidityof 85-90%foranother8-10days(tillcaserun) (**Singh*etal.* 2016**).

FruitingandHarvesting

The mycelium emerged on casing soil after 10 days, the environmental conditions werechanged in cropping room by providing fresh air through ventilation and light for 6-8hrs,relativehumidity90-95%weremaintainedbysprayingofthewaterthriceaday.Thetemperature of cropping room was maintained at 16±2˚C. Low CO_2 concentration (0.08-0.15%) is favorable for reproductive growth at this stage. Pin heads were appeared between13-16daysafter casingand theybecamereadyfor harvestingwithin nextoneweek.

Biologicalefficiency:

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

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

Statisticalanalysis:

In this experiment Complete Randomized Design (CRD) was followed. The analysis ofvariance (ANOVA) technique was applied for drawing conclusion from data. The calculatedvalues were compared, the tabulated values at 5% level of probability for the appropriatedegreeof freedom.

RESULTSANDDISCUSSION

Invitro*effectofdifferentconcentrationsofneemleafextractonradialgrowth(mm)of *Trichodermaharzianum

Neemleafextractmoreorlesssignificantlyinhibitedmycelialgrowthof*Trichodermaharzianum*at all the tested concentrations. The maximum mycelial inhibition percentage andleast mycelial growth of *T. harzianum*(64.59%, 31.85mm) were observed in T_5 (neem leafextract@4.5%)followed by(57.22%,37.80 mm)inT_4 (Neem leaf extract@3.5%)(Table1).

In vivo* effect of neem leaf powder supplementation in casing mixture on the growth andyieldparametersof*Agaricusbisporus

It is evident from (Table 2) the minimum days taken for mycelium run and pin head initiationin casing layer were observed in T_2 [FYM + Sawdust + Cocopeat (1:1:1) + Neem leafpowder@ 1.5%(15.14, 18.28)]. It was further observed that the maximum stalk length(2.81cm), stalk diameter (1.85 cm), pileus diameter (5.64 cm) and maximum number offruiting bodies (14.71), weight of fruiting bodies (22.54 g) were recorded in T_2 [FYM +Sawdust+Cocopeat (1:1:1) +Neem leafpowder@ 1.5%].

Significant difference was recorded in the effect of neem leaf powder supplemented in thecasing mixture on the yield of *Agaricus bisporus*(Table 2). The maximum yield (470.09g)with(27.11%)biologicalefficiencywasrecordedinT_2[FYM+Sawdust+Cocopeat(1:1:1)
+Neem leaf powder @1.5%].

Table1:*Invitro*effectofdifferentconcentrationsofneemleafextractonradialgrowth(mm)of*Trichoderma harzianum*

Treatment number	Treatmentname	Concentration(%)	Mean colonydiameter (mm)	Percentinhibition(%)
T_0	Control		90.00	00.00
T_1	Neemleafextract	0.5%	85.11	5.96
T_2	Neemleafextract	1.5%	62.04	30.96
T_3	Neemleafextract	2.5%	45.51	49.41
T_4	Neemleafextract	3.5%	37.80	57.22
T_5	Neemleafextract	4.5%	31.85	64.59
	C.V.		1.88	
	CD(0.05)		1.96	

Table 2: Effect of neem leaf powder supplementation in casing mixture on the growthandyieldparametersof whitebuttonmushroom[*Agaricusbisporus*(Lange)Imbach

Tr no.	Treatments	Case run(days)	Pinheadinitiation (days)	Number offruiting bodies	Weight offruiting bodies	Stalk length (cm)	Stalkdiameter(cm)	Pileusdiameter(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 =Neemleafpowder

DISCUSSION

The probable reason for such result may be due to the inhibitory effect of neem on themycelium growth of *Trichoderma harzianum*due to which maximum inhibition per cent at4.5%concentrationofneemleafextract(**Kumar*etal.*,2023)**.Thismaybeduetopresenceof activebiologicalcompoundssuch asquercetin, sitosterol,polyphenolic flavonoidandmany other important compoundscause the pathogen's cell to rupture, restrict the activity ofcertainenzymesthatarefoundinproliferatingfungiandultimatelycausethefungitodie**(Jha *et al.*, 2023**) Neem leaf powder at 1.5% concentration promoted faster mycelial run andthusresultedinrequiringleastdaysformyceliumrunandpinheadinitiationandprovidedthe sufficient level of nutrients for *Agaricus bisporus***Sidhu *et al.* (2021).** Similar findingshave 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 leafmore or less inhibited the growth of *Trichoderma harzianum*, the probable reason might bethe presence ofanti-microbial properties in neem leafwhich inhibitedthegrowthgreenmould disease cause by *Trichoderma harzianum.* The casing had a positive effects on theyield,size,andqualityof buttonmushrooms. The mixtureofT_2 [FYM+Cocopeat+Sawdust
+ Neem leaf powder@ 1.5%] performed best in terms of yield and growth parameters andbiologicalefficiencyofwhitebutton mushroom.

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