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Evaluation of crude extracts of oyster and button mushroom, spent mushroom substrate of *Pleurotus ostreatus* and spent mushroom compost of *Agaricus bisporus* against major soil borne pathogens of chickpea

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Wilt, Collar rot and Dry root rot respectively caused by *Fusarium oxysporum* f. sp. *ciceri*, *Sclerotium rolfsii* and *Rhizoctonia bataticola* are major challenging soil borne diseases of chickpea in India. Antifungal efficacy of methanol and acetone extracts of oyster mushroom (*Pleurotus ostreatus*) and button mushroom (*Agaricus bisporus*), and water extract of spent mushroom substrate (SMS) of *P. ostreatus* and spent mushroom compost (SMC) of *A. bisporus* were evaluated against pathogenic fungus of wilt, collar rot and dry root rot disease in chickpea. Significant mycelial growth inhibition of all the tested pathogen was recorded from both mushroom crude and SMS extracts at three concentrations (5, 10 and 15 %). Significant highest mean per cent growth inhibition of *F. oxysporum* f. sp. *ciceri* (46.15, 39.7 and 35.36%), *S. rolfsii* (40.44, 36.21 and 31.29 %) and *R. bataticola* (43.92, 36.25 and 32.64 %) was recorded from methanol button mushroom extract, followed by methanol oyster and acetone oyster mushroom extract, respectively. Similarly water extract of SMS of both mushroom showed significant mycelial growth inhibition of all of three pathogens at 15% concentration. In this case SMC of button mushroom was superior than SMS of Oyster mushroom. In a pot experiment, significant disease reduction was also recorded from soil mix with different ratio of SMS of both mushrooms in comparison to soil without SMS. The lowest mean incidence of wilt (38.91 %), collar rot (40.30 %) and dry root rot (37.14 %) was recorded from treatment of 75 SMS: 25 Soil in comparison to soil without SMS (65.10, 80.25 and 56.85%). SMC of Button mushroom was comparatively superior in reducing incidence of all three disease than SMS of oyster mushroom. Hence, concluded that crude mushroom extracts and their SMS and SMC can be applied as an organic supplement to minimize inoculum load of these soil borne fungal pathogensin chickpea and other crop fields facing similar problem.

Keywords: *Agaricus bisporus, Pleurotus ostreatus,* SMS, SMC*, Fusarium oxysporum*f. sp.*ciceri, Rhizoctonia bataticola*, *Sclerotium rolfsii.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops of India. Its production is hampered by many diseases across the country. Among this, wilt, collar rot and dry root rot caused by *Fusarium oxysporum* f. sp*. ciceri, Sclerotium rolfsii, Rhizoctonia bataticola,* respectively are the major ones in the country (Dubey *et al*. 2010; Ghosh *et al.* 2013). Madhya Pradesh has been key state of chick pea cultivation and all these three diseases are prevalent in the states that influence production of chickpea seriously (Singh *et al.* 2022; Bankoliya *et al*. 2022). Due to soil borne nature of disease effective management is limited which argue the necessity to integrate different management practices.

Further, today's agriculture practices is focused on cost effective and sustainable management of diseases. Earlier studies have mainly focused on management of these devastating diseases using strategies such as change in sowing date, use of beneficial microorganisms, organic amendments, other chemicals, and fungicides. Disease can be managed by growing resistant varieties but this strategy is not always applicable

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because there are no complete resistant source against *F. oxysporum* f. sp. *ciceri*, *S.rolfsii* and *R. bataticola*. Due to soil borne nature of these pathogens chemical management is not practically applicable and not reported to be significant.

The edible mushrooms (*Agaricus bisporus* and *Pleurotus sajor-caju*) have high nutritional value, enhance the immune system, potential of host mediated response and may be act as an antimicrobial agent (Hawksworth, 2001, Thakur and Singh, 2020). Mushroom has medicinal property due to the presence of several antimicrobial compounds and are used to treat various diseases (Smith *et al.* 2002; Patel *et al*. 2012). Mushroom extracts were also found significant in reducing the intensity of disease as well as several pathogens. Eryngin, an antifungal peptide isolated from the fruiting bodies of *P. eryngii*, showed efficacy against *F. oxysporum* and *Mycosphaerella arachidicola* (Wang *et al*. 2004). Leftover mushroom substrate can also be utilized as mulch for controlling weeds in vegetables (Ozores- Hampton *et al.* 2001). The mycelia of mushrooms found in SMS are plentiful sources of elicitors, suggesting that applying spent mushroom substrate (SMS) to plants might help manage plant diseases (Shibuya and Minami 2001).The autoclaved SMS of *Lyophyllum decastes* (hatakeshimeji) and autoclaved water extract from SMS protected cucumber (*Cucumis sativus* L.) against anthracnose (*Colletotrichum orbiculare*) (Parada *et al.* 2011). In case of organic management mushroom extracts and SMS extracts can also be used as an organic amendment for management of soil borne diseases of chickpea. The use of spent mushroom substrate (SMS) and spent mushroom compost (SMC) in growing agricultural crop has been recognized in recent times as a possible means of enhancing substantiable agriculture (Barman *et al.* 2015). Spent mushroom compost (SMC) of *A. bisporus* has been demonstrated for management of root rot disease of mandarin (*Citrus reticlulata*) caused by *Fusarium oxysporum* (Barman *et al*. 2022). Considering all these facts antifungal activities of oyster and button mushroomswereevaluated *in-vitro* as well as SMS of *P. sajorcaju* and SMC of *A. bisporus*were evaluated in pot experiment against major soil borne pathogens of chickpea.

MATERIALS AND METHODS

Isolation of pathogen

The infected samples of wilt, collar rot and dry root rot were collected from the research field of J. N. K. V. V. Jabalpur. The pathogens were isolated on PDA medium by following standard method of isolation. It was purified by single spore, single sclerotia and hyphal tip techniques. The pathogens were identified based on their typical cultural characters (Sajeena *et al.* 2004; Amrate*et al.* 2019). The pathogenicity of the fungus was demonstrated using the soil inoculation method (Padmodaya and Reddy, 1996).

Mass multiplication of pathogen for pot experiment

Fusarium oxysporum f.sp.*ciceri* was mass multiplied in 250 ml conical flasks on sand maize meal medium (90 g sand, 10 g maize meal, and 40 ml water). The flasks were autoclaved at 15 psi for 20 mins. Two discs of 1.0 cm diameter mycelial growth from a three-day-old culture of *F. oxysporum* f. sp. *ciceri* grown on PDA were injected into the medium., The flasks were incubated at 28 ±2°C. Inoculum was applied to soil in pots at 100 g kg^{-1} after 3 weeks of incubation and chickpea were seeded at the same time as pathogen inoculation keeping an un-inoculated control. The plants were examined for signs of wilt. Each treatment was replicated thrice. Similarly, *Sclerotium rolfsii* inoculum was mass multiplied on sterilized sorghum which were soaked in water overnight. Sorghum grains weighing 250 g were packed into bags and sterilized in an autoclave. The sterilized mixture was infected with 7-day-old pathogen culture and allowed to grow for 25 days at 25°C. Sclerotia and fungal mycelium grew profusely and densely as a result. At 10 g/ kg soil, the inoculum was completely mixed in sterilized soil (sand + soil) (1:1). After inoculation, the soil was incubated for 15 days at room temperature. Seeds were surface sterilized with mercuric chloride 1:1000 for 30 seconds before being sown in pot. Chickpea seeds put in uninoculated sterile soil

served as a control in these pots, which were kept in their natural state.

Mass multiplication of *Rhizoctonia bataticola* was done on sorghum grain seeds which had been soaked overnight. The sorghum seed flask was autoclaved for 20 mins at 15 psi. The flasks were then inoculated with four 5.0 mm diameter mycelial growth discs from a three-day-old *R. bataticola* culture grown on PDA plate. The flasks were incubated for twenty-one days at 28 ±2°C. The inoculum was then combined with sterilized soil at a concentration of 10 g kg^{-1} soil. Chickpea seeds were seeded at the same time as pathogen inoculation, at a rate of 7 seeds per pot, with an uninoculated control. Root rot signs were seen on the plants. Each treatment was replicated thrice.

Preparation of crude mushroom extract

Fresh fruiting bodies of oyster and button mushrooms were dried in the shade, and the dried materials (50g) were crushed in a blender separately to get coarse powder, which was then immersed separately in 300 ml of methanol and acetone in Erlenmeyer flasks for methanol and acetone extracts. For extraction, the flasks were covered with aluminum foil and let to stand for 7 days. These extracts were filtered using Whatman filter paper no. 1 and evaporated using a rotary evaporator at 40°C (Jonathan and Fasidi, 2003; Bala kumar *et al.* 2011). The extracts were collected, and a conc.10 mg/ml stock solution was made.

Preparation of water extract from spent mushroom substrate

Extracts of spent mushroom substrate (SMS) of oyster and spent mushroom compost (SMC) of button mushroom weredone in water. The SMS/ SMC (300 g) were shaken at 150 rpm for 2 hrs with 900 ml distilled water. Following that, the mixture was filtered through two layers of Mira cloth (Calbiochem, La Jolla, CA, USA). The filter was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was utilized to make the water extract.

Evaluation of antimicrobial activity of crude mushroom extracts and SMS/SMC

The antimicrobial activity of *A. bisporus* and *P. ostreatus* extracts was tested using the poisoned food method (Nene *et al.* 2000). In pre-sterilized Petri plates, 20 mL of sterilized cooled growth medium (PDA) were poured. Following that, the required concentrations (5%, 10%, 15%) of all the methanol and acetone extracts were applied to the plates, which were then rotated clockwise and anticlockwise to achieve a uniform dispersion of the extract into the medium. No extract was used in the control plates. After the agar medium had set, 5mm disc of 7-day-old test fungal culture was aseptically deposited in the center of each plate. After that, the plates were incubated for 7 days at 28±2 °C. The experiment was repeated three times. The results were obtained in percentage of mycelial inhibition.

The antimicrobial activity of spent mushroom substrate extracts from *A. bisporus* and *P. ostreatus* were tested using the poison food technique (Nene *et al.* 2000). In pre-sterilized Petri plates, 20 mL of disinfected and cooled growth medium (PDA) were poured. After that, the plates were filled with the required concentrations (5 %, 10%, 15%) of all the extracts, and the plates were turned clockwise and anticlockwise to obtain a uniform dispersion of the extract into the medium. No extract was used in the control plates. After the agar medium had set, a little amount of 7-day-old test fungal culture was aseptically deposited in the center of each plate. Afterwards, the plates were then incubated for 7 days at 28±2 °C. The process was repeated three times. The results were obtained in percentage of mycelial inhibition colony growth in each treatment were recorded and compared with control full growth (90mm).

Fungal toxicity against *S. rolfsii, F. oxysporum* f. sp*. ciceri,* and *R. bataticola* were expressed as percentage of radial growth of test pathogens by the following formula (Vincent 1947):

$$I = \frac{(C-T)}{C} \times 100$$

Where,
I = percent growth inhibition,
C = colony diameter in control (mm),
T = colony diameter in respective treatment

Evaluation of SMS/SMC in pot experiment against soil borne diseases

Pot culture experiment was carried out for studying antagonistic activity of SMS/SMC against *Fusarium oxysporum* f. sp. *ciceri, Sclerotium rolfsii* and *Rhizoctonia bataticola.* Mass multiplied inocula of pathogens were mixed in pot soil. These were mixed with soil in different combination of T1 - 25 per cent SMS + 75 per cent soil, T2- 50 per cent SMS + 50 per cent soil, T3- 75 per cent SMS + 25 per cent soil and T4 - 0 per cent SMS + 100 per cent soil. Disease incidence in each trial was recorded till 45 days sowing. The separate trial was conducted for each treatment with the replication of three. Percent disease incidence was calculated based on plant died over total number of plants (Shitole *et al.* 2013).

RESULTS AND DISCUSSION

Efficacy of mushroom extracts

Results revealed that mushroom extracts of both fungi showed inhibitory effect on all the three-soil borne pathogen of chickpea.Percent growth inhibition was significantly reduced by mushroom extracts at 5%, 10%and 15% concentrations (Table 1). Against *F. oxysporum* f. sp. *ciceri.* at 5% percent, inhibition ranged between 19.73 % (AEBM-Acetone extract of button mushroom) to 34.83 %(MEBM- Methanol extract of button mushroom) like wise, it ranged from 29.47% in AEBM to 47.21% in MEBM at 10%. In case of 15 % concentration it ranged from 38.19% (AEBM) to 56.41% (MEBM). Overall mean revealed that the highest inhibition of growth percent inhibition of *F. oxysporum* f. sp. *ciceri* was recorded from MEBM (46.15%) followed by methanol oyster mushroom (39.76%) and acetone oyster mushroom (35.36%)(Fig.1).

In case of *S. rolfsii* the significant inhibition was also reported by mushroom extract in Table1. In 5% (Fig.1), concentration it was ranged 16.25% (AEBM) to 31.95% (MEBM). Similarly, it ranged from 20.72% (AEBM) to 39.29% (MEBM) at 10%. In case of 15 % concentration, it ranged from 34.61% to 50.08% in the Acetone and Methanol extracts. Overall mean revealed that the prominent inhibition of growth percent inhibition o*f S. rolfsii* was recorded from methanol extract of button mushroom (40.44%) followed by methanol extract of oyster mushroom (36.21%) and acetone extract of oyster mushroom (31.29%)(Fig.2).

The mushroom extracts also reported significant efficacy in inhibiting of *R. bataticola* through poison food technique(Table 1). In 5% concentration it ranged from 17.26% (AEBM) to 26.25% (MEOM) and it also ranged from 21.54% to 32.67% respectively at 10%. In case of 15% concentration, it ranged from 28.48%to 36.25% in the acetone and methanol extracts (Fig. 3). Overall mean revealed that the prominent inhibition of growth percent inhibition o*f R. bataticola* was recorded from MEBM (43.92%) followed by MEOM (36.25%) and AEOM (32.64%).In our finding mushroom extracts of *Pleurotus ostreatus* and *Agaricus bisporus* have shown antifungal activity of against major soil borne pathogen of chick pea. These of results maybedue to the fact that different mushroom species release various bioactive compounds such as terpenoids, flavonoids, tannins, alkaloids, and polysaccharides (Vamanu *et al.* 2018). Owaid *et al.* (2017) reported that *Pleurotus* sp. showed antifungal activity against *Trichoderma harzianum, Verticillium* sp. and *pythium* sp.

Antifungal efficacy of SMS extract

Percent growth inhibition of these three pathogens was recorded from SMS/SMC water extracts (Table 2).The growthof *F. oxysporum* f. sp. *ciceri* was significantly inhibited by SMS extracts of oyster and SMC extracts of button mushroom. Maximum growth was inhibited at 15% (41.85%) followed by 10% (27.19%) and 5% (22.36%). SMC of button mushroom was significantly superior in inhibition the growth of *F. oxysporum* f. sp. *cicero* as compared to SMS of oyster mushroom. SMS/ SMC extract of both mushrooms (oyster and button) dramatically reduced the growth of the *S. rolfsii*. Maximum growth was inhibited at 15% (19.87%) followed by 10% (14.27%) and 5% (11.86%) (Fig. 4). SMC of button mushroom was superior as compared to SMS of oyster mushroom in inhibiting the growth of *S. rolfsii*. *R. bataticola* development was also significantly

Table 1: Per cent mycelia growth inhibition of soil borne fungal pathogen of chickpea by organic solvent mushrooms extracts

Treatment details	*Fusarium oxysporum* f. sp. *ciceri*				*Sclerotium rolfsii*				*Rhizoctonia bataticola*			
	5%	10%	15%	Mean	5%	10%	15%	Mean	5%	10%	15%	Mean
Acetone extract of oyster mushroom	25.05	36.23	44.82	35.36	23.46	28.31	42.11	31.29	20.74	27.24	32.64	32.64
Methanol extract of oyster mushroom	28.12	41.53	49.63	39.76	27.18	36.18	45.27	36.21	26.25	32.67	36.25	36.25
Acetone extract of button mushroom	19.73	29.47	38.19	29.13	16.25	20.72	34.61	23.86	17.26	21.54	28.48	28.48
Methanol extract of button mushroom	34.83	47.21	56.41	46.15	31.95	39.29	50.08	40.44	22.31	29.75	35.32	43.92
Mean	26.93	38.61	47.26	-	24.71	31.12	43.02	-	31.14	38.37	43.41	-
CD(p=0.05)	A = 0.94, B = 1.08, A×B= NS				A = 1.49, B = 1.72, A×B= NS				A = 1.41, B = 1.63, A×B= 2.82			
SE(m)±	A = 0.32, B = 0.37, A×B= 0.64				A = 0.50, B = 0.58, A×B= 1.01				A = 0.48, B = 0.55, A×B= 0.96			

A= Concentrations, B= Treatment, A x B = Concentrations x Treatment

Table 2: Evaluation of antifungal activity of SMS of oyster and SMC of button mushroom extracts against major soil borne pathogens of chickpea

Concentration of extracts	Percent inhibition of mycelial growth								
	Fusarium oxysporum f. sp. *ciceri*		Mean	*Sclerotium rolfsii*		Mean	*Rhizoctonia bataticola*		Mean
	SMS oyster	SMC button		SMS oyster	SMC button		SMS oyster	SMC button	
5%	20.61	24.12	22.36	10.47	13.26	11.86	15.51	17.32	16.41
10%	26.61	27.76	27.19	14.18	14.37	14.27	19.96	21.76	20.86
15%	40.52	43.18	41.85	19.57	20.16	19.87	24.56	24.65	24.60
Mean	29.24	31.69		14.74	15.93		20.01	21.24	
CD(p=0.05)	A = 2.01, B = 2.46, A×B = NS			A = NS, B= 2.04, A×B = NS			A = NS, B = 2.07, A×B = NS		
SE(m)±	A = 0.67, B = 0.82, A×B = 1.16			A = 0.55, B = 0.68, A×B = 0.96			A = 0.56, B = 0.69, A×B = 0.97		

Table 3: Effect of SMS of oyster and SMC of button mushrooms on soil borne pathogens of chickpea under pot conditions

Treatments (SMS/SMC and Soil ratio)	Percent disease incidence								
	Sclerotium rolfsii.		Mean	*Fusarium oxysporum* f. sp. *ciceri*		Mean	*Rhizoctonia bataticola*		Mean
	SMS Oyster	SMC Button		SMS Oyster	SMC Button		SMS Oyster	SMC Button	
T1- 25:75 (SMS/SMC: Soil)	70.00	65.26	67.26	54.75	44.45	49.60	51.51	47.20	49.35
T2- 50:50 (SMS/SMC: Soil)	54.00	52.45	53.22	45.25	40.04	42.64	44.24	44.62	44.43
T3- 75:25 (SMS/SMC: Soil)	43.75	36.86	40.30	42.36	35.45	38.91	39.32	34.96	37.14
T4- 00:100 (SMS/SMC: Soil)	80	80.50	80.25	65.10	65.10	65.10	56.85	56.85	56.85
Mean	61.93	58.76		51.86	46.26		47.98	45.90	
CD(p = 0.05)	A =2.72, B = 3.85, A×B = NS			A =3.04, B = 4.30, A×B = NS			A = NS, B = 3.89, A×B = NS		
SE(m) ±	A =0.92, B = 1.30, A×B = 1.83			A =1.02, B = 1.45, A×B = 2.05			A = 0.92, B = 1.31, A×B = 1.85		

A = Mushrooms SMS/SMC, B = treatments, A x B = SMS/SMC x Concentrations

reduced by the extracts of oyster and button mushrooms. Maximum growth was inhibited at 15% (24.60%) followed by 10% (20.86%) and 5% (16.41%). Button mushroom SMC extract compared to oyster mushroom SMS extract was considerably more effective at inhibiting the growth of *R. bataticola.* In case of SMS our results revealed that water extract of spent mushroom substrate of button and oyster mushroom extracts showed potential in inhibiting the growth of *F. oxysporum* f. sp. *ciceri, S. rolfsii* and *R. bataticola*. Similar kind of result was also reported by Ocimati (2021 that spent substrate of *Pleurotus ostreatus* showed potential inhibition of *F. oxysporum* f. sp*. cubense* (Adedeji *et al.* 2016). It was observed that aqueous extract of spent mushroom substrate of *Pleurotus ostreatus* showed antifungal activity against *F. oxysporum* f. sp*. lycopersici.*

Efficacy of SMS and SMC in reducing disease

Results of pot experiment revealed that SMS of oyster and SMC of button mushrooms were significantly effective in reducing collar rot incidence of chickpea (Table 3). The lowest *S. rolfsii* incidence (43.75% and 36.86%) was recorded from 75 SMS/SMC: 25 Soil (T3) in both SMS of oyster and SMC of button mushroom respectively. In untreated (without SMS) the incidence was 80% and 80.50%. Overall mean incidence was lowest (40.30%) from 75 SMS/ SMC: 25 Soil (T3) followed by (53.23%) 50 SMS/ SMC: 50 Soil (T2) and (67.26%) 25 SMS/SMC: 75 Soil (T1) and untreated (T4) (80.25%). Similarly, the wilt incidence was also reduced by SMS of oyster and SMC of button mushrooms. The lowest *F. oxysporum* f. sp. *ciceri* incidence (42.36% and 35.45%) was recorded from T3 in both SMS of oyster and SMS of button mushroom respectively. In untreated (without SMS) the incidence was 65.10% and 65.10%. Overall mean incidence was lowest (38.91%) from T3 followed by (42.64%) T2 and (49.60%) T1 and highest in T4 (untreated -65.10%). Dry root rot was incidence significantly reduced by both the extracts. The lowest *R. bataticola* incidence (39.32% and 34.96%) was recorded from T3 in both SMS of oyster and SMC of button mushroom respectively. In untreated (without SMS) the incidence was 56.85% and 56.85%. Overall mean incidence was lowest (37.14%) from T3 followed by (44.43%) T2 ,(49.35%) T1 and untreated (56.85%) T4. Shitole (2013) showed that combination (75 per cent SMS + 25 per cent soil) was found effective, resulting in seed germination

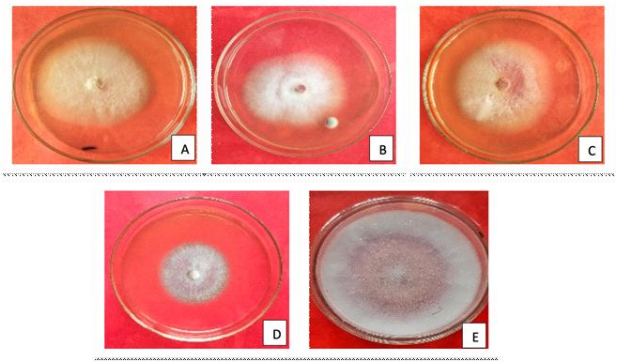

Fig.1 : Antifungal activity of organic solvent mushroom extracts against *Fusarium oxysporum* f. sp. *ciceri* at 15% concentration. A- Acetone extract of oyster mushroom; B-Methanol extract of oyster mushroom; C- Acetone extract of button mushroom. D- Methanol extract of button mushroom; E- Control

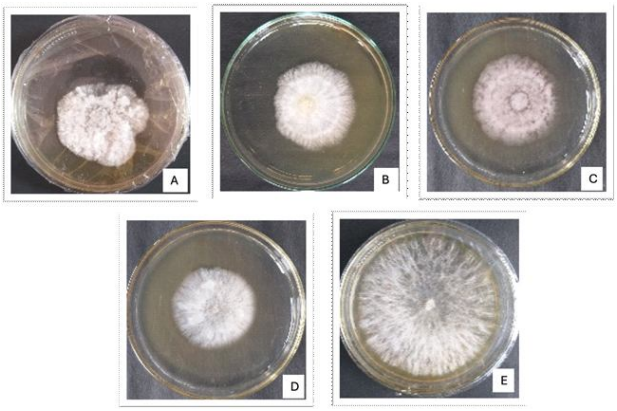

Fig. 2 : Antifungal activities of organic solvent mushroom extracts against Sclerotium rolfsii at 15 % concentration. A- Acetone extract of oyster mushroom; B-Methanol extract of oyster mushroom; C- Acetone extract of button mushroom. D- Methanol extract of button mushroom; E- Control

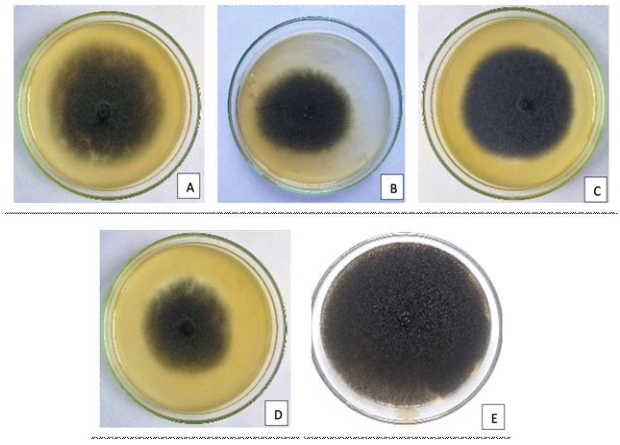

Fig.3 : Antifungal activities of organic solvent mushroom extracts against *Rhizoctonia bataticola* at 15 % concentration. A- Acetone extract of oyster mushroom; B-Methanol extract of oyster mushroom; C- Acetone extract of button mushroom . D- Methanol extract of button mushroom; E- Control

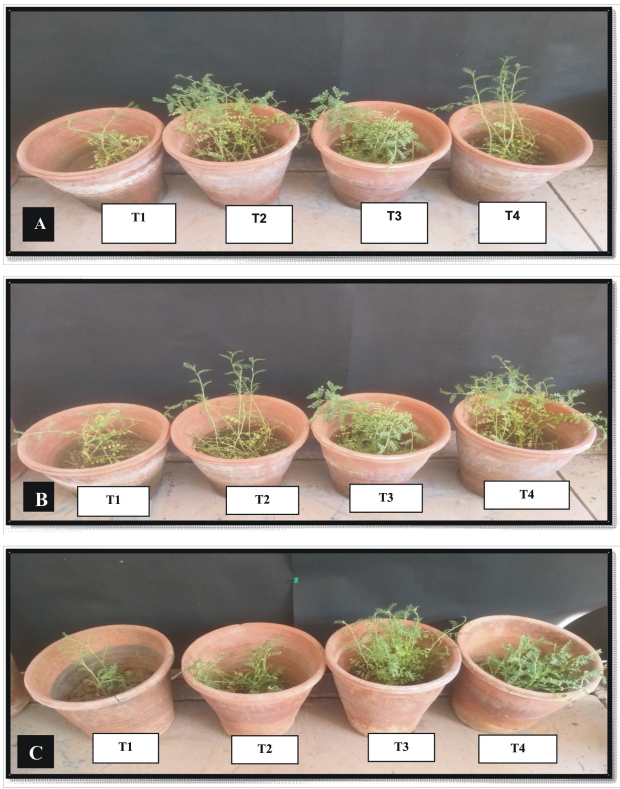

Fig. 4: Effect of SMC of button mushrooms on soil borne pathogens of chickpea under pot conditions(A) *S. rolfsii,* (B) *F. oxysporum* f. sp. *ciceri and* (C) *R. bataticola*

T1= 25 SMC + 75 soil, T= 50 SMC + 50 soil, T3= 75 SMC + 25 soil and T4 = 0 SMC + 100 soil

and minimum pre-emergence and post emergence per cent damping off disease of tomato. The efficiency of SMS treatments boosted plant growth and yield while also minimizing disease incidence caused by various fungi. Spent mushroom substrate (SMS) of *Pleurotus ostreatus* and its use as organic manure for improvement of *Capsicum chinense* has been documented (Roy *et al*.2015). Use of SMS of *Calocybe indica* has been demonstrated for growth improvement of leafy vegetables (Barman *et al.* 2015). Severity of root rot disease in *Citrus reticulata* caused by *F. oxysporum* was reduced by the treatment of SMC of *A. bisporus* in potted condition. Induced resistance in mandarin plants against *F. oxysporum* following SMC treatment of was confirmed by immunolocalization of defense enzymes both in root and leaf tissues (Barman *et al.* 2022). The spent mushroom substrate (SMS) of oyster and milky mushroom as well as spent mushroom compost (SMC) left after final crop harvest of button mushroom be utilized for agricultural

productivity . It can also overcome the problem of solid waste disposal in the mushroom industry.

DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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