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 terrus. 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

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. It is an important and cheap source of vitamins and an indispensable condiment in any meal preparation. Tomato can be eaten fresh or in processed forms and its consumption is associated with the prevention of several diseases due to the fact that it contains antioxidants including carotenes, (Lycopene as well as β-carotene), ascorbic acid, and phenolic compounds. 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 of tomato. This disease is deficient, and as a result, the rise of new fungicide-resistant pathogens species is of major concern. Management strategies for fusarium wilt are generally focused on preventive measures as no effective control measures are available yet. 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 cultivar. 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. 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. 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. 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.

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
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Collection of spent mushroom compost from oyster mushroom cultivation: A. Oyster Mushroom (Pleurotus saju 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 air oven (at 1600°C) for at least 2 hours. Metals including inoculating needles, wire loops, forceps, spreader, scalpel were always sterilized by exposure to the blue part of incandescent bulb at 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 15 minutes. The medium is allowed to cool to around 450°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 2 minutes and rinsed in three changes of sterile distilled water then dried on sterilized filter paper before placing on solidified 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±2°C) for 7 to 10 days as described by Norhito et al.,14 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 homogenously mixed soil samples was dispensed 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 9 ml of sterile distilled water in test tubes. 0.1 ml of dilutions of 102 to 106 was spread on solidified 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, chlamydospores 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±2°C) for 10 days. After which 50ml of sterile distilled water was dispensed into the pure cultures on Petri plates and the surface of the cultures was scraped 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 0.0025mm^2 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:

\[ \text{Number of conidia} = \frac{\text{N}}{100} \]

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. Seeds 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 30 minutes. 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. Twenty-one day old tomato (cv Kerewa and Roma) seedlings were maintained in each treatment (treatments consisted of the spent mushroom compost fungus and the pathogens from the centre of disc towards the edge of the plates). All plates were incubated at 25±2°C. Data were collected on mycelia growth and recorded from 3 days 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:

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

Where 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 indigenous fungi. Plates were incubated at 28±2°C for 5 days. 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:

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

Where I = percentage inhibition of mycelia growth

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**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 chlamydospores (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, epyctical, kidney- shaped microconidia gradually pointed with curved edges (pointed end) like a boat shape, they are hyaline and, transparent and had no septa. The chlamydospores 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 Fsp. lycopersici* since they were pathogenic specifically to tomato. Tomato cv kerewa was more susceptible than cv Roma. Other Fungi such as Penicillin 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 Chlamydospores of *F. oxysporum*.

**Plate 3B** Microscopic view of macroconidia and microconidia of *F. oxysporum*.

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

All the fungal isolates obtained from spent mushroom compost (Trichoderma viridae, Penicillum oxalicum, *P. chysogenum* and *Aspergillus terrus*) except the residual mushroom mycelia showed antagonistic property on *Fusarium oxysporum* fsp lycopersici in vitro at 7days after inoculation as shown in Figure 1 and Table 3 with *T. viridae* and *A. terrus* showing significantly the highest antagonistic effects. Plate 5A shows the hyper-parasitic effect of *Trichoderma viridae* on *Fusarium oxysporum Fsp. lycopersici* and Plate 5B shows...
the interaction of Aspergillus terrus 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 3 days after inoculation (Figure 2), but a few more days after, T. viridae over grew on F. oxysporum and preyyed on the mycelia of F. oxysporum showing its hyper-parasitic effect on the pathogen (Plate 5A). However, in the interaction between A. terrus and F. oxysporum, the growth of A. terrus preceded that of F. oxysporum and as it grew it pushed the A. terrus towards the centre of inoculation thus A. terrus only inhibited the growth of F. oxysporum based on its faster growth rate than pathogen.

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 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 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 7 days of inoculation. Standard error of the mean is indicated by vertical line. DAI, Days after inoculation](image)

![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 5 days after inoculation. Standard error of the mean is indicated by vertical line.](image)

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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Location</th>
<th>Colour on PDA</th>
<th>Mycelia growth pattern on PDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW1</td>
<td>NIHORT</td>
<td>White</td>
<td>adherent smooth growth</td>
</tr>
<tr>
<td>FW2</td>
<td>OGBOMOSO</td>
<td>Grey</td>
<td>Floccose</td>
</tr>
</tbody>
</table>

Table 2 Microorganisms isolated from spent mushroom compost

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

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

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

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

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

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.\(^4\) 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 bioactive 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.\(^9\) More so, Microbial activity has been used as an indicator of the disease-suppressive properties of compost according to Boem et al.\(^{20,21}\) Spent mushroom compost from *Pleurotus sajor-caju* used in this study harboured fungal and bacterial population including *Aspergillus* *tertus*, *Trichoderma viridae*, *Penicillium* spp., *Bacillus* *licheniformis* and *Pseudomonas fluorescens*. This is in support of the findings of Ashlawat 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 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.

**Acknowledgements**

None.

**Conflict of interest**

The author declares no conflict of interest.

**References**

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