

***In Vitro* Assessment of Botanical Compatibility with *Agaricus bisporus* for Sustainable Disease Management**

Abstract

Button mushroom (*Agaricus bisporus*) cultivation is a significant aspect of the global mushroom industry, the production of fruiting bodies is severely affected by fungal, bacterial and viral pathogens that can have an effect on yield and quality. Botanicals, such as plant extracts, have been reported to possess various bioactive compounds with potential benefits for mushroom growth and disease control. A lab experiment was carried out in the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, to evaluate the compatibility of various botanicals viz., *Allium Sativum* (garlic), *Coriandrum sativum* (coriander), *Cuminum cyminum* (jeera), *Mentha spicata* (pudina) with *Agaricus bisporus* in a Completely Randomised Design. In vitro evaluation was carried out using poison food technique at 10, 20 and 30% concentrations and 24, 48 & 72 hours of incubation. The maximum radial growth of *Agaricus bisporus* was observed in T₀-Control (*Agaricus bisporus*) (17.86 mm), (16.82 mm) and (16.53 mm) and minimum in T₃-*Cuminum cyminum* (jeera) (2.23 mm), (0.98 mm) and (0.39 mm) at 10, 20 and 30 percent concentration respectively. Maximum percentage of inhibition was observed in T₃ - *Cuminum cyminum* (jeera) (87.51 %), (94.11 %) and (98.24 %) at 10, 20 and 30 percent concentration respectively.

Keywords: *Agaricus bisporus*, *Allium Sativum*, *Coriandrum sativum*, *Cuminum cyminum*, *Mentha spicata*

INTRODUCTION

Agaricus bisporus is an edible basidiomycete mushroom native to grasslands in Europe and North America. *Agaricus bisporus* is one of the most important mushrooms that cultivated in the world. Historical evidence indicates that it was first cultivated in France and that cultivar strains originated in Western Europe [1,2,3]. Mushrooms are considered to be heterotrophic (saprophytic) organisms and thus possess no chlorophylls, but decompose organic materials to feed off [4]. The initial step of mushroom cultivation is the compost production and it is a complex microbial process in which microorganisms decompose and stabilize the organic substrates under controlled conditions [5]. Mesophilic microorganisms present in Phase I digest easily degradable polysaccharide, which raise the temperature and cause the shifting of mesophilic microorganisms to thermophilic microbial community, during Phase II heat treatment is given to the compost which stimulates the growth of thermophilic microbial community and protects the crop from its parasites [6,7]. Light brown colored compost having no smell of ammonia should be allowed to cool down before spawning [8]. In phase III during spawn run there is decrease in Actinobacteria and Firmicutes and increase in Proteobacteria [9,10]. Mushrooms such as *A. bisporus* contained high amounts of protein, minerals, B vitamins group, D and K vitamin and sometimes A and C vitamins. Against, fat amount, calorie, sodium and cholesterol are low [11]. The six major constituents of mushrooms are water, proteins, carbohydrates, fiber, fat, and ash along with minerals and essential amino acids [12,13]. Out of the total mushroom produced, white button mushroom share is 73% followed by oyster mushroom (16%), paddy straw mushroom (7%) and milky mushroom (3%) [14].

Mushrooms are mainly subjected to bacterial, fungal and viral diseases. The production of fruiting bodies is severely affected by fungal, bacterial and viral pathogens that can cause diseases which have an effect on yield and quality [15]. Control of myco- pathogens is based on the use of chemicals, cultural practices, and sanitation. Some workers have recommended fungicides for management. But growers hardly use the fungicides for the treatment of this disease. They often found fungicidal treatment as non-economical and hazardous to health. Botanicals have demonstrated their value as a beneficial reservoir of fungicidal compounds that are comparatively safer than synthetic fungicides, which frequently carry unwanted adverse effects. Numerous plant species have been identified as containing compounds that are toxic to microbial pathogens and act as a defensive shield against infections. With this perspective in mind, an attempt was undertaken to assess the effectiveness of leaf and seed extracts in managing pathogenic fungi which hinder the mushroom cultivation, in *in vitro* condition.

MATERIALS AND METHODS

The study was conducted at the laboratory of the department of plant pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh. The culture of *Agaricus bisporus* (NBS-5) was procured from Directorate of Mushroom Research, Chambaghat Solan, Himachal Pradesh. The different botanicals were evaluated under *in vitro* to test their compatibility with *Agaricus bisporus* viz., *Allium Sativum* (garlic), *Coriandrum sativum* (coriander), *Cuminum cyminum* (jeera), *Mentha spicata* (pudina). The fresh plant leaves were washed in distilled water and were separately homogenized with sterile distilled water at 1:1 w/v, (100g fresh leaves/seed with 100 ml of sterile distilled water) in a pestle and mortar separately of each plant and seed and filtered through a muslin cloth followed by sterilized Whatman No.1 Filter paper [16]. This formed 100 percent plant extract solution. The plant extracts so prepared were heated at 40°C for 10 minutes to avoid contamination. Aqueous extract of 10, 20, 30 % was prepared according to the treatment by mixing 10, 20 and 30 ml of aqueous extract with 90, 80, 70ml PDA respectively in separate conical flask. The media in conical flask were sterilized in an autoclave at temperature of 121°C for 20 minutes. The botanicals were evaluated *in vitro* through poison food technique [17]. The 20 ml sterilized media with botanical extract was poured into 90mm petriplates under aseptic conditions in laminar air flow. After solidification of media 5mm disc of 7 days old subculture of *Agaricus bisporus* were placed in the centre of the petriplates and one control plate which has only the PDA medium inoculated with culture disc and used as check. Three replicates were maintained for each test and those plates were incubated at 27±1 °c in incubator. The radial growth of mycelium was measured at different intervals of 24, 48 and 72 hrs. The radial growth of mycelium of each plate was measured by taking average of the two diameters taken at right angles for each colony. Percent inhibition in growth was calculated in relation to growth in control using the formula of Vincent (1947). The experiment was conducted in completely randomized block design (CRD) with three replications in each treatment. The variance ratio test at the 5% level of probability was used to determine the significance of treatment differences. The observation of percent inhibition of mycelial growth, were transformed in to “Arc sin Transformation” = $\sin^{-1} \sqrt{p/100}$ used for statistical analysis.

Mycelial inhibition = (Radial growth in control – Radial growth in treatment) / (Radial growth in control) ×100

Table 1. List of botanicals and their scientific names with part used

Serial No.	Common name	Botanical name	Plant part used
1	Garlic	<i>Allium Sativum</i>	leaves

2	Coriander	<i>Coriandrum sativum</i>	leaves
3	Jeera	<i>Cuminum cyminum</i>	seed
4	Pudina	<i>Mentha spicata</i>	leaves

Results and discussion

***In vitro* effect of botanicals on radial growth(mm) and percent inhibition of *Agaricus bisporus* at 10 % concentration at 24hrs, 48hrs and 72hrs**

As shown in Table 2 and depicted in Figure 1 reveals that at 10% concentration, after 24hrs, 48hrs and 72hrs incubation, the maximum radial growth of *Agaricus bisporus* was observed in T0- control (*Agaricus bisporus*) (17.86mm) followed by T1 –*Allium Sativum* (garlic) (5.29mm), T2- *Coriandrum sativum* (coriander) (3.69mm), T4- *Mentha spicata* (pudina) (2.46mm) and T3 - *Cuminum cyminum* (jeera) (2.23mm). The treatment T0- control (*Agaricus bisporus*) was significant over all the treatments. The result showed that maximum percentage of inhibition was observed in T3 - *Cuminum cyminum* (jeera) (87.51%) followed by T4- *Mentha spicata* (pudina) (86.23%), T2- *Coriandrum sativum* (coriander) (79.32%), T1 –*Allium sativum* (garlic) (70.39 %) and T0- control [*Agaricus bisporus* (alone)] (0.00 %). (Table 3).

***In vitro* effect of botanicals on radial growth(mm) and percent inhibition of *Agaricus bisporus* at 20 % concentration at 24hrs, 48hrs and 72hrs**

As shown in Table 2 and depicted in Figure 1 reveals that at 20% concentration, after 24hrs, 48hrs and 72hrs incubation, the maximum radial growth of *Agaricus bisporus* was observed in T0- control (*Agaricus bisporus*) (16.82mm) followed by T1–*Allium Sativum* (garlic) (3.97mm), T2- *Coriandrum sativum* (coriander) (2.10mm), T4- *Mentha spicata* (pudina) (1.13mm) and T3 - *Cuminum cyminum* (jeera) (0.98mm). The treatment T0- control (*Agaricus bisporus*) was significant over all the treatments. The result showed that maximum percentage of inhibition was observed in T3 - *Cuminum cyminum* (jeera) (94.16%) followed by T4- *Mentha spicata* (pudina) (93.29%), T2-*Coriandrum sativum* (coriander) (87.52%), T1 –*Allium Sativum* (garlic) (76.40%) and T0- control [*Agaricus bisporus* (alone)] (0.00 %). (Table 3).

***In vitro* effect of botanicals on radial growth(mm) and percent inhibition of *Agaricus bisporus* at 30 % concentration at 24hrs, 48hrs and 72hrs**

As shown in Table 2 and depicted in Figure 1 reveals that at 30% concentration, after 24hrs, 48hrs and 72 hrs incubation, the maximum radial growth of *Agaricus bisporus* was observed in T0- control (*Agaricus bisporus*) (16.53mm) followed by T1–*Allium Sativum* (garlic) (2.95mm), T2- *Coriandrum sativum* (coriander) (1.23mm), T4-*Mentha spicata* (pudina) (0.41mm) and T3 -*Cuminum cyminum* (jeera) (0.39mm). The treatment T0- control (*Agaricus bisporus*) was significant over all the treatments. The result showed that maximum percentage of inhibition was observed in T3 - *Cuminum cyminum* (jeera) (98.24%) followed by T4- *Mentha spicata* (pudina) (97.54%), T2- *Coriandrum sativum* (coriander) (92.57%), T1 – *Allium sativum* (garlic) (82.16%) and T0- control [*Agaricus bisporus* (alone)] (0.00 %).(Table 3).

Sailaja and Radhika (2020) [18] reported that fruits of *Coriandraum sativum* at 5 and 10% concentration produced more mushroom, but the growth was slow. **Jahan et al. (2013)** [19] found that botanicals like garlic and henna reduced the radial colony diameter of pathogen appreciably at different concentrations. **Kousar et al. (2018)** [20] evaluated different botanicals against *Verticillium fungicola* at 1/5 and 3% con- centration which revealed that *Allium sativum* (garlic) exhibited maximum inhibition (100%) of mycelial growth. **Singh et al. (2017)** [21]

reported 13.69 percent inhibition over control in *Allium sativum* (garlic). Garlic extract has antimicrobial activity against many genera of bacteria, fungi and viruses (**Gebreyohannes and Gebreyohannes, 2015**) [22].

Table 2. Mycelial growths of *Agaricus bisporus* at 10 %, 20 % and 30 % concentration along with 24, 48 and 72 hours of incubation

Treatments	Mycelial growth(mm)								
	10%			20%			30%		
	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours
<i>Agaricus bisporus</i> (Alone)	6.23	9.89	17.86	5.65	10.51	16.82	6.12	10.36	16.53
<i>Allium Sativum</i> (Garlic)	2.11	3.14	5.29	1.51	2.70	3.97	1.42	2.15	2.95
<i>Coriandrum sativum</i> (Coriander)	1.61	2.35	3.69	0.9	1.49	2.10	0.84	1.07	1.23
<i>Cuminum cyminum</i> (Jeera)	1.10	1.56	2.23	0.61	0.83	0.98	0.29	0.30	0.39
<i>Mentha spicata</i> (Pudina)	1.13	1.53	2.46	0.66	1.01	1.13	0.35	0.41	0.48
F-test	S	S	S	S	S	S	S	S	S
S.Em	0.011	0.010	0.013	0.013	0.010	0.009	0.011	0.009	0.007
CD (0.05)	0.036	0.034	0.042	0.041	0.033	0.030	0.036	0.030	0.023

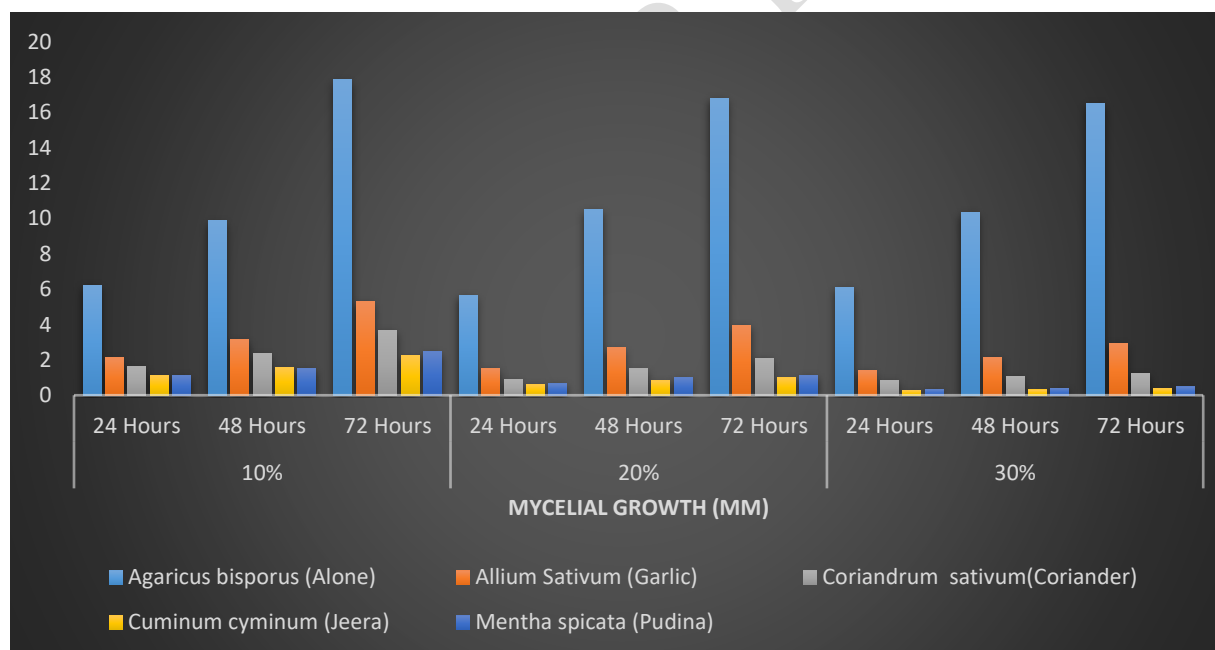


Fig. 1. Mycelial growth of *Agaricus bisporus* at 10 %, 20 % and 30 % concentration along with 24, 48 and 72 hours of incubation.

Table 3. Mycelial growth inhibition of *Agaricus bisporus* at 10 %, 20 % and 30 % concentration along with 24, 48 and 72 hours of incubation

Treatments	Percent inhibition of mycelial growth(mm)								
	10%			20%			30%		
	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours
<i>Agaricus bisporus</i> (Alone)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Allium Sativum</i> (Garlic)	66.15(54.41)	68.24(55.72)	70.39(57.02)	73.32(58.87)	74.32(59.54)	76.39(60.93)	76.82(61.20)	79.21(62.89)	82.15(65.00)
<i>Coriandrum sativum</i> (Coriander)	74.18(59.41)	76.26(60.82)	79.32(62.96)	84.13(66.47)	85.79(67.88)	87.51(69.30)	86.31(68.25)	89.62(71.25)	92.57(74.17)
<i>Cuminum cyminum</i> (Jeera)	82.29(65.15)	84.19(66.60)	87.51(69.30)	89.13(70.81)	92.11(73.61)	94.15(76.03)	95.11(77.21)	96.24(78.81)	98.23(82.39)
<i>Mentha spicata</i> (Pudina)	81.83(64.79)	84.51(66.83)	86.23(68.21)	88.29(70.01)	90.42(71.94)	93.28(74.97)	94.21(76.16)	95.32(77.57)	97.53(80.94)
CD (0.05)	0.48	0.19	0.11	0.60	0.28	0.13	0.59	0.31	0.17

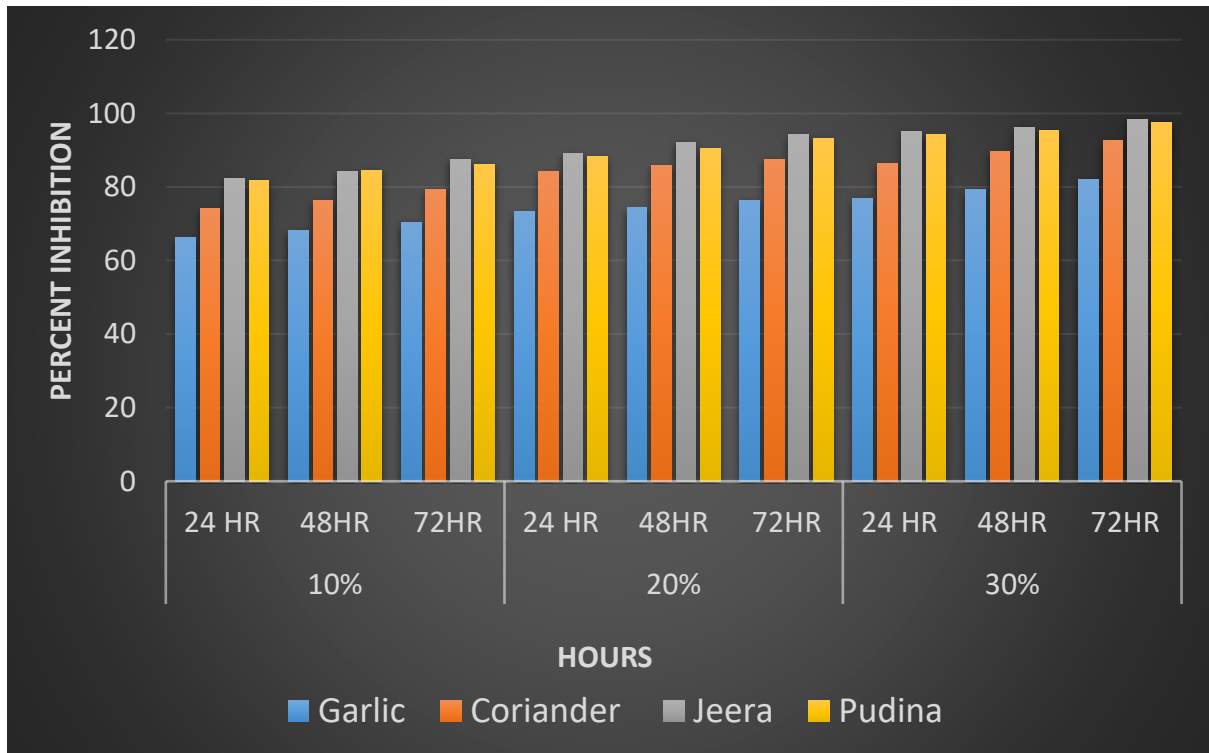


Fig. 2. Mycelial growth inhibition of *Agaricus bisporus* at 10 %, 20 % and 30 % concentration along with 24, 48 and 72 hours of incubation

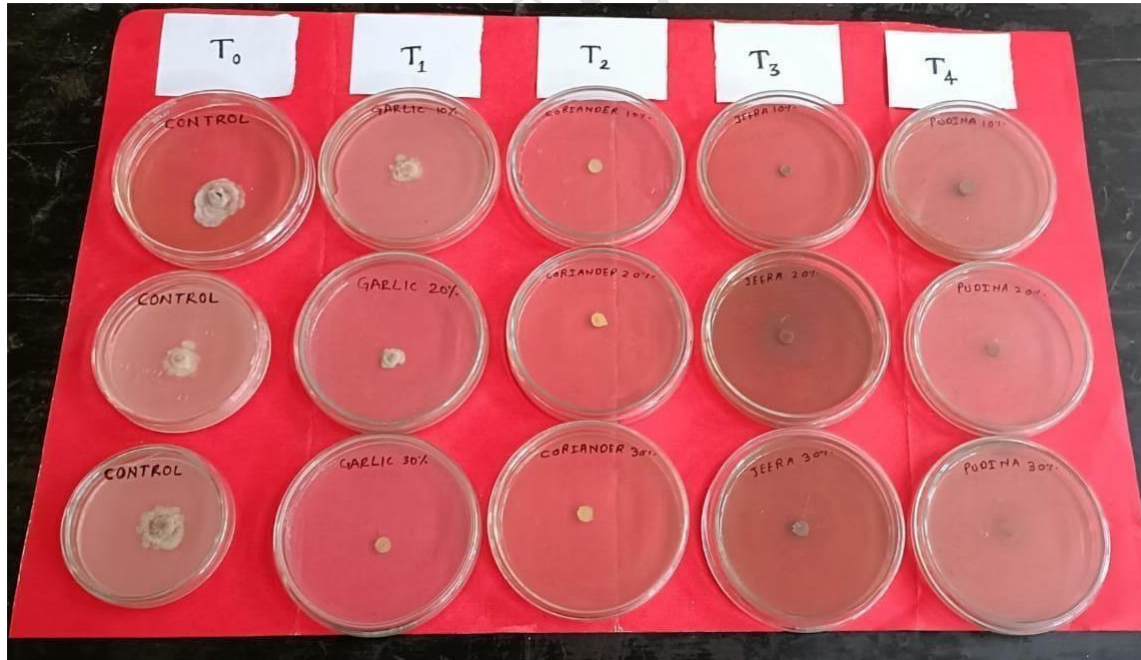


Plate 1: Effect of selected botanicals on radial growth of *Agaricus bisporus*

4. CONCLUSION

The evaluation of various botanical extracts indicated that each one inhibited the growth of *Agaricus bisporus* to varying degrees when compared to the control group (*Agaricus bisporus* alone). An increase in the concentration of these botanicals corresponded with a greater inhibition of mycelial growth. Thus, the findings demonstrate that under *in vitro* conditions, these botanical extracts are not compatible with *Agaricus bisporus*.

REFERENCES

1. Atkins, F. C. (1974). Guide to Mushroom Growing. London: Faber and Faber, (12): 122-125.
2. Kerrigan, R. W. (1995). Global genetic resources for *Agaricus* breeding and cultivation. *Journal of Botany*, (73): 973-975.
3. Jeong, S. C., Koyyalamudi, S. R., Jeong, Y. T., Song, C. H. and Pang, G. (2012). Macrophage immunomodulating and antitumor activities of polysaccharides isolated from *Agaricus bisporus* white button mushrooms. *Journal of Medicinal Food*, (15): 58-65.
4. Miles, P. G. and Chang, S. T. (2004). Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. CRC press, (7): 12-13.
5. Johri, B. N. and Rajni. (1999). Mushroom compost: microbiology and application. In *Modern approaches and innovation in soil management*, (24): 345–358.
6. Mouthier, T. M. B., Kilic, B., Vervoort, P., Gruppen, H. and Kabel, M. A. (2017). Potential of a gypsum-free composting process of wheat straw for mushroom production. *PLoS One*, (10): 108-111.
7. Vieira, F. R. and Pecchia J. A. (2018). An exploration into the bacterial community under different pasteurization conditions during substrate preparation (composting phase II) for *Agaricus bisporus* cultivation. *Microbial Ecology*, (75): 318-330.
8. Netam, R. S., Yadav, S. C., Mukherjee, S. C. and Kumari, P. (2018). Cultivation of button mushroom (*Agaricus bisporus*) under controlled condition: An initiative in Bastar Plateau of Chhattisgarh. *International Journal of Current Microbiology and Applied Sciences*, (10): 782-786.
9. Kertesz, M., Safianowicz, K. and Bell, T. (2016). New insights into the microbial communities and biological activities that define mushroom compost. In *Proceedings of the 19th International Society for Mushroom Science (ISMS) Conference*, (8): 161-165.
10. Carrasco, J., Tello M. L., Tkacz, A., Poole, P., Clavijo, M. and Preston, G. (2019). Casing microbiome dynamics during button mushroom cultivation: implications for dry and wet bubble diseases. *Microbiology*, (165): 611–624.
11. Saiqa, S., Nawaz, B. H. and Asif, H. M. (2008). Studies on chemical composition and nutritive evaluation of wild edible mushrooms. *Iran Journal of chemistry and Chemical Engineering*, (3): 151-154.
12. Heleno, S. A., Barros, L., Sousa, M. J., Martins, A. and Ferreira, I. C. F. R. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, (119): 1443-1450.
13. Alispahic, A., Sapcanin, A., Salihovic, M., Ramic, E., Dedic, A. and Pazalja, M., (2015) Phenolic content and antioxidant activity of mushroom extracts from Bosnian market. *Bulletin of the Chemists and Technologist of Bosnia and Herzegovina*, (44):5-8.
14. Sharma, V. P., Annepu, S. K., Gautam, Y., Singh, M. and Kamal, S. (2017). Status of mushroom production in India. *Mushroom research*, (2): 111-20.

15. Potocnik, I., Vukojevic, J., Stajic, M., Tanovic, B. and Todorovic, B. (2008b). Fungicide sensitivity of selected *Verticillium fungicola* isolates from *Agaricus bisporus* farms. *Archives of Biological Sciences*, (60): 151-158.
16. Kumar, R., Bhaskar, R., Mapari, P. N., Datre, S., Ahammad, F., Patil, P. and Simon, S.(2023). Compatibility analysis of mushroom with different botanicals in in-vitro condition. *International Journal of Environment and Climate Change*. 13(12): 1017-1023.
17. Nene, Y. L. and Thapliyal, P. N. (2000). *Fungicides in Plant Disease Control*. Oxford and IBH Publishing Company, (12): 531-533.
18. Sailaja, B. and Radhika, B. (2020). Production of Value-Added Oyster Mushrooms. *International Journal of Pharmaceutical Sciences and Drug Research*, 12(6): 652-659
19. Jahan, B., Ali, M. A., Alam, S., Moni, Z. R. and Alam, M. A. (2013). In vitro evaluation of antifungal activity of plant extracts against *Rhizoctonia oryzae-sativae* causing aggregated sheath spot of rice. *Bangladesh Journal of Plant Pathology*, (29): 13-18.
20. Kousar, S., Rasool, F., Aafia, S., Mushtaq, N. and Nazim, N. (2018). Evaluation of different botanicals against *Verticillium fungicola* causal pathogen of dry bubble disease of button mushroom. *The Pharma Innovation Journal*, 7(4): 34-36.
21. 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. *An international Quarterly Journal of Life Sciences*, (4): 2969-2973.
22. Gebreyohannes, G. and Gebreyohannes, M. (2015). Medicinal values of garlic: A review. *International Journal of Medicine and Medical Sciences*, (9): 401-408.

UNDER PEER REVIEW