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From Farm to Field: Spent Mushroom Substrate as Potential Anti-Fungal Agents and Plant Growth Enhancer

RACHMI YUNISAASTARI[1], IVAN PERMANA PUTRA[2*]

[1]*Microbiology Study Program, Biology Department, Faculty of Natural Sciences and Mathematics, Graduate School, IPB University, Bogor 16680, Indonesia*
[2]*Biology Department, Faculty of Mathematics and Natural Sciences, IPB University, IPB Darmaga Campus, Bogor 16680, Indonesia*

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Spent mushroom substrate (SMS) is a result of mushroom growing utilizing mushroom substrate media, such as substrate. Unhandled SMS garbage demands a subscription and poses an environmental risk. The SMS was obtained from oyster mushrooms (*Pleurotus ostreatus*) in Jonggol, Bogor, West Java, using a mixture of sawdust, lime, and bran. The purpose of this research was to examine the effects of using SMS as a planting medium and biofertilizer for the growth of maize (*Zea mays*), also on determining the optimum concentration of SMS as an anti-pathogen for the plant pathogens *Fusarium oxysporum* and *Colletotrichum gloeosporioides*. This research utilized a completely randomized design. The antifungal test results for SMS water extract against *Fusarium oxysporum* showed that the fungus was inhibited from growing at 60 to 100% extract concentrations. The use of SMS as a growing medium gave the best results on the growth of the number of plant leaves. Microscopic observation of the morphology of fungal pathogens *Fusarium oxysporum* and *Colletotrichum gloeosporioides* showed that there was damage to the structure of fungal hyphae in the treatment of SMS water extract with 100% extract concentration. SMS might offer an eco-friendly solution for enhancing agricultural productivity and promoting waste valorization in Indonesia.

Key words: *Colletotrichum gloeosporioides, Fusarium oxysporum, Pleurotus ostreatus*, Spent Mushroom Substrate (SMS)

INTRODUCTION

As the mushroom industry progresses, it generates a by-product known as spent mushroom substrate (SMS). The necessity to identify an appropriate method of disposal, the ensuing vast volumes of SMS which are now regarded as waste products present a significant problem to mushroom farmers. Furthermore, the management and disposal of SMS pose a significant environmental risk because of the greenhouse gas emissions from leachate drainage to water receptors that causes pollution and eutrophication, foul odours, and spontaneous anaerobic digestion, which frequently occurs in piles formed during temporary storage (Beyer 2011; Martin *et al.* 2023; Ma *et al.* 2025).

Fungal plant diseases show the ability to severely damage natural ecosystems as well as inflict significant ecological and financial losses on agricultural and forestry sectors (Fisher *et al.* 2012; Hyde *et al.* 2018). The agricultural with the biggest commodities, request, and food demands is maize and chili worldwide (Erenstein *et al.* 2022). Massive production of maize and chilies followed by the spread of pathogenic diseases that can lead to reduced yields (Zhan *et al.* 2022; Singh *et al.* 2025). *Fusarium* is a prevalent fungus which causes rots, wilts, cankers, and blights on plants (O'Donnell *et al.* 2018; Jayawardena *et al.* 2019). One of the best instances of a cryptic species in plant diseases is *Colletotrichum. Colletotrichum gloeosporioides* was thought to be a common tropical fruit disease causing anthracnose, and it has been associated with more than 400 different host genera (Cannon *et al.* 2012).

SMS waste can be used to manage plant diseases (Suess and Curtis 2006; Parada *et al.* 2011). As reported by Łopusiewicz (2018), button mushroom (*Agaricus bisporus*) medium waste has been reported to inhibit *Phytophthora drechsleri* caused seedling lodging disease in celery. The number of *Heterodera schachtii* cysts on sugar beet plants can be reduced by composting oyster mushroom waste made of *Pleurotus ostreatus* and *P. sajor-caju* (Palizi *et al.* 2009). *Ralstonia solanacearum* induced bacterial wilt disease in tomato plants can be inhibited by *Hericium erinaceus* fungal waste substrate (Kwak *et al.* 2015).

Waste mushroom growth medium has the potential to be utilised as fertilizer since it includes a variety of nutrients that plants require. The percentage of total nitrogen (N) in SMS trash is still 0.6%, in addition to

*Corresponding author:
E-mail: ivanpermanaputra@apps.ipb.ac.id

0.7% for phosphor (P), 0.2% for potassium (K), an 83 C/N ratio, and 49% C-organic (Kwak *et al.* 2017; Hunaepi *et al.* 2018). The National Standardisation Agency (2004) indicates that a minimum standard of compost quality must include a C/N ratio of 10–20, a C-organic 27–58%, a phosphor (P) of 0.1%, potassium (K) of 0.2%, and nitrogen (N) of up to 0.4%. Based on the nutrients that are still present, SMS waste can still be utilised as an organic fertilizer. The purpose of this study was to determine the optimal concentration of SMS as an antimicrobial for the plant pathogen *Fusarium oxysporum* and *Colletotrichum gloeosporioides* and to determine the effect of using SMS as a planting medium and biofertilizer for maize (*Zea mays*) growth.

MATERIALS AND METHODS

SMS (Spent Mushroom Substrate) Water Extract. The research was carried out from February to April 2024 at the Mycology Laboratory, Department of Biology, IPB University. The SMS was obtained from oyster mushroom cultivation (*P. ostreatus*) with the composition of sawdust, lime and bran from Jonggol, Bogor, West Java. The SMS was taken approximately 300 g, then mixed with 900 mL of distilled water by shaking at 150 rpm for 2 hours. The mixture was filtered through two layers using Whatman filter paper no 41. The filtrate was centrifuged for 20 min at 4,000 rpm, and the supernatant was used as SMS water extract following Kwak *et al.* (2015).

Antifungal Activity Assay. Antifungal activity was evaluated with agar-based disk diffusion method to determine various antifungals. SMS extract was made with different concentrations of 0% (negative control), 60%, 70%, 80%, 90% and 100%. *Fusarium oxysporum* and *Colletotrichum gloeosporioides* isolates were obtained from collection of Mycology Laboratory, IPB University. *Fusarium oxysporum* and *C. gloeosporioides* were taken using a sterile straw and then inoculated into a Petri dish at 3 cm at the right side of the dish. Commercially available discs (6 mm diameter) preloaded with SMS extract and then inoculated into a Petri dish in the left side (3 cm from edge) of the dish and incubated at 28°C for 3 days and the inhibition zone was measured (Kwak *et al.* 2015). The percentage of inhibition of pathogen growth was calculated using the following equation:

$$\text{Inhibition percentage} = \frac{\text{Control diameter} - \text{Treatment diameter}}{\text{Control diameter}} \times 100\%$$

Treatment of SMS on *Zea mays*. Seeds were germinated in cell trays containing compost soil. To determine the effect of SMS in soil on plants, seedlings were transplanted after seven days into plastic pots (9 cm) containing a mixture of SMS and compost soil (1:2, v/v). The same experiment was conducted using SMS autoclaved at 121 C for 30 minutes (ASMS). Seedlings were also planted in pots containing only compost as a control. All treatment were done for 4 weeks.

Effect of SMS Water Extract on The Fungal Morphological Structure. According to Putra *et al.* (2023), a 7-day-old mycelium with a diameter of 5 mm was put into a sterile cover glass that was placed over the PDA medium in a Petri dish. An amount of 100 μL of SMS water extract (WESMS100%, WESMS 90%, WESMS 80%, WESMS 70%, WESMS 60%, and WESMS 0%) was dripped onto the mycelial disk after a two-day incubation period. The cultures were kept in an incubator for five days at 27±3°C. Following incubation, the mycelial disk was observed using light microscope and stained with lactophenol cotton blue. Hyphae were observed and measured for width, and measurements of cytoplasmic density, clamp connection, and cell wall.

Statistical Analysis. The assay was replicated three times. All experiments were counted for height and number of leaves grown each day and the data were combined into an average value and presented in the same table or figure. Significant differences from control values were analysed using a one-way analysis of variance (ANOVA) and compared using Tukey's test at the 5% level.

RESULTS

Antifungal Activity. The inhibitory activity of SMS water extract showed inhibition on the growth of plant pathogens *F. oxysporum* and *C. gloeosporioides* (Figure 1). The application of SMS water extract to *F. oxysporum* showed the largest inhibition size value by SMS water extract with 100% concentration with 28.16% of inhibition. On the other hand, SMS water extract to *C. gloeosporioides* showed the biggest inhibition by giving SMS water extract on 70% concentration with 31% of inhibition (Table 1).

Treatment with SMS on Plants. A suitable ratio of SMS compost to organic fertilizer was determined to provide a biofertilizer for the growth of maize (*Zea mays*). The effects of SMS compost in combination with organic fertilizer at different levels

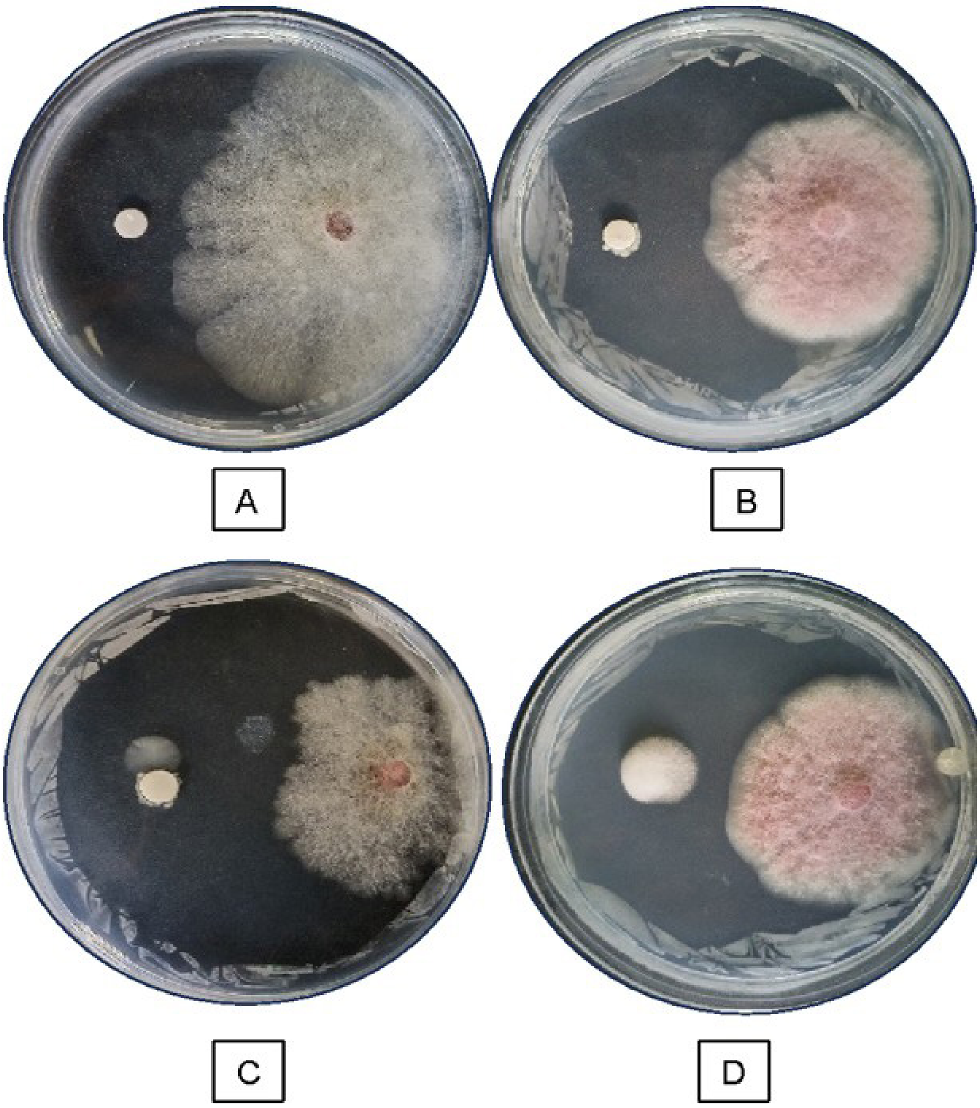

Figure 1. Antimicrobial activity of SMS extract against *Fusarium oxysporum* and *Colletotrichum gloeosporioides*: (A) 0% of SMS water extract on *C. gloeosporioides,* (B) 0% of SMS water extract on *F. oxysporum*, (C) inhibition and hyphal growth of *Pleurotus ostreatus* on discs treated with extract concentrations of 70% on *C. gloeosporioides*, (D) inhibition and hyphal growth of *P. ostreatus* on discs treated with extract concentrations of 60% and *Fusarium oxysporum*

Table 1. Antimicrobial activities from SMS water extract to *Fusarium oxysporum* and *Colletotrichum gloeosporoides*

Extract concentration (%)	% of inhibition	
	Fusarium oxysporum	*Colletotrichum gloeosporioides*
60	5.1	0.0
70	8.8	31.0
80	22.5	8.9
90	23.5	0.0
100	28.16	7.0

were evaluated on maize. The treatment of maize planting on planting media containing compost soil as a control gave the best results in plant height growth variables compared to the treatment of planting media combined with ASMS and those combined with SMS (Figure 2). The planting media which combined with ASMS and those combined with SMS showed the best results in the number of leaves possessed by maize plants (Table 2). The treatment of planting media with ASMS and SMS was carried out without additional fertiliser so that the effect of SMS planting media could be clearly seen.

Effect of SMS Water Extract on the Morphological Structure of Fungal Pathogen. The morphological structure characteristics of hyphae of the plant pathogens *F. oxysporum* and *C. gloeosporioides* treated with SMS water extract with various concentrations observed under a light microscope showed alteration in hyphal structure (Figure 3). The hyphal structure of *F. oxysporum* and *C. gloeosporioides* were damaged by the treatment of SMS water extract (Table 3). The application of distilled water (0% of SMS water extract) to the hyphae of *F. oxysporum* and *C. gloeosporioides* used

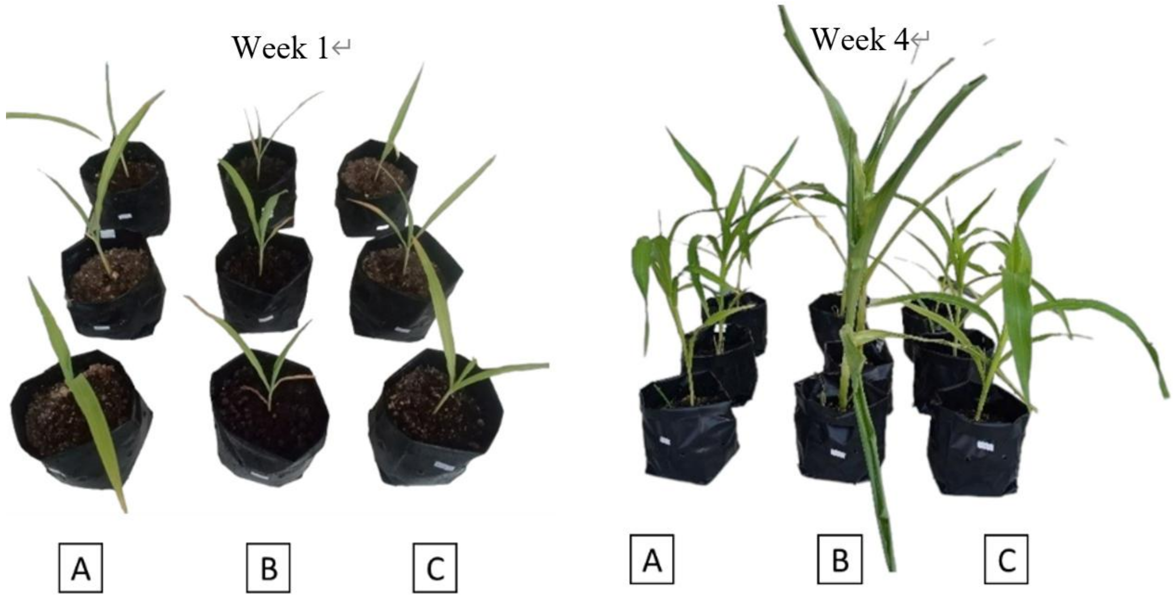

Figure 2. Treatment of spent mushroom substrate (SMS) and autoclaved spent mushroom substrate (ASMS) (three replications) on maize plant growth: (A) SMS treatment, (B) control treatment (0% SMS water extract), (C) ASMS treatment

Table 2. Measurements of maize plants treated with spent mushroom substrate extract

Treatment	Number of leaves	Leaf length (cm)	Leaf height (cm)
Control M1	3.00±0.000[a]	13.06±1.066[a]	22.20±1.606[a]
ASMS M1	3.00±0.000[a]	14.76±1.530[ab]	22.33±0.189[a]
SMS M1	3.00±0.000[a]	18.30±1.725[bc]	25.26±2.068[ab]
Control M4	4.67±0.471[b]	19.03±0.850[c]	28.67±4.143[b]
ASMS M4	6.67±1.247[c]	19.40±0.163[c]	30.00±1.414[b]
SMS M4	6.67±0.471[c]	33.05±3.058[d]	41.50±4.190[c]

as a control showed no damage on hyphal structure and its septate (Figure 3A and B). Concentration of 100% SMS water extract showed a change in the hyphal structure *F. oxysporum* which was seen that the hyphae had lost their structure (Figure 3D). Giving 70% concentration of SMS water extract on *C. gloeosporioides* hyphae experienced changes in the structure of hyphae that were destroyed (Figure 3C).

DISCUSSION

In the current study, we found that SMS of the oyster mushroom wastes inhibit the fungal pathogen which in line to previous study worldwide. Notably, the extract exhibited a concentration-dependent response, with *F. oxysporum* showing the highest inhibition (28.16%) at full strength (100% concentration), suggesting that higher extract concentrations may be necessary to suppress this pathogen effectively. Ahlawat and Sagar (2007), reported that oyster mushroom waste has a higher microbial density than straw mushroom (*Volvariella volvaceae*) waste which might pose a role as pathogen-opposing bacteria. In the antifungal test using discs treated with SMS water extract, there was hyphal growth on the discs after 7 days incubation (Figure 1C and D). Hyphal growth on the discs is known to occur because the SMS water extract still contains spores that have escaped from the filtration. This is attributed to the extract being sourced from the residual substrate used in cultivating oyster mushrooms. Fungal spores are generally not considered the principal agents responsible for pathogen inhibition. Instead, the antimicrobial efficacy of oyster mushrooms is primarily attributed to secondary metabolites synthesized by the actively growing mycelium and fruiting bodies, including phenolic compounds, terpenoids, and polysaccharides (Fogarasi *et al.* 2024). These bioactive constituents exhibit direct antifungal and antibacterial properties (Fogarasi *et al.* 2024). Nevertheless, under favourable conditions, residual spores may germinate and form vegetative mycelium (Feofilova *et al.* 2011), which could contribute indirectly to pathogen suppression through competitive substrate colonization and secretion of antimicrobial compounds. In the context of SMS water extract, we suggest that any spores that escape filtration may germinate and exert supplementary antifungal effects.

The application of SMS compost in maize revealed differential effects on plant growth parameters depending on the treatment composition in the current study. The media supplemented with ASMS and SMS showed enhanced leaf development, suggesting a

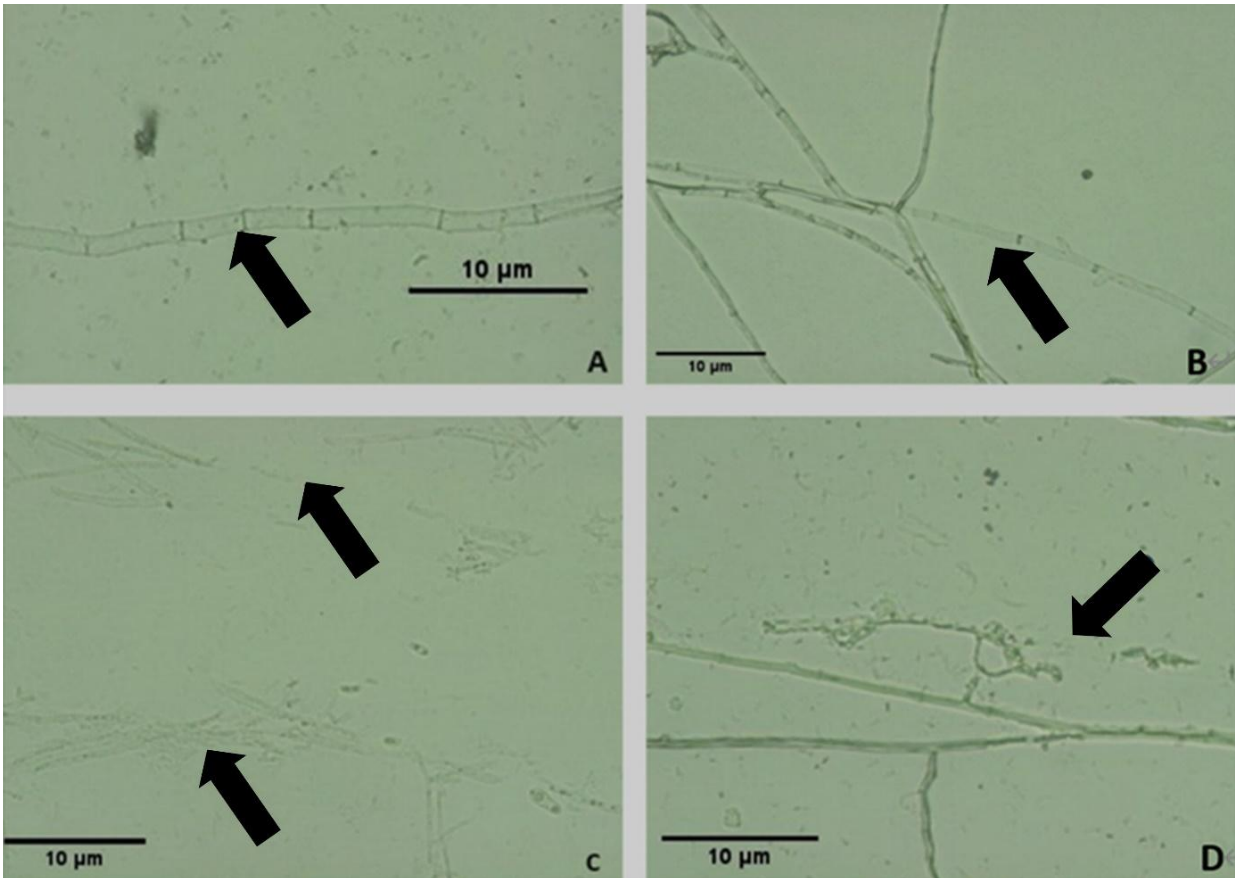

Figure 3. Effect of SMS water extract on fungal hyphae of *Fusarium oxysporum* and *Colletotrichum gloeosporioides*: (A) 0% of SMS water extract on *C.gloeosporioides* indicating no hyphal damages, (B) 0% of SMS water extract on *F. oxysporum* indicating no hyphal damages, (C) hyphal damages at 70% SMS water extract concentration on *C. gloeosporioides*, (D) hyphal damage at 100% SMS water extract concentration on *F. oxysporum*

Table 3. Effect of spent mushroom substrate water extract on hyphal structure changes of pathogens *Fusarium oxysporum* and *Colletotrichum gloeosporioides*

Extract concentration (mL)	Hyphal structure damaged	
	Fusarium oxysporum	*Colletotrichum gloeosporioides*
Control	-	-
60%	-	-
70%	-	+
80%	+	-
90%	+	-
100%	+	+

(-): no damage, (+): damage

positive influence on vegetative biomass accumulation. Previously, Ahlawat and Sagar (2007) discovered that composting SMS increased hardness and ascorbic acid concentration and increased fruit weight, ascorbic acid content, dry matter, total soluble solids, and acidity of plant. Composted SMS has demonstrated numerous benefits in enhancing crop output and quality, making it an excellent growing medium for field crops and vegetables (Zhao and Zhang 2023; Mwangi *et al.* 2024). According to Köninger *et al.* (2021), organic manure activates a wide variety of living species, causing them to emit phytohormones that may promote plant growth and nutrient absorption. Because organic inputs contain substantially less nutrients than inorganic fertilizers, they cannot cover the nutritional needs of crops on their own. In order to improve crop output, it is necessary to combine the two types of inputs. When combined with deficient garden soil, SMS compost of *Pleurotus pulmonarius* generally improved all the growth characteristics evaluated (Jonathan *et al.* 2014; Muchena *et al.* 2021). Notably, the treatments incorporating ASMS and SMS in this study were applied in the absence of supplemental fertilizer inputs, thereby enabling a more accurate evaluation of the inherent biofertilizer properties of SMS in the further research.

In the current study, several hyphal alteration phenomena of fungal pathogens can be observed after treated with SMS water extract. Compared to the control group treated with distilled water, which maintained intact hyphal and septate structures, exposure to SMS extract at varying concentrations resulted in visible hyphal damage. Changes in hyphal structure may occur due to changes in cytoplasmic density as a response of the fungus to the changed environmental conditions or secondary metabolites due to the application of SMS water extract (Ishihara *et al.* 2018; Ocimati *et al.* 2021). Since hydrostatic pressure, also known as turgor, is isotropic, the force acting on it is uniform in all directions. Actin cytoskeletal disruption in growing hyphae leads to isotropic swelling of hyphal tips, a clear indication of the isotropic type of turgor (Bronkhorst *et al.* 2022). Variations in density can have an impact on macromolecular concentrations as well as the physical characteristics of the cytoplasm, including viscosity levels. Therefore, a number of cellular processes, including phase transitions, enzyme transport and protein interactions, can be significantly affected by these changes (Molines *et al.* 2022). Our findings suggest that bioactive compounds present in SMS extract compromise fungal hyphae, and might has potential role as a natural antifungal agent.

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