

In vitro evaluation of spent mushroom compost on growth of *Fusarium Oxysporium* F. Sp *Lycopersici*

Abstract

Aqueous extracts of Spent Mushroom Compost (SMC) were screened in vitro for antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in the laboratory using standard procedures. Potato Dextrose Agar (PDA) were amended with 1%, 5%, 10% and 15% of autoclaved or non-autoclaved SMC extract in Petri dishes before inoculating with 5mm mycelia disc of the pathogen. The mycelia growth was monitored for five days after inoculation. Data were taken on the radial mycelia length and used to calculate percentage mycelia inhibition. Microorganisms present in the spent mushroom compost were also screened against Fol in a dual culture experiment. Observations were recorded on the relationships that existed between the different organisms and percentage inhibition of Fol. Analysis was done using ANOVA and means were separated at 5% level of probability. Radial growth of the mycelia of the pathogen on PDA amended with 15% of autoclaved SMC extracts were significantly reduced ($P=0.05$) when compared with the PDA lacking the extract. However, 1%, 5%, 10%, or 15% of the non-autoclaved extracts significantly inhibited the growth of the pathogen by 69.7%, 88.1%, 80.3%, or 85.5%, respectively. The SMC of oyster mushroom contained diverse microorganisms including fluorescent *Pseudomonas* spp., *Trichoderma viridae*, *Bacillus* spp., *Penicillium* spp., and *Aspergillus terreus*. These fungal isolates showed a strong antagonism to Fol. The findings suggest that the SMC extracts could be exploited as a biological control measure for *Fusarium* wilt of tomato.

Keywords: fusarium oxysporum fsp, lycopersici, spent mushroom compost, mycelia growth inhibition

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Introduction

Tomato (*Solanum lycopersicon* L.) is one of the most widely consumed fruit and the second most important vegetable crop after potato in the world.^{1,2} It is an important and cheap source of vitamins and an indispensable condiment in any meal preparation.³ Tomato can be eaten fresh or in processed forms and its consumption is associated with the prevention of several diseases⁴ due to the fact that it contains antioxidants including carotenes, (Lycopene as well as β -carotene), ascorbic acid, and phenolic compounds.⁴ There has been a high demand for tomato production worldwide due to its aforementioned nutritional and medicinal importance. In 2012, the world production of tomato was 145.8 metric tons with Nigeria ranked as the fourth largest producing country in Africa and first in West Africa sub-region with an estimated output of 1.10 metric tons and average yield of 10 tons per ha.^{4,5} However, Nigeria as a country still struggles to meet the local demand for the crop and in fact till date Nigeria still import tomato in form of paste due to major production constraints especially pests and diseases infestation. Though several abiotic and biotic factors predisposes tomato to diseases infestation, but the impact of the soil-borne pathogen *Fusarium oxysporium* f. sp. *lycopersici* (Fol) that causes fusarium wilt of tomato cannot be overemphasized as it can result in 10-50 % yield losses in tomatoes grown in greenhouses and field.^{6,7} This fungus penetrates through the roots and proliferates in the vascular tissue, impeding water transport and causing spectacular wilting, stunted seedlings, drooping and yellowing leaves and rapid death of the plant.⁸ The interaction between Fol and tomato is race-cultivar-specific, making the pathogen difficult to manage; and three host-specific races of Fol have been described to date.⁹

Management strategies for fusarium wilt are generally focused on preventive measures as no effective control measures are available yet.¹ These include the use of resistant cultivars, soil fumigation treatments, pesticide application and biological methods. The use of resistant tomato cultivars provides some degree of control, however, the occurrence and development of new pathogenic races are a recurring challenge resulting in no commercially acceptable tomato cultivars.^{1,6} The use of soil fumigants, especially sulphur based, only suppresses the disease. Similarly, the pesticides that is available for this disease is deficient, and as a result, the rise of new fungicide-resistant pathogens species is of major concern.¹⁰ Furthermore, the risks of non-target effects of the pesticides and the resulting adverse environmental impact have attracted concern. Therefore, there is an interest in technologies that will reduce dependency on chemical pesticides, which are predominantly synthetic.¹¹ Biological method of managing plant diseases, including the use of composts and biological control agents, has received attention as a better alternative to the intensive use of chemically synthesized products. Biological method of approach is generally safer and has a minimal environmental impact.¹¹ Compost is biodegradable, and less expensive to develop, compared to fungicides. Effectiveness of composts including Spent Mushroom Compost against plant diseases caused by a broad range of pathogens, such as bacteria, fungi and nematode species, has been demonstrated in various studies.¹²⁻¹⁵

Spent Mushroom Substrate Compost - the substrate left after a full mushroom crop harvest is a by-product of mushroom production. It is rich in diverse microorganisms, such as disease antagonistic bacteria and fungus. It is biodegradable, safe to apply and less expensive to develop. It naturally suppresses pathogens in the soil that cause plant

damage and decline in yields. More so, its use in organic agriculture, zero waste integrated farming system as well as integrated disease management is receiving attention lately as its accumulation in mushroom production industries can become a nuisance to the environment if more alternative uses are not found for it, hence this study proposes its use to combat the pathogen- *Fusarium oxysporum f.sp. lycopersici*(Fol).

Materials and methods

Experimental site

All experiments in this study were conducted at the roof top garden and in the Laboratory of the Plant Pathology unit, Crop Protection and Environmental Biology Department, Faculty of Agriculture and Forestry, at the University of Ibadan.

Sources of materials

Spent Mushroom Composts were collected from a mushroom farm (LTC Mushroom farm) in Osogbo. Tomato seedlings showing vascular wilts symptoms were obtained from field sampling of different tomato growing areas including farms in: NIHORT, Ibadan and Ogbomoso, Oyo State, Nigeria.

Field sampling

Random sampling was done to collect diseased seedlings in the field. After thorough examination through visual observation of wilted, chlorotic tomato seedlings, leaf curling, marginal necrosis etc. And some diagnostic tests such as cutting the back of the stems for brown colorations. The samples were packed in separate envelopes with labeling indicating date of collection and observations noted. Soil samples around the rhizosphere were also randomly collected, bulked and packed in separate envelopes. All envelopes were brought into the laboratory and used for the isolation of the causal pathogens.

Preparation of spent mushroom compost

The spent mushroom composts collected (Plate 1) after a full harvest of mushroom crop were bulked, mixed homogeneously, air dried, blended, and then stored for subsequent use.



Plate 1 Collection of spent mushroom compost from oyster mushroom cultivation: A. Oyster Mushroom (*Pleurotus sajou caju*) on its substrate. B. Spent Mushroom Substrate (Compost).

Sterilization of Materials

Petri dishes and other glassware such as beakers, conical flask, measuring cylinders, and test tubes, etc. were washed with detergent, rinsed in clean tap water, dried and sterilized in an hot air-oven (at

1600C) for at least 2hours. Metals including inoculating needles, wire loops, forceps, spreader, scapel were always sterilized by exposure to the blue part of burning flame till red hot. Working table tops and hands were always surface sterilized with 75% ethanol.

Preparation of growth media

Potato Dextrose Agar (PDA) medium was prepared by dispensing 39g of PDA in 1L distilled water in a conical flask, plugged with cotton wool, properly wrapped, and sterilized at 121°C for 15minutes. The medium is allowed to cool to around 45°C then acidified by adding 1ml of lactic acid. The cooled agar was dispensed aseptically into sterile glass Petri-dishes inside the inoculating chamber and allowed to cool down to solidify.

Isolation of *Fusarium oxysporum f.sp. lycopersici*

The stem and root parts of infected tomato plants collected were cut into pieces to include asymptomatic and symptomatic parts with a sterile scalpel. The pieces were surface sterilized with 0.5% bleach solution (1% sodium hypochlorite) for 2minutes and rinsed in three changes of sterile distilled water then dried on sterilized filter papers before placing on sterilized PDA medium in Petri dishes in order to isolate the fungal pathogen. The inoculated petri dishes were sealed and labeled, then incubated at room temperature ($28\pm 2^\circ\text{C}$) for 7 to 10days as described by Norhito et al.,¹⁶ in Nirmaladevi et al.¹⁷ Fungal colonies were purified by sub culturing, and resulting pure cultures were maintained on slants. For isolation through serial dilution of soil samples, 1 g of homogeneously mixed soil samples was dispense into 10ml of sterile distilled water in a beaker and mixed thoroughly to make the stock solution from which several dilutions (101 to 109) was made by dispensing 1 ml of the preceding solution into 9ml of sterile distilled water in test tubes. 0.1ml of dilutions of 102 to 106 was spread on sterilized PDA and other procedures followed as for dilution plating method.

Identification of *Fusarium oxysporum f.sp. lycopersici*

Cultural and microscopic characteristics on PDA were used for the identification of the fungal pathogens to species level. The races and formae speciales of the pathogen were determined by pathogenicity tests according to Schaad et al.¹⁸ Colonies exhibiting the taxonomic features of *F. oxysporum* were identified according to Barnett et al.¹⁹ Cultural and microscopic identification was based on the colour and the mycelia growth pattern of the pathogen on PDA as well as characteristics such as shape, size, structure and diversity of the macroconidia, phialides, microconidia, chlamydo spores and colony growth traits were examined under the compound microscope.

Pathogenicity of *Fusarium oxysporum* on healthy tomato plants

Conidia suspensions of *F. oxysporum* were prepared by culturing the fungus on PDA at ($28\pm 2^\circ\text{C}$) for 10days. After which 50ml of sterile distilled water was dispensed into the pure cultures on Petri plates and the surface of the cultures was scrapped slightly with a sterile scalpel to dislodge the conidia from the mycelium then filtered through a double layer of sterilized cheese cloth to remove mycelia fragments, into sterile beakers, then mixed thoroughly and re-suspended in sterile distilled water. The micro conidia were counted with a hemocytometer and the concentration was adjusted to 106 conidia per ml. For each count, 0.01ml of the conidial suspension was one the hemocytometer covered with its cover slip via the V groove

using a sterile syringe, the loaded hemocytometer I was then placed on the compound microscope and the counting grid is brought to focus at X40 magnification. Conidial counts were taken from the small squares (with area of about 0.0025mm² and depth of 0.1mm). Conidia were counted in five separate squares containing 16 small squares each. The formula below was used to calculate the final concentration of the micro conidia suspension used for inoculation:

The total number of conidia suspended in 1 ml (1000mm³) of sterile distilled water

$$NX100$$

Where N = the mean number of conidia counted in chosen square and V = volume of suspension between the cover slip and above the square

Twenty-one day old tomato (cv Kerewa and Roma) seedlings were inoculated by standard root dip inoculation method. Seedlings were removed from the pots in the nursery, shaken to remove the adhering particles, and washed carefully under running water. The roots were trimmed with a sterile scissors and were submerged in the micro conidial suspension contained in a beaker for 30minutes. The inoculated seedlings were then transplanted to sterilized pots of 13cm diameter and 12.5cm height, containing soil and sand mix in 1:1 ratio. There were four replicated pots of two plants each for each fungal strain and for the different cultivars of tomato. Control seedlings were treated with water alone. Plants were placed in same growth conditions. The percentage incidence of wilt disease was estimated using the formula by Michel et al.²⁰

The severity of the disease was assessed every week starting from 2weeks after inoculation to up to 45days. Symptoms were recorded according to a 1 to 5 scale.^{17,21}

- i. No symptoms.
- ii. Slight chlorosis, wilting or stunting of the plant.
- iii. Moderate chlorosis, wilting or stunting of the plant.
- iv. Severe chlorosis, wilting or stunting of the plant.
- v. Death of the plant.

The discoloration of the vascular tissue was confirmed by splitting the stem. To re-isolate the pathogen, stems were collected, surface sterilized, sectioned, and the stem sections plated onto PDA as described above. Strains producing disease severity index scores above 2 with typical wilt symptoms were considered tomato fusarium wilt pathogens and those that did not were considered nonpathogenic. More so, cultivars of tomato used were also referred to as very susceptible and moderately susceptible.

Effect of SMC on the radial growth of *Fusarium oxysporum fsp Lycopersci* in vitro

Poisoned food technique was used to test the effect of SMC on the growth of *F. oxysporum f.sp. lycopersici* in vitro under laboratory conditions. To prepare the aqueous extracts of SMC: 1g, 5g, 10g, or 15g of the SMC prepared (Section 3.4) were each suspended in 100ml of sterile distilled water to obtain the concentrations of 1% (0.01g/ml), 5% (0.05g/ml), 10% (0.1g/ml), or 15% (0.15g/ml), respectively. Each suspension was agitated by shaking very well to obtain even particle distribution. Two portions were prepared for each of the

concentrations, one portion was sterilized by autoclaving at 121°C for 15minutes and the other portion was not.

One ml each of the portions (sterilized and unsterilized) of the different concentrations of SMC suspension was then poured into sterile petri dishes. Ten ml of cooled molten, PDA was aseptically poured into each petri dish and rotated gently to ensure even dispersion of SMC in the agar. Control plates (0g/ml SMC) had 1ml of sterile distilled water mixed with cooled molten PDA. The plates were left to solidify. Inoculation was done by placing a 5mm diameter mycelia disc taken from the edge of a fresh culture (5-day old) of the fungal pathogen in the centre of the plates. All plates were incubated at 25±2°C. Data were collected on mycelia growth and recorded from 3days after inoculation till when control plates were fully covered with test pathogen's growth. Per cent inhibition in mycelia growth was determined using the formula below:

$$I=(C-T)CX100$$

I=percentage inhibition of mycelia growth

C=radial growth of pathogen in control

T=radial growth of pathogen in dual culture.

Isolation and identification of fungi in spent mushroom compost

Serial dilutions of SMC were made by dispensing 1g of SMC in 10ml of sterile distilled water to make the stock solution and then by making several dilutions (ranging from 10-1 to 10-5) from it with sterilized distilled water. 0.1ml of each dilution was then plated separately on potato dextrose agar to isolate the inherent fungi. Plates were incubated at 28±2°C for 5days. Well isolated colonies were further purified by repeated sub-culturing until pure cultures were obtained then maintained and stored for subsequent use by growing on the respective media slants and stored at 4°C.

Antifungal properties of isolated microorganisms from spent mushroom compost

The fungal isolates obtained from SMC were tested for their antagonistic property against the fungal wilt pathogens on PDA medium in a dual culture assay. Five-mm diameter disc of actively growing culture of the fungal pathogen was taken from the culture plates and placed at one end of the petri dish containing solidified PDA. Similarly, mycelia disc of the spent mushroom compost fungus was placed at the centre of the petri-plate in such a way that the distance between the pathogen and the spent mushroom compost fungus was about 4cm (Plate 2). The plates containing PDA medium inoculated with pathogen alone served as control. Three replications were maintained in each treatment (treatments consisted of the different isolated fungi from spent mushroom compost). The plates were incubated at (28±2°C). The radial growth of the spent mushroom compost' fungus and the pathogens from the centre of disc towards the centre of the plate were recorded from three days after incubation until the control plates were completely covered by the pathogens when the assay was terminated. Mycelia inhibition percentage of the pathogen was determined using the formula:

$$I=(C-T)CX100$$

Where I=percentage inhibition of mycelia growth

C = radial growth of pathogen in control

T = radial growth of pathogen in dual culture.

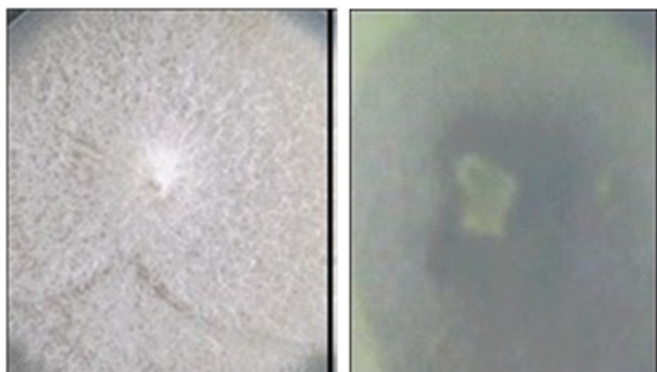


Plate 2 Cultural characteristics of *Fusarium oxysporum* on PDA
Plate 2A Grey Floccose mycelia growth of FW2.
Plate 2B White adherent mycelia growth of FW1.

Data Analysis

All experiments were conducted in a completely randomized design with three replicates and data recorded were individually subjected to ANOVA. Significant differences from the control values were determined using Fisher's LSD at 5% and 1% level of significance.

Results

Isolation and identification of *Fusarium oxysporum* f.sp. *lycopersici*

Most typical symptoms peculiar of *Fusarium* wilt including leaf chlorosis, marginal necrosis and browning of the vascular system as a result of necrosis of the vessels in a cross-section of the stem appeared on tomato plants in the fields where they were collected. The diseased leaves wilted and dried up. In many cases one side of the plants was affected first. The Presence of *Fusarium oxysporum* was then confirmed by microscopic examination of the formed conidia (a structure which bear *Fusarium* spores) from the pure cultures of the isolates on PDA.

Two different isolates designated as FW1 and FW2 were obtained (Table 1), each peculiar to the various locations from which infected seedlings were sourced and with its own distinct cultural characteristics on PDA (Plate 2) but with similar microscopic view of the conidia (Plate 3A) and chlamydoconidia (Plate 3B) typical of *Fusarium oxysporum*. The colour of the isolates on PDA ranged from pure floccose white, grey, to purple. The mycelia growth pattern ranged from fluffy (floccose) growth to adherent smooth growth (the isolates from Ogbomoso has floccose growth while the one from NIHORT had adherent smooth growth as shown in Table 1). All observed isolates under the compound microscope formed a hyaline, branching mycelium and macroconidia which were short and had three septa and they formed a large number of unicellular, elliptical, kidney-shaped microconidia gradually pointed with curved edges (pointed end) like a boat shape, they are hyaline and, transparent and had no septa. The chlamydoconidia were also present but at the terminal or intercalary positions, occurring singly (Plate 3). All these

characteristics confirmed the isolates to be *F. oxysporum*. The result of the pathogenicity test further confirmed the isolates being *Fusarium oxysporum* f.sp. *lycopersici* since they were pathogenic specifically to tomato. Tomato cv kerewa was more susceptible than cv Roma. Other Fungi such as *Penicillium* spp. and *Aspergillus* spp. were also obtained from first isolation with very low frequency of occurrence, thus they were termed contaminating fungi and disregarded. Microorganisms present in spent mushroom compost (Plate 4).

Table 2 shows the different microbes isolated from Spent Mushroom Compost and their cultural characteristics on PDA and NA for fungi and bacteria respectively. A total of 9 different isolates were obtained comprising 4 bacterial isolates and 5 fungal isolates. The identities of the five fungal isolates and their cultural characteristics are shown in Table 2 while the biochemical characteristics of the bacterial isolates are presented in Table 2.

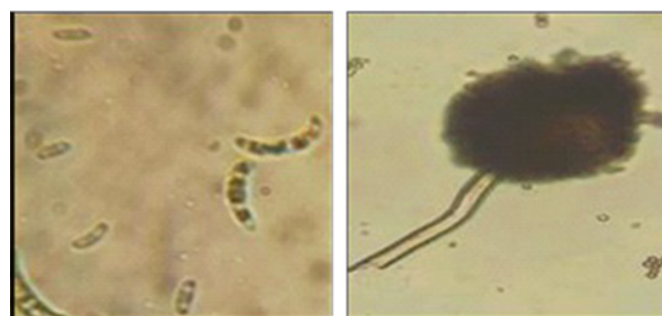


Plate 3 Microscopic characteristics of *Fusarium oxysporum* (Magnification: X400)
Plate 3A Microscopic view of Chlamydoconidia of *F. oxysporum*.
Plate 3B Microscopic view of macroconidia and microconidia of *F. oxysporum*.



Plate 4 Symptoms of *Fusarium* wilt of tomato
Plate 4A Tomato seedlings showing wilt symptoms on Nihort Research farm.
Plate 4B Tomato seedlings showing wilt symptoms with whitish mycelia on fruits in Ogbomoso farm.

Effect of fungal isolates in spent mushroom compost on *Fusarium oxysporum* f.sp. *lycopersici*

All the fungal isolates obtained from spent mushroom compost (*Trichoderma* viridae, *Penicillium* oxalicum, *P. chrysogenum* and *Aspergillus* terreus) except the residual mushroom mycelia showed antagonistic property on *Fusarium oxysporum* f.sp. *lycopersici* in vitro at 7 days after inoculation as shown in Figure 1 and Table 3 with *T. viridae* and *A. terreus* showing significantly the highest antagonistic effects. Plate 5A shows the hyper-parasitic effect of *Trichoderma* viridae on *Fusarium oxysporum* f.sp. *lycopersici* and Plate 5B shows

the interaction of *Aspergillus terreus* and *Fusarium oxysporum* f.sp. *lycopersici*. In the interaction between *T. viridae* and *F. oxysporum* f.sp. *lycopersici*, *F. oxysporum* initially grew at a faster rate than *T. viridae* resulting in a stimulation rather than the inhibition of the fungi on the pathogen at 3days after inoculation (Figure 2), but a few more days after, *T. viridae* over grew on *F. oxysporum* and preyed on the mycelia of *F. oxysporum* showing its hyper-parasitic effect on the pathogen (Plate 5A), However, in the interaction between *A. terreus* and *F. oxysporum*, the growth of *A. terreus* preceded that of *F. oxysporum* and as it grew it pushed the *A. terreus* towards the centre of inoculation thus *A. terreus* only inhibited the growth of *F.oxysporum* based on its faster growth rate than pathogen.

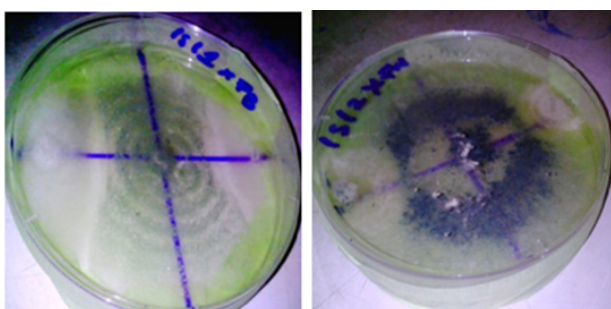


Plate 5 Dual culture plate of fungal isolates in spent mushroom compost and *Fusarium oxysporum* f.sp. *lycopersici*.
Plate 5A *Aspergillus terreus* and *Fusarium oxysporum* in dual culture experiment.
Plate 5B Hyperparasitic effect of *Trichoderma viridae* on *F. oxysporum*.

significantly higher than the lowest concentration (0.01g/ml), however, for sterilized spent mushroom compost, only the highest concentration (0.15g/ml) significantly inhibited the mycelia growth, the lower concentrations of 0.01 and 0.05g/ml rather stimulated the growth of the pathogen. Figure 3 & Figure 4 compares the effects of sterilized and unsterilized spent mushroom compost on *F. oxysporum* f. sp. *lycopersici* and indicated that unsterilized spent mushroom compost exhibited maximum inhibition in mycelia growth of *F. oxysporum* f.sp. *lycopersici* compared to sterilized spent mushroom compost for all the concentrations tested.

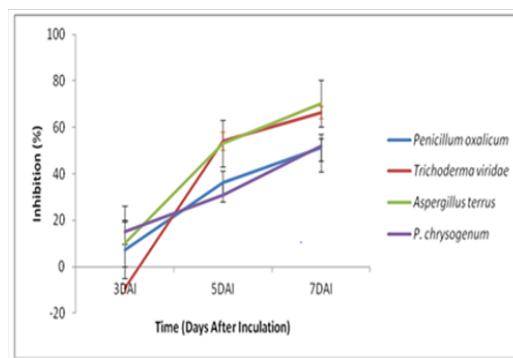


Figure 2 Trend of the inhibitory effects of the fungal isolates in spent mushroom compost on *Fusarium oxysporum* f.sp. *lycopersici* over 7days of inoculation. Standard error of the mean is indicated by vertical line. DAI, Days after inoculation

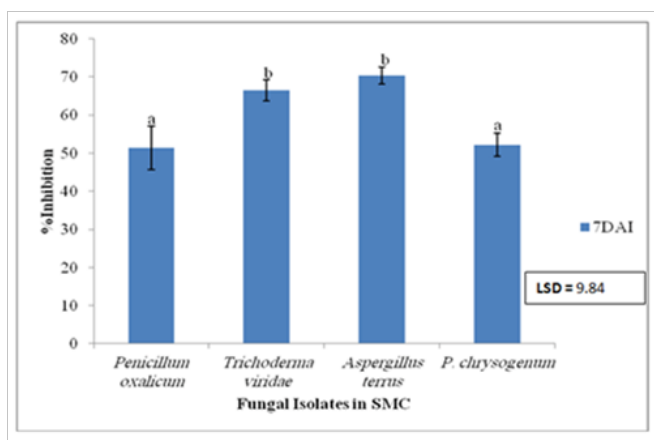


Figure 1 Effect of fungal isolates in spent mushroom compost on the inhibition of *Fusarium oxysporum* f.sp. *lycopersici* 7 days after inoculation. Bars with the same letter were not significantly different according to LSD at P(0.05). Standard error of the mean is indicated by vertical line.

Effect of spent mushroom compost on the growth of *Fusarium oxysporum* f.sp. *lycopersici*

Table 4 shows the means of percentage inhibition of mycelia growth of *Fusarium oxysporum* f. sp. *lycopersici* on PDA incorporated with Spent Mushroom Compost (sterilized and unsterilized) at the different concentrations from 3 days to 5 days after inoculation. A high inhibition of mycelium growth of *F. oxysporum* f. sp. *lycopersici* was recorded for all the tested concentrations of unsterilized spent mushroom compost, with 0.05, 0.10 and 0.15g/ml concentrations

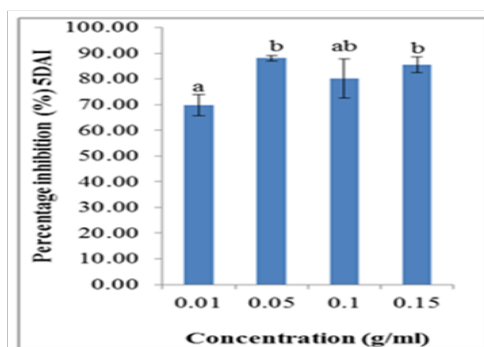
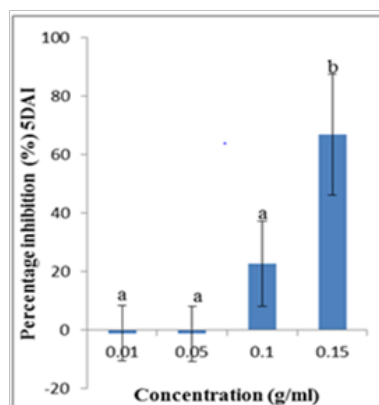


Figure 3 A comparison of the effect of Sterilized (A) and Unsterilized (B) Spent Mushroom Compost on the growth of *Fusarium oxysporum* f.sp. *lycopersici* at 5days after inoculation. Bars with the same letter were not significantly different according to LSD at P(0.05). Standard error of the mean is indicated by vertical line.

Table 1 List of fungal pathogens from vascular wilt diseased tomato seedlings

Isolates	Location	Colour on PDA	Mycelia growth pattern on PDA
FW1	NIHORT	White	adherent smooth growth
FW2	OGBOMOSO	Grey	Floccose

Table 2 Microorganisms isolated from spent mushroom compost

Isolate No.	Class of organism	Cultural Characteristics	Organism identified
1	Fungi	Mycelia is fluffy white with light greenish concentric dots turning grey as it ages	<i>Aspergillus terrus</i>
2	Fungi	Mycelia initially white but covered with dark green spores as it ages.	<i>Trichoderma viridae</i>
3	Fungi	Mycelia is fluffy white with very thick end and dispersed light greenish dots in the centre	<i>Penicillium chysogenum</i>
4	Fungi	Fluffy disperse white mycelia producing pinkish exudates	<i>Penicillium oxalicum</i>
5	Fungi	Fluffy disperse white mycelia	<i>Pleurotus saju caju</i>

Table 3 Effect of fungal isolates in spent mushroom compost on *Fusarium oxysporu*

	% inhibition		
	3DAI	5DAI	7DAI
<i>Penicillium oxalicum</i>	4.77±12.22a	36.16±5.21ab	51.43±5.74a
<i>Trichoderma viridae</i>	-9.35±1.72a	54.16±4.50b	66.51±2.78b
<i>Aspergillus terrus</i>	4.107±11.24a	53.2±3.99b	70.36±2.16b
<i>P. chrysogenum</i>	14.53±11.33a	30.93±5.32a	52.16±4.36a
Control	0.00	0.00	0.00
LSD (0.05)	26.86	12.45	9.84

Table 4 Effects of Sterilized and Unsterilized Spent Mushroom Compost on *Fusarium oxysporum f.sp. lycopersici* growth

Concentration (g/ml)	Average Percentage Inhibition (%)			
	3DAI		5DAI	
	SSMC	USMC	SMC	USMC
0.01	10.47±5.47a	32.68±6.61a	-4.12±9.46a	69.71±4.11a
0.05	4.112±17.91a	68.29±1.81b	-1.23±9.52a	88.10±1.13b
0.1	34.71±7.25a	56.83±13.86b	22.76±14.72a	80.27±7.56ab
0.15	49.59±27.10a	68.29±1.81b	66.93±20.58b	85.45±3.11b
Control (0.00)	0	0	0	0
LSD (0.05)	44.91	20.73	38.13	12.28

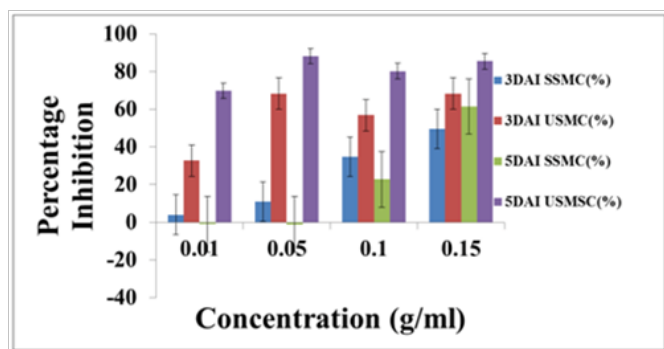


Figure 4 Effect of SMC inputs on the inhibition of *Fusarium oxysporum f.sp. lycopersici*.

Discussion

The inhibition of *F. oxysporum f.sp. lycopersici* by both sterilized and unsterilized spent mushroom compost show that spent mushroom compost extracts can be used for combating the economically devastating disease - fusarium wilt of tomato, which has gotten no safe effective control measures till date. In this study, we discovered that the inhibitory effect of spent mushroom compost on vascular wilt pathogens was affected by sterilization; this was in contradiction to the findings of Yohalem et al.²² that the inhibitory properties of spent mushroom substrate remained unaffected even after autoclaving and filter sterilization of extract. Unsterilized spent mushroom compost had a better inhibition potential than sterilized compost, this suggested that the pathogen inhibitory properties of spent mushroom compost could be more due to the biotic components than the abiotic components, i.e., more due to the activities of the inherent microorganisms rather than the chemical properties or the organic matter content. This is in support of the findings of Suárez et al.²³ More so, Microbial activity has been used as an indicator of the disease-suppressive properties of compost according to Boem et al.^{24–26} Spent mushroom compost from *Pleurotus sajor-caju* used in this study harboured fungal and bacterial population including *Aspergillus terreus*, *Trichoderma viridae*, *Penicillium spp.*, *Bacillus. licheniformis* and *Pseudomonas fluorescens*. This is in support of the findings of Ahlawat et al. on the microbial composition of spent mushroom compost of *Pleurotus sajor-caju*. Some of these microorganisms (*Trichoderma viridae*, *Penicillium oxalicum*, and *Bacillus subtilis*) have been reported in various studies to possess antagonistic property (6) and this was confirmed in this study from the result obtained from the dual culture assay involving the spent mushroom compost microorganisms and vascular wilt pathogens. Spent mushroom composts contained *Trichoderma viridae* that is easy to isolate and culture, grow rapidly on many substrates, and affect a wide range of plant pathogens, are rarely pathogenic to higher plants, act as myco parasites, compete well for food and site, produce antibiotics, and have enzyme system capable of attacking a wide range of plant pathogens.

Conclusion and recommendation

Spent Mushroom Compost that is discarded in mushroom industries in Nigeria offers a potential benefit to combat fusarium wilt disease of tomato thus the compost could be rather recycled for use after adequate preparation and treatment and/or fortified with additional

nutrients before use. In essence, spent mushroom compost can be used for biopesticides formulation to combat fusarium wilt of tomato. This will provide great benefits, involve very low cost and establish appropriate, environment friendly, sustainable and farmer friendly control measures against fusarium wilt disease of tomato. However, further *in vitro* studies are needed on the mechanism involved in the inhibition process so as to aid further *in vivo* studies in determining the mode and rates of application of spent mushroom compost in the tomato fields to suppress fusarium wilt disease without a significant reduction in plant growth.

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None.

Conflict of interest

The author declares no conflict of interest.

References

- Borrero C, Ordova's J, Trillas MI, et al. Tomato Fusarium wilt suppressiveness, the relationship between the organic plant growth media and their microbial communities as characterized by Biolog. *Soil Biology and Biochemistry*. 2006;38(7):1631–1637.
- Panthee DR, Chen F. Genomics of Fungal Disease Resistance in Tomato. *Curr Genomics*. 2010;11(1):30–39.
- Olaniyi JO, Akanbi WB, Adejumo TA, et al. Growth, fruit yield and nutritional quality of tomato varieties. *African Journal of Food Science*. 2010;4(6):398–402.
- Isah AS, Amans EB, Odion EC, et al. Growth rate and yield of two tomato varieties (*Lycopersicon esculentum* Mill) under green manure and NPK Fertilizer Rate Samaru Northern Guinea Savanna. *International Journal of Agronomy*. 2014;2014:8.
- <http://faostat.fao.org/site/339/default.aspx>
- Larkin RP, Fravel DR. Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. *Plant Dis*. 1998;82:1022–1028.
- Borrero C, Trillas MI, Ordovás J, et al. Predictive factors for the suppression of Fusarium wilt of tomato in plant growth media. *Phytopathology*. 2004;94(10):1094–1101.
- Horinouchi H, Watanabe H, Taguchi Y, et al. Biological control of Fusarium wilt of tomato with Fusarium equiseti GF191 in both rock wool and soil systems. *BioControl*. 2011;56(6):915–923.
- Hemming MN, Basuki S, McGrath DJ, et al. Fine mapping of the tomato I-3 gene for Fusarium wilt resistance and elimination of a co-segregating resistance gene analogue as a candidate for I-3. *Theor Appl Genet*. 2004;109(2):409–418.
- Dekker J. Resistance to fungicides in plant pathogens:abstracts of papers. *Netherland Journal of Plant Pathology*. 1981;87(6):233–255.
- Brimmer T, Boland G. A review of the non-target effects of fungi used to biologically control plant diseases. *Agricultural Ecosystem and Environment*. 2003;100(1):3–16.
- Litterick AM, Harrier L, Wallace P, et al. The role of un composted materials, composts, manures, and composts extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production—a review. *Critical Reviews in Plant Sciences*. 2004;23(6):453–479.

13. Avile's M, Borrero C, Trillas MI. Review on compost as an inducer of disease suppression in plants grown in soilless culture. In: Special Issue Compost III—dynamic plant, dynamic soil. *Global Science Books*. 2011;5(2):1–11.
14. Noble R, Coventry E. Suppression of soil-borne plant diseases with composts: A review. *Biocontrol Science Technology*. 2005;15(1):3–20.
15. Bonanomi G, Antignani V, Capodilupo M, et al. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry*. 2010;42(2):136–144.
16. Norhito Y, Jyuichi S, Mamoru S, et al. Physiological races and vegetative compatibility groups of butterhead lettuce isolates of *Fusarium oxysporum* f.sp. *lactucae* in Japan. *Journal of Genetics and Plant Pathology*. 2004;70(6):308–313.
17. Nirmaladevi D, Srinivas C. Cultural, Morphological, and Pathogenicity Variation in *Fusarium oxysporum* f. sp. *lycopersici* Causing Wilt of Tomato. *Batman University Journal of Life Sciences*. 2012;2(1):1–16.
18. Schaad NW, Jones JB, Chun W. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. 3rd ed. Monnesota, USA: APS Press; 2001. p. 155–156.
19. Barnett HL, Hunter BB. *Illustrated genera of imperfect fungi*. APS; 1998. 218 p.
20. Michel VV, Hartman GL, Midmore DJ. Effect of previous crop on soil populations of *Burkholderia solanacearum* Bacterial Wilt, and yield of tomatoes in Taiwan. *Plant Disease*. 1996;80(12):1367–1372.
21. Marlatt ML, Corell JC, Kaufmann P. Two genetically distinct populations of *Fusarium oxysporum* f.sp. *lycopersici* race 3 in the United States. *Plant Disease*. 1999;80(12):1336–1342.
22. Yohalem DS, Harris RF, Andrews JH. Aqueous extracts of spent mushroom substrate for foliar disease control. *Compost Science & Utilization*. 1994;2(4):67–74.
23. Suárez EF, Bustamante MA, Moral R, et al. *In vitro* control of fusarium wilt using agroindustrial Subproduct-based composts. *Journal of Plant Pathology*. 2012;94(1):59–70.
24. Boehm MJ, Madden LV, Hoitink HA. Effect of organic matter decomposition level on bacteria species diversity and composition in relationship to Pythium damping-off severity. *Appl Environ Microbiol*. 1993;59(12):4171–4179.
25. Chen W, Hoitink HAJ, Madden LV. Microbial activity and biomass in container media predicting suppressiveness to damping-off caused by *Pythium ultimum*. *Phytopathology*. 1988;78(11):1447–1450.
26. Choi HW, Chung IM, Sin MH, et al. The effect of spent mushroom sawdust compost mixes, calcium cyanamide and solarization on basal stem rot of the cactus *Hylocereus trigonus* caused by *Fusarium oxysporum*. *Crop Protection*. 2007;26(2):162–168.