

## Natural bio-agents in casing mixture for yield improvement of *Agaricus bisporus*

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### ABSTRACT

*Agaricus bisporus* (Imbach) commonly known as white button mushroom, is the most widely accepted food globally with nutritional and medicinal properties. In the present study, different natural bioagents viz., neem (*Azadiracta indica*), castor (*Ricinus communis*), natural vinegar, *Bacillus thuringiensis* commercial formulation Dipel-8L and *Beauveria bassiana* commercial formulation Biojaal-1.15% SC were evaluated *in vitro* to record their effect on mycelial growth of *A. bisporus*. Maximum radial (57.1mm) and linear growth (31.1mm) were recorded in neem @0.5% on the potato dextrose agar medium and in compost-filled tubes, respectively. Further, field studies were conducted to evaluate different concentrations ranging from @0.5% to @4.5% of these natural bioagents on the quality and yield of *A. bisporus*. The cultivation trials of *A. bisporus* recorded a maximum number of fruit bodies (1606) and maximum yield (19.91 kg/100kg of compost) in neem powder treatment @1.5%. The maximum percent increase in yield over control (41.91%) was also recorded in neem powder treatment @1.5%. These results indicated that incorporating neem powder @1.5% in casing mixture improves the yield of *A. bisporus* and is preferred over synthetic chemicals as it is ecofriendly and reduces environmental pollution.

**Keywords:** *A. bisporus*, neem powder, castor powder, potato dextrose agar medium

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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. These are wonderful sources of proteins, vitamins, minerals and low in calories and cholesterol (Sharma *et al.*, 2017). Cultivation of mushrooms is well established and useful alternative to decrease hunger and malnutrition in the world of increasing prices (Lakshmipathy *et al.*, 2017). Mushrooms are now becoming an innate part of innumerable cultures' cooking (Khan *et al.*, 2017). In Punjab, commonly cultivated varieties of mushroom are white button mushroom (*A. bisporus*), oyster mushroom (*Pleurotus* spp.), shiitake mushroom (*Lentinus*

*edodes*), paddy straw mushroom (*Volvariella* spp.) and milky mushroom (*Calocybe indica*). Among these, the white button mushroom is favourable across the world and is of economic importance (Maheshwari, 2013). *A. bisporus* occurs in class Basidiomycetes and order Agaricales (Khan *et al.*, 2017). Composting and casing are considered the essential steps for the cultivation of *A. bisporus*. The most important step for the cultivation of white button mushroom is the casing layer (Murmu and Lal, 2016). The compost is covered by a casing layer that helps in holding moisture, gas exchange and maintaining a microbial population that releases hormones like substances required to initiate fruiting bodies. The yield and quality of mushrooms are dependent on casing mixtures

mainly affected by a wide variety of biotic and abiotic factors. The major biotic factors, *viz.*, nematodes, mites, insects, pests, and flies, lead to a decrease in mushrooms' yield and quality. Various chemicals are used to control the biotic factors, which leads to a decrease in the yield of button mushroom also. The utilization of these chemicals creates many secondary effects in resistance to pesticides, environmental pollution, and a decrease of non-target organisms, including beneficial microbes. The ingestion of formaldehyde may lead to nausea, vomiting, diarrhea with bloody stool, renal failure, liver failure and tumor development and irreversible neurotoxicity (Nowshad *et al.*, 2018). Moreover, these days people are more concerned about organic food. Therefore, the present study was planned to evaluate natural bioagents *viz.*, neem powder, castor powder, vinegar, *B. thuringiensis* and *B. bassiana* formulations during casing to study their effect on mycelial growth and yield potential of *A. bisporus* U3 strain.

## MATERIALS AND METHODS

The local strain of *A. bisporus* (U3) was procured from Germ Plasm Collection Bank of Dr. H.S. Garcha Mushroom Laboratories, Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab. The selected strain of *A. bisporus* was maintained on potato dextrose agar medium with potato 250g/L, dextrose 20g/L and agar 20g/L at 25°C for 15 days and stored at 4°C till further use. The natural bioagents used were: **1.** The botanicals *viz.*, neem leaves (*Azadiracta indica*) and castor leaves (*Ricinus communis*) procured from the Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana. **2.** Apple vinegar collected from Department of Microbiology, Punjab Agricultural University, Ludhiana. **3.** The *B. thuringiensis var. kurstaki* commercial formulation Dipel-8L manufactured by Sumitomo Chemical Pvt. Ltd., India & *B. bassiana* commercial formulation Biojaal-1.15% SC manufactured by Pest control. Pvt. Ltd., Mumbai, India, was used in the present study.

### Preparation of leaf extracts

To prepare a 20% neem leaf extract and castor leaf extract, the leaves of neem and castor were washed, dried and crushed separately for preparation of fine powder. Twenty gram of homogenized fine neem leaf powder and castor leaf powder were separately diluted with 100ml of distilled water and was kept overnight. The next day the extract was filtered through muslin cloth and volume was made up to 100ml to make a stock solution. Further, it was diluted to 0.5% concentration with distilled water for further use *in vitro* studies (Jatav *et al.*, 2014).

### *In vitro* study of natural bioagents on the growth of *A. bisporus* (U3 strain)

Different natural bioagents, *i.e.*, neem leaf powder, castor leaf powder, apple vinegar, *B. thuringiensis var. kurstaki* and *B. bassiana* @0.5%, were studied for their effect on the mycelial growth of *A. bisporus* U3 strain on Potato dextrose agar medium. Two ml of prepared stock solution of neem leaf extract and castor leaf extract was added separately in Petri plates to which potato dextrose agar was poured. Apple vinegar, *B. thuringiensis var. kurstaki* and *B. bassiana* were also supplemented @0.5% concentration in Potato dextrose agar plates. There were six treatments with three replications for each treatment. The Potato dextrose agar plates without supplementation of bioagents were used as control. These Petri plates were inoculated with a circular mycelial disc of 5 mm diameter cut from the master plate of *A. bisporus* U3 strain with the help of a cork borer and placed inverted on all solidified Potato dextrose agar plates so that mycelial layer directly touched the agar surface. These inoculated Petri plates were incubated at 25°C for one month and recorded the radial growth at a weekly interval up to one month.

### ***In vitro* study of linear growth of *A. bisporus* in compost-filled race tubes**

Compost was prepared by using supplements like wheat straw, poultry manure, wheat bran, urea and gypsum as per the short method of composting (Khanna *et al.*, 2007). The race tubes were filled 3/4<sup>th</sup> with prepared compost and autoclaved at 121°C at 15-20 psi for 30 minutes. Afterward, natural bioagents, *i.e.*, neem leaf powder, castor leaf powder, apple vinegar, *B. thuringiensis* var. *kurstaki* and *B. bassiana*, were supplemented @0.5% concentration in separate compost tubes in triplicates to study the linear growth of *A. bisporus* U3 strain. Under aseptic conditions, the *A. bisporus* bit was placed onto autoclaved compost and incubated at 25°C for one month and recorded linear mycelial growth at weekly intervals till one month.

### **Mushroom yield experiment**

The cultivation of button mushroom and treatments in casing mixture was carried out during winter season 2019-2020 at Mushroom Research Complex, Punjab Agricultural University, Ludhiana, India. The cultivation experiment included spawn preparation, compost preparation, casing mixture and casing mixture with different concentrations of natural bioagents, crop management, and harvesting.

According to standard methodology, wheat grain spawn was prepared to cultivate button mushroom (Garcha, 1997). According to this methodology, wheat grains were boiled in water for two hours, followed by mixing 2% CaCO<sub>3</sub> and 4% CaSO<sub>4</sub>. After mixing these components, wheat grains were filled in glucose bottles (500ml capacity) and autoclaved at 20 psi for 1.5 hrs. After cooling, these bottles were inoculated with a master culture of *A. bisporus* U3 strain by placing mycelial bit along with agar on the surface of grains in bottles in such a way that mycelial surface was in close with grains. These bottles were incubated at 25°C till the mycelia completely covered the grains. Compost for the cultivation of *A. bisporus* U3 strain was prepared according to the short composting

method (Khanna *et al.*, 2007). The bags were filled with compost and a top layer of bags was levelled. The bags were kept in the growing room for complete mycelial growth in compost. The compost bags impregnated with mycelial growth were cased for pinhead formation. A standard casing mixture was prepared by mixing farmyard manure and sandy soil (4:1) (v/v). For the experiment the casing mixture was supplemented with natural bioagents *viz.*, neem leaf powder (w/w), castor leaf powder (w/w), apple vinegar (v/w), *B. thuringiensis* commercial formulation Dipel-8L (v/w) and *B. bassiana* commercial formulation Biojaal-1.15% SC (v/w) at different concentrations *viz.*, 0.5%, 1.5%, 2.5%, 3.5%, 4.5%. In control, the casing mixture was supplemented with 4% formaldehyde for 100 kg casing. There were three bags per replicate. Bags were kept at room temperature (25°C) and relative humidity 80 to 90% with proper cross ventilation up to harvesting. Comparison between natural bioagents treated bags and control bags were made to determine the yield of *A. bisporus*. For this comparison, yield data were recorded for days of spawn run, case run, first harvesting, last harvesting, along with weight and number of fruiting.

### **Statistical analysis**

All the experiments were analyzed statistically by analysis of variance (ANOVA) and the Critical Difference (CD) was calculated at 5% level of probability for comparison between treatments and control.

## **RESULTS AND DISCUSSION**

### **Radial growth**

The radial growth of *A. bisporus* U3 strain on Potato dextrose agar medium supplemented with natural bioagents (neem powder, castor powder, vinegar, *B. thuringiensis* and *B. bassiana*) @0.5% was recorded at weekly interval. First reading was recorded on seventh day after inoculation and incubation. On 7<sup>th</sup> day, maximum radial growth

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(11.2mm±0.17) was recorded in neem supplemented Potato dextrose agar plates followed by vinegar (10.2mm±0.25) which was at par with control (10.0mm ±0.21). On 21<sup>st</sup> day, Potato dextrose agar supplemented with neem powder recorded maximum radial growth (49.1mm±0.29) followed by vinegar supplemented with Potato dextrose agar medium (46.2mm±0.22), while the growth in Potato dextrose agar supplemented castor powder (38.1mm±0.19) and *B. bassiana* (39.0mm±0.34) was significantly less than control (43.3mm±0.19). On 28<sup>th</sup> day, neem powder treatment recorded maximum mycelial growth (57.1mm±0.25) followed by growth in *B. thuringiensis* treatment (53.0mm±0.25) and vinegar treatment (50.3mm±0.21). However, radial growth in castor powder (46.0mm±0.30) and *B. bassiana* (44.1mm±0.42) was significantly lesser than control (48.4mm±0.21) (Table 1).

### Linear growth

Linear growth of *A. bisporus* on compost was recorded at weekly interval upto 28 days. On 7<sup>th</sup> day, maximum linear growth was recorded in compost tubes supplemented with *B. bassiana* (15.0mm±0.31) followed by *B. thuringiensis* (14.0mm±0.77) and neem powder (13.4mm±0.23) which was at par with each other. On 21<sup>st</sup> day, maximum linear growth was recorded in *B. thuringiensis* supplemented compost

(30.1mm±0.26) which was at par with neem powder supplemented compost (29.0mm±0.35). However, *B. bassiana* supplemented compost recorded maximum growth (19.0mm±0.35) and was at par with control (19.9mm±0.34) and castor (20.1mm±0.32). On 28<sup>th</sup> day, maximum linear growth was recorded in compost supplemented with neem powder (31.1mm±0.66) which was at par with *B. thuringiensis* (30.0mm±0.70) and were significantly better than control (22.1mm±0.66). However, minimum growth (20.2mm±0.67) was recorded in *B. bassiana* supplemented compost (Table 1).

### Effect on natural bio-agents on mushroom yield

Neem powder was supplemented at different concentrations ranging from @0.5% to @4.5% and recorded yield data ranged between 12.31 (kg/100kg of compost) to 19.91 (kg/100kg of compost). The results indicated that neem powder treatment @1.5% recorded maximum number of fruit bodies (1606) and maximum mushroom yield (19.91 kg/100kg of compost) in comparison to other treatments (Table 2). Neem powder @1.5% showed the period of 19 days for spawn run and 20 days for case run. However, pinhead appearance and first harvesting took 22 days and 25 days as compared to 24 days and 27 days in control respectively. In association with other treatments, castor powder treatment @3.5% recorded

**Table 1.** Effect of natural bioagents on the mycelial growth of *A. bisporus* U3 on potato dextrose medium and compost

Natural Insecticides Treatment	Colony diameter (mm) on Potato Dextrose Agar Days				Linear growth (mm) in compost filled race tubes Days			
	7d	14d	21d	28d	7d	14d	21d	28d
Neem powder	11.2* ±0.17	33.0† ±0.32	49.1* ±0.29	57.1* ±0.25	13.4** ±0.23	22.7* ±0.35	29.0* ±0.35	31.1* ±0.66
Castor powder	07.1 <sup>£</sup> ±0.21	29.3 <sup>§</sup> ±0.40	38.1 <sup>£</sup> ±0.19	46.0 <sup>£</sup> ±0.30	10.1 <sup>‡</sup> ±0.24	13.1 <sup>§</sup> ±0.64	20.1 <sup>‡</sup> ±0.32	23.1 <sup>†</sup> ±0.71
Vinegar	10.2 <sup>†</sup> ±0.25	36.1 <sup>†</sup> ±0.23	46.2 <sup>†</sup> ±0.22	50.3 <sup>†</sup> ±0.21	12.2 <sup>†</sup> ±0.51	15.0 <sup>‡</sup> ±0.90	22.2 <sup>†</sup> ±1.04	23.7 <sup>†</sup> ±0.76
<i>B. thuringiensis</i>	09.3 <sup>‡</sup> ±0.36	31.1 <sup>‡</sup> ±0.36	42.0 <sup>§</sup> ±0.34	53.0 <sup>±</sup> 0.25	14.0 <sup>**</sup> ±0.77	19.2 <sup>†</sup> ±0.59	30.1 <sup>*</sup> ±0.26	30.0 <sup>*</sup> ±0.70
<i>B. bassiana</i>	08.1 <sup>§</sup> ±0.40	29.2 <sup>§</sup> ±0.25	39.0 <sup>£</sup> ±0.34	44.1 <sup>†</sup> ±0.42	15.0 <sup>*</sup> ±0.31	18.2 <sup>†</sup> ±0.40	19.0 <sup>‡</sup> ±0.35	20.2 <sup>‡</sup> ±0.67
Control	10.0 <sup>**</sup> ±0.21	31.0 <sup>‡</sup> ±0.15	43.3 <sup>‡</sup> ±0.19	48.4 <sup>§</sup> ±0.21	10.1 <sup>‡</sup> ±0.21	11.1 <sup>£</sup> ±0.56	19.9 <sup>‡</sup> ±0.34	22.1 <sup>**</sup> ±0.66
CD at 5%	0.86	0.92	1.01	0.86	2.08	1.78	1.60	2.19

Data within the same symbols and columns are not significantly different (a=\*, b=†; c=‡; d=§; e=£; f=")

**Table 2.** Effect of Neem powder supplementation in casing mixture on the yield of *A. bisporus* (U3 Strain)

Neem powder concentration (%)	Spawn run (d)	Case run (d)	Pinning after casing (d)	First Harvest (d)	Yield (kg/100kg Compost)	NFB (no./100kg compost)	AV. Wt of FB (grams)
0.5%	19	22	23	27	14.76 <sup>‡</sup> ±1.728	1200 <sup>‡</sup> ±113.0	12.3
1.5%	19	20	23	25	19.91 <sup>*</sup> ±2.246	1606 <sup>*</sup> ±96.9	12.4
2.5%	19	21	23	26	14.63 <sup>‡</sup> ±1.468	1190 <sup>‡</sup> ±114.8	12.3
3.5%	19	21	23	27	13.91 <sup>‡</sup> ±1.337	1169 <sup>‡</sup> ±61.0	11.9
4.5%	19	21	23	27	12.31 <sup>§</sup> ±1.211	1044 <sup>§</sup> ±117.0	11.8
Control	19	22	24	27	18.29 <sup>†</sup> ±1.767	1487 <sup>†</sup> ±115.7	12.3
CD (5%)					1.28	96.7	

Data within the same symbols and columns are not significantly different (a=\*, b=†; c=‡; d=§)

**Table 3.** Effect of Castor powder supplementation in casing mixture on the yield of *A. bisporus* (U3 Strain)

Castor powder concentration (%)	Spawn run (d)	Case run (d)	Pinning after casing (d)	First Harvest (d)	Yield (kg/100kg Compost)	NFB (no./100kg compost)	AV. Wt of FB (grams)
0.50%	19	22	24	27	11.39 <sup>§</sup> ±1.238	1064 <sup>†</sup> ±66.1	10.7
1.50%	19	20	24	27	12.00 <sup>§</sup> ±1.255	1101 <sup>†</sup> ±56.3	10.9
2.50%	19	22	23	26	14.20 <sup>†</sup> ±0.687	1291 <sup>†</sup> ±60.6	11
3.50%	19	21	23	26	15.93 <sup>†</sup> ±1.839	1374 <sup>†</sup> ±129.3	11.6
4.50%	19	20	24	27	10.40 <sup>§</sup> ±1.697	1000 <sup>†</sup> ±55.4	10.4
Control	19	22	24	27	12.90 <sup>†</sup> ±1.132	1152 <sup>†</sup> ±60.3	11.2
CD (5%)					1.91	123.6	

Data within the same symbols and columns are not significantly different (a=\*, b=†; c=‡; d=§)

**Table 4.** Effect of Vinegar supplementation in casing mixture on the yield of *A. bisporus* (U3 Strain)

Vinegar concentration (%)	Spawn run (d)	Case run (d)	Pinning after casing (d)	First Harvest (d)	Yield (kg/100kg Compost)	NFB (no./100kg compost)	AV. Wt of FB (grams)
0.50%	19	21	24	27	13.14 <sup>‡</sup> ±1.592	1289 <sup>†</sup> ±57.1	10.2
1.50%	19	20	24	27	13.80 <sup>†</sup> ±0.622	1327 <sup>†</sup> ±108.5	10.4
2.50%	19	21	23	26	15.98 <sup>†</sup> ±0.490	1415 <sup>†</sup> ±47.1	11.3
3.50%	19	20	23	26	17.76 <sup>†</sup> ±0.927	1480 <sup>†</sup> ±163.3	12
4.50%	19	21	24	26	14.75 <sup>†</sup> ±0.821	1250 <sup>†</sup> ±104.0	11.8
Control	19	21	24	27	13.10 <sup>‡</sup> ±1.484	1129 <sup>‡</sup> ±163.0	11.6
CD(5%)					2.4	167.1	

Data within the same symbols and columns are not significantly different (a=\*, b=†; c=‡; d=§)

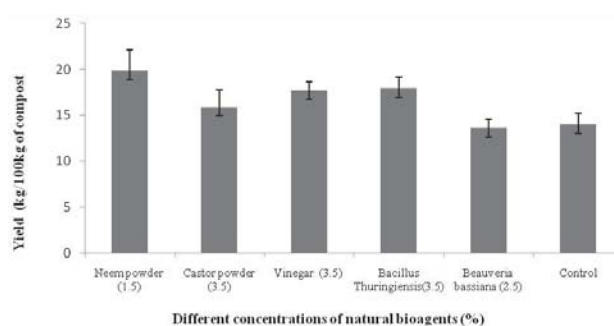
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maximum number of fruit bodies (1374) and maximum mushroom yield (15.93 kg/100kg of compost) (Table 3). The castor treatment @3.5% showed the period of 19 days for spawn run, 21 days for case run, 23 days for pinning and 26 days for first harvesting. Apple vinegar was supplemented at different concentrations ranging from @0.5% to @4.5% and recorded yield data ranged between 13.10 (kg/100kg of compost) to 17.76 (kg/100kg of compost). Vinegar treatment @3.5% recorded maximum number of fruit bodies (1480) and maximum mushroom yield (17.76 kg/100kg of compost) (Table 4). The vinegar treatment @3.5% recorded period of 19 days for spawn run and 20 days for case run. However, pinhead appearance and harvesting took 23 days and 26 days respectively as compared to control (24days and 27days).

*B. thuringiensis* treatment yield data ranged from 11.12 (kg/100kg of compost) to 17.94 (kg/100kg of compost). Maximum number of fruit bodies (1483) and maximum yield (17.94 kg/100 kg of compost) were recorded @3.5% concentration (Table 5). The field data showed period of 19 days for spawn run and 20 days for case run. However, pinhead appearance and harvesting took 23 days and 26 days in comparison to 24 days and 27 days in control respectively. *B. bassiana* supplementation in casing mixture showed no significant change in harvested

mushrooms and number of fruit bodies in comparison to the control (Table 6).

The best concentration of natural bioagents from this experiment were summarized. The selected treatments viz., neem powder @1.5%, castor powder @3.5%, vinegar @3.5%, *B. thuringiensis* @3.5% and *B. bassiana* @2.5% were screened to identify best treatment which gave maximum mushroom yield (Fig.1). Casing mixture supplemented with neem powder @1.5% recorded maximum mushroom yield (19.91 kg/100kg of compost). The yield recorded in *B. thuringiensis* @3.5% (17.94 kg/100kg of compost) and vinegar @3.5% (17.76 kg/100kg of compost) concentration were at par with each other. However, *B. bassiana* recorded yield (13.67 kg/100kg



**Fig. 1.** Effect of natural bioagents in casing mixture on the yield of *A. bisporus* (U3 Strain)

**Table 5.** Effect of *B. thuringiensis* Supplementation in casing mixture on the yield of *Agaricus bisporus* (U3 strain)

<i>B. thuringiensis</i> concentration (%)	Spawn run (d)	Case run (d)	Pinning after casing (d)	First Harvest (d)	Yield (kg/100kg Compost)	NFB (no./100kg compost)	AV. Wt of FB (grams)
0.50%	19	20	24	27	11.12 <sup>§</sup> ±0.911	1080 <sup>§§</sup> ±62.4	10.3
1.50%	19	22	25	27	12.80 <sup>†‡</sup> ±1.039	1143 <sup>†‡§</sup> ±120.3	11.2
2.50%	19	20	24	27	14.20 <sup>†</sup> ±1.330	1246 <sup>†</sup> ±175.8	11.4
3.50%	19	21	23	26	17.94 <sup>*</sup> ±1.279	1483 <sup>*</sup> ±62.0	12.1
4.50%	19	20	23	26	14.11 <sup>†</sup> ±1.242	1217 <sup>†‡</sup> ±161.6	11.6
Control	19	22	24	27	12.39 <sup>†‡</sup> ±1.539	1050 <sup>§</sup> ±125.3	11.8
CD (5%)					1.88	155.4	

Data within the same symbols and columns are not significantly different (a=\*; b=†; c=‡; d=§)

**Table 6.** Effect of *B. bassiana* Supplementation in casing mixture on the yield of *Agaricus bisporus* (U3 strain)

<i>B. bassiana</i> concentration (%)	Spawn run (d)	Case run (d)	Pinning after casing (d)	First Harvest (d)	Yield (kg/100kg Compost)	NFB (no./100kg compost)	AV. Wt of FB (grams)
0.50%	19	20	25	28	13.05±1.10	1155±39.4	11.3
1.50%	19	21	25	28	13.38±1.11	1174±55.1	11.4
2.50%	19	20	24	28	13.67±0.86	1189±75.8	11.5
3.50%	19	21	25	28	12.29±0.50	1098±106.2	11.2
4.50%	19	21	25	28	11.48±0.61	1025±64.4	11.2
Control	19	21	24	27	13.48±1.01	1183±59.7	11.4
CD(5%)					NS	NS	

Data within the same symbols and columns are not significantly different (a=\*, b=†; c=‡; d=§)

of compost) at par with the control (14.03 kg/100kg of compost). It was also observed that first harvest was completed at 25 days in neem supplemented bags as compared to 27 days in control. The percent increase in yield over control was maximum (41.91%) in neem powder @1.5%. This was followed by percent increase in yield over control in *B. thuringiensis* @3.5% (27.86%) and vinegar @3.5% (26.58%) which were at par with each other. The average weight of fruit body ranged between 11.5 to 12.4 grams.

The results indicated that natural bioagents have significant effect on radial growth and yield of *A. bisporus* and can be used for improvement of yield of button mushroom. It has been observed that the addition of natural bioagents in casing provided a sufficient level of nutrients to obtain maximum yield potential. It was recorded that neem powder @1.5% recorded maximum fruit bodies and yield as compared to control. Inam-ul-haq *et al.* (2010) also recorded maximum yield of *A. bisporus* in neem powder at 2% to 4% concentration. This may be due to antimicrobials against pathogens such as flavonoids, triterpenoids, liminoids, quercetinm, and nutrients (N, P, K) for a better quality of fruiting bodies. Nagesh & Reddy (2000) also reported that 3% concentration of castor oil increased the number of thermophiles and mesophiles and decreased pathogenic molds, leading

to high productivity. Yoshimura *et al.* (1995) recorded that increase in yield of *A. bisporus* in vinegar treatment might be due to active components such as 3,5-dimethylphenol, 2-methoxy phenol and butanoic acid, which shortened the time required for primordium formation. Singh *et al.* (2018) also reported the essential components of wood vinegar, such as butanoic acid, 2-methoxyphenol, 3,5-dimethylphenol and 1-pentanol responsible for increase in yield and number of fruiting bodies of *A. bisporus*. The yield of the *A. bisporus* was also increased after supplementation of *B. thuringiensis* which might be due to decrease in loss caused by attack of insect pest and increase in supplementation of proteins and lipids for nutrition due to increase in metabolic activity of enzymes secreted by *Bacillus* species (Keil, 1991). Young *et al* (2013) isolated a number of plant growth promoting bacteria (*Bacillus* species) which enhanced the yield of *Agaricus subrufescens* (21.5%) due to nitrogen fixing, phosphate solubilizing as well as mycelial growth promoting ability.

So we can conclude from our studies that incorporation of neem powder @1.5% in casing mixture improves the number of fruiting bodies and yield of *A. bisporus* and is preferred over synthetic chemicals as it is eco-friendly and reduces environmental pollution.

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