

Original Research Article

OPTIMIZATION, CHARACTERIZATION, & EVALUATION OF SPENT MUSHROOM-BASED BIOFUNGICIDE FOR TOMATO DISEASE MANAGEMENT

Comment [R1]: Give the tomato its scientific name (*Lycopersicon esculentum* Mill. or *Solanum lycopersicon* L., whichever suits you)

ABSTRACT

Aims: This study evaluates the potential of Spent Mushroom Substrate (SMS) as an organic biofungicide for managing tomato diseases effectively.

Study design: The optimization tests employed ANOVA to evaluate the effects of fermentation periods and SMS-to-water ratios on the biofungicide's effectiveness. Significant differences among treatments were further analyzed using the LSD test to determine the optimal fermentation period and SMS-to-water ratio for biofungicide preparation. For physico-chemical analysis, a T-test was conducted to compare the physical and chemical properties of non-fermented and fermented SMS-based biofungicide. Additionally, their *in vitro* evaluation of the SMS-based biofungicide against various fungal diseases of tomato utilized ANOVA, with significant means compared through Tukey's HSD test, ensuring accurate identification of effective treatments.

Place and Duration of 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.

Methodology: To optimize SMS preparation, 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) were evaluated to identify conditions that maximize the production of beneficial microbes. The physico-chemical properties of both fermented and non-fermented SMS-based biofungicides were analyzed by a third-party testing laboratory to ensure comprehensive characterization. Additionally, the bio-efficacy of SMS-based biofungicide against tomato diseases was assessed under *in vitro* conditions, providing insights into its potential for effective disease management.

Results: ANOVA and LSD analyses revealed that microbial populations, including *Trichoderma spp.*, *Penicillium spp.*, actinomycetes, and *Bacillus spp.*, were significantly influenced by fermentation duration and SMS concentration. The optimal formulation—1.0 kg SMS per 20 L water fermented for 7 days—yielded the highest beneficial microbial growth. Fermentation significantly altered the physico-chemical properties, increasing nutrient levels such as P, Ca, Mg, Fe, and Cu while reducing N, Zn, and Mn. These changes underscore fermentation's role in optimizing microbial activity and nutrient availability in SMS-based biofungicides. *In vitro* tests demonstrated that fermented SMS biofungicides, applied at 20 mL/100 mL water, effectively controlled soil-borne, foliar, and post-harvest tomato diseases, outperforming non-fermented formulations.

Comment [R2]: I would recommend omitting this paragraph in the abstract of the article. I consider it superfluous here.

Comment [R3]: According to which analysis methods?

Conclusion: The findings emphasize SMS as a sustainable and eco-friendly biofungicide, advancing circular economy principles in mushroom production while offering an innovative, environmentally responsible approach to disease management. Further validation under *in vivo* and field conditions, as well as trials on other crops, are recommended to maximize its potential in sustainable agriculture.

Keywords: *Bio-fungicide, Diseases, Fermentation, Spent Mushroom Substrate, Tomato*

1. INTRODUCTION

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. 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 (United Nations Environment Programme, 2022). These negative impacts have become a growing concern, especially in rural areas where agricultural practices rely heavily on chemicals. 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; Lee 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. This study aims to explore the bio-efficacy of SMS in managing tomato diseases, advancing crop resilience in the Philippines, and contributing to responsible, sustainable agricultural practices that support local farmers and ecosystems.

OBJECTIVES OF THE STUDY

General Objective:

Comment [R4]: Replace "Biofungicide" or "Bio-fungicide" throughout the document. Only one form of writing should be chosen.

Comment [R5]: You did not discuss the tomato from a botanical perspective (scientific name, botanical family, etc.). I recommend it.

Comment [R6]: References?

Comment [R7]: The model plant of your study is the tomato and only this plant should arouse your interest. It is therefore not necessary to evoke the pathological problems relating to other plants as you have done in this paragraph with the banana. It is like jumping from one thing to another. To this end, I recommend that you kindly revise the interest of this paragraph or simply delete it.

Comment [R8]: References?

To optimize, characterize, and evaluate the bio-efficacy of spent mushroom substrate (SMS)-based biofungicides for effective management of tomato diseases.

Specific Objectives:

1. To determine the optimal fermentation time and SMS-to-water ratio for maximizing the bio-efficacy of SMS-based biofungicides based on the quantified microbial composition of SMS-based biofungicides;
2. To characterize the physical and chemical properties of SMS-based bio-fungicide that potentially contribute to its biocontrol properties; and
3. To assess the efficacy of various concentrations of SMS-based biofungicides in inhibiting the growth of selected fungal pathogens of tomato under *in vitro* conditions.

Comment [R9]: It would be more digestible when writing a research article to make this part a simple paragraph of your Introduction and not a subsection of it.

2. MATERIAL AND METHODS

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.

Comment [R10]: The geographical coordinates of the study location must be mentioned.

Optimization of SMS-Based Biofungicide 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 biofungicides, 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) were tested using an improvised extraction-fermentation equipment. These variations were evaluated for their efficacy in promoting the growth of beneficial microbes.

Comment [R11]: What equipment is this specifically? Mention some details about it (brand, operating mechanism).

Comment [R12]: References that allowed you to develop this optimization method?

Physico-chemical Characterization of SMS-based Biofungicides

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

Comment [R13]: Describe the method that allowed to examine and analyze the optimal preparation of biofungicides based on fermented SMS for its physicochemical properties. This, supported by references.

In Vitro Evaluation of SMS-based Biofungicides Against Selected Fungal Diseases of Tomato

Different concentrations of SMS-based biofungicides (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 biofungicides 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 BIOFUNGICIDES	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 biofungicide.

TYPE OF DISEASE	DISEASE	CAUSAL AGENT
Soil-borne diseases	Fusarium wilt	<i>Fusarium spp.</i>
Foliar diseases	Leaf spot	<i>Septoria spp.</i>
	Anthracnose	<i>Colletotrichum spp.</i>
Post-harvest diseases	Fruit anthracnose	<i>Colletotrichum spp.</i>
	Fruit rot	<i>Fusarium spp.</i>

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 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 biofungicide under laboratory conditions, the poisoned food technique, as described by Burgess et al. (2008), was employed. SMS-based biofungicides, 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 biofungicides 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.

Data Gathered

The following data were gathered in this experiment:

Optimization of SMS-Based Biofungicide Formulation Through Enhanced Beneficial Microbial Population Density

Comment [R14]: This part is really irrelevant to the logical structuring of your ideas.

To quantify the microbial population in SMS-based biofungicides, a spread plating technique was employed. One milliliter of well-mixed composite SMS-based biofungicide 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 biofungicide were calculated using the following formula:

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

Physico-chemical Characterization of SMS-based Biofungicide

The physico-chemical analyses of the optimum fermented SMS-based biofungicide was 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). These analyses provided a comprehensive profile of the SMS-based biofungicide's chemical composition, which is essential for assessing its suitability as a sustainable biocontrol agent.

Inhibitory Effects of SMS-based Biofungicide Against Selected Fungal Diseases of Tomato

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 after 7 days of incubation.

2. Zone of Inhibition (ZI)

Zone of inhibition (ZI) of the different rates of SMS-based biofungicides 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):

$$\text{Inhibition (\%)} = \frac{(C-T)}{C} \times 100$$

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

Statistical Analysis

For the optimization tests, data were analyzed using Analysis of Variance (ANOVA) to evaluate the overall effects of different fermentation periods and SMS-to-water ratios on the effectiveness of the SMS-based biofungicides. 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 biofungicide preparation.

Comment [R15]: Merge this text with the one devoted to "Optimization of SMS-Based Biofungicide Formulation Through Enhanced Beneficial Microbial Population Density" mentioned above. This will allow you to avoid redundancies and back and forth in the presentation of your ideas. This will improve the writing quality of your article and make it more digestible.

Comment [R16]: Merge this text with the one on "Physico-chemical Characterization of SMS-based Biofungicide" mentioned above. This will allow you to avoid redundancies and back-and-forths in the presentation of your ideas. This will improve the editorial quality of your article and make it more digestible.

Comment [R17]: Missing description of analysis method (referenced)

Comment [R18]: References?

Comment [R19]: What threshold was applied to the tests? 5% threshold?

For the characterization of two SMS-based biofungicide 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 biofungicide against selected fungal diseases of tomato, data were analyzed using ANOVA 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

Optimization of SMS-Based Biofungicide Formulation Through Enhanced Beneficial Microbial Population Density

Microbial population density present in SMS-based biofungicide 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 biofungicide 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 biofungicide'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 biofungicide were significantly highest in extracts fermented for 7 days. The biofungicide 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 biofungicide prepared with 0.50 kg of SMS per 20 L of water. Similar trends were noted in biofungicide 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 biofungicide fermented for only 2 days yielded the lowest fungal populations among the fermentation durations tested, and unfermented biofungicide 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 demonstrate a strong positive correlation between fermentation duration and fungal population density in SMS extracts. Extended fermentation periods, particularly 7 days, created conditions conducive to higher fungal growth compared to shorter durations. Additionally, the substrate concentration was a critical factor in determining fungal population. The preparation using 1.0 kg of SMS per 20 L of water consistently supported the highest fungal growth across all fermentation durations, suggesting it as the optimal concentration. This result underscores the role of nutrient availability in enhancing fungal proliferation during biofungicide preparation.

Bacterial Population. Beneficial bacteria, such as actinomycetes and *Bacillus spp.* (Fig. 2), were significantly more abundant in SMS-based biofungicide 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 biofungicide 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 biofungicides 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 biofungicides 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 biofungicides 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.

Comment [R20]: There are methods to highlight the levels of correlation between two given factors. This is not done scientifically just by deduction. For example, the straight use of linear regression between two factors can effectively provide information on this (Tsala et al. 2024).

Reference:
Ramses Tsala, Zacharie Mounkéné Bounyahré, Marlyse Mboudou Belibi, Élysée Djedoubouyom Namé, Abba Maïmouna, Lucien Tchuenteu Tatchum and Clotilde Megueni. *In vitro* evaluation of the phytopharmaceutical potential of aqueous extracts of *Jatropha curcas* seeds, *Thevetia peruviana* seeds and *Cyperus rotundus* rhizomes against *Phytophthora infestans* (Mont) de Bary. *J Pharmacogn Phytochem* 2024; 13(5): 717-728. DOI: 10.22271/phyto.2024.v13.i5j.15145

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. 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, Zhang 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. Zhang 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.

Comment [R21]: So name some metabolites that are considered valuable and say how they are valuable in the context of your study.

The current study's findings mirror these results, with the 7-day fermentation period producing the highest bacterial and fungal populations in SMS-based biofungicides. 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 Zhang et al.'s (2015?) observation that organic substrates enhance microbial activity. Together, these findings reinforce the critical roles of fermentation time, substrate concentration, and organic substrates in maximizing microbial populations and biofungicide efficacy.

Table 3. Population density (cfu/ml¹) of microbes present in SMS-based biofungicide in different fermentation period and SMS-to-water ratio.

RATIO (SMS:20 L Water)	NO FERMENTATION		WITH FERMENTATION					
	Fungi**	Bacteria**	2 DAYS		5 DAYS		7 DAYS	
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.

¹Colony 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

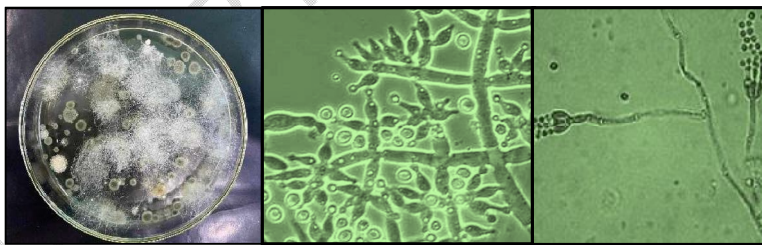


Fig. 1. Fungal organisms found in SMS-based biofungicides after 7 days of incubation (left) and their morphological structures under microscope (100x): *Trichoderma* spp. (middle) and *Penicillium* spp. (right).



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

Physico-chemical Characterization of SMS-based Biofungicide

The results of the physico-chemical composition of both fermented and unfermented SMS-based biofungicides 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. To assess the effects of the fermentation process on the physical and chemical properties of the SMS-based biofungicide, both fermented and non-fermented formulations were analyzed. Statistical analysis revealed significant differences in the properties of the formulations ($p < 0.01$).

Regarding hydrogen ion concentration (pH), the SMS-based biofungicide 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 biofungicide falls within a favorable range for microbial activity and biocontrol efficacy.

Comment [R22]: You can omit this sentence. It is not really necessary and also, it makes the sentence heavy.

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

PARAMETERS	NON-FERMENTED SMS-BASED BIOFUNGICIDE	FERMENTED SMS-BASED BIOFUNGICIDE	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
	<i>ppm</i>		
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

The primary macronutrients, including Nitrogen (N), Phosphorus (P), and Potassium (K), showed significant differences between the fermented and non-fermented SMS-based biofungicides, 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 biofungicide 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 biofungicide, 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 biofungicide 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 biofungicide (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 biofungicide 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 biofungicide (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 biofungicide 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 biofungicide. 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 biofungicide, 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 biofungicides for biocontrol and plant health.

Inhibitory Effects of SMS-based Biofungicide Against Selected Fungal Diseases of Tomato

The inhibitory effect exhibited by different levels of non-fermented and fermented SMS-based biofungicide 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 biofungicide is presented in Fig. 3.

Soil-borne disease- *Fusarium spp.* The bio-efficacy evaluation of SMS-based biofungicide 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 biofungicide 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. In contrast, the application of 30 mL/100 mL water fermented biofungicide 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 biofungicide may have supported some fungal survival or growth. The non-fermented SMS-based biofungicide 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 biofungicide 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.

Comment [R23]: In relation to what treatment? It is scientifically more judicious to make this clarification.

These results suggest that the optimum application rate for the fermented SMS-based biofungicide 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 biofungicide were also highly effective, but efficiency at rates beyond 20 mL appeared comparable. Moreover, the non-fermented SMS-based biofungicide was less effective, suggesting that fermentation enhances the biofungicide's bio-efficacy, likely due to the increased microbial activity and metabolite production. These findings highlight the potential of fermented SMS-based biofungicides as an effective organic alternative for controlling *Fusarium spp.* and improving tomato crop health. The optimum rate of 20 mL fermented SMS-based biofungicide 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 biofungicides against *Septoria spp.*, complete inhibition (100%) was achieved at an optimum level of 15 mL of non-fermented biofungicide per 100 mL water and at all application levels of fermented SMS-based biofungicide. The lowest inhibition was observed with the lowest concentration of non-fermented biofungicide, 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 biofungicide and all tested levels of fermented biofungicide are highly effective in significantly inhibiting *Septoria spp.*, a causative agent of foliar diseases in tomato.

Comment [R24]: You should compare your results and interpretations with similar work as was the case above in your analyses.

For *Colletotrichum spp.*, complete inhibition (100%) was achieved at the optimum level of 20 mL/100 mL water of fermented SMS-based biofungicide, 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 biofungicide, 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 biofungicide, which had a comparable effect to non-fermented biofungicides 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 biofungicides (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 biofungicide is the optimum application rate for controlling foliar diseases caused by *Colletotrichum spp.* in tomato.

The findings demonstrate the effectiveness of SMS-based biofungicides, 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 biofungicides at 20 mL/100 mL 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 biofungicides 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 biofungicides.

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 biofungicide after seven days of incubation.

SMS-BASED BIOFUNGICIDE FORMULATION AND RATES PER 100 WATER	SOIL BORNE DISEASES		FOLIAR DISEASES				POST-HARVEST DISEASES			
	<i>Fusarium spp.</i>		<i>Septoria spp.</i>		<i>Colletotrichum spp.</i>		<i>Fusarium spp.</i>		<i>Colletotrichum spp.</i>	
	CD (mm)	ZI (%)	CD (mm)	ZI (%)	CD (mm)	ZI (%)	CD (mm)	ZI (%)	CD (mm)	ZI (%)
T ₁ - untreated (SDW only)	58.67 ⁱ	0.00 ⁱ	20.00 ^e	0.00 ^c	85.67 ⁱ	0.00 ⁱ	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	100.0 ^{0a}	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	100.0 ^{0a}	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	100.0 ^{0a}	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	100.0 ^{0a}	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	100.0 ^{0a}	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	100.0 ^{0a}	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	100.0 ^{0a}	0.00 ^a	100.00 ^a	0.00 ^a	100.0 ^{0a}	0.00 ^a	100.00 ^a
T ₁₀ -25ml fermented	0.00 ^a	100.00 ^a	0.00 ^a	100.0 ^{0a}	0.00 ^a	100.00 ^a	0.00 ^a	100.0 ^{0a}	0.00 ^a	100.00 ^a
T ₁₁ -30ml fermented	0.00 ^a	100.00 ^a	0.00 ^a	100.0 ^{0a}	0.00 ^a	100.00 ^a	0.00 ^a	100.0 ^{0a}	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

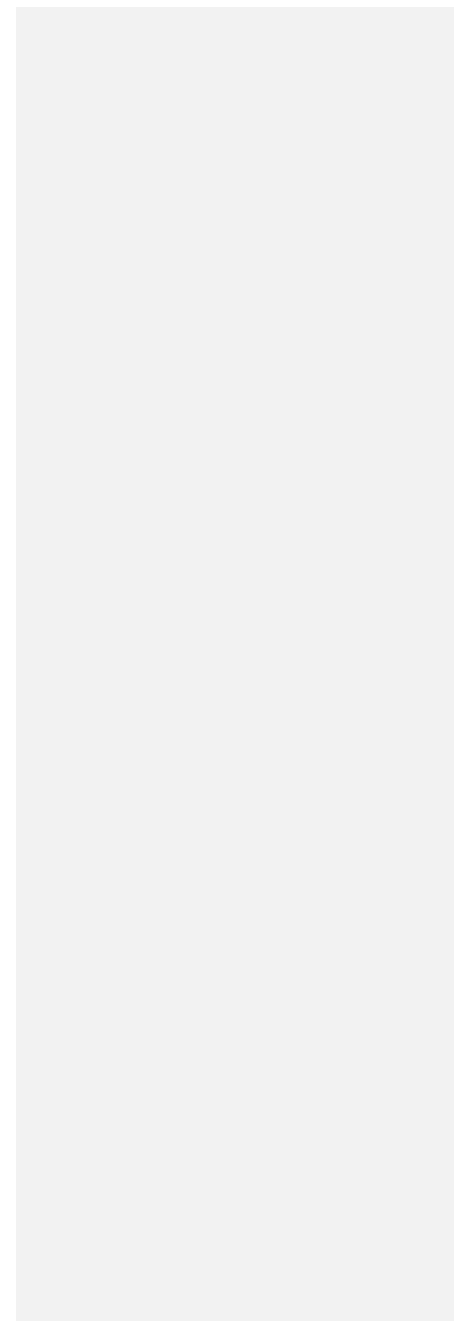
**= significant at 1% level, THSD. Data were transformed using Arc Sine transformation prior to data analysis.





























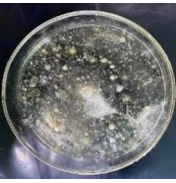

¹Colony in diameter was recorded by measuring the average of two longest and shortest radial growth diameter of the pathogen using a ruler after seven days of incubation.

²Zone 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

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SMS-BASED BIOFUNGICIDE 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					
T ₄ – 20ml non-fermented					
T ₅ – 25ml non-fermented					
T ₆ – 30ml non-fermented					

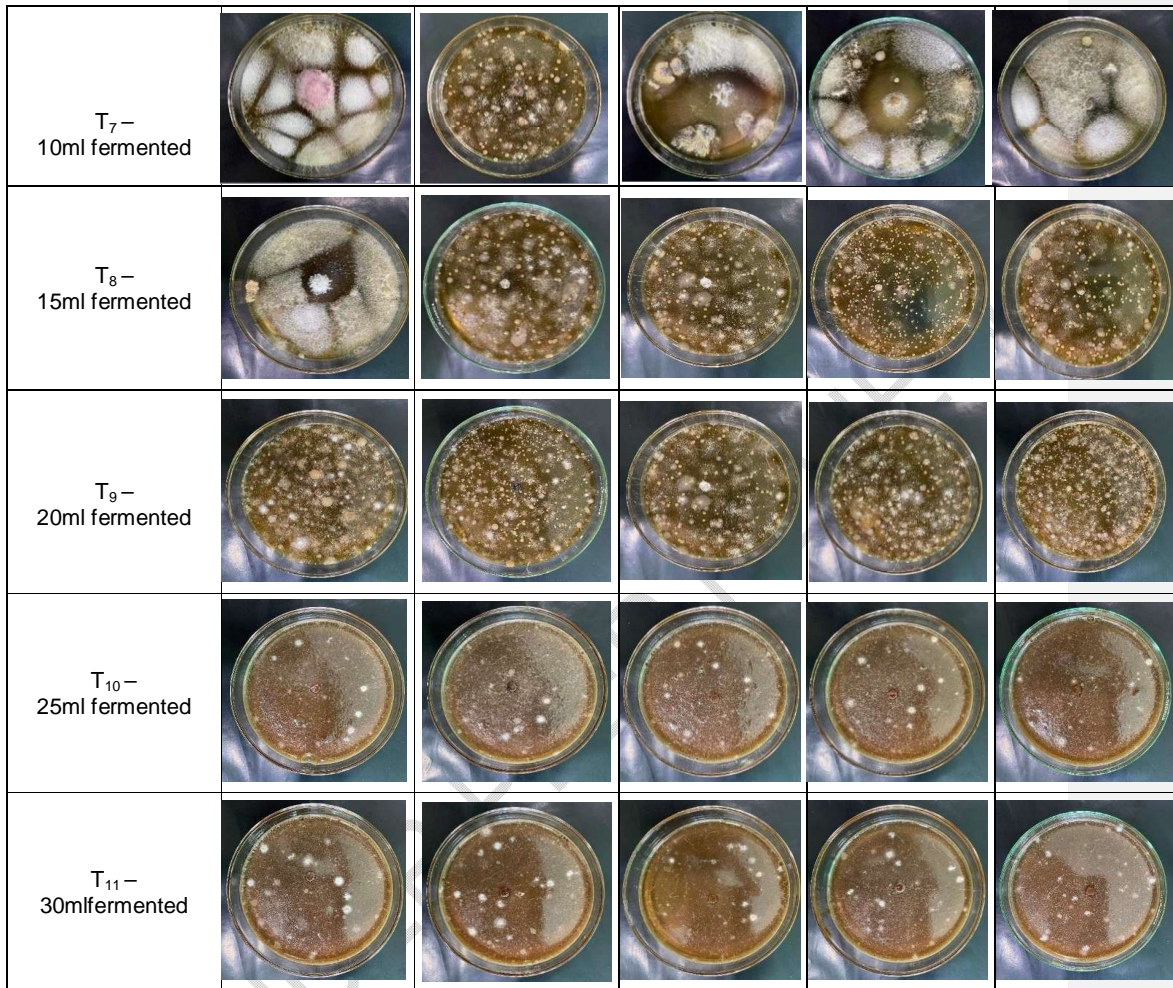


Fig. 3. The inhibitory effects exhibited by different levels of fermented and non-fermented SMS-based biofungicides on the growth of fungal diseases of tomato after 7 days of incubation.

Post-harvest diseases- *Fusarium spp.* and *Colletotrichum spp.* Among the rates of fermented and non-fermented SMS-based biofungicides 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 biofungicide at 10 mL/100 mL water (38.72% inhibition) and non-fermented biofungicide 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 biofungicide at 20 mL/100 mL water is the most effective application rate for controlling *Fusarium spp.*, 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 biofungicide. 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 biofungicide at 15 mL/100 mL water is the optimum rate for effectively controlling *Colletotrichum spp.*, achieving complete inhibition. Fermentation consistently enhanced the biofungicide's efficacy, making it a promising solution for managing post-harvest diseases in tomatoes.

The findings highlight the superior performance of fermented SMS-based biofungicides 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 biofungicide 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* (Lee et al., 2015). 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.

Additionally, bioactive compounds found in SMS, as noted by Martín et al. (2023), possess antimicrobial properties, making SMS an effective tool for suppressing plant disease incidence. 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 biofungicides 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 biofungicide 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 biofungicides 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 biofungicide preparation. The fermentation process significantly modifies the physico-chemical properties of the SMS-based biofungicide, 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 biofungicides.

Additionally, bio-efficacy evaluations against various fungal diseases of tomatoes indicate that fermented SMS-based biofungicide, 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 biofungicides 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.

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