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Research Paper

Screening of *Agaricus bisporus* strains against green mould (*Trichoderma harzianum*) disease of white button mushroom

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ABSTRACT

Green mould (*Trichoderma harzianum*) is a fungal pathogen which poses a significant threat to mushroom cultivation. Seven *Agaricus bisporus* strains *viz*., RRL-89, S-737, U-3, S-11, S-176, NCS-102 and RRL-80 were evaluated against green mould disease. RRL-80 strain recorded comparatively lesser disease intensity (56.67%) and the strain U3-6 the maximum intensity (71.95%). The yield of mushroom per quintal compost was highly influenced by the disease in all the test strains. The yield was maximum (5.47 kg q^{-1} compost) in strain RRL-80 compared to all other test strains which yielded 2.12-4.85 kg q^{-1} compost. The highest numbers of fruit bodies per kg mushroom were present in RRL-80 (79.67/kg mushroom) and largest weight of single fruit body was present in S-11(21.83g).

Key words: *Agaricus bisporus*, Green mould, Screening, Strains, *Trichoderma harzianum*

Mushroom cultivation is a commercially relevant microbiological method for recycling agro-waste. Regulated mushroom cultivation relieves demand on arable land. Mushrooms are saprobic fungi that grow on decaying organic materials. The scientists and the public have long been interested in macrofungi due to their beneficial and detrimental impacts on plants, use in the food and medicinal sectors, and role in bio-degradation (Stojchev *et al*., 1998). *Agaricus bisporus* (Lange.). Imbach, a commercially cultivated fungus also called as white button mushroom or European mushroom is grown all over the world. It is produced in the US, Canada, China, Western Europe, Indonesia, Korea, Taiwan and India on large scale. In India, the annual production of mushrooms is estimated to be around 1,20,000 metric tonnes with 85 per cent of this production being of button mushrooms (Annonymous, 2010). Jammu and Kashmir's annual mushroom production is 1478 metric tonnes, with the valley contributing 513 metric tonnes (Annonymous, 2021). Mushroom and truffle total output have been on the rise in recent years. In the previous 20 years, annual output volume has increased by an average of 5.5 percent, nearly doubling to close to 11.9 million MT in 2019 (FAO Stat, 2021). *Agaricus bisporus* is rich in protein, minerals, vitamin B group, vitamins D and K, and sometimes vitamins A and C. This fungus possesses hypocholesterolemic, hypoglycemic, hypotensive, anticancer (carcinostatic), hypolipidemic, immune modulatory, haematoprotective properties, blood sugar and blood pressure reduction properties, antiviral, antibacterial and antifungal activities. However, numerous factors, such as the ratio of carbon and nitrogen content, temperature, luminosity, water activity, air composition, humidity, pH as well as the casing material and quality of growing substrate/compost play key roles in mushroom cultivation, having substantial influence on the formation and development of fruiting bodies (Bellettini *et al*.,2019; Allaga *et al*., 2021). Besides these factors, mushroom diseases constitute major constraint to successful mushroom production in Union Territory of Jammu and Kashmir.

The crop becomes infested with a wide variety of bacterial, fungal, and viral infections, which leads incomplete to complete destruction of the crop and reduction in the quality of the food. Diseases that are caused by fungi, poses a significant risk to the mushroom industry across the country. Crop losses of varied degrees

have been reported to be caused by major fungal diseases such as wet bubble, cob web dry bubble, green mould, and competitive moulds such as false truffle, brown plaster mould, olive green mould, yellow moulds, and ink caps, amongst others (Forer *et al*., 1974; Singh *et al*., 2010). Of these fungal diseases and competitor moulds, the green mould disease caused by *Trichoderma harzianum* has been reported to cause extensive damage, resulting in losses of approximately 63-65 per cent in cultivated mushrooms. (Bhatt and Singh, 2000). Although, defensive response of mushrooms to the attack of some fungal parasites has been studied, such as the production of laccase to fight green mold pathogen (Sjaarda *et al*., 2015), the immune response of mushrooms to mycoparasite action still remains broadly unresolved (Largeteau and Savoie 2010), which represents a shortcut to design resistant strains through breeding programs (Sonnenberg *et al*., 2017). White button mushroom, Strain No. 703, having cream coloured fruiting body has been reported less sensitive to *T. harzianum* compared to other strains (You *et al.,* 1978). Although several aspects of this disease have been studied elsewhere in the world, yet meager information is available, particularly in Kashmir valley, about the susceptibility/tolerance level of available strains against the green mould disease. An attempt was therefore made to evaluate some white button mushroom strains, which included some new strains collected from Directorate of Mushroom Research, Solan, against green mould disease.

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MATERIALS AND METHODS

Pathogenicity test: The pathogenicity of the isolated fungus was established by proving Koch's postulates on white button mushroom grown in 5 kg compost capacity plastic baskets. The baskets were cleaned with 2% formalin and then washed with distilled water three times. The pasteurized compost-filled baskets were spawned and held at 24±2ºC. Conidial suspension (1×10^4 spores ml^{-1}) was sprayed on sterilized casing mixture during casing. After inoculation, the baskets were kept at 21±1ºC temperature, more than 85% relative humidity and stored in the isolated room. Similar conditions were maintained for un-inoculated baskets to serve as checks and to avoid contamination, were kept separate. The fungus was re-isolated from the compost and the resulting cultures were compared to the initially inoculated one to confirm the koch's postulates.

Screening of various White button mushroom strains against green mould (*Trichoderma harzianum*) disease: Seven strains of white button mushroom (*Agaricus bisporus*) *viz*., RRL-89, S-737, U-3, S-11, S-176, NCS-102 and RRL-80, obtained from Mushroom Research and Training Centre, SKUAST-K and Directorate of Mushroom Research, Solan were tested for their susceptibility/tolerance level to the green mould disease caused by *Trichoderma harzianum*. The spawn from these strains was produced on wheat grains as per the protocol, adopted by Munjal (1973). Long method of composting (Mantel *et al.*, 1972) was adopted and the ingredients used were as per SKI-4 formula (wheat straw 300 kg; chicken manure 150 kg; rice bran, 50 kg; molasses 12 ½ kg; linseed meal 12.5 kg; urea 5 kg; potash 2 kg and gypsum 15 kg). The final ready compost was weighed and then placed in polythene bags at a rate of 10 Kgs per bag. Layers were created at a rate of 0.6 per cent spawning per layer. After the spawning process was finished, a layer of 3-4 cm casing was placed down on each bag. The mixture of sick soil and peat in a ratio of 2:1 was used to make the casing. The sick soil was made by adding 10 ml of a spore suspension of the pathogen (1×10^4 spores ml^{-1}) to 1 kg sterilized soil. Three replications were kept for each treatment and each replication consisted of 10 bags with each bag of 10 Kg compost capacity. After the casing process, the bags were stored in an incubation room at 23±2ºc temperature and between 75 to 80 percent relative humidity. After the sixth day of incubation, the temperature was kept between 10 to 20º c, and the relative humidity maintained at 75 per cent throughout the experiment. The data on the per cent disease intensity, per cent loss in fruit body weight due to the disease, mushroom yield per quintal of compost and other quality parameters *viz*., No. of fruit bodies Kg^{-1} mushroom, weight of single fruit body, diameter of pileus (cm), weight of stipe (g) and diameter of stipe (cm) were recorded.

For working out the disease intensity, following 0-5 scale was devised which was based on the visual observations of approximate surface are covered by the mould.

Category	Compost area infected (%)
0	0
1	1-5
2	6-10
3	11-25
4	26-50
5	More than 50

Per cent disease intensity was calculated by using the following formula:

$$\text{Disease intensity (\%)} = \frac{\Sigma \text{ disease score}}{\text{Total No. of observations} \times \text{Maximum grade value}} \times 100$$

Per cent Yield loss was calculated by using the formula:

$$\text{Per cent yield loss} = \frac{\text{Expected yield-yield obtained}}{\text{Expected yield}} \times 100$$

Expected yield was taken as 10kg per quintal compost

Statistical analysis: The data collected were subjected to statistical analysis wherever needed. The difference exhibited by the treatments in various experiments were tested for their significance as per the methods suggested by Gomez and Gomez (1984). The 'Minitab' computer software was used for data analysis.

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RESULTS AND DISCUSSION

Pathogenicity: The pathogenicity test of the isolated fungus, *Trichoderma harzianum*, indicated the pathogenic behavior of the fungus as it developed typical symptoms of green mould disease (Fig. 1). The symptoms started appearing as white to grayish mycelium on casing surface after 3 days of inoculation which latter turned to dark green color due to heavy sporulation of causal agent. The mushroom fruit bodies in inoculated bags produced enlarged stipes and caps with discoloured tissue. The fungus obtained on re-isolation from the infected bags, was similar to the initially inoculated one and thus confirmed Koch's postulates.



Inoculated Uninoculated (Control)

Fig. 1: Pathogenicity test of green mould (*Trichoderma harzianum*)

Screening of *A. bisporus* strains: All the seven tested button mushroom strains, screened against green mould disease (*T. harzianum*) were found susceptible (Table 1). Disease intensity ranged from 56.67 to 71.95 percent among the strains, with lowest in RRL-80 and highest in U-3. The date presented in Table 2 depicts that the disease affected mushroom output per quintal compost of all the tested strains. The highest production (5.47 kg q-1 compost) was from RRL-80 as against 2.12-4.85 kg q-1 compost in other tested strains. Yield loss due to green mould ranged from 66.55 to 79.85 per cent with least yield loss of 66.55 per cent in RRL-89 and highest of 79.85 per cent in U-3 strain. The highest number of fruit bodies per kg mushroom was present in RRL-80 (79.67/kg mushroom) followed by U-3 (77.15/kg mushroom) and minimum in NCS-102 (68.74/kg mushroom). Largest weight of single fruit body was present in S-11 (21.83g) followed by RRL-89 (20.67g). The highest weight (11.83g) and diameter (6.93cm) of pileus was present in S-11. The highest weight (6.55g) and diameter (1.38cm) of stipe was present in RRL-80.

Table 1:Evaluation of different strains of *Agaricus bisporus* against green mould (*Trichoderma harzianum*)

*A. bisporus*Strain	Green mouldintensity (%)	Button yield (kg q^{-1} compost)	Yield loss (%)
RRL-89	66.65	3.07[d]	69.33
S-737	68.55	2.98[d]	70.20
U-3	71.95	2.12[e]	78.80
S-11	58.35	4.85[b]	51.50
S-176	61.85	3.85[c]	61.50
NCS-102	62.25	3.15[d]	68.50
RRL-80	56.67	5.47[a]	45.30
CD (p≤0.05)	**(0.058)**	**(0.034)**	(0.041)

Means followed by similar letter(s) are statistically identical

In the present study, yield loss ranged from 45.30 to 78.80 across all the tested strains. Although susceptible, RRL-80 with least disease intensity and highest button yield proved to be the best strain among all the tested strains. Sjaarda *et al*. (2015) found three strains *viz*., RRL-80, S-11 and S-176 that exhibited highest yield and less crop damage due to some evidence of a defensive response to the attack of fungal parasites, such as the noted increased production of laccase to fight green mold. Resistance associated with laccase activity as a defense response of *A. bisporus* to *T. aggressivum* toxins has been also described, particularly associated with *A. bisporus lcc2* gene (Sjaarda *et al*., 2015). Similar result with *Agaricus bisporus* strains have also been reported by Abubaker *et al. (*2013) who reported *in vitro* increased laccase production in U1 and SB65 strains of *A. bisporus* to confront toxic metabolites produced by *T. aggressivum*. Our results indicated clear differences in yield losses between the tested *A. bisporus* strains due to green mould disease.

Table 2:Quality parameters of different strains of button mushroom (*Agaricus bisporus*)

A. bisporus strain	Fruit bodies (No./kg mushroom) *	Weight of single fruit body (g) *	Weight of pileus(g) *	Diameter of pileus (cm) *	Weight of stipe (g)*	Diameter of stipe (cm) *
RRL-89	69.37	20.67	10.66	6.25	6.06	1.28
S-737	69.25	19.55	10.15	6.05	6.45	1.34
U-3	77.15	20.33	10.73	6.74	6.25	1.31
S-11	76.67	21.83	11.83	6.93	6.35	1.33
S-176	75.47	19.87	10.25	6.25	5.95	1.32
NCS-102	68.74	19.33	10.15	5.76	5.25	1.25
RRL-80	79.67	18.55	10.67	6.67	6.55	1.38
CD(p≤0.05)	**(0.041)**	**(0.035)**	**(0.035)**	**(0.035)**	**(0.035)**	**(0.035)**

*Means of three replication

CONCLUSION

The current findings on screening *Agaricus bisporus* strains against *Trichoderma harzianum* showed that RRL-80 exhibited comparatively the least disease intensity and highest button production of all the tested strains. It also had higher quality metrics such as pileus weight, pileus diameter, stipe weight and stipe diameter. Further studies regarding the use of this strain in future breeding programs for stable resistance and genetic studies of *A. bisporus* strains are required. As the resistance to the green mould disease is rarely found in mushroom strains,

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we suggest that RRL-80 can be utilized in future breeding programmes as the strain although found susceptible produced comparatively better yield and was less affected by the disease.

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