Compatibility Analysis of Mushroom with Different botanicals in *in vitro* condition

Abstract

White button mushroom [*Agaricus bisporus* (Lange) Imbach] is the most popular cultivated edible mushroom, fetching high price and still dominating in Indian and International market. However, the limiting factor for its successful cultivation is the occurrence of competitor moulds.The present investigations were carried out in the laboratory Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh.In this experiment different botanical (seed extract)*viz.,Trachyspermum ammi* (Ajwain), *Foeniculum vulgare* (Saunf), *Anethum grveolens* (Soa), *Trigonella foenumgraecum* (Methi)were evaluated to test their compatibilitywith Agaricus bisporus. The botanicals (seed extract) were evaluated *in vitro* through 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 T_0-Control (*Agaricus bisporus*) (16.45mm), (17.12 mm) and (17.22 mm) and minimum in T1 - *Trachyspermum ammi* (Ajwain) (1.46mm), (0.83mm) and (0.60mm) at 10, 20 and 30percent concentration respectively. Maximum percentage of inhibition was observed in T1 - *Trachyspermum ammi* (Ajwain) (91.11%), (95.13%) and (96.50%) at 10, 20 and 30 percent concentration respectively.

Keywords:Agaricus bisporus, *Anethum grveolens*, *Foeniculum vulgare*, *Trachyspermum ammi*

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INTRODIUCTION

The white button mushroom (*Agaricus bisporus*) is very popular throughout the world and is the most important mushroom of commercial significance in Indian (Maheshwari, 2013). Mushrooms, classified in Phylum Basidiomycota, class Agaricomycetes, are revered as the "Flower of God" in India, known by diverse names. Thriving in varied agro-climatic conditions, these saprophytic fungi absorb nutrients from organic matter, flourishing in natural settings like decaying matter, leaves, droppings, and dead wood. (Kumar *et. al* ., 2020). A Frenchman achieved the initial success in commercial mushroom production in 1780 by cultivating *A. bisporus* underground in quarries near Paris (Beyer 2003). Significant

advancements in mushroom cultivation occurred in France around 1600 A.C., with the first cultivation of *Agaricus bisporus* on specially prepared agricultural media (Chang and Miles 2004). Compost, essential for growing white button mushrooms, results from breaking down organic waste by tiny organisms. Composting involves these organisms breaking down organic material, making protein, and preparing fibrous materials to hold water. Microbes not only change compost's properties but also limit the growth of other competing microbes. China initiated the first cultivation of mushrooms, and in India, commercial production began in hilly areas like Chail (Himachal Pradesh), Kashmir, and Ooty (Tamil Nadu) (Jain *et. al* ., 2011). In the northern region, commercial cultivation primarily occurs during the winter season (Maheshwari, 2013). India has approximately 198,000 hectares under mushroom cultivation, yielding an annual production of 487,000 metric tons (Anonymous, 2017). Among the total mushroom production in India, white button mushrooms constitute 73% (Sharma *et. al* ., 2017). In the 2021-2022 period, Bihar leads in mushroom production, followed by Maharashtra, Orissa, Haryana, and Uttarakhand, as per the Agricultural and Processed Food Products Export Development Authority of India (APEDA), 2021. Fungal pathogens such as Lecanicillium (Verticillium) fungicola, *Mycogoneperniciosa*, Cladobotryum (Dactylium) spp. and *Trichoderma spp*. afflict the cultivated mushroom *Agaricus bisporus* (Lange) Imbach, causing its most serious fungal diseases, dry and wet bubble, cobweb disease and green mould, respectively (Potocnik *et. al* ., 2008a, 2008b, 2010a, 2010b). 4 Several diseases of cultivated mushrooms are caused by bacteria and viruses (Grogan, 2008; Geels *et. al* ., 2008). The most common bacterial disease, distributed worldwide, is the bacterial brown blotch caused by *Pseudomonas tolaasii*(Todorovic *et. al* ., 2012). Various botanicals have proved useful source of fungitoxic substances that are rather harmless compared to synthetic chemical fungicides, which often impose undesirable side effects. Several plants have been reported to posses' substances, which are toxic to microbial pathogens and serve as protective barrier to infection. Keeping above in view, an effort was made to evaluate the efficacy of leaf extracts of angiospermic plants as compared to control of pathogenic fungi of mushrooms under field conditions.

MATERIALS AND METHODS

The present investigations were carried out in the laboratory, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh(Year 2022-23) to test the compatibility of different treatments with*Agaricus bisporus* under *in vitro* conditions. In order of find out the compatibility of various plants extracts*viz.,*

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Trachyspermum ammi (Ajwain), *Foeniculum vulgare* (Saunf), *Anethum grveolens* (Soa), *Trigonella foenumgraecum* (Methi)with*Agaricus bisporus* were used. Seeds of all botanicalswere collected and washed thoroughly in clean water. Equal amount of washed seeds were grinded in mortar and pestle by adding same amount of sterilized water (1:1 w/v) and boiled at 80^0 C for 10 minutes in hot water bath. The extract was filtered by double layer muslin cloth followed by sterilized Whatman No.1 Filter paper. (Bhaskar *et. al* ., 2023)Aqueous extract of 10, 20, 30 % was prepared according to the treatment by mixing 10, 20 and 30 ml of spices seed extract with 90, 80, 70 ml PDA respectively in separate conical flask. The flasks were thoroughly shaken to ensure an even mix of the extract under aseptic conditions.Twenty ml of sterilized melted PDA was aseptically poured in sterilized Petri dishes and allowed to solidify. After solidification of media 5mm disc of 7 days old subculture of *Agaricus bisporus* were placed in the centre of the Petri plates and one control plate which has only the PDA medium inoculated with culture disc and used as control. Each treatment and control were repeated three times to make three replications. Replicates were maintained for each test and those plates were incubated at 27 ± 1^0C at incubator. The radial growth of mycelium was measured at different intervals of 24, 48 and72 hrs. The radial growth of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony. Percent inhibition in growth was calculated in relation to growth in control using the following formula of Vincent (1947). The experiment was conducted in completely randomized block design (CRD) with three replications in each treatment. The conclusion was arrived at after statistical analysis of the data. The Completely Randomized Design (CRD) method recommended by Goon *et. al* . was used to conduct the statistical analysis of laboratory experiments (1931). The variance ratio test at the 5% level of probability was used to determine the significance of treatment differences. The observation of per cent inhibition of mycelial growth, were transformed in to “Arc sin Transformation” $=^{\sin-1} \sqrt{p}/100$ used for statistical analysis.

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

Table 1. List of botanicals and their scientific names

S. No.	Common name	Botanical name	Plant part used
1	Ajwain	*Trachyspermumammi*	Seed

2	Saunf	*Foeniculumvulgare*	Seed
3	Soa	*Anethumgrveolens*	Seed
4	Methi	*Trigonellafoenumgraecum*	Seed

RESULTS AND DISCUSSION

***In vitro* effect of botanicals (seed extract) on mycelial growthand per-cent inhibition of *Agaricus bisporus* at 10 % concentration at 24hrs, 48hrs and 72hrs.**

As shown in table-2 and depicted in fig-1 reveals that at 10% concentration, after 24hrs, 48hrs and 72hrs incubation, the maximum radial growth of *Agaricus bisporus* was observed in T_0- Control (*Agaricus bisporus)* (16.45mm) followed by T_{2-} *Foeniculum vulgare* (Saunf) (4.90mm), T_4 - *Trigonella foenumgraecum* (Methi) (2.11mm) T_3- *Anethum grveolens (Soa)* (1.95mm), and T_1 – *Trachyspermum ammi* (Ajwain) (1.46 mm).The treatment T_0-*Agaricus bisporus* (Alone) was significant over all the treatments. The result showed that maximum percentage of inhibition was observed in T_1 – *Trachyspermum ammi* (Ajwain) (91.12%) followed by T_3- *Anethum grveolens (Soa)* (88.17%),T_4 - *Trigonella foenumgraecum* (Methi) (88.15%), T_{2-}*Foeniculum vulgar* (Saunf) (70.21%) and T_0-Control (*Agaricus bisporus*)Table-3.

***In vitro* effect of botanical (seed extract) on mycelial growth and per-cent inhibition of *Agaricus bisporus* at 20 % concentration at 24 hrs, 48 hrs and 72 hrs.**

As shown in table-2 and depicted in fig-1 reveals that at 20% concentration, after 24hrs, 48hrs and 72hrs incubation, the maximum radial growth of *Agaricus bisporus* was observed in T_0- Control(*Agaricus bisporus*)(Alone) (17.12 mm) followed by T_{2-} *Foeniculum vulgare* (Saunf) (3.54 mm), T_4 - *Trigonella foenumgraecum* (Methi) (1.17 mm), T_3- *Anethum grveolens (Soa)* (0.96 mm), and T_1 – *Trachyspermum ammi* (Ajwain) (0.83 mm).The treatment T_0-*Agaricus bisporus* (Alone)was significant over all the treatments. The result showed that maximum percentage of inhibition was observed in T_1 – *Trachyspermum ammi* (Ajwain) (96.04%) followed by T_3- *Anethum grveolens (Soa)* (95.55%), T_4 - *Trigonella*

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foenumgraecum (Methi) (93.63%), T_{2-} *Foeniculum vulgare* (Saunf) (80.12%) and T_0- Control (*Agaricus bisporus*)(0.00 %).Table-3.

***In vitro* effect of botanical (seed extract) on mycelial growth and per-cent inhibition of *Agaricus bisporus* at 30% concentration at 24 hrs, 48 hrs and 72 hrs.**

As shown in table-2 and depicted in fig-1 reveals that at 30% concentration, after 24 hrs, 48 hrs and 72 hrs incubation, the maximum radial growth of *Agaricus bisporus* was observed in T_0-Control (*Agaricus bisporus*)(17.22 mm) followed by T_{2-} *Foeniculum vulgare* (Saunf) (2.56 mm), T_4 - *Trigonella foenumgraecum* (Methi) (0.84 mm), T_3- *Anethum grveolens (Soa)* (00.60 mm), and T_1 – *Trachyspermum ammi* (Ajwain) (0.50 mm).The treatment T_0-*Agaricus bisporus* (Alone) was significant over all the treatments. The result showed that maximum percentage of inhibition was observed in T_1 – *Trachyspermum ammi* (Ajwain) (97.10%) followed by T_3- *Anethum grveolens (Soa)* (96.52%),T_4 - *Trigonella foenumgraecum* (Methi) (95.12%), T_{2-} *Foeniculum vulgare* (Saunf) (85.13%) and T_0-*Agaricus bisporus* (0.00 %).Table-3.

Similar findings of botanicals, and fungicides on mycelial growth and percent inhibition were reported byJahan *et. al* **.,** Singh *et. al .,*Sailja and Radhika.

Table No: 2Mycelial growth of *Agaricus bisporus* at 10 %, 20% and 30 % concentration along with 24, 48 and 72 hours of incubation.

	Mycelialgrowth (mm)								
	10 %			20 %			30 %		
	24 Hours	**48 Hours**	**72 Hours**	**24 Hours**	**48 Hours**	**72 Hours**	**24 Hours**	**48 Hours**	**72 Hours**
Agaricusbisporus (Alone)	5.92	10.72	16.45	6.35	10.98	17.12	6.12	10.87	17.22
Trachyspermumammi (Ajwain)	0.7	1.08	1.46	0.49	0.63	0.83	0.32	0.47	0.50
Foeniculumvulgare (Saunf)	1.96	3.38	4.9	1.58	2.48	3.54	1.03	1.72	2.56

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Anethumgrveolens (Soa)	0.93	1.59	1.95	0.56	0.67	0.96	0.34	0.50	0.60
Trigonella foenumgraecum(Methi)	0.93	1.58	2.11	0.51	0.8	1.17	0.42	0.63	0.84
F-test	S	S	S	S	S	S	S	S	S
S. Em	0.010	0.007	0.011	0.013	0.008	0.013	0.012	0.009	0.012
CD(0.05)	0.033	0.023	0.035	0.040	0.026	0.041	0.039	0.027	0.040

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

Table No: 3.Mycelial growth inhibition of *Agaricus bisporus* at 10 %, 20 % and 30 % concentration along with 24, 48 and 72 hours of incubation.

Treatments	**Per-cent inhibition of mycelialgrowth (mm)**								
	10%			20%			30%		
	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours
Agaricusbi sporus (Alone)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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Trachysper mumammi (Ajwain)	88.18(69.86)	89.93 (71.46)	91.12 (72.63)	92.28 (73.84)	94.26(76.11)	96.04(78.49)	94.77(76.75)	95.40(77.58)	97.10(80.15)
Foeniculu mvulgare (Saunf)	66.89(54.84)	68.47 (55.81)	70.21 (56.90)	75.12 (60.05)	77.41 (61.59)	80.12(63.50)	83.17(65.75)	84.18(66.53)	85.13(67.29)
Anethumgrv eolens (Soa)	84.29(66.62)	85.26 (67.39)	88.17 (68.98)	91.18 (72.69)	93.90(75.66)	95.55(77.79)	94.44(76.34)	95.68(77.96)	96.52(79.21)
Trigonella foenumgrae cum(Methi)	84.29(66.62)	85.17 (67.32)	88.15 (69.83)	92.07 (73.61)	92.71(74.31)	93.63(75.35)	93.14(74.78)	94.20(76.04)	95.12(79.21)
C.D	0.35	0.14	0.14	0.54	0.22	0.96	0.71	0.17	0.32

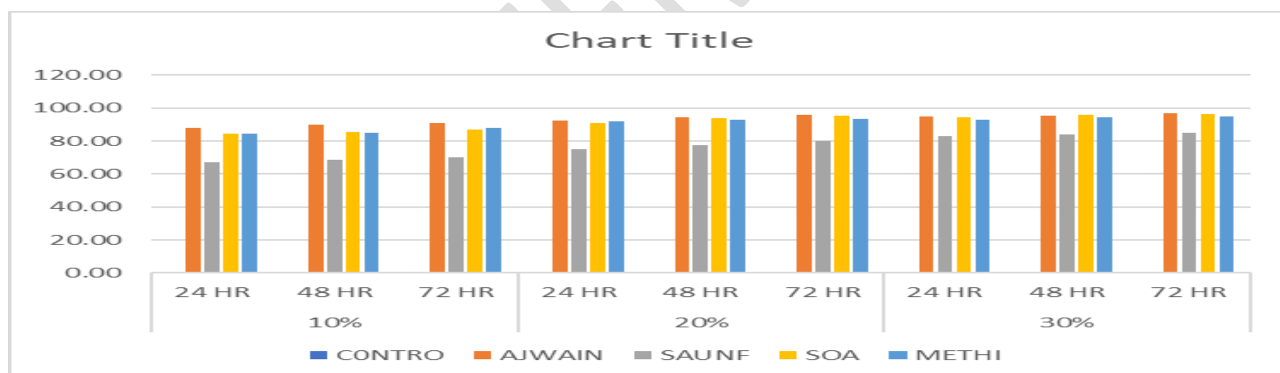

Fig.2. Mycelial growth inhibition of *Agaricus bisporus* at 10 %, 20 % and 30 % concentration along with 24, 48 and 72 hours of incubation

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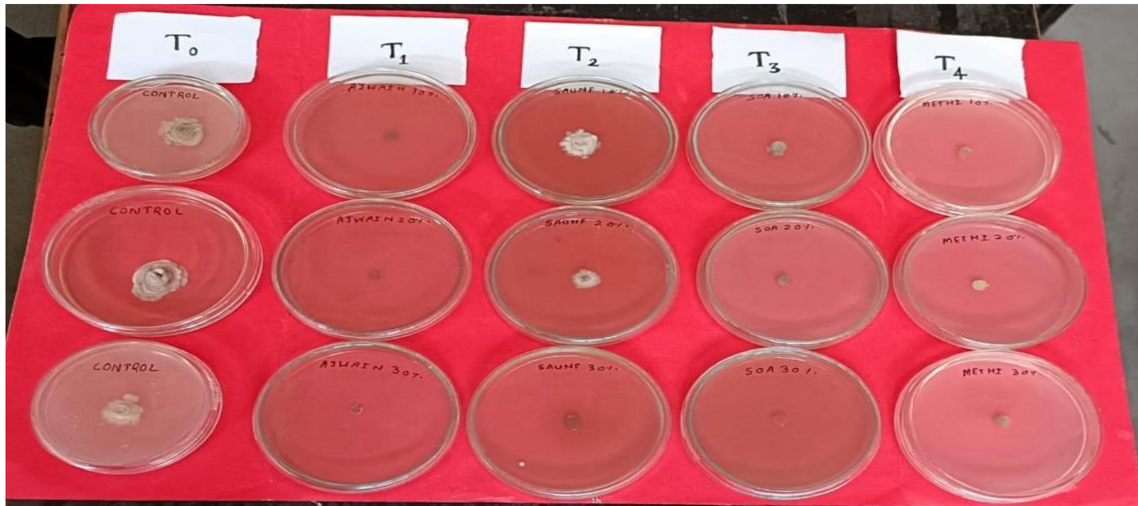

Plate 1. Response of botanicals against *Agaricus bisporus* on mycelial growth

CONCLUSION

Among the tested botanicals, it was found that all the botanical moreor less inhibited the growth of *Agaricus bisporus* as compared to growth in control-T_0[*Agaricus bisporus* (Alone)]. With the increase in concentration there was increase in the inhibition ofmycelialgrowthof*Agaricusbisporus.*Theresultsrevealedthatbotanicalseedextractswerefoundincompatiblewith*Agaricus bisporus* under*in vitro*condition.

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