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RESEARCH ARTICLE

Bioefficacy of aqueous phytoextract for management of common contaminants in straw mushroom bed and yield enhancement

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Abstract

The main obstacle to increased production of paddy straw mushroom (*Volvariella volvacea*) is the frequent contamination of the mushroom growing beds with micro-flora. An experiment was conducted to develop an eco-friendly management practice against competitor moulds of straw mushroom in an eco- friendly manner. Eight different botanicals were tested along with control against the competitor mould fungi both *in vitro* and *in vivo*. *Azadirachita indica* (neem) showed the highest efficacy among all the botanicals tested and exhibited the maximum inhibitory effect (88.15 % to 93.71 %) against *Trichoderma* spp., *Coprinus* spp. and *Penicillium* spp. A mean reduction of 79.93 % to 84.07 % in the incidence of *Sclerotium* spp. and *Aspergillus spp.* was recorded with turmeric leaf extracts. Among all the botanicals *Ocimum sanctum* (tulsi) showed maximum radial mycelia growth of competitor moulds (20.52 to 70.09 mm) in mushroom beds with the lowest percentage of inhibition. Application of 20 % leaf extract of *A. indica* was significantly superior among all treatments in giving yield of 1060.50 g/bed, followed by karanja (1051.30 g/bed). A yield increase of 11.39 % and 10.42 % over normal practices was observed in beds sprayed with *A. indica* and *Pongamia pinnata* (karanja). These results provide an effective alternative approach for managing competitor moulds in straw mushroom cultivation while enhancing yield compared to control practices.

Keywords: competitor moulds; contaminants; paddy straw mushroom; phytoextract; *Volvariella volvacea*

Introduction

Paddy straw mushroom (*Volvariella volvacea*), also known as the Chinese mushroom or straw mushroom, is commonly found in the tropics and subtropics. This mushroom grows quickly and has a short cropping cycle. This mushroom requires a C:N ratio of 40 to 60, which is relatively high, compared to other cultivated mushrooms and it can use a variety of cellulosic materials (1). It requires an ideal temperature of 34 ℃-38 ℃ for spawning and 32 ± 1 ℃ for fruiting, along with 80 %-90 % relative humidity (2). Many diseases and competing fungi infest the mushroom crop, causing it to fail partially or completely or at the very least, lowering the quality of the output. The growth and reproduction of mushrooms are hampered by competitor moulds competing for nutrients and space in the culture substrate (3). Many competitive moulds attack fruit bodies at different crop stages, but most hinder spawn run and reduce yield. Poor bed management further increases the contamination risk.

The infestation must be controlled, otherwise productivity could be seriously threatened. *Coprinus* spp. was the predominant in both outdoor and indoor farming situations. However, compared to indoor farming (27 %), outdoor farming had higher bed contamination rates (46.8 %) (4). *Trichoderma* species are capable of frequently producing green mould disease and can lower straw mushroom yields (5). Studies on *Volvariella* spp. report major contaminants such as *Coprinus* spp., *Sclerotium rolfsii*, *Aspergillus* spp., *Trichoderma* spp., *Penicillium* spp. and *Rhizopus* spp. (4-8). In the east and southeast coastal plains, *Coprinus* spp. is the primary contaminant in paddy straw mushroom beds (9). It is well recognized that chemicals have harmful impacts on both human health and the environment. In addition to these issues, persistent use of the same compounds might cause resistance to develop in contaminants. The growth of competitor moulds in mushroom beds can be checked by using different plant extracts (9, 10). These plant extracts offer a viable choice, as they are non-persistent in the environment and safer to use.

Keeping these points in view, an attempt was made to develop a suitable management practice by using plant extracts against the competitor moulds of *V. volvacea* in an eco- friendly manner.

Materials and Methods

Survey, isolation and purification of competitor moulds

During the straw mushroom growing season at CTMRT (Centre of Tropical Mushroom Research and Training), OUAT, a study was conducted to determine the prevalence of competitor moulds. Forty beds were prepared every month and examined for different types of mould problems. Each month, the data on contaminants were collected from the damaged beds in sterile Petri plates and then transferred to PDA plates under *in vitro* conditions. To obtain the pure culture, a single colony was separated from the PDA plate and then transferred to additional PDA plates. For preservation, pure cultures were stored in a refrigerator at 4 ºC. Identification of fungi was carried out through microscopic examination of morphological parameters.

Preparation of plant extracts

Various phytoextracts were used in this context to examine their respective impacts on competing moulds. Eight plant species from different families were tested for their antifungal properties (Table 1). Healthy, fresh leaves, rhizomes and cloves were collected from several suitable plants, thoroughly cleansed with sterile distilled water and then chopped into small pieces. A total of 100 g chopped plant products were homogenized with an equivalent volume of sterile distilled water. Each extract was crushed and then centrifuged at 4000 rpm after passing through three layers of sterile muslin cloth. The supernatant, after being filtered through Whatman No. 1 filter paper, was considered the stock solution. For a 10 % concentration 10 mL of stock solution was added to 90 mL of PDA medium. Similarly, for 15 % and 20 % concentrations, 15 mL and 20 mL of stock solution were added to 85 mL and 80 mL of basal medium, respectively (11).

In vitro antifungal study of phytoextracts against common contaminants of *V. volvacea*

Plant extracts were added to the basal media in varying amounts using the poison food technique (12). The media were autoclaved for 20 min at 15 psi for sterilization. Sterile Petri plates were aseptically filled with 20 mL of sterile molten basal media, which was then allowed to solidify. A control consisting of a Perti plate filled with molten basal media without any phytoextracts was also used, along with three replicates for each treatment. Each plate was inoculated with fungal discs of the same size (5 mm) and the plates were thereafter incubated in a BOD incubator at 30 ± 2 ℃. The growth rate in the contaminated media was measured six days after inoculation. The percentage of growth inhibition was calculated using the following method after noting the average colony diameters in every treatment along with the control.

Table 1. List of plants and their arts used in management of competitor moulds

Common name	Scientific name	Family	Plant parts used
Neem	*Azadirachta indica*	Meliaceae	Leaf
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome
Black turmeric	*Curcuma caesia*	Zingiberaceae	Rhizome
Tulsi	*Ocimum sanctum*	Lamiaceae	Leaf
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
Garlic	*Allium sativum*	Amaryllidaceae	Cloves
Marigold	*Tagetus erecta*	Asteraceae	Leaf
Karanja	*Pongamia pinnata*	Fabaceae	Leaf

$$\text{Percent inhibition} = \frac{dc - dt}{dc} \times 100 \qquad \text{(Eqn.1)}$$

dc = colony diameter of test fungus in control

dt = colony diameter of test fungus in treated plate

In vivo application of phytoextract on straw mushroom bed

Contamination during the growing season lowered straw mushroom yield, highlighting the need to test phytoextracts for controlling contaminants and improving harvests. The most effective extracts (20 %) were sprayed on substrates during bed preparation, with two applications at seven and nine days after spawning. Control beds received only water. Beds were monitored and maintained until maturity and data were recorded for fruit body number, average weight, pinhead emergence time, first harvest time and total yield over the three-week cropping period.

Statistical analysis

Experimental data were analysed and analysis of variance (ANOVA) was calculated statistically using a completely randomized design (CRD) for lab work and randomized block design (RBD) for field work with OPSTAT software. Inferences were made based on critical difference (CD) between the means at the 5 % level of significance.

Results

Prevalence of competitor moulds in straw mushroom beds

Since competitor moulds pose a major threat to straw mushroom cultivation, a study was carried out at CTMRT to determine their prevalence throughout the cropping season (Table 2, Fig. 1). The data revealed ten competitor moulds contaminating the straw mushroom beds during the fruiting stage. *Coprinus* spp. was the predominant species in all farming situations. Out of 40 beds, 30 beds were affected by *Coprinus* spp., 26 beds were affected by *Sclerotium* spp. and 25 by *Rhizopus* spp. *Coprinus* spp. caused higher bed contamination (75.00 %). The incidence of other contaminants viz., *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., *Sclerotium* spp., *Trichoderma* spp., *Fusarium* spp. and *Neurospora* spp. was in the range of 17.50 % to 65.00 %. It was observed that the appearance of *Coprinus* spp., *Sclerotium* spp. and *Rhizopus* spp., was more predominant in mushroom beds (Fig. 2).

Effect of phytoextract against *Trichoderma* spp.

At 10 % and 15 % concentrations, it was discovered that none of the plant extracts could totally block mycelial growth of *Trichoderma* spp. However, complete mycelial inhibition of *Trichoderma* spp. was observed by *A. indica, P. pinnata*, *Tagetes erecta* (marigold) and *Curcuma longa* (turmeric) at 20 % concentration (0 mm). *O. sanctum* treatment at 10 % and 15 % induced mycelial growth of 47.00 mm and 33.23 mm, respectively (Table 3). *A. indica* had the highest percentage of mean mycelial inhibition (93.69 %) followed by *T. erecta* (87.78 %). Even at 20 % dosage, *O. sanctum* (showed the least amount of mycelial inhibition (Fig. 3).

Effect of phytoextract against *Aspergillus flavus*

Radial mycelial growth of *A. flavus* was ranged from 11.40 mm to 75 mm at 15 % concentration (Table 4, Fig. 4). Radial mycelial growth

Table 2. Survey of common contaminants of straw mushroom bed

Name of the contaminants	Total number of beds observed	Number of beds found to be affected	Incidence (%)
Coprinus spp.	40	30	75.00
Rhizopus spp.	40	25	62.50
Sclerotium spp.	40	26	65.00
Trichoderma spp.	40	20	50.00
Penicilium spp.	40	9	22.50
Mucor spp.	40	18	45.00
Aspergillus flavus	40	11	27.50
Aspergillus niger	40	22	55.00
Neurospora spp.	40	10	25.00
Fusarium spp.	40	7	17.50

Fig. 1. Straw mushroom bed affected by common contaminants A, H. *Coprinus* spp.; B, I. *Sclerotium* spp.; C. *Aspergillus* spp.; D. *Rhizopus* spp.; E, G. *Trichoderma* spp.; F. *Neurospora* spp..

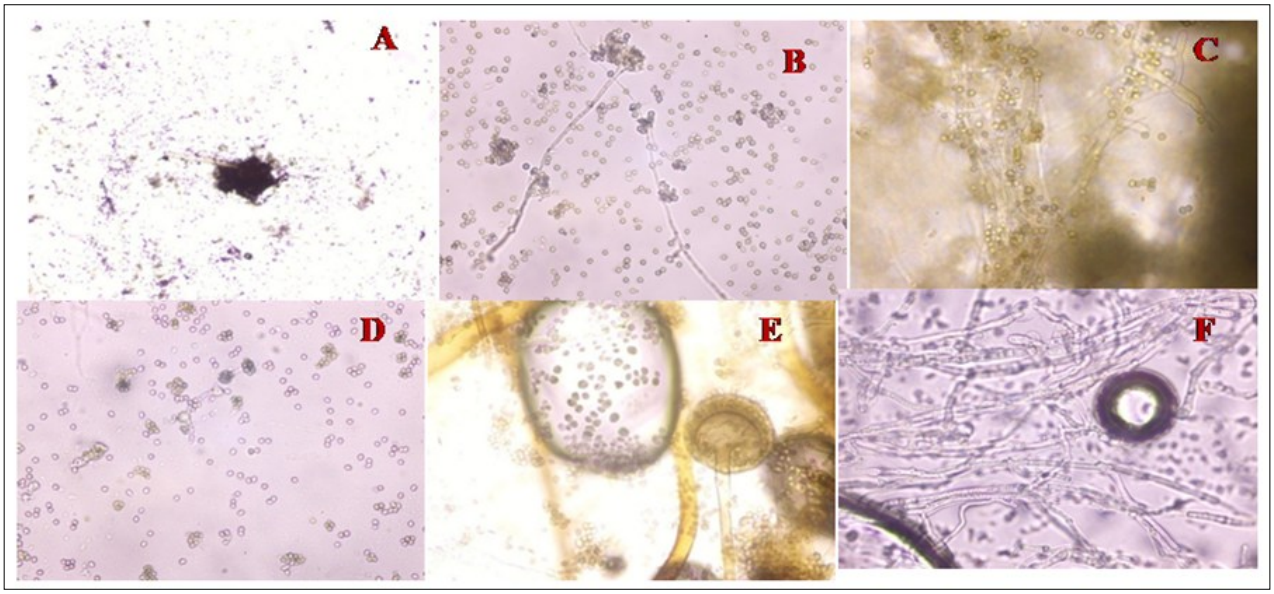

Fig. 2. Microphotograph of common contaminants found in straw mushroom bed, A. *Aspergillus niger*; B. *Penicillium* spp.; C. *Aspergillus flavus*; D. *Trichoderma* spp.;E. *Rhizopus* spp.; F. *Sclerotium* spp..

Table 3. Effect of phytoextract against *Trichoderma* spp.

Effect Treatments	Common name	Plant parts used	Radial mycelial growth (mm)			Mean inhibition (%)
			10 %	15 %	20 %*	
T_1	Neem	Leaf	13.00	4.00	0.00 (0.71)	93.69
T_2	Turmeric	Rhizome	25.00	13.10	0.00 (0.71)	85.91
T_3	Black turmeric	Rhizome	27.00	15.40	0.11 (0.78)	84.06
T_4	Tulsi	Leaf	47.00	33.23	20.52 (4.58)	61.11
T_5	Ginger	Rhizome	30.00	21.27	11.30 (3.44)	77.02
T_6	Garlic	Cloves	26.00	16.48	9.00 (3.08)	81.10
T_7	Marigold	Leaf	30.00	30.11	0.00 (0.71)	87.78
T_8	Karanja	Leaf	35.00	16.21	0.00 (0.71)	82.59
T_9	-	-	82.00	80.00	79.00 (8.92)	0
SE(m)±			**3.08**	**2.56**	**0.28**	**-**
CD (0.05)			**9.15**	**7.62**	**0.85**	**-**

T_1: *A. indica,* T_2: *C. longa*, T_3: *C. caesia*, T_4: *O. sanctum*, T_5: *Z. officinale*, T_6: *A. sativum*, T_7: *T. erecta*, T_8: *P. pinnata* and T_9: control. *Figures in the parentheses indicate corresponding $\sqrt{(x+0.5)}$ transformed value

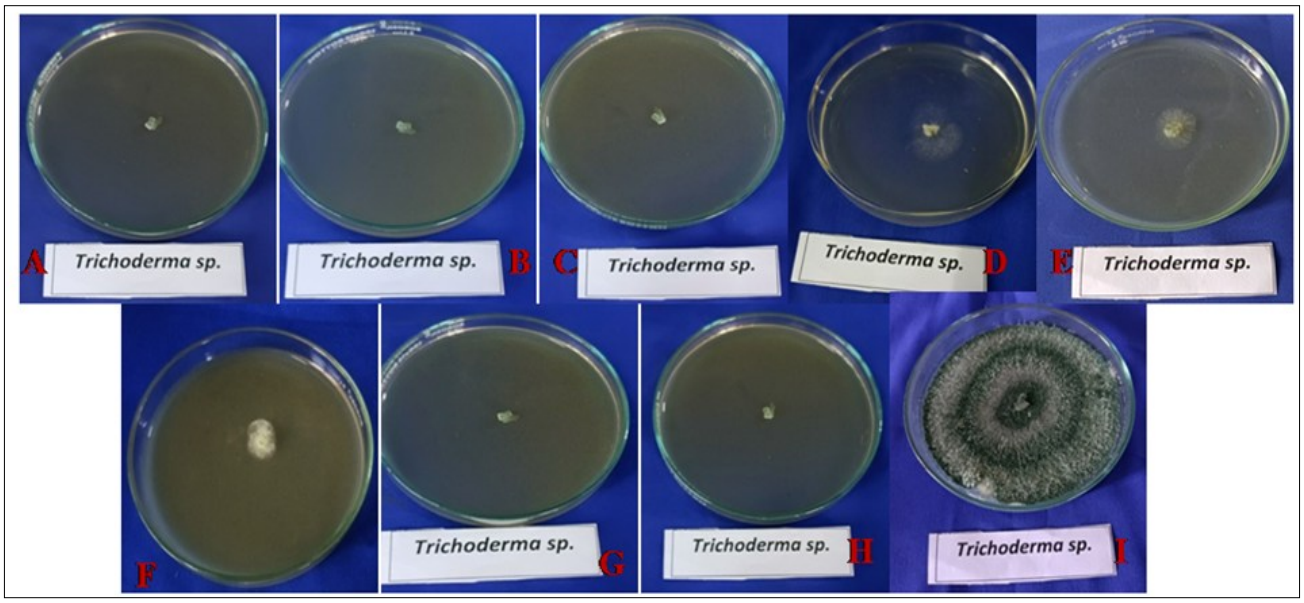

Fig. 3. Effect of phytoextract (20 %) against. *Trichoderma* spp., A. neem; B. turmeric; C. black turmeric; D. Tulsi; E. ginger; F. garlic; G. marigold; H. karanja; I. control.

Table 4. Effect of phytoextract against *Aspergillus flavus*

Treatments	Common name	Plant parts used	Radial mycelial growth (mm)			Mean inhibition (%)
			10 %	15 %	20 %*	
T_1	Neem	Leaf	30.16	22.80	14.00 (3.81)	66.09
T_2	Turmeric	Rhizome	23.00	11.40	0.00 (0.71)	79.93
T_3	Black turmeric	Rhizome	23.99	12.11	0.00 (0.71)	79.29
T_4	Tulsi	Leaf	48.89	29.71	27.82 (5.32)	46.02
T_5	Ginger	Rhizome	43.00	32.99	25.29 (5.08)	52.12
T_6	Garlic	Cloves	46.81	35.10	24.30 (4.98)	50.37
T_7	Marigold	Leaf	32.00	15.29	80.40 (8.99)	71.53
T_8	Karanja	Leaf	30.21	21.04	2.00 (1.58)	72.33
T_9	-	-	78.00	75.00	73.00 (8.57)	0.00
SE(m)±			**3.32**	**2.62**	**0.30**	
CD (0.05)			**9.87**	**7.80**	**0.90**	

T_1: *A. indica,* T_2: *C. longa*, T_3: *C. caesia*, T_4: *O. sanctum*, T_5: *Z. officinale*, T_6: *A. sativum*, T_7: *T. erecta*, T_8: *P. pinnata* and T_9: control. *Figures in the parentheses indicate corresponding $\sqrt{(x+0.5)}$ transformed value.

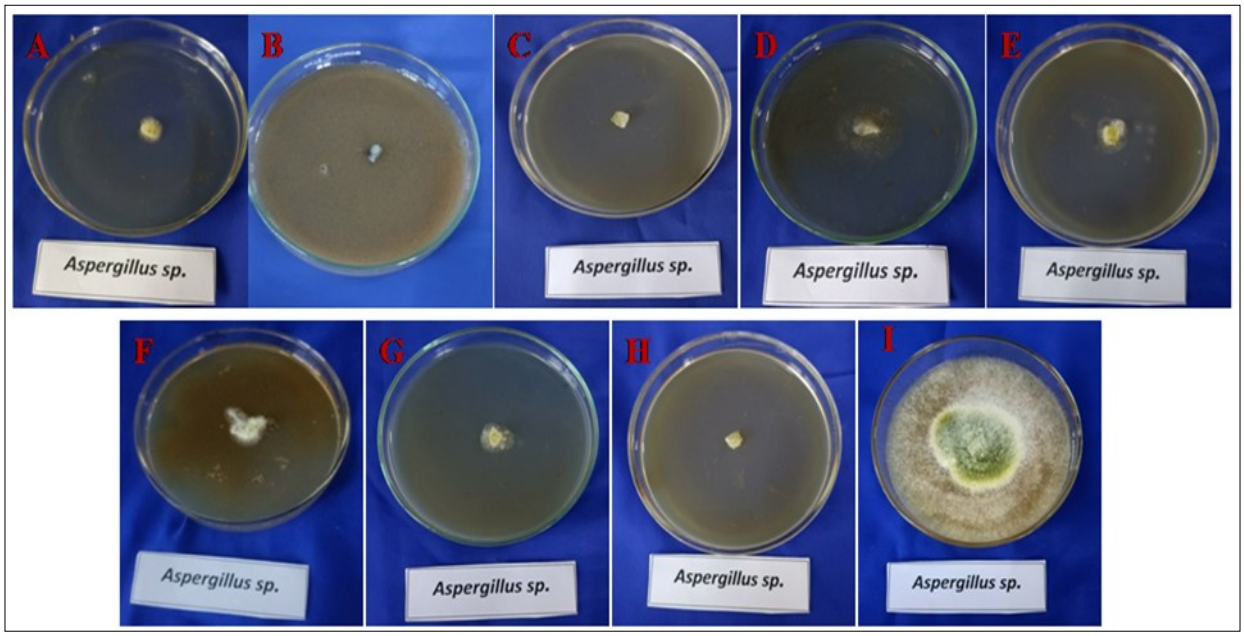

Fig. 4. Effect of phytoextract (20 %) against *Aspergillus flavus*; A. neem; B. turmeric; C. black turmeric; D. Tulsi; E. Ginger; F. garlic; G. marigold; H. karanja; I. control.

of these contaminants was 0 mm in *C. longa* and *C. cassia,* while values of 25.29 mm and 27.82 mm were observed *in Z. officinale, O. sanctum*, respectively. The maximum mean percentage of inhibition was observed at 79.93 % against *A. flavus* with treatment using *C. longa* at a 20 % concentration. The minimum mean percentage of inhibition, 46.02 % was obtained with phytoextract *O. sanctum*.

Effect of phytoextract against *Coprinus* spp.

Mycelium of *Coprinus* spp. was completely inhibited by *P. pinnata* and *A. indica* at 20 % concentration (Table 5, Fig. 5). The mean percentage of inhibition for *A. indica* at a 20 % concentration was 88.15 %. While *O. sanctum* had the lowest mean inhibition percentage (47.04 %). At 10 %, 15 % and 20 % concentrations, the radial mycelial growth of *Corpinus* spp. was measured to be 60.45 mm, 49.27 mm and 37.18 mm, respectively. This implies that *O. sanctum* was unable to prevent *Coprinus* spp. from growing. It may be due to the fact that bioactive compounds in *O. sanctum* are ineffective against fast-growing moulds such as *Coprinus*, *Trichoderma*, *Aspergillus* or *Rhizopus*, which are common competitors in mushroom beds. Moreover, many contaminant fungi produce enzymes (like laccases, oxidases) that can detoxify or tolerate the phytochemicals of *O. sanctum*, allowing them to continue growing despite its presence.

Effect of phytoextract against *Rhizopus* spp.

Mycelial inhibition of *Rhizopus* spp. was identified and presented below (Table 6 and Fig. 6). This result suggested that only *P. pinnata* at a 20 % concentration helped in the complete suppression *of Rhizopus* spp. However, the maximum mean percentage of mycelial inhibition was recorded as 75.30 % with the phytoextract of *A. indica. Rhizopus* spp. showed radial mycelial growth of 10.00 mm and 0.00 mm in response to *A. indica* and *P. pinnata* phytoextract at 20 %, respectively. The best phytoextracts against

Table 5. Effect of phytoextract against *Coprinus* spp.

Treatments	Common name	Plant parts used	Radial mycelial growth (mm)			Mean inhibition (%)
			10 %	15 %	20 %*	
T_1	Neem	Leaf	21.00	11.04	0.00 (0.71)	88.15
T_2	Turmeric	Rhizome	48.12	37.11	26.01 (5.15)	58.89
T_3	Black turmeric	Rhizome	49.25	38.35	26.03 (5.15)	58.15
T_4	Tulsi	Leaf	60.45	49.27	37.18 (6.14)	47.04
T_5	Ginger	Rhizome	48.34	40.06	35.09 (5.97)	54.45
T_6	Garlic	Cloves	56.91	40.00	27.22 (5.26)	58.15
T_7	Marigold	Leaf	46.45	31.67	25.24 (5.07)	63.33
T_8	Karanja	Leaf	26.38	16.33	0.00 (0.71)	84.44
T_9	-	-	79.00	78.00	78 (8.86)	0.00
SE(m)±			**3.96**	**3.27**	**0.27**	-
CD (0.05)			**11.79**	**9.71**	**0.82**	-

T_1: *A. indica,* T_2: *C. longa*, T_3: *C. caesia*, T_4: *O. sanctum*, T_5: *Z. officinale*, T_6: *A. sativum*, T_7: *T. erecta*, T_8: *P. pinnata* and T_9: control. *Figures in the parentheses indicate corresponding $\sqrt{(x+0.5)}$ transformed value.

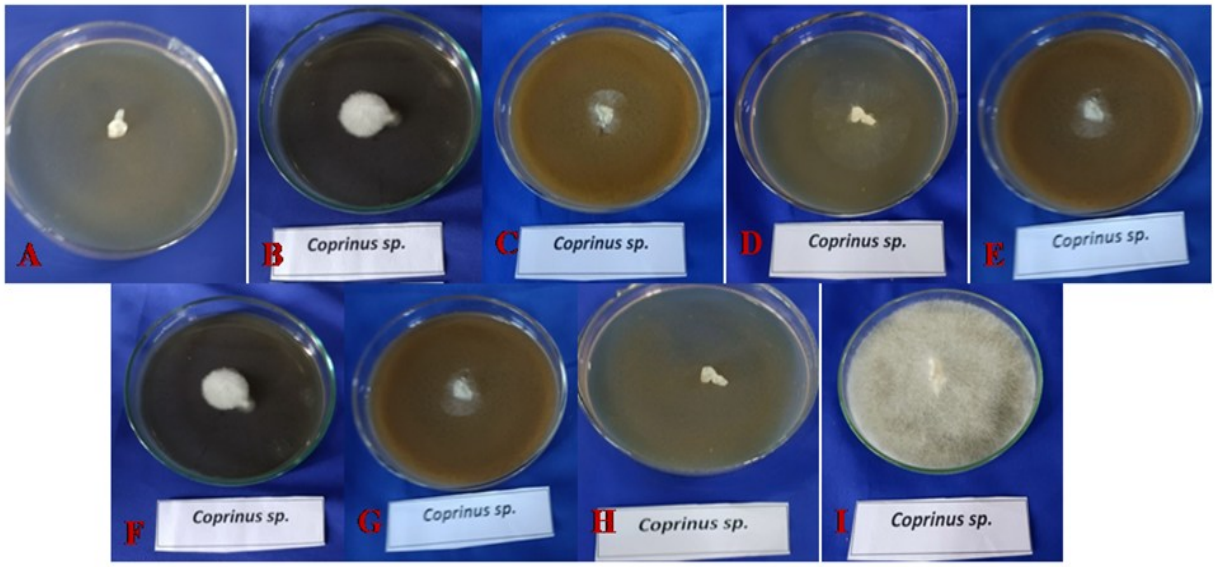

Fig. 5. Effect of phytoextract (20 %) against *Coprinus* spp.; A. neem; B. turmeric; C. black turmeric; D. Tulsi; E. ginger; F. garlic; G. marigold; H. karanja; I. control.

Table 6. Effect of phytoextract against *Rhizopus* spp.

Treatments	Common name	Plant parts used	Radial mycelial growth (mm)			Mean inhibition (%)
			10 %	15 %	20 %*	
T_1	Neem	Leaf	41.11	26.08	10 (3.24)	75.3
T_2	Turmeric	Rhizome	51.04	36.00	14.09 (3.82)	59.5
T_3	Black turmeric	Rhizome	50.01	35.12	13.05 (3.68)	60.7
T_4	Tulsi	Leaf	60.23	55.07	36.11 (6.05)	39.1
T_5	Ginger	Rhizome	56.64	43.60	35.09 (5.97)	45.9
T_6	Garlic	Cloves	60.21	54.35	34.71 (5.93)	39.9
T_7	Marigold	Leaf	48.18	33.06	29.99 (5.52)	67.7
T_8	Karanja	Leaf	44.85	34.12	0.00 (0.71)	72.1
T_9	-	-	86.00	82.00	80.00 (2.92)	0.0
SE(m)±			**4.40**	**3.66**	**0.26**	
CD (0.05)			**13.09**	**10.90**	**0.77**	

T_1: *A. indica,* T_2: *C. longa*, T_3: *C. caesia*, T_4: *O. sanctum*, T_5: *Z. officinale*, T_6: *A. sativum*, T_7: *T. erecta*, T_8: *P. pinnata* and T_9: control.

*Figures in the parentheses indicate corresponding $\sqrt{(x+0.5)}$ transformed value.

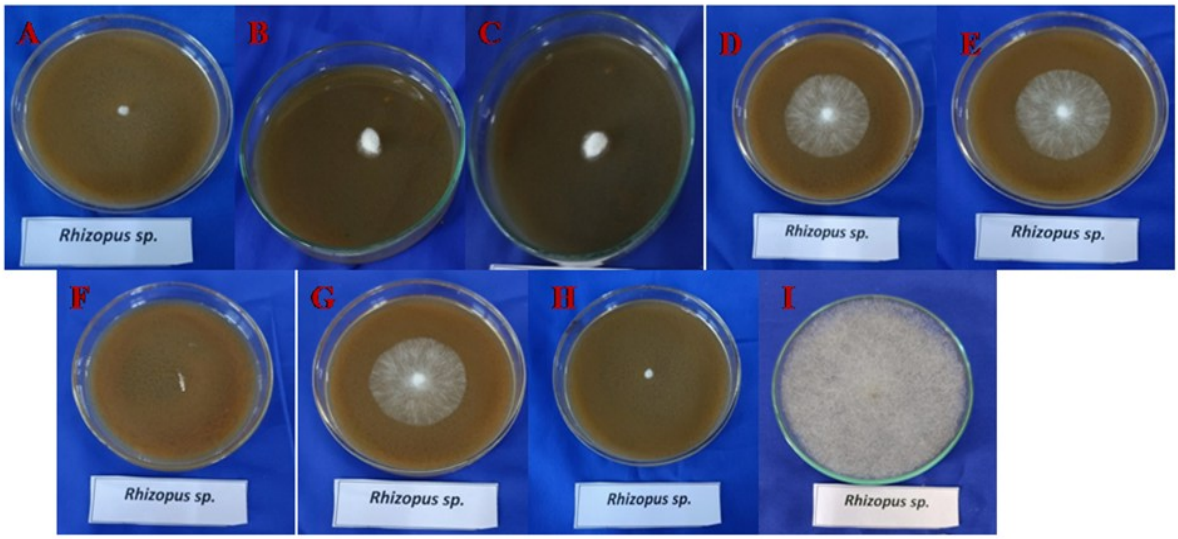

Fig. 6. Effect of phytoextract (20 %) against *Rhizopus* spp.; A. neem; B. turmeric; C. black turmeric; D. Tulsi; E. ginger; F. garlic; G. marigold; H. karanja; I. control.

Rhizopus spp. were discovered to be *A. indica* and *P. pinnata*.

Effect of phytoextract against *Sclerotium* spp.

The application of eight phytoextracts against *Sclerotium* spp. was documented, together with the mean percentage of inhibition and radial mycelial growth of these fungi. (Table 7, Fig. 7). At a 20 % concentration *C. longa*, *A. indica*, *C. caesia* and *P. pinnata* exhibit total suppression activity against *Sclerotium* spp.. Against these four phytoextracts, a 0.00 mm radial mycelia growth was observed. At a 20 %, turmeric and black turmeric recorded the highest inhibition, with mean values of 84.07 % and 83.70 %, respectively. In all eight phytoextract treatments, the radial mycelial growth of *Sclerotium* spp. at 10 % concentration ranged from 25.00 mm to 70.09 mm. *O. sanctum* phytoextract showed a 48.54 % mean inhibition rate against *Sclerotium* spp., while a 20 % *O. sanctum* dosage resulted in radial mycelial growth of 29.96 mm.

Effect of phytoextract against *Penicillium* spp.

The mean percentage of inhibition, along with the radial mycelial growth of *Penicillium* spp. against eight phytoextracts was assessed (Table 8, Fig. 8). Complete inhibition (0.00 mm growth) was observed at 20 % concentration with *A. indica*, *P. pinnata* , *C. longa*, *C. caesia* and *T. erecta*. None of the plant extracts could totally suppress *Penicillium* spp. mycelium at concentrations of 10 % and 15 %. When *A. indica* and *T. erecta* were used, the maximum mean percentage of inhibition of *Penicillium* spp. were 93.71 % and 87.78 %, respectively. The highest radial mycelial development was observed at *O. sanctum*: 47.05 mm at 10 % concentration, 32.18 mm at 15 % concentration and 26.00 mm at 20 % concentrations. However, a 20 % *A. indica* concentration resulted in the highest level of inhibition (93.71 %).

Bioefficacy of application of aqueous phytoextract of medicinal plants for management of competitor moulds and enhancing yield of *V. volvacea*

Selected phytoextracts, including *A. indica*, *P. pinnata*, *C. longa*, *C. caesia*, *Z. officinale*, *O. sanctum*, *Allium sativum* (garlic) and *T. erecta*, were evaluated *in vitro* and then tested *in vivo* on paddy

Table 7. Effect of phytoextract against *Sclerotium* spp.

Treatments	Common name	Plant parts used	Radial mycelial growth (mm)			Mean inhibition (%)
			10 %	15 %	20 %*	
T_1	Neem	Leaf	45.11	20.01	0.00 (0.71)	75.93
T_2	Turmeric	Rhizome	25.17	18.00	0.00 (0.71)	84.07
T_3	Black turmeric	Rhizome	25.00	18.04	0.00 (0.71)	83.70
T_4	Tulsi	Leaf	70.09	40.01	29.96 (5.52)	48.54
T_5	Ginger	Rhizome	48.05	33.34	23.41 (4.89)	61.48
T_6	Garlic	Cloves	62.30	29.18	24.22 (4.97)	57.41
T_7	Marigold	Leaf	31.17	17.06	9.13 (3.10)	78.89
T_8	Karanja	Leaf	32.06	18.00	0.00 (0.71)	81.48
T_9	-	-	85.00	83.00	80.00 (8.97)	0
SE(m)±			**3.90**	**2.85**	**0.25**	
CD (0.05)			**11.80**	**8.46**	**0.74**	

T_1: *A. indica,* T_2: *C. longa*, T_3: *C. caesia*, T_4: *O. sanctum*, T_5: *Z. officinale*, T_6: *A. sativum*, T_7: *T. erecta*, T_8: *P. pinnata* and T_9: control.

*Figures in the parentheses indicate corresponding $\sqrt{(x+0.5)}$ transformed value.

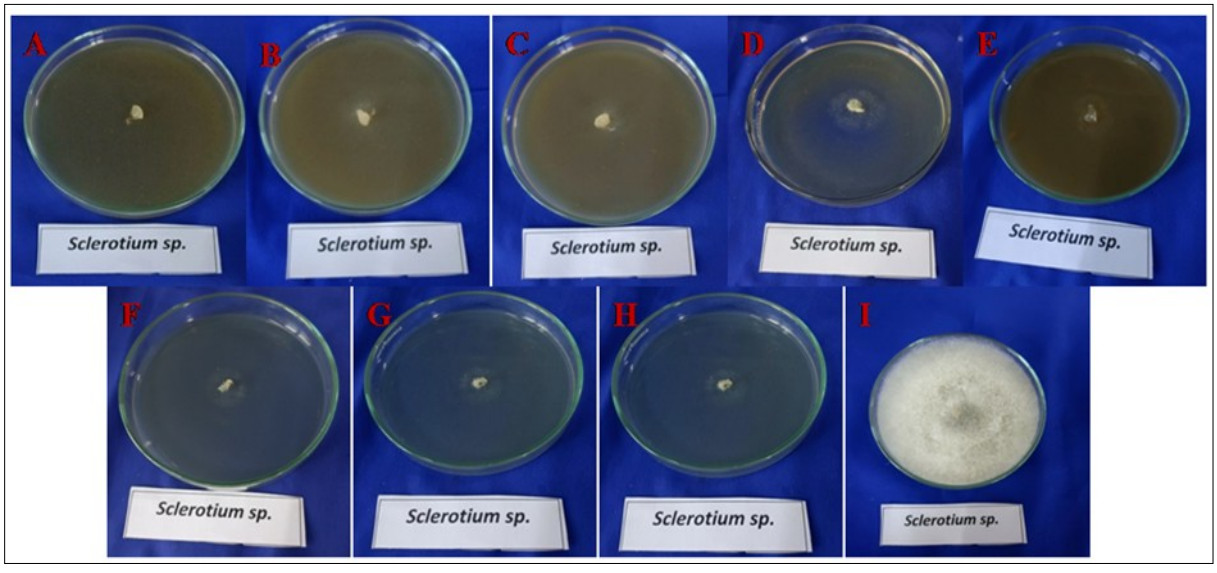

Fig. 7. Effect of phytoextract (20 %) against *Sclerotium* spp; A. neem; B. turmeric; C. black turmeric; D. Tulsi; E. ginger; F. garlic; G. marigold; H. karanja; I. control.

Table 8. Effect of phytoextract against *Penicillium* spp.

Treatments	Common name	Plant parts used	Radial mycelia growth (mm)			Mean inhibition (%)
			10 %	15 %	20 %*	
T_1	Neem	Leaf	11.05	4.00	0.00 (0.71)	93.71
T_2	Turmeric	Rhizome	23.4	12.11	0.00 (0.71)	87.04
T_3	Black turmeric	Rhizome	24.29	12.16	0.00 (0.71)	86.67
T_4	Tulsi	Leaf	47.05	32.18	26.00 (5.15)	61.11
T_5	Ginger	Rhizome	28.05	19.27	10.02 (3.24)	78.89
T_6	Garlic	Cloves	25.01	17.00	9.07 (3.09)	81.11
T_7	Marigold	Leaf	29.00	4.03	0.00 (0.71)	87.78
T_8	Karanja	Leaf	45.06	17.06	0.00 (0.71)	77.04
T_9	-	-	78.00	75.00	73.00 (8.57)	0.00
SE(m)±			**3.04**	**2.30**	**0.18**	
CD (0.05)			**9.05**	**6.85**	**0.54**	

T_1: *A. indica,* T_2: *C. longa*, T_3: *C. caesia*, T_4: *O. sanctum*, T_5: *Z. officinale*, T_6: *A. sativum*, T_7: *T. erecta*, T_8: *P. pinnata* and T_9: control.

*Figures in the parentheses indicate corresponding $\sqrt{(x+0.5)}$ transformed value

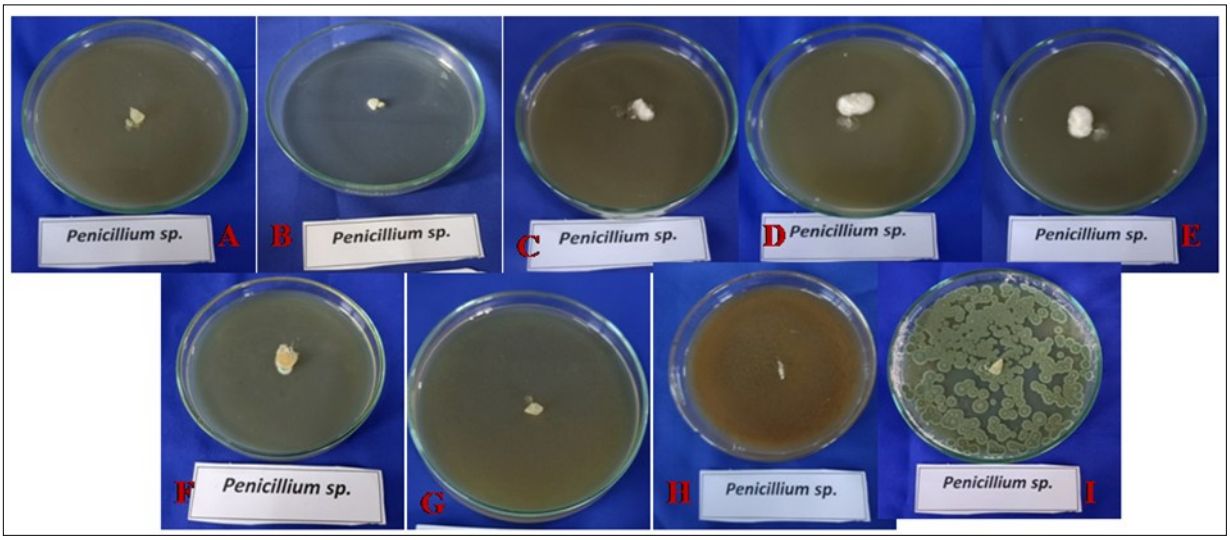

Fig. 8. Effect of phytoextract (20 %) against *Penicillium* spp; A. neem; B. turmeric; C. black turmeric; D. Tulsi; E. ginger; F. garlic; G. marigold; H. karanja; I. control.

straw mushroom beds to see how they affected the number of contaminants and the growth of mushrooms. Data on biological efficiency (%), average number of mushrooms, yield per bed (g), days to pin head formation, days to first harvest and incidence of pollutants were recorded during the experiment (Table 9, Fig. 9). Nine days after spawning, pinhead initiation was noted in a bed sprayed with black turmeric, karanja, ginger and neem. First harvest occurred between 13 and 14 days after spawning. The beds sprayed with *A. indica* leaf extracts produced the highest yield of mushrooms (1060.50 g/bed), followed by *P. pinnata* (1051.30 g/bed) at a 20 % concentration. *A. indica* and *P. pinnata* phytoextracts sprayed on beds yielded biological efficiencies of 15.15 % and 15.02 %, respectively. Moreover, *C. longa, C. caesia* and *T. erecta* also had higher biological efficiency than that of untreated beds. Percentage increases over normal practices of 11.39 % and 10.42 % were observed in bed sprayed with *A. indica* and *P. pinnata.* This trial demonstrated that neem, karanja, marigold, turmeric and black turmeric enhanced yields while inhibiting competitor moulds.

Discussion

The occurrence of competitor moulds and diseases has been observed at different stages of straw mushroom (*V. volvacea*) production and their incidence often leads to serious yield reduction and poor-quality harvests. Among the various factors influencing contamination, the initial condition of the substrate plays a crucial role. Pasteurization of substrates prior to bed preparation is generally recommended to minimize infection by competitor moulds; however, the hot and humid climatic conditions typical of mushroom-growing environments, coupled with improper bed management practices, create favorable conditions for the rapid proliferation of contaminants. These issues highlight the urgent need for developing sustainable, eco-friendly and non-chemical (organic) management strategies to improve the overall productivity of paddy straw mushrooms. With this objective, a detailed survey was undertaken during the cropping season of paddy straw mushrooms to document the diversity and extent of competitor mould infections.

Table 9. Bio-efficacy of application of aqueous phytoextract of medicinal plants for management of competitor moulds and enhancing yield of *V. volvacea*

Treatments	Doses (20 %)	Days of pinhead emergence	Days after 1st harvest	No. of sporophores	Avg. weight of fruiting body (g)	Yield (g)	Biological efficiency (%)	Percentage of increase over normal practices
T_1	20	9	14	77	50	1060.50	15.15	11.39
T_2	20	10	14	72	39	990.40	14.15	4.02
T_3	20	9	13	81	42	980.30	14.00	2.96
T_4	20	10	14	53	29	615.80	8.80	-35.32
T_5	20	9	13	75	32	656.70	9.38	-31.03
T_6	20	10	14	60	30	723.40	10.33	-24.02
T_7	20	10	14	68	41	1000.20	14.29	5.05
T_8	20	9	13	79	44	1051.30	15.02	10.42
T_9	-	10	14	74	36	952.10	13.60	0.00
SE(m)±		**0.04**	**0.33**	**0.72**	**0.54**	**10.13**		
CD(0.05)		**0.12**	**1.01**	**2.17**	**1.63**	**30.51**		**-**

T_1: *A. indica,* T_2: *C. longa*, T_3: *C. caesia*, T_4: *O. sanctum*, T_5: *Z. officinale*, T_6: *A. sativum*, T_7: *T. erecta*, T_8: *P. pinnata* and T_9: control.

Fig. 9. Yield of straw mushroom (VV-3 isolate) influenced by phytoextract (20 % doses).

The survey revealed the presence of several common fungal contaminants such as *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Coprinus* spp., *Sclerotium* spp. and *Rhizopus* spp. in the straw mushroom beds. Among these, a higher prevalence of *Coprinus* spp., *Sclerotium rolfsii* and *Aspergillus* spp. was consistently recorded, particularly in non-pasteurized substrates under conventional methods of cultivation. This observation is in agreement with previous findings reported by several workers (6, 13-15), who emphasized that unpasteurized substrates are highly vulnerable to infection. Additionally, the common occurrence of *Aspergillus* spp., *Coprinus* spp., *Rhizopus* spp. and *Sclerotium* spp. in straw mushroom beds has also been documented by other researchers confirming the widespread nature of these contaminants (16).

In the current study, comparative evaluations of different phytoextracts were carried out to assess their inhibitory effect on fungal contaminants. Interestingly, minimum mycelial inhibition and maximum percentage of contamination were observed in treatments involving *O. sanctum* extract, indicating its relatively poor efficacy against competitor moulds. In contrast, *A. indica* extract showed the highest inhibitory effect, particularly against *Coprinus* spp., *Penicillium* spp., *Sclerotium* spp. and *Trichoderma* spp. (17). Similar inhibitory activity of *A. indica* against mushroom contaminants has been previously reported by various workers, further validating its potential as a biocontrol agent (9, 18-21). The role of plant extracts in the management of mushroom contaminants has also been highlighted by earlier research, with significant emphasis placed on their antifungal efficacy and yield-enhancing properties (10, 18).

The yield-promoting effect of *A. indica* in mushroom cultivation was also recognized in earlier reports (18, 22). The observed increase in mushroom yield in neem-treated beds could be attributed not only to the nutrient-rich substrate (protein and carbohydrate content) but also to the antifungal and antibacterial properties of *A. indica*. The bioactive molecules present in neem, such as azadirachtin, limonoids and terpenoids, are believed to interfere with the release of ammonium during the early stages of fruiting body formation in contaminants like *Coprinus* spp., thereby restricting their growth and favoring the establishment of straw mushroom mycelia. Similarly, *P. pinnata* extract also exhibited remarkable anti-fungal activity. Its leaves and oil contain bioactive compounds such as karanjin, oleic acid, karanjic acid and their esters, along with karanj ketone and its oxime derivatives, all of which contribute to its antifungal properties. The excellent inhibitory responses of both *A. indica* and *P. pinnata* extracts against most of the common fungal contaminants in mushroom beds can be attributed to the combined detrimental effects of these antifungal molecules. Their application not only minimized the growth of competitor moulds but also contributed positively to mushroom production parameters, such as pinhead initiation (observed as early as 9 d) and biological efficiency (15.15 % and 15.02 %, respectively).

Conclusion

Plant extracts have shown significant potential as eco-friendly alternatives to chemical treatments in managing competitor moulds and diseases in straw mushroom cultivation. The extract of *A. indica* was reported to be excellent in inhibiting the mycelial growth of *Penicillium* spp., *Trichoderma* spp. and *Coprinus* spp. at a 20 % concentration. The phytoextract *C. longa* was found to be most effective in reducing the mycelial growth of two contaminants, such as *Sclerotium* spp. and *Aspergillus* spp., while *P. pinnata* was most effective against *Rhizopus* spp. *In vivo* application of these extracts also led to a yield increase of 11.39 % and 10.42 %, respectively over the control. These findings highlight the dual benefits of plant extracts in enhancing mushroom yield while reducing environmental and health risks associated with chemical use.

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Authors' contributions

SB, MKM, NC, AKS and PRM were involved in conceptualization of the research work including data curation, formal analysis, investigation and methodology development. They also contributed in visualization and writing of the research paper. BRS and UB carried out the statistical analysis and assisted in the editing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflicts of interests to declare.

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