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Optimization, Characterization, & *In vitro* Evaluation of Spent Mushroom-Based Bio-fungicide for Tomato (*Solanum lycopersicon L.*) Disease Management

Mary Amor G. Figueroa [a++*], Romel R. Sajorga, Jr. [a#], Rowena M. Cahagna-an [a#], Rosemarie P. Caser [a#] and Reymon Z. Ybañez [a#]

[a] Surigao del Norte State University – Mainit Campus, Magpayang, Mainit, Surigao del Norte, Philippines.

Authors' contributions

This work was carried out in collaboration among all authors. Author MAGF spearheaded the design and overall management of the project, including the statistical analysis, interpretation of results, and finalization of the manuscript. Author RRSJ contributed to data analysis and prepared the initial draft of the manuscript, with support from other research assistants. Authors RMC and RPC conducted the In vitro bioassay of selected pathogenic fungi. Author RZY performed microbial population testing. All authors read and approved the final manuscript.

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[++] *Faculty Researcher;*
[#] *Research Assistants;*
**Corresponding author: Email: mafigueroa03@gmail.com;*

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ABSTRACT

Aims: This study evaluated the potential of spent mushroom substrate (SMS) as an organic bio-fungicide for managing tomato diseases effectively while promoting sustainable agricultural practices.

Study Design: Optimization tests utilized ANOVA ($p \leq 0.05$) to assess the effects of fermentation periods and SMS-to-water ratios on the bio-fungicide's effectiveness. Significant differences among treatments were analyzed using LSD, identifying optimal preparation conditions. A T-test compared the physico-chemical properties of fermented and non-fermented SMS-based bio-fungicides. Additionally, *In vitro* efficacy against various fungal diseases of tomato was evaluated using ANOVA and Tukey's HSD test for precise treatment comparisons.

Place and Duration of Study: The study was conducted at the Mushroom and Crop Protection Laboratory, Surigao del Norte State University (SNSU) – Mainit Campus, Magpayang, Mainit, Surigao del Norte, Philippines.

Methodology: Fermentation periods (2, 4, and 7 days) and SMS-to-water ratios (0.5 kg, 1.0 kg, and 2.0 kg per 20 L) were evaluated to optimize beneficial microbial growth. Physico-chemical properties of both fermented and non-fermented SMS-based bio-fungicides were analyzed to determine nutrient composition and microbial activity. *In vitro* efficacy trials assessed the bio-fungicide's ability to control soil-borne, foliar, and post-harvest tomato diseases.

Results: ANOVA and LSD analyses identified 1.0 kg SMS per 20 L water fermented for 7 days as the optimal formulation, achieving the highest microbial population (41,666.67 CFU/mL) dominated by *Trichoderma* spp., *Penicillium* spp., actinomycetes, and *Bacillus* spp. Fermentation significantly enhanced the nutrient profile, increasing levels of P, Ca, Mg, Fe, and Cu, while reducing N, Zn, and Mn, thereby optimizing microbial activity and nutrient availability. *In vitro* assays demonstrated superior efficacy of fermented SMS bio-fungicides at 20 mL/100 mL water in managing tomato diseases, significantly outperforming non-fermented formulations.

Conclusion: This study highlights SMS as a sustainable and eco-friendly bio-fungicide, aligning with circular economy principles in mushroom production. Its effectiveness in controlling tomato diseases underscores its potential as an innovative solution for sustainable agriculture. Further field validation and application to other crops are recommended to maximize its broader utility.

Keywords: Bio-fungicide; diseases; fermentation; spent mushroom substrate; tomato.

1. INTRODUCTION

Tomato (*Solanum lycopersicon* L.) is one of the most important vegetables cultivated worldwide for commercial purposes. Belonging to the Solanaceae family, commonly known as the nightshade family, the tomato shares its lineage with over 3,000 species, including eggplant (aubergine), peppers, tobacco, and potatoes. Globally, tomatoes rank as the second most widely cultivated and consumed non-starchy vegetable, surpassed only by potatoes. Annually, approximately 159 million tons of tomatoes are produced worldwide. In recent years, tomatoes have gained significant importance in human diets, with consumption steadily increasing (Padmanabhan et al., 2016). They are an excellent dietary source of antioxidants, as well as a rich supply of essential vitamins, minerals, and dietary fiber. Beyond their nutritional value, tomatoes serve as a model organism in scientific research, particularly in studies on functional genomics and fruit development (Giovanni, 2004).

Tomatoes are the second most important vegetable crop globally, with production reaching approximately 170 million metric tons in 2014, led by major producers such as China, the USA, India, and Turkey (FAOSTAT, 2017). In the Philippine context, tomatoes are also a crucial crop, particularly in the country's agriculture industry, contributing to local food systems and the economy (Department of Agriculture, 2021). However, similar to global challenges, the yield and quality of tomatoes in the Philippines are significantly impacted by pathogens that affect the crop both in the field and during post-harvest processing (Wyenandt & Nitzsche, 2020). Filipino tomato farmers often face these challenges, compounded by limited access to advanced pest management technologies and rising concerns over chemical pesticide residues.

Just like tomatoes, other essential crops in the Philippines, such as bananas, also face similar challenges with diseases like crown rot, caused by *Colletotrichum* and *Fusarium* species, which lead to substantial economic losses for local

farmers. These diseases impact both smallholder and commercial producers, further stressing the need for sustainable and effective disease management strategies (Trevorrow, 2018).

Historically, chemical pesticides have been the primary solution for managing crop diseases in the Philippines. While effective, their widespread use has led to environmental concerns, including soil degradation, nutrient depletion, and contamination of water resources. These negative impacts have become a growing concern, especially in rural areas where agricultural practices rely heavily on chemicals (United Nations Environment Programme, 2022). This scenario highlights the need for sustainable agricultural practices that minimize harm to the environment and protect the long-term health of the land.

Recognizing the importance of sustainable practices, there is an increasing demand for environmentally friendly alternatives to chemical pesticides in the Philippines. The shift towards such practices aligns with the United Nations Sustainable Development Goals, particularly SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land), which emphasize sustainable food production, responsible resource use, and ecosystem protection.

In line with these global and local challenges, recent studies indicate that biological control agents, such as compost-based treatments, can effectively manage plant diseases (Ko and Ao, 2016; Kwak et al., 2015; Martín et al., 2023). Spent Mushroom Substrate (SMS), a byproduct of mushroom cultivation, contains beneficial microorganisms that support further decomposition while inhibiting soil pathogens. SMS, which is locally available in the Philippines due to the growing mushroom farming industry, presents a promising, sustainable alternative to chemical pesticides.

Hence, this study aimed to investigate the bio-efficacy of spent mushroom substrate (SMS)-based bio-fungicides for managing tomato diseases, enhancing crop resilience in the Philippines, and promoting sustainable agricultural practices. To achieve this, the study focused on optimizing, characterizing, and evaluating the effectiveness of SMS-based bio-fungicides in controlling tomato diseases under *in vitro* test conditions.

2. MATERIALS AND METHODS

2.1 Location of the Study

The study was conducted at the Mushroom/Crop Protection Laboratory of the Surigao del Norte State University (SNSU) – Mainit Campus, Magpayang, Mainit, Surigao del Norte, Philippines (9.7874° N, 125.4944° E).

2.2 Optimization of SMS-Based Bio-fungicide Formulation Through Enhanced Beneficial Microbial Population Density

Six-month-old spent mushroom substrates (SMS) were collected from the mushroom laboratory at SNSU-Mainit Campus. To optimize the preparation of SMS-based bio-fungicides, different fermentation periods (2, 4, and 7 days) and SMS-to-water ratios (0.5 kg, 1.0 kg, and 2.0 kg per 20 liters) (Zakaria et al., 2023) were tested using an improvised extraction-fermentation equipment designed and developed by the researcher. These variations were evaluated for their efficacy in promoting the growth of beneficial microbes.

2.3 Physico-chemical Characterization of SMS-based Bio-fungicides

The optimum fermented SMS-based bio-fungicides preparation was thoroughly examined and analyzed by a reliable testing laboratory for its physico-chemical properties as compared to the non-fermented bio-fungicides formulation at the Regional Soils Laboratory, located at P-8, Taguibo, Butuan City, 8600 Agusan del Norte, Philippines.

2.4 *In Vitro* Evaluation of SMS-based Bio fungicides Against Selected Fungal Diseases of Tomato

Different concentrations of SMS-based bio-fungicides (as outlined in Table 1) were tested for their efficacy against selected fungal diseases of tomato (as listed in Table 2). The study utilized the optimized SMS-based bio-fungicides formulation, which was compared to non-fermented SMS and a control group to evaluate its effectiveness in disease management.

Table 1. Treatments used in the study

Code	SMS-Based Bio-Fungicides	Rates (ml) Per 100ml Water
1	Control (SDW only)	-
2	Non-fermented SMS	10
3	Non-fermented SMS	15
4	Non-fermented SMS	20
5	Non-fermented SMS	25
6	Non-fermented SMS	30
7	Fermented SMS	10
8	Fermented SMS	15
9	Fermented SMS	20
10	Fermented SMS	25
11	Fermented SMS	30

Table 2. Selected tomato diseases tested against SMS-based bio-fungicide

Type of Disease	Disease	Causal Agent
Soil-borne diseases	Fusarium wilt	Fusarium spp.
Foliar diseases	Leaf spot	Septoria spp.
	Anthracnose	Colletotrichum spp.
Post-harvest diseases	Fruit anthracnose	Colletotrichum spp.
	Fruit rot	Fusarium spp.

Fungal pathogens were isolated from infected tomato plant tissues collected from identified infested areas in Surigao del Norte province. To confirm the identity of the fungal species, a microscopic examination was conducted. The isolation protocols for fungal pathogens, as described by Perez-Vicente et al. (2014), were followed in this experiment. Infected plant tissues were cut into small sections (3-6 mm in length) from the advancing portion of the lesion. The cut tissues were then dipped in a 70% ethyl alcohol solution for approximately one minute, followed by rinsing in three separate plates containing sterile distilled water (SDW). Afterward, the tissues were blotted dry using blotting paper and equidistantly plated onto culture media. The media used included ¼ strength potato dextrose agar (PDA) or water agar (WA), both supplemented with an antibacterial agent (streptomycin sulfate at a concentration of 1.2 mL per 240 mL of PDA) to inhibit bacterial contamination. Pure culture of fungal pathogens was then mass-produced on a half-strength potato dextrose agar and were incubated for seven days for fungal pathogens, and two days for bacterial pathogens.

To evaluate the efficacy of different concentrations of SMS-based bio-fungicide under laboratory conditions, the poisoned food technique, as described by Burgess et al. (2008), was employed. SMS-based bio-fungicides, as outlined in Table 1, were prepared by calibrating the extract to a 100 mL water solution. This solution was then mixed with previously sterilized culture media and shaken thoroughly before pour-plating. Pure culture disks of each fungal pathogen were obtained using a sterilized cork borer and placed at the center of the treated medium. The plates were incubated at room temperature for seven days. After incubation, the zone of inhibition around the fungal cultures was measured to assess the efficacy of the SMS-based bio-fungicides in preventing fungal growth. Observations were recorded based on the size of the colony and inhibition zones, which indicate the effectiveness of the SMS treatments.

2.5 Data Gathered

2.5.1 Microbial population density (CFU/ml) in SMS-based bio-fungicide

To quantify the microbial population in SMS-based bio-fungicides, a spread plating technique was employed. One milliliter of well-mixed composite SMS-based bio-fungicides sample was plated to determine the bacterial and fungal population. The sample was first diluted by placing one milliliter into 9 milliliters of sterile distilled water (SDW) in sterile test tubes, followed by serial dilution to achieve a 2-fold dilution series (1:100 or 10^2) for fungi and a 3-fold dilution series (1:1000 or 10^3) for bacteria. One milliliter from each dilution was pipetted and transferred into 9 milliliters of SDW to reach the

desired dilution. From these solutions, 0.3 milliliters were spread-plated on Potato Dextrose Agar (PDA) to cultivate fungi and bacteria. The plates were then sealed, covered, and incubated under suitable conditions for microbial growth.

The colony-forming units (CFUs) of the fungi and bacteria per milliliter of SMS-based bio-fungicide were calculated using the following formula:

$$CFU\ per\ milliliter = \frac{Number\ of\ colonies\ that\ developed\ on\ each\ plate \times dilution\ factor}{Volume\ of\ culture\ plate}$$

2.5.2 Physico-chemical properties of SMS-based bio-fungicide

The physico-chemical analyses of the optimum fermented SMS-based bio-fungicide were compared from the non-fermented formulation based on the following determinants: pH, and levels of primary macronutrients (nitrogen, phosphorus, and potassium), secondary macronutrients (calcium and magnesium), and micronutrients (iron, manganese, sulphur, copper, and zinc) (Mortada et al., 2020; Tajbakhsh et al., 2008). These analyses provided a comprehensive profile of the SMS-based bio-fungicide's chemical composition, which is essential for assessing its suitability as a sustainable biocontrol agent.

2.6 Inhibitory Effects of SMS-based Bio-fungicide Against Selected Fungal Diseases of Tomato under *In Vitro* Test Condition

2.6.1 Diameter of colony

The diameter of the fungal colony was recorded by taking the average of two long and two short diameters taken at right angles for each colony measured in millimeter (mm) using a ruler (Karthik et al., 2017) after 7 days of incubation.

2.6.2 Zone of inhibition (ZI)

Zone of inhibition (ZI) of the different rates of SMS-based bio-fungicides was determined by measuring the growth of fungi after 7 days of incubation and % inhibition was calculated using the formula below (Alwathnani and Perveen, 2012):

Inhibition (%) = (C-T/C) 100

Where, C is the average colony diameter in control plate and T is the average colony diameter in treated plates.

2.7 Statistical Analysis

For the optimization tests, data were analyzed using Analysis of Variance ($p \leq 0.05$) to evaluate the overall effects of different fermentation periods and SMS-to-water ratios on the effectiveness of the SMS-based bio-fungicides. Significant differences among treatment groups were further examined using the Least Significant Difference (LSD) test to identify specific variations and determine the optimal fermentation period and SMS-to-water ratio for bio-fungicides preparation.

For the characterization of two SMS-based bio-fungicide formulations, a t-test was applied to compare the differences between the treatments and assess their distinct characteristics.

To evaluate the bio-efficacy of the SMS-based bio-fungicide against selected fungal diseases of tomato, data were analyzed using ANOVA ($p \leq 0.05$) under a Completely Randomized Design (CRD). The differences among treatment means were then compared using Tukey's Honest Significant Difference (THSD) test to identify the most effective treatment for controlling fungal diseases.

3. RESULTS AND DISCUSSION

3.1 Optimization of SMS-Based Bio-fungicide Formulation Through Enhanced Beneficial Microbial Population Density

Microbial population density present in SMS-based bio-fungicide in different fermentation periods and SMS-to-water ratios are shown in Table 3. Results of the ANOVA revealed significant differences among treatment means ($p \geq 0.01$). The optimization of the SMS-based bio-fungicide focused on enhancing its capacity to promote the proliferation of beneficial microbes, crucial for effective biocontrol and overall plant health improvement. This approach aimed to maximize the microbial population density, particularly beneficial fungi and bacteria, thereby increasing the bio-fungicide's efficacy in managing plant diseases and supporting sustainable agricultural practices.

Fungal Population: The colony-forming units (CFU) of fungal populations per milliliter of SMS-based bio-fungicide were significantly highest in extracts fermented for 7 days. The bio-fungicide prepared with 1.0 kg of SMS per 20 L of water achieved the highest population count at 41,666.67 CFU/ml, followed by the formulation using 2.0 kg of SMS per 20 L of water with a count of 12,222.22 CFU/ml. A comparable population of 10,555.56 CFU/ml was observed in the bio-fungicide prepared with 0.50 kg of SMS per 20 L of water. Similar trends were noted in bio-fungicide fermented for 5 and 2 days, where the 1.0 kg SMS/20 L water preparation consistently exhibited the highest fungal population. Conversely, SMS-based bio-fungicide fermented for only 2 days yielded the lowest fungal populations among the fermentation durations tested, and unfermented bio-fungicide exhibited the least fungal populations, ranging from 0.00 to 9,698.37 CFU/ml. These fungal populations primarily comprised *Trichoderma spp*. and *Penicillium spp.* (Fig. 1), both of which are known for their beneficial effects on crop health.

These findings align with the results of Putri (2008), who reported that efficient nutrient utilization and metabolic adaptability drive microbial populations to peak during the optimal fermentation period. The increased fungal population density observed in SMS extracts on the seventh day of fermentation supports the role of this adaptive phase in promoting microbial growth. Furthermore, the 1.0 kg SMS/20 L water formulation provided an optimal nutrient-rich environment, corroborating Putri's assertion that nutrient availability is critical for microbial proliferation. Beyond the seventh day, microbial growth stabilized due to resource depletion and a balance between reproduction and mortality, consistent with population dynamics observed during extended fermentation periods.

Bacterial Population: Beneficial bacteria, such as actinomycetes and Bacillus spp**.** (Fig. 2), were significantly more abundant in SMS-based bio-fungicide fermented for 7 days and prepared with the optimal substrate concentration of 1.0 kg SMS per 20 L of water, achieving a bacterial population of 672,222.22

CFU/ml. This was followed by the bio-fungicide prepared with 2.0 kg SMS/20 L water, which recorded 277,777.78 CFU/ml, while the 0.50 kg SMS/20 L preparation had the lowest bacterial population at 89,444.44 CFU/ml. A similar trend was observed in bio-fungicides fermented for 5 days, where the 1.0 kg SMS/20 L formulation maintained the highest bacterial population, followed by the 2.0 kg SMS/20 L and 0.50 kg SMS/20 L preparations. For bio-fungicides fermented for 2 days, bacterial populations were notably lower, with 1.0 kg SMS/20 L recording 670,370.37 CFU/ml, while the 0.50 kg SMS/20 L preparation showed no bacterial growth. Unfermented SMS-based bio-fungicides had the lowest bacterial populations overall, ranging from 0 to 553,227.78 CFU/ml. These results suggest that the optimal substrate concentration for promoting bacterial growth in SMS extracts is 1.0 kg SMS per 20 L of water.

These findings align with the observations of Xu et al. (2023), who reported that longer fermentation periods, mimicking co-culture environments, not only enhanced fungal populations but also promoted the production of valuable secondary metabolites such as peptides and proteins, sesquiterpenes and other terpenes, steroids, organic acids and quinoline, have been shown to have antimicrobial activity (Alves et al., 2012). Similarly, Sharma et al. (2020) demonstrated that microbial density and auxin (IAA) production peaked after 10 days of fermentation, emphasizing the significance of fermentation duration in maximizing microbial populations. Additionally, Wang et al. (2015) highlighted the critical role of substrate concentration and treatment duration in promoting microbial growth during aerobic co-composting of penicillin fermentation fungi residue (PFFR) with pig manure. Their study revealed a 40–80% increase in microbial populations, particularly actinomycetes and bacteria, due to the favorable conditions created by the composting process. Wang et al. (2015) also found that a 7-day composting period was sufficient to degrade over 99% of penicillin in PFFR, further underscoring the importance of optimal treatment duration in enhancing microbial activity.

The current study's findings mirror these results, with the 7-day fermentation period producing the highest bacterial and fungal populations in SMS-based bio-fungicides. Furthermore, the optimal substrate concentration (1.0 kg SMS per 20 L water) in this study created a nutrient-rich environment conducive to microbial growth, paralleling Wang et al.'s (2015?) observation that organic substrates enhance microbial activity. Together, these findings reinforce the critical

Table 3. Population density (cfu/ml[1]) of microbes present in SMS-based bio-fungicide in different fermentation period and SMS-to-water ratio

Ratio (SMS:20L water)	No Fermentation		With Fermentation					
			2 days		5 days		7days	
	Fungi**	Bacteria**	Fungi**	Bacteria**	Fungi**	Bacteria**	Fungi**	Bacteria**
0.50kg	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	1666.67^{c}	11666.66^{c}	10555.56^{c}	89444.44^{c}
1.0kg	6484.89^{b}	381375.67^{b}	29444.44^{a}	670370.37^{a}	33888.89^{a}	635000.00^{a}	41666.67^{a}	672222.22^{a}
2kg	9698.37^{a}	553227.78^{a}	23888.89^{b}	261111.11^{b}	6111.11^{b}	212777.78^{b}	12222.22^{b}	277777.78^{b}
CV (%)	**13.41**	**18.17**	**15.93**	**20.31**	**17.89**	**21.64**	**23.98**	**14.97**

***=Significant at 1% level, LSD.*

[1]Colony forming units per milliliter (cfu/ml) was computed using the formula: cfu/ml = (Number of colonies that developed on each plate x dilution factor)/Volume of culture plate

Table 4. Physico-chemical composition of non-fermented and fermented SMS-based bio-fungicides

Parameters	Non-Fermented SMS-Based Bio-Fungicide	Fermented SMS-Based Bio-Fungicide	Lsd ($p \leq 0.05$)
pH	5.81 ± 0.03	5.94 ± 0.02	0.002
		%	
Nitrogen	0.42 ± 0.02	0.39 ± 0.01	0.009
		ppm	
Phosphorus	10.24 ± 0.74	54.34 ± 3.58	<0.001
Potassium	1543.17 ± 72.86	617.67 ± 118.75	<0.001
Calcium	3046.67 ± 178.79	7366.67 ± 580.04	<0.001
Magnesium	529.83 ± 20.82	1243.83 ± 100.38	<0.001
Iron	21.00 ± 6.61	86.28 ± 2.43	<0.001
Manganese	97.50 ± 4.33	68.65 ± 2.21	0.001
Copper	0.72 ± 0.03	3.82 ± 0.13	<0.001
Zinc	77.50 ± 4.33	16.90 ± 0.58	<0.001

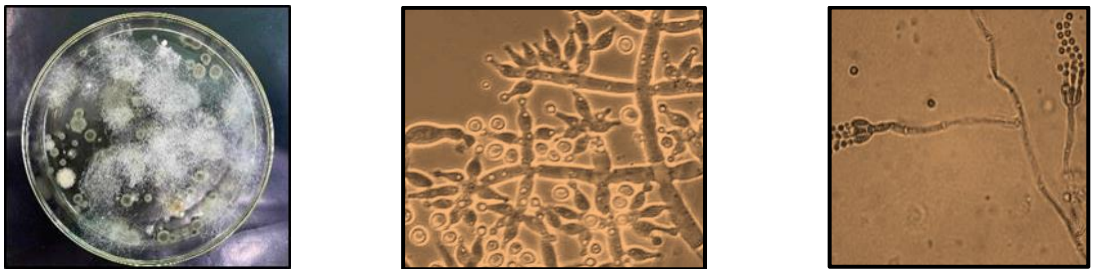


Fig. 1. Fungal organisms found in SMS-based bio-fungicides after 7 days of incubation (left) and their morphological structures under microscope (100x): *Trichoderma spp*. (middle) and *Penicillium spp.* (right)

Fig. 2. Bacterial organisms found in SMS-based bio-fungicides after 7 days of incubation (left) and their colony morphology in culture media: Actinomycetes (middle) and *Bacillus spp.* (right)

roles of fermentation time, substrate concentration, and organic substrates in maximizing microbial populations and bio-fungicide efficacy.

3.2 Physico-chemical Properties of SMS-based Bio-fungicide

The results of the physico-chemical composition of both fermented and unfermented SMS-based bio-fungicides are presented in Table 4, characterized based on the optimal fermentation period and SMS-to-water ratio identified to promote the growth of beneficial microbes. Statistical analysis revealed significant differences in the properties of the formulations ($p < 0.01$).

Regarding hydrogen ion concentration (pH), the SMS-based bio-fungicide was found to be slightly acidic, with an average pH of 5.85. The fermented formulation exhibited a slightly higher pH of 5.94, while the non-fermented formulation had a pH of 5.81. This pH range is consistent with the findings of Paredes et al. (2006) and Sendi et al. (2013), who reported that the optimal pH range for SMS in oyster mushroom cultivation lies between 5.1 and 7.4, suggesting that the pH of the bio-fungicide falls within a favorable range for microbial activity and biocontrol efficacy.

The primary macronutrients, including Nitrogen (N), Phosphorus (P), and Potassium (K), showed significant differences between the fermented and non-fermented SMS-based bio-fungicides, as presented in Table 4. The N content was higher in the non-fermented formulation (0.42%) compared to the fermented formulation (0.39%). The P content of the non-fermented bio-fungicide was 10.24 ppm, which increased significantly to 54.34 ppm in the fermented formulation. In contrast, the K content was 1,543.17 ppm in the non-fermented bio-fungicide, but it decreased to 617.67 ppm after fermentation. This decrease in N and K content can be attributed to microbial consumption for growth during fermentation. As for P, the initial low levels in the non-fermented bio-fungicide are consistent with the findings of Bellettini et al. (2019), who noted that lignocellulosic materials like sawdust typically have low mineral content. After fermentation, the sawdust-based SMS exhibited a significant increase in P content, aligning with the results of Hanafi et al. (2018), who found that fermentation enhances the availability of phosphorus in SMS.

The secondary macronutrients Calcium (Ca) and Magnesium (Mg) also showed significant differences between the formulations. The Ca content was notably higher in the fermented SMS-based bio-fungicide (7,366.67 ppm) compared to the non-fermented formulation

(3,046.67 ppm). Similarly, the Mg content was higher in the fermented formulation (1,243.83 ppm) than in the non-fermented one (529.83 ppm). These findings align with Sendi et al. (2013), who reported that SMS contains Ca and Mg values of 0.51% and 0.15%, respectively. The study's results for the fermented SMS-based bio-fungicide translate to 0.74% for Ca and 0.12% for Mg, confirming the increases in these secondary macronutrients post-fermentation.

Micronutrients, including Zinc (Zn), Manganese (Mn), Iron (Fe), and Copper (Cu), were also tested. The Zn content in the non-fermented formulation was higher (77.50 ppm) than in the fermented formulation (16.90 ppm), while the Mn content was higher in the non-fermented bio-fungicide (97.50 ppm) than in the fermented (68.65 ppm). The Fe content increased significantly from 21.00 ppm in the non-fermented formulation to 86.28 ppm after fermentation. Similarly, the Cu content increased from 0.72 ppm in the non-fermented bio-fungicide to 3.75 ppm after fermentation. These differences suggest that fermentation increased the availability of certain micronutrients like Fe and Cu, while the Zn and Mn contents decreased. The Fe content reported by Medina et al. (2009) for SMS was 337 mg/kg, which is significantly higher than the 86.28 ppm observed in this study's fermented SMS-based bio-fungicide. However, the Cu, Mn, and Zn values observed in this study (3.82 mg/kg, 68.65 mg/kg, and 16.90 mg/kg, respectively) fall within the range reported by Medina et al. (2009), reinforcing the consistency of the findings across studies. In summary, fermentation significantly altered the physico-chemical properties of the SMS-based bio-fungicide, enhancing certain nutrient concentrations, particularly P, Ca, Mg, Fe, and Cu, while decreasing others like Zn and Mn. These changes highlight the complex interactions between fermentation, nutrient availability, and microbial activity, further supporting the optimization of SMS-based bio-fungicides for biocontrol and plant health.

3.3 Inhibitory Effects of SMS-based Bio-fungicide Against Selected Fungal Diseases of Tomato under *In Vitro* Test Condition

The inhibitory effect exhibited by different levels of non-fermented and fermented SMS-based bio-fungicide on various fungal diseases of tomato under *in vitro* test condition is shown in Table 5. Analysis of Variance revealed significant differences among treatment means of various plant pathogenic fungi of tomato. Colony growth of these microorganisms causing tomato diseases as inhibited by the SMS-based bio-fungicide is presented in Fig. 3.

Soil-borne disease- *Fusarium spp*.: The bio-efficacy evaluation of SMS-based bio-fungicide against *Fusarium spp.*, a causal agent of tomato wilt, demonstrated significant inhibition at various application rates for both fermented and non-fermented formulations. Complete inhibition of *Fusarium spp.* was observed at an optimum application rate of 20 mL fermented SMS-based bio-fungicide per 100 mL water, as well as at higher concentrations of 25 mL and 30 mL/100 mL water. These treatments exhibited 100% fungal growth inhibition. At a slightly lower rate of 15 mL fermented SMS extract per 100 mL water, the colony growth was reduced to 6.67 mm, corresponding to 88.65% inhibition as compared to the untreated plates. In contrast, the application of 30 mL/100 mL water fermented bio-fungicide showed reduced efficacy with a colony diameter of 15.57 mm, translating to 73.28% inhibition. This decrease at higher concentrations could be due to a saturation effect, where excess nutrients in the bio-fungicide may have supported some fungal survival or growth. The non-fermented SMS-based bio-fungicide showed less inhibitory activity compared to the fermented formulation. At 25 mL and 20 mL/100 mL water, colony diameters were 19.33 mm and 26.50 mm, respectively, with corresponding percent inhibitions of 66.96% and 54.71%. Lower application rates of 10 mL and 15 mL/100 mL water for the non-fermented bio-fungicide resulted in less effective control, with colony diameters of 46.67 mm and 39.67 mm, corresponding to 32.33% and 19.19% inhibition, respectively.

These results suggest that the optimum application rate for the fermented SMS-based bio-fungicide was identified as 20 mL per 100 mL water, achieving complete fungal inhibition. Higher rates (25 mL and 30 mL/100 mL water) of fermented bio-fungicide were also highly effective, but efficiency at rates beyond 20 mL appeared comparable. Moreover, the non-fermented SMS-based bio-fungicide was less effective, suggesting that fermentation enhances the bio-fungicide's bio-efficacy, likely due to the increased microbial activity and metabolite production. These findings highlight the potential of fermented SMS-based bio-fungicides as an

effective organic alternative for controlling *Fusarium spp.* and improving tomato crop health. The optimum rate of 20 mL fermented SMS-based bio-fungicide per 100 mL water provides a sustainable and practical solution for managing tomato wilt.

Foliar diseases- *Septoria spp.* and *Colletotrichum spp*.: Among the tested rates of fermented and non-fermented SMS-based bio-fungicides against *Septoria spp.*, complete inhibition (100%) was achieved at an optimum level of 15 mL of non-fermented bio-fungicide per 100 mL water and at all application levels of fermented SMS-based bio-fungicide. The lowest inhibition was observed with the lowest concentration of non-fermented bio-fungicide, which resulted in 2.67 mm colony growth and 73.33% inhibition. These findings suggest that 20 mL/100 mL water of non-fermented SMS-based bio-fungicide and all tested levels of fermented bio-fungicide are highly effective in significantly inhibiting *Septoria spp.*, a causative agent of foliar diseases in tomato.

This work supports the findings of Bose et al. (2012), who found that fermentation at 20 µg/ml considerably improves the bioactivity of *Rhizoma coptidis*. Similar to the improved potency of fermented RC (FRC) reported in their investigation, the fermented SMS-based bio-fungicide created in this study was more effective in inhibiting *Septoria* spp. than the non-fermented bio-fungicide. The efficiency of the tested SMS-based bio-fungicide levels lends credence to fermentation's function in bioactive component enrichment. Furthermore, the dose-dependent effect found in both experiments highlights the need of identifying appropriate concentrations to maximize biological activity, whether in the suppression of inflammatory mediators or fungal infections.

For *Colletotrichum spp.*, complete inhibition (100%) was achieved at the optimum level of 20 mL/100 mL water of fermented SMS-based bio-fungicide, as well as at higher application rates of 25 mL and 30 mL/100 mL water. A slightly lower inhibition (89.89%) was observed at 15 mL/100 mL water of fermented bio-fungicide, which resulted in 8.67 mm colony growth. Among the non-fermented formulations, the highest inhibition (82.52%) was recorded at 30 mL/100 mL water, with a colony growth of 15.00 mm. Conversely, the lowest inhibition was observed with the 10 mL/100 mL water application of fermented bio-fungicide, which had a comparable effect to non-fermented bio-fungicides at 15 mL, 20 mL, and 25 mL, showing colony diameters of 47.17 mm, 67.17 mm, 37.50 mm, and 30.17 mm, respectively, with percent inhibitions ranging from 21.53% to 64.88%. Lower levels of non-fermented bio-fungicides (10 mL and 15 mL/100 mL water) exhibited the least inhibition, ranging from 14.82% to 21.53%. These results suggest that 20 mL/100 mL water of fermented SMS-based bio-fungicide is the optimum application rate for controlling foliar diseases caused by *Colletotrichum spp.* in tomato.

The findings demonstrate the effectiveness of SMS-based bio-fungicides, both fermented and non-fermented, against *Septoria spp.* and *Colletotrichum spp.*, which are common foliar pathogens of tomato. However, the study highlights the superior efficacy of fermented SMS-based bio-fungicides at 20 mL/100mL water, as it consistently achieved complete inhibition against both *Septoria spp.* and *Colletotrichum spp.* This application rate is recommended as the optimum level for managing foliar diseases in tomato. Non-fermented bio-fungicides were effective but required higher concentrations to achieve comparable results. These findings emphasize the importance of fermentation in enhancing the bio-pesticidal properties of SMS-based bio-fungicides.

Post-harvest diseases- *Fusarium spp.* and *Colletotrichum spp*.: Among the rates of fermented and non-fermented SMS-based bio-fungicides tested against *Fusarium spp.* in post-harvest tomatoes, complete inhibition (100%) was achieved at the optimum application rate of 20 mL/100 mL water of the fermented formulation. Higher concentrations (25 mL and 30 mL) also achieved 100% inhibition, while 15 mL/100 mL water of the fermented formulation resulted in 96.37% inhibition. The most effective non-fermented treatment was 30 mL/100 mL water, which achieved a colony growth of 15.67 mm and 78.66% inhibition. Lower inhibition levels were noted with fermented bio-fungicide at 10 mL/100 mL water (38.72% inhibition) and non-fermented bio-fungicide at 20 mL and 25 mL, with inhibition rates of 57.16% and 49.01%, respectively. The lowest efficacy was observed with non-fermented formulations at 10 mL and 15 mL/100 mL water, showing inhibition rates of 9.74% to 18.46%. The results demonstrate that fermented SMS-based bio-fungicide at 20 mL/100 mL water is the most effective application rate for controlling *Fusarium spp.*,

Table 5. Average colony diameter (mm)[1] and zone inhibition (%)[2] of selected fungal diseases in tomato as affected by non-fermented and fermented SMS-based bio-fungicide after seven days of incubation.

SMS-based Bio-fungicide Formulation and Rates PER 100 Water	Soil Borne Diseases		Foliar Diseases				Post-harvest Diseases			
	Fusarium spp.		*Septoria* spp.		*Colletotrichum* spp.		*Fusarium* spp.		*Colletotrichum* spp.	
	CD (mm)	ZI (%)	CD (mm)	ZI (%)	CD (mm)	ZI (%)	CD (mm)	ZI (%)	CD (mm)	ZI (%)
T_1 - untreated (SDW only)	58.67^{f}	0.00^{f}	20.00^{c}	0.00^{c}	85.67^{f}	0.00^{f}	73.50^{e}	0.00^{e}	52.50^{e}	0.00^{e}
T_2 – 10ml non-fermented	46.67^{e}	19.19^{e}	2.67^{b}	73.33^{b}	72.83^{e}	14.82^{e}	60.00^{de}	18.46^{de}	42.00^{d}	19.79^{d}
T_3 – 15ml non-fermented	39.67^{e}	32.33^{e}	0.00^{a}	100.00^{a}	67.17^{e}	21.53^{e}	66.33^{e}	9.74^{e}	43.33^{d}	17.30^{d}
T_4 – 20ml non-fermented	26.50^{d}	54.71^{d}	0.00^{a}	100.00^{a}	37.50^{cd}	56.13^{cd}	37.50^{c}	49.01^{c}	22.83^{c}	56.50^{c}
T_5 – 25ml non-fermented	19.33^{cd}	66.96^{cd}	0.00^{a}	100.00^{a}	30.17^{c}	64.88^{c}	31.50^{bc}	57.16^{bc}	21.67^{c}	58.68^{c}
T_6 – 30ml non-fermented	15.57^{bc}	73.28^{bc}	0.00^{a}	100.00^{a}	15.00^{b}	82.52^{b}	15.67^{ab}	78.66^{ab}	13.67^{b}	73.98^{b}
T_7 – 10ml fermented	23.83^{cd}	59.14^{cd}	0.00^{a}	100.00^{a}	47.17^{d}	44.74^{d}	45.00^{c}	38.72^{c}	6.83^{ab}	86.95^{ab}
T_8 – 15ml fermented	6.67^{ab}	88.65^{ab}	0.00^{a}	100.00^{a}	8.67^{ab}	89.89^{ab}	2.67^{ab}	96.37^{ab}	0.00^{a}	100.00^{a}
T_9 – 20ml fermented	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}
T_{10} – 25ml fermented	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}
T_{11} – 30ml fermented	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00$_{a}$	100.00^{a}	0.00^{a}	100.00^{a}
CV (%)	**12.34**	**5.48**	**15.12**	**0.93**	**11.69**	**4.66**	**12.98**	**10.47**	**13.02**	**8.69**

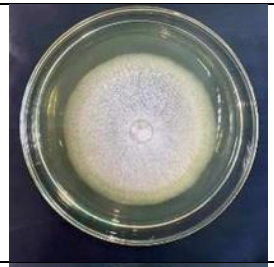
***= significant at 1% level, THSD. Data were transformed using Arc Sine transformation prior to data analysis.*

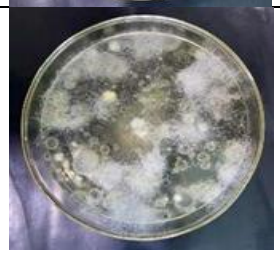
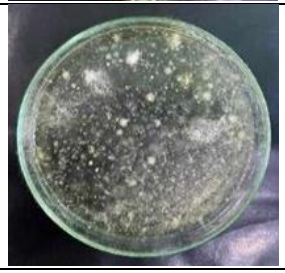
[1]Colony in diameter was recorded by measuring the average of two longest and shortest radial growth diameter of the pathogen using a ruler after seven days of incubation.

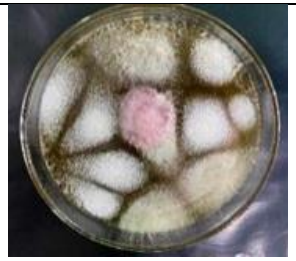
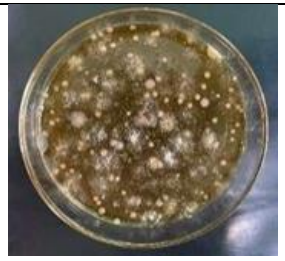
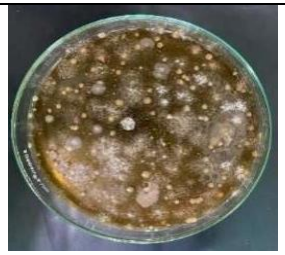
[2]Zone Inhibition was calculated using the formula: Inhibition (%) = (C-T/C) X 100, where C =control, and T = treated plates

CD = Colony diameter in millimeter; ZI (%) = Percent zone of inhibition

SMS= Spent Mushroom Substrate

SMS-based Bio-fungicide Formulation and Rate Per 100ml Water	Soil-borne Diseases	Foliar Diseases		Post-harvest Diseases	
	***Fusarium* spp.**	***Septoria* spp.**	***Colletotrichum* spp.**	***Fusarium* spp.**	***Colletotrichum* spp.**
T_1 – untreated (SDW only)					
T_2 – 10ml non-fermented					
T_3 – 15ml non-fermented					

SMS-based Bio-fungicide Formulation and Rate Per 100ml Water	Soil-borne Diseases	Foliar Diseases		Post-harvest Diseases	
	***Fusarium* spp.**	***Septoria* spp.**	***Colletotrichum* spp.**	***Fusarium* spp.**	***Colletotrichum* spp.**
T_4 – 20ml non-fermented					
T_5 – 25ml non-fermented					
T_6 – 30ml non-fermented					

SMS-based Bio-fungicide Formulation and Rate Per 100ml Water	Soil-borne Diseases	Foliar Diseases		Post-harvest Diseases	
	***Fusarium* spp.**	***Septoria* spp.**	***Colletotrichum* spp.**	***Fusarium* spp.**	***Colletotrichum* spp.**
T_7 – 10ml fermented					
T_8 – 15ml fermented					
T_9 – 20ml fermented					

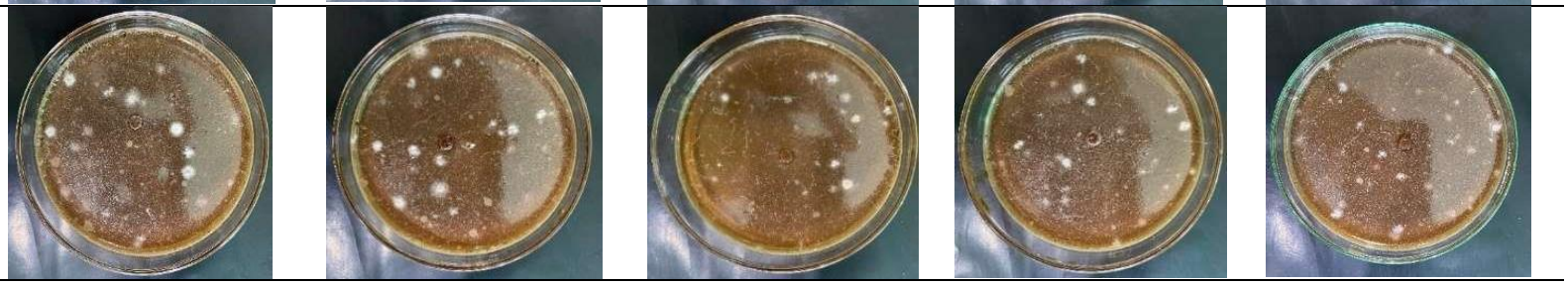
SMS-based Bio-fungicide Formulation and Rate Per 100ml Water	Soil-borne Diseases	Foliar Diseases		Post-harvest Diseases	
	***Fusarium* spp.**	***Septoria* spp.**	***Colletotrichum* spp.**	***Fusarium* spp.**	***Colletotrichum* spp.**
T_{10} – 25ml fermented					
T_{11} – 30ml fermented					

Fig. 3. The inhibitory effects exhibited by different levels of fermented and non-fermented SMS-based bio-fungicides on the growth of fungal diseases of tomato after 7 days of incubation

achieving complete inhibition. Fermentation significantly enhanced the biocontrol efficacy compared to non-fermented formulations, highlighting its potential as a sustainable post-harvest treatment for tomato fruit rot.

For *Colletotrichum spp.* in post-harvest tomatoes, complete inhibition (100%) was achieved with 15 mL/100 mL water of fermented SMS-based bio-fungicide. Similar effects were observed with higher doses (20 mL, 25 mL, and 30 mL per 100 mL water) of the fermented formulation. A lower concentration of 10 mL/100 mL water of fermented formulation demonstrated significant efficacy with 86.95% inhibition and 6.83 mm colony growth. Among non-fermented formulations, the most effective treatment was 30 mL/100 mL water, with 73.98% inhibition and a colony growth of 13.67 mm. Comparable inhibition was noted at 20 mL and 25 mL/100 mL water of non-fermented formulations, with inhibition rates of 56.50% and 58.68%, respectively. The least effective treatments were 10 mL and 15 mL/100 mL water of non-fermented formulations, with inhibition rates of 19.79% and 17.30%, respectively. These results indicate that fermented SMS-based bio-fungicide at 15 mL/100 mL water is the optimum rate for effectively controlling *Colletotrichum spp.*, achieving complete inhibition. Fermentation consistently enhanced the bio-fungicide's efficacy, making it a promising solution for managing post-harvest diseases in tomatoes.

The findings highlight the superior performance of fermented SMS-based bio-fungicides compared to non-fermented ones in inhibiting *Fusarium spp.* and *Colletotrichum spp.* in post-harvest tomatoes. The optimum application rates were 20 mL/100 mL water for *Fusarium spp.* and 15 mL/100 mL water for *Colletotrichum spp.*, both achieving 100% inhibition. Fermentation enhances the biocontrol efficacy, demonstrating its potential for sustainable disease management in tomato production.

The results generally suggest that the fermented SMS-based bio-fungicide at an optimum application rate of 20 mL/100 mL water exhibited superior performance in controlling various diseases of tomatoes, including soil-borne, foliar, and post-harvest diseases. This effectiveness can be attributed to the inherent properties of Spent Mushroom Substrate (SMS) as a biological control measure. For instance, Spent Mushroom Compost (SMC) has demonstrated the ability to inhibit *Fusarium spp.* growth, contributing to disease suppression rates ranging from 69.7% to 88.1%, as reported by Ko and Ao (2016). Similarly, the water extract from *Hericium erinaceus* SMS has been shown to inhibit the mycelial growth of multiple pathogenic fungi, including *Phytophthora capsici* and *Ralstonia solanacearum* (Kwak et al., 2015; Kang et al., 2017). Furthermore, microbial antagonists derived from SMS have proven effective against *Colletotrichum musae*, a significant post-harvest pathogen in bananas, emphasizing SMS's potential as a broad-spectrum biocontrol agent (Inagaki and Yamaguchi, 2009; Ranathunge et al., 2016).

Additionally, bioactive compounds found in SMS, possess antimicrobial properties, making SMS an effective tool for suppressing plant disease incidence (Martín et al., 2023; Hoitink and Fahy, 1986). Fermentation further enhances these benefits. According to Skowron et al. (2022), fermentation introduces several advantages, such as inhibiting the growth of pathogenic microorganisms, improving the organoleptic properties and digestibility of products, and providing a valuable source of functional microbes. The dominance of fermenting microorganisms, coupled with their metabolites and the resulting pH changes, significantly inhibits pathogen growth. Therefore, while SMS in its raw form is already effective in pathogen suppression, fermentation amplifies its efficacy by fostering beneficial microbial activity.

These findings underscore the promise of fermented SMS-based bio-fungicides as an innovative and sustainable approach to managing tomato diseases. By leveraging the natural properties of SMS and the additional benefits introduced through fermentation, this bio-fungicide presents a practical and environmentally friendly solution for disease management in tomato production.

4. CONCLUSION

Based on the results, it can be concluded that fermenting SMS-based bio-fungicides for 7 days using 1.0 kg of SMS per 20 L of water supports the highest bacterial and fungal growth, identifying this as the optimal concentration and fermentation period for enhancing the proliferation of beneficial microbes during bio-fungicide preparation. The fermentation process significantly modifies the physico-chemical properties of the SMS-based bio-fungicide,

enhancing specific nutrient concentrations such as P, Ca, Mg, Fe, and Cu, while reducing others like N, Zn, and Mn. These changes underscore the intricate interactions between fermentation, nutrient availability, and microbial activity, emphasizing the importance of optimization in developing effective SMS-based bio-fungicides.

Additionally, bio-efficacy evaluations against various fungal diseases of tomatoes indicate that fermented SMS-based bio-fungicide, applied at an optimum rate of 20 mL/100 mL water, demonstrate superior performance in controlling a range of diseases, including soil-borne, foliar, and post-harvest diseases. This highlights the potential of fermented SMS-based bio-fungicides as a sustainable and effective solution for disease management in tomato cultivation. Hence, the study provides valuable insights into leveraging spent mushroom substrate to advance the circular economy in mushroom production, promote environmentally sustainable practices, and offer an eco-friendly alternative for managing diseases in tomato cultivation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models (ChatGPT) have been used during the editing of this manuscript.

Details of the AI usage are given below:

ChatGPT was utilized solely to enhance sentence structure and grammatical organization. The formulation of results, data analysis, and interpretation were conducted entirely by the researchers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Alves, M. J., Ferreira, I. C., Dias, J. F., Teixeira, V., Martins, A., & Pintado, M. (2012). A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta Med.*, 78, 1707–1718. https://doi.org/10.1055/s-0032-1315370

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., & Ribani, R. H. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal of Biological Sciences, 26*(4), 633–646. https://www.sciencedirect.com/science/article/pii/S1319562X16301814

Bose, S., Jeon, S., Eom, T., Song, M., & Kim, H. (2012). Evaluation of the *in vitro* and in vivo protective effects of unfermented and fermented *Rhizoma coptidis* formulations against lipopolysaccharide insult. *Food Chemistry, 135*(2), 452–459. https://doi.org/10.1016/j.foodchem.2012.05.007

Burgess, L. W., Knight, T. E., Tesoriero, L., and Phan, H. T. （2008）Diagnostic manual for plant diseases in Vietnam, pp. 126-133, ACIAR, Canberra

Department of Agriculture. (2021). *Investment guide for tomato*. https://www.da.gov.ph/wp-content/uploads/2021/04/Investment-Guide-for-Tomato.pdf

FAOSTAT. (2017). "Production – Crops – Area harvested/ Production quantity – Tomatoes – 2014". FAO Statistics online database, Food and Agriculture Organization, Rome. http://www.fao.org/faostat/en (accessed 22 Sept. 2017).

Giovanni, J. J. (2004). Genetic regulation of fruit development and ripening. *The Plant Cell, 16*(suppl_1), S170–S180. https://doi.org/10.1105/tpc.019158

Hanafi, F. H. M., Rezania, S., Taib, S. M., Din, M. F. M., Yamauchi, M., Sakamoto, M., & Ebrahimi, S. S. (2018). Environmentally sustainable applications of agro-based spent mushroom substrate (SMS): An overview. *Journal of Material Cycles and Waste Management, 20*(3), 1383–1396.

Hoitink, H. A. J., & Fahy, P. C. (1986). Basis for the control of soil-borne plant pathogens with composts. *Annual Review of Phytopathology, 24*, 93–114.

Inagaki, R., & Yamaguchi, A. (2009). Spent substrate of shiitake (*Lentinula edodes*) inhibits symptoms of anthracnose in cucumber. *Mushroom Science and Biotechnology, 17*, 113–115.

Kang, D., Min, K., Kwak, A., Lee, S., & Kang, H. (2017). Defense response and

suppression of *Phytophthora* blight disease of pepper by water extract from spent mushroom substrate of *Lentinula edodes*. *Plant Pathology Journal, 33*(3), 264–275. https://doi.org/10.5423/ppj.oa.02.2017.0030

Ko, A., & Ao, A. (2016). *In vitro* evaluation of spent mushroom compost on growth of *Fusarium oxysporum* f. sp. *Lycopersici*. *Advances in Plants and Agriculture Research, 4*. https://doi.org/10.15406/APAR.2016.04.00147

Kwak, A. M., Kang, D. S., Lee, S. Y., & Kang, H. W. (2015). Effect of spent mushroom substrates on *Phytophthora* blight disease and growth promotion of pepper. *Journal of Mushrooms, 13*, 16–20. https://doi.org/10.14480/JM.2015.13.1.16

Martín, C., Zervakis, G. I., Xiong, S., Koutrotsios, G., & Strætkvern, K. O. (2023). Spent substrate from mushroom cultivation: Exploitation potential toward various applications and value-added products. *Bioengineered, 14*(1). https://doi.org/10.1080/21655979.2023.2252138

Medina, E., Paredes, C., Pérez-Murcia, M. D., Bustamante, M. A., & Moral, R. (2009). Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. *Bioresource Technology, 100*(18), 4227–4232.

Mortada, A. N., Bolhassan, M. H., & Wahi, R. (2020). Physicochemical composition of spent oyster mushroom substrate. *Malaysian Journal of Analytical Sciences, 24*(6), 848–854.

Padmanabhan, P., Cheema, A., & Paliyath, G. (2016). Solanaceous fruits including tomato, eggplant, and peppers. https://www.sciencedirect.com/referencework/9780123849533/encyclopedia-of-food-and-health

Paredes, C., Moral, R., Pérez-Murcia, M. D., Moreno-Caselles, J., & Pérez-Espinosa, A. (2006). Agricultural value of the spent mushroom substrate. In *Technology for recycling of manure and organic residues in a whole-farm perspective* (pp. 301–304). Danish Institute of Agricultural Sciences, Ministry of Food, Agricultural Sciences, Tjele.

Perez-Vicente, L., Dita, M.A., Martinez-dela Parte, E. (2014). Technical Manual Prevention and Diagnostic of Fusarium Wilt (Panama disease) of Banana caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (TR4). Conference Paper. https://www.researchgate.net/publication/273632807_Technical_Manual_Prevention_and_diagnostic_of_Fusarium_WiltPanama_Disease_of_banana_caused_by_Fusarium_oxysporum_f_sp_cubense_Tropical_Race_4TR4.

Putri, N. A. (2008). The effect of fermentation duration of liquid organic fertilizer combining banana stems, banana peels, and bitter melon on the test for macro nutrient content of phosphorus (P) and total calcium (Ca) with the addition of EM4 bioactivator. *Bachelor Thesis*, Sanata Dharma University, Yogyakarta.

Ranathunge, N., Rajapaksha, R., Yogarajah, K., & Preethikumara, M. (2016). Isolation, screening and *in vitro* evaluation of bacterial antagonists from spent mushroom substrate against *Colletotrichum musae*. *Tropical Agricultural Research and Extension, 17*, 69–76. https://doi.org/10.4038/TARE.V17I2.5309

Sendi, H., Mohamed, M. T. M., Anwar, M. P., & Saud, H. M. (2013). Spent mushroom waste as a media replacement for peat moss in Kai-Lan (*Brassica oleracea* var. *Alboglabra*) production. *The Scientific World Journal, 2013*, 258562.

Sharma, R., Garg, P., Kumar, P., Bhatia, S. K., & Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. *Fermentation, 6*(4), 106. https://doi.org/10.3390/fermentation6040106

Skowron, K., Budzyńska, A., Grudlewska-Buda, K., Wiktorczyk-Kapischke, N., Andrzejewska, M., Wałecka-Zacharska, E., & Gospodarek-Komkowska, E. (2022). Two faces of fermented foods—the benefits and threats of its consumption. *Frontiers in Microbiology, 13*, 845166. https://doi.org/10.3389/fmicb.2022.845166

Tajbakhsh, J., Abdoli, M. A., Goltapeh, E. M., Alahdadi, I., & Malakouti, M. J. (2008). Trend of physico-chemical properties change in recycling spent mushroom compost through vermicomposting by epigeic earthworms *Eisenia foetida* and *E. andrei*. *Journal of Agricultural Technology, 4*, 185–198.

Trevorrow, P. (2018). The cause and management of crown rot of banana. Hort Innovation. 1 Chifley Square, Sydney NSW 2000.

https://www.horticulture.com.au/globalassets/laserfiche/assets/project_reports/ba13011/ba13011-final-report-386.pdf

United Nations Environment Programme. (2022). *Synthesis report on the environmental and health impacts of pesticides and fertilizers and ways to minimize them*. Geneva.

Wang, P., Mao, R., Zhang, L., Cai, L., Zhao, G., Liang, J., & Zuo, C. (2015). Evaluation of aerobic co-composting of penicillin fermentation fungi residue with pig manure on penicillin degradation, microbial population dynamics, and composting maturity. *Waste Management, 46*, 169–175. https://doi.org/10.1016/j.wasman.2015.07.033

Wyenandt, A., & Nitzsche, P. (2020). Diagnosing and controlling fungal diseases of tomato in the home garden. Rutgers New Jersey Agricultural Experiment Station Fact Sheet. https://njaes.rutgers.edu/fs547/

Xu, S., Li, M., Hu, Z., Shao, Y., Ying, J., & Zhang, H. (2023). The potential use of fungal co-culture strategy for discovery of new secondary metabolites. *Microorganisms, 11*(2), 464. https://doi.org/10.3390/microorganisms11020464

Zakaria, A. A., Malik, K., & Muhammad, I. (2023). The effect of fermentation duration on nutrition composition of seaweed (*Sargassum* sp.) liquid organic fertilizer. *Journal of Aquaculture and Fish Health, 12*(1), February 2023. https://doi.org/10.20473/jafh.v12i1.25669

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