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Role of Mushroom Pathogen *Trichoderma harzianum* Rafai in the Reduction of Growth Yield, Nutrient Quality, and Morphometric Measurements of the Fruiting Body of *Pleurotus ostreatus* EM-1 From Seven Oyster Mushroom Cultivation Farms in Ghana.

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Abstract

The cultivation of edible mushrooms such as Pleurotus ostreatus commonly known as oyster mushroom has been undertaken by both commercial and cottage industry entrepreneurs in Ghana with appreciable success. However, the presence of competitors and potential pathogens in the substrate militate against increased yield and quality of fruiting bodies. The most prevalent pathogen Trichoderma harzianum in the compost for cultivating P. ostreatus is responsible for the green mould disease. In this study, in vitro pathological effects of spiking Triplochiton scleroxylon sawdust substrate with T. harzianum on pinhead emergence, percentage formation of fruiting bodies, weight of fruit body, yield, Biological Efficiency as well as morphometric data of stipe length, stipe diameter, cap diameter as criteria for reduced growth performance of P. ostreatus were investigated. The effect of the pathogen on the nutritional and elemental composition of the oyster mushroom were determined with the view of assessing the quantitative changes of nutritional and elemental composition of the mushroom using standard methods. Growth substrates inoculated with T. harzianum reduced pinhead formation; number of fruiting bodies formed, percentage conversion of pinheads, weight of fruiting bodies, and reduced morphometric measurements of stipe length, stipe diameter, cap diameter with all three flushes of the mushroom. Total yield of fruiting bodies in all three flushes in the T. harzianum-inoculated bags decreased by 67.4%, 68.5% and 83.3% respectively with the concomitant reduction of total yield by 52.3% and Biological Efficiency by 52.8%. Some of the control bags (uninoculated bags) also performed poorly as a result of contamination by T. harzianum, indicative of inadequate sterilization. Fruiting bodies of P. ostreatus grown in uninoculated compost contained Ca, Cu, Fe, K, Mg, Na, P, and Zn in contrast with the absence of Na, and Zn in the infected bags. There was a reduction in fat, crude fibre, crude protein, and carbohydrates of the fruiting bodies grown in the contaminated bags. On the other hand, total ash content was doubled in the carpophore from the T. harzianum spiked contaminated bags. The pathogen therefore has adverse effect on the production and marketing quality as well as the nutritional value of the fruiting bodies.

Keywords : *Trichoderma harzianum, Pleurotus ostreatus, Fruiting bodies*, Nutritional and Elemental Composition; Total Yield, Biological Efficiency, Morphometric Measurement.

Introduction

Mushrooms are macro-fungi with distinctive fruiting body, which may be above-ground (epigeous) or below ground (hypogenous) of sufficient size to be seen by the naked eye and to be picked by hand (Miles and Chang, 1997). Most mushrooms belong to the Class Ascomycetes (hypogenous) or Basidiomycetes (epigeous). Like plants, fungi have a distinct cellular structure, but they lack the most important feature of high plants, i.e., the possession of chlorophyll which enables higher plants to use the energy from the direct sunlight (Chen, 1981). Since mushrooms do not possess chlorophyll, they depend on the plant material, the substrate for their food. They are simply referred to as saprophytic, parasitic, or symbiotic in nature (Emuh, 2009).

Saprophytic mushrooms depend on and grow in plant and animal remains and waste such as animal dropping, dead animals, twigs, fallen leaves, dead wood, stumps, and other lignocellulose waste from agricultural production (Obinna-Echem and Chukunda, 2018; Emuh, 2009; Oei, 1996). Thus, they form the first menu in the food chain or food web by secreting acids and enzymes which degrade plant and animal remains, breaking down large and insoluble molecules e.g., carbohydrates, lipids, and proteins into smaller molecules that can be absorbed (Stamets, 2006; Alexopoulos et al., 2003).

Pleurotus ostreatus is a saprophytic macro-fungus which exploit polysaccharides (cellulose, hemicellulose) usually from a wide range of lignocellulose to produce expensive protein for human consumption (Frimpong-Manso et al., 2011). This mushroom has high nutritional value as an important source of protein, carbohydrates, vitamins, mineral elements and is among the most favourite mushrooms of the world. Global production of mushrooms was 11.9 million metric tons in 2019 (Market Intelligence Team, 2021). *P. ostreatus* is one among the 40 known species of the genus *Pleurotus* commonly called "oyster mushroom" grown widely in tropical and subtropical areas artificially cultivated (Deepalakshimi and Mirunalini, 2014). The nutritional and several medicinal properties of mushrooms of the genus *Pleurotus* has been reviewed by Deepalakshimi and Mirunalini, 2014; Khan and Tania, 2012.

There is an old saying that "medicines and food have a common origin". Mushrooms are the manifestation of this idea in constituting both a nutritionally functional food and a source of physiologically beneficial compounds with possible medicinal applications (Asaduzzaman Khan and Tania, 2012). Recent studies have shown that medicinal actions of mushrooms, including *Pleurotus* are: antitumour, immunomodulating, antioxidant, radical scavenging, cardiovascular protector, anti-hypercholesterolemia, antiviral, antibacterial, antiallergic, antiparasitic, antifungal, detoxification, hepatoprotective, antidiabetic effects and antiallergic effect (Ichinohe et al., 2010; Khan 2010; Wasser, 2010; Dai et al., 2009; Zhang et al., 2007; Sullivan et al., 2006; Didukh et al., 2003; Gao et al., 2002; 2003; 2004; Rowan et al., 2003; Kaul 2001; Wasser and Weiss, 1999).

In many developing countries, small-scale cottage and commercial farms for cultivating *P. ostreatus* exist (Muchane, 2016). In Ghana oyster mushroom cultivation is on the ascending taken on as part of the poverty alleviation strategy for rural folks. Mushroom Growers and Exporters Association of Ghana exist manned by entrepreneurs coming together to popularise consumption and production of *P. ostreatus* in addition to other cultivatable mushrooms such as *Volvariella volvacea* (popularly called 'domo') (https://yen.com.gh; https://myhealthbasics.site). The major problem militating against sustained production is the presence of fungal competitors and pathogens of the genus *Trichoderma* in the compost bags leading to a drastic reduction in Biological Efficiency and total yield of the crop. Sharma et al. (2013) reiterated that in many instances, there is a complete crop failure of mushrooms depending on the extent of competition by residual resident fungi and the mycological quality of substrate after pasteurization. The most popular substrate used for cultivation of *P. ostreatus* in Ghana was wawa sawdust (*Triplochiton scleroxylon*) until recently when other workers e.g., Wiafe-Kwagyan et al. (2015; 2018; 2022) and Frimpong-Manso et al. (2011) showed that rice wastes abounding in Ghana can be used as substrate for successful cultivation of *Pleurotus* species to ameliorate environmental pollution by discarded lignocelluloses with prospective economic benefits.

The method of commercial and cottage industry cultivation of *P. ostreatus* is on sterilized substrates (sawdust, sugarcane baggase, cornstalks, waste cotton, banana leaves, etc.) (Thongklang and Luagham, 2016; Chang and Miles, 2004). In some instances, the substrate may not be sterilized before inoculation with spawn, though a pasteurization step may be included in the process and the mushroom grows in competition with other microorganisms on the substrate (Kertesz and Thai, 2018). Mushrooms grown in properly sterilized substrates, the rate of mycelial growth is dependent on enzymatic degradation of lignocellulose by the mushroom itself and is independent of other microbes (Kertesz and Thai, 2018). For *Pleurotus* by contrast, growth of the mycelium and production of commercial fruiting body are dependent not only on the mushroom itself but also on bacteria and other fungi in the substrate.

These bacteria play key roles at several different stages of production like

- conversion of the lignocellulose into a selective nutrient-rich compost for mushroom growth
- interaction with fungal mycelium during hyphal elongation and proliferation through the substrate
- induction of fruiting body formation during cropping.

Additionally, several bacterial and fungal taxa act as pathogens of the mushroom crop causing either a reduction in yield or severe loss of quality (Kertesz and Thai, 2018). The composting and the subsequent growing process in mushroom substrate e.g., wawa sawdust has been reviewed by Obodai and Odamtten (2012) on the fungal aspect of the phenology and attendant changes in agricultural lignocellulose waste used for mushroom cultivation. One of the principal fungal contaminants in mushroom composts causing marked decline in production are in the genus *Trichoderma*.

Trichoderma species are known to be pathogenic to *Pleurotus* cultivation by the process of antibiosis. Antibiosis is the inhibition of one microorganism by another through chemical means; lysis is the final destruction and degradation of mycelium by enzymes from the antagonist (Goltapeh et al., 2000; Mumpuni & Maryanto, 1994) Alternatively, antagonism may be expressed by competition for nutrients in a micro-ecological environment which is a well-known phenomenon in mushroom compost during preparation of substrate for lignocellulose bioconversion of mushroom mycelium into fruiting body. Some members of the genus *Trichoderma* (*T. koningii, T. harmatatum, T. crissum, T. sporula, T. harzianum, T. viride*) have been isolated from mushroom composts antagonistic to

P. ostreatus, P. eous, and *P. sajor-caju* (Wiafe-Kwagyan et al. 2015; Ospina - Geraldo et al., 1999; Jandaik and Guleria, 1999; Castle et al., 1998). *T. aggressivum* caused extensive crop losses in both America and Europe in the 1990's and is problematic worldwide caused by two slightly different strains in America and Europe (*T. aggressivum* and *T. aggressivum* f. european (Hatvani et al., 2017; Samuels et al., 2002). *Pleurotus ostreatus* is also affected by a green mould disease but the disease is caused by a related but phylogenetically different species named T. harzianum (Kredics et al., 2009). Previous aggressive colonization of mushroom compost causing epidemic outbreak of green mould in *P. ostreatus* was also attributed to *T. harzianum* (Seaby, 1996, 1987; Doyle, 1994; Morris et al. 1995). Jayalal and Adikaran (2007), however isolated *T. harzianum* from mushroom compost causing green mould disease in *P. ostreatus* resulting in considerable inhibition of growth of mycelium and formation of fruitbodies and consequently lowering substantially the yield of the crop. The culture metabolites of *Aspergillus flavus, Penicillium citrinum* and *Trichoderma harzianum* were antagonistic to dry matter accumulation by the mycelium of *P. ostreatus* and *P. eous* (Wiafe- Kwagyan et al., 2015). *T. harzianum* metabolites was the most potent in the antibiosis effect on the two *Pleurotus* species (Wiafe-Kwagyan et al., 2015).

Oyster mushroom cultivation in Ghana is faced with a plethora of constraints including the presence of strong fungal competitors and pathogens especially *T. harzianum*. Furthermore, the environmental health quality in the incubation and cropping houses as well as the aeromycoflora exacerbates the problem of cropping losses. Kortei (2015), reported that about 64% of the Ghanaian mushroom farmers of oyster mushrooms expressed dissatisfaction about the efficacy of their steam sterilization process of the compost in oil drums and blamed the insufficient sterilization on the subsequent proliferation of pathogens particularly *T. harzianum* and other fungal competitors in the compost during the cultivation of oyster and other mushrooms. This often led to heavy losses of crops economic setbacks owing to the green mould disease.

Recently, Adalete (2020) surveyed seven (7) mushroom farms in two Regions of Ghana with the view to understanding and also obtaining baseline data on the mycobiota quality of the spawn compost substrate ('wawa' sawdust), fruiting body, and aeromycoflora of the incubation / cropping rooms where the mushroom is produced. Across all the seven farms, 20 fungal species belonging to 11 genera were isolated from the compost substrate and predominated by *Aspergillus species* (*A. candidus, A. flavus, A. fumigatus, A. niger, A. oryzae, A terreus, A. penicillioides*) followed by *Fusarium* (*F. oxysporum, F. poae, F. solani*) and *Cladosporium* (*C. herbarum, C. macrocarpum*). Although the mushroom farms were widely separated by distance (and not juxtaposition) *T. harzianum* was the most frequently encountered and isolated fungal species. In addition, potential mycotoxigenic *Aspergillus* species (*A. flavus, A. niger, A. terreus, A. fumigatus*) were resident in the substrate used on the farms (Adalete, 2020).

Eighteen (18) different fungal species belonging to ten (10) genera were isolated from the mature fruiting body of *P. ostreatus* across all the seven farms (Adalete, 2020). Again, *Aspergillus* species (*A. alutaceus, A. candidus, A. flavus, A. fumigates, A. niger, A terreus*) predominated over the other genera encountered resident in the fruiting body. This was followed by *Cladosporium (C. macrocarpum, C. herbarum), Fusarium (F. poae, F. oxysporum)* and *Penicillium (P. brevicompactum, P. roqueforti)*. In general, *T. harzianum* was predominant and was isolated from the fruiting body from all the seven mushroom farms causing the green mould disease (Adalete, 2020). *Aspergillus* species with mycotoxigenic potential resident on the fruiting bodies were *A. flavus, A. fumigatus, A. niger, A. terreus and A. alutaceus (= A. ochraceus)*.

The aeromycoflora in the farmhouses belonged to nineteen (19) fungal species belonging to twelve (12) genera predominated by *Aspergillus species* (*A. candidus, A flavus, A. niger, A. oryzae, A parasiticus*) all with mycotoxigenic potential, followed by *Fusarium (F. oxysporum, F. poae), Penicillium (P. brevicompactum, P. citrinum), Cladosporium (C. herbarum, C. macrocarpum)*. The potential pathogenic *T. harzianum* was isolates from 57% (4/7) of the cropping / storage houses surveyed.

In this paper, we report the pathological influence of the prevalent *T. harzianum* on some growth and developmental parameters of the developing mushroom (pinhead formation, pinhead conversion to fruitbody, weight of fruits harvested, morphometric measurements of stipe, and pileus of fruiting body) in the composts used in the farmhouses. Finally, the influence of the antibiosis effect of *T. harzianum* on the proximate analysis, elemental composition, and nutrient composition with or without infection are presented.

Materials and Methods

Substrate Bags for Pathogenicity Test

Ten Substrates bags were supplied by the Kwesi-Babs Mushroom Farm at Kasoa in the Central Region of Ghana (GPS Coordinates; Satellite Map 5' 32'4.16 M (Lat) 0°25'0 44 E (Long.) in the Awutu Senya East Municipal District. This Farm supplies already inoculated and sterilized substrate bags to most Mushroom Farmers in Accra for commercial production of oyster mushrooms.

Pathogenicity test and Maintenance of Culture

Culture of *Trichoderma harzianum* was same as studied by Wiafe-Kwagyan (2015) and authenticated by molecular sequence method of Kredics et al. (2009) and Hatvani et al. (2007) and cultural and morphological and molecular characteristics Wiafe-Kwagyan et al. (2022). Stock cultures were maintained on slants of PDA in Macartney tubes and 90 cm Petri dishes and kept in a refrigerator at 8°C until needed.

The sterilized 1(21°C for 15 min at 1.05 kg /cm3) and inoculated substrate bags were divided into two batches of five. One batch of five bags were spiked (inoculated) with 3 mm discs of the

test fungus *T. harzianum* and the remaining five bags served as control (without inoculation). The bags were incubated in the conventional way for the fruiting bodies to develop and mature. Pathogenic effect of the *T. harzianum* was ascertained by estimating the underlisted parameters after thirty-five (35) days of growth through 1st, 2nd and 3rd flushes of the fruiting bodies.

Assessment of yield of fruiting bodies

The following parameters were assessed:

1. The total number of pinheads formed at each flushing time (1st, 2nd, 3rd)
2. Total number of fruiting bodies formed at each flushing tine (1st, 2nd, 3rd)
3. Fresh weight of fruiting bodies formed at each flushing time using digital electric weighing balance.

Biological Efficiency (BE) at each harvesting time and final total

Percentage (%) Biological Efficiency was determined using the method prescribed by Pathmashini et al. (2008) and Patra and Panu (1995).

$$BE = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} X\ 100\ \%$$

Determination of Moisture Content

This parameter was determined by the gravimetric method (Black, 1965). A gram of dried powdered mushroom was measured separately into previously measured cans in triplicate. The sample was dried in GallenKamp Oven at 105°C for 6 h, cooled in a desiccator and then reweighed. The cooled sample was returned to the oven for further 1 h heating until constant weight was obtained. The weight of the moisture loss was determined and expressed as a percentage.

Proximate Analysis of fruiting bodies

The proximate compositions of the mushroom from the Farms were analysed using the standard analytical methods (AOAC, 2012). Ash was determined gravimetrically in a muffle furnace at 150°C for 6 h as described by Van Svest et al. (1991) using 2.0 g of the sample.

$$\text{Ash content} = \frac{W_2 - W_1}{W} \text{ x } 100\%$$

Where W_1 = weight of Empty crucible
W_1 = weight of crucible + ashes.
W = weight of sample

Protein was determined by the standard Kjedahl method. After distillation and titration, the nitrogen was corrected using a factor of 4.58 for mushrooms (Miles and Chang. 1997).

Lipids were estimated by exhaustive extraction of 2 g sample with petroleum ether in a Soxhlet extraction apparatus (AOAC, 2012).

Percentage fat was obtained from the mathematical relations:

$$\%\ \text{Ether extract (DMB)} = \frac{W_3 - W_2}{W_1} \text{ x } 100$$

$$\%\ \text{Ether extract (DMB)} = \frac{\%\ \text{Ether extract}}{\%DM} \text{ x } 100\%$$

$$\%\ \text{Fat} = 100\ (W_2\text{-}W_1)\ X\ 100$$

When W_1 = weight of sample; W_2= Initial Weight of extraction flask and content
W_3 = Oven dry weight of flask + oil (fat) extract

Crude fibre was obtained as the difference from the weight of ash subtracted from the increase of weight on the paper due to the insoluble material after the acid hydrolysis of the fat free sample and the filtration into ashless filter paper (Obinna-Echem and Chukunda, 2018).

Carbohydrates was obtained by difference:

Total Carbohydrate (%% DW) = 100% – protein content (% DW) – Lipid content (% DW) – ash (% DW).

Carbohydrate (%) = 100 – moisture – total ash – fibre – protein – fat (Nielson, 2010).

Mineral Assay

The Atomic Absorption Spectrophotometer method was employed for the mineral element assay. Mineral elements of *Pleurotus ostreatus* was determined by preparing the solutions of their ashes. The quantitative measurement of each element Fe, Mg, Na, Ca, P, K, Zn, Cu was carried out using UNICAM 929 Atomic Absorption Spectrophotometer AAS, Model RinAAcle 900T. Phosphorus was determined on the ash solution using the molybdenum blue method (AOAC, 2012).

Statistical Analysis

Where necessary, data was analysed statistically using T-test and ANOVA and the results are quoted at 5% level of significance ($P \leq 0.05$).

Results

Assessment of some growth and development parameters (i.e., no. of pinheads, percentage pinhead conversion to fruitbodies, weight of fruit, stipe length, stipe width, cap diameter) of *Pleurotus ostreatus* EM-1 grown on sawdust composted treated with *Trichoderma harzianum*.

Pinhead Formation

The ten (10) bags of compost bags for the experiment were divided into two groups; 5 bags were left uninoculated (control) and 5 bags spiked or inoculated with *T. harzianum* spores. This set of experiments was repeated twice. The results obtained is summarised as follows:

The bags in each group of inoculated and uninoculated samples behaved independently at 1st, 2nd and 3rd flushes (Fig. 4) in terms of the number of pinheads (primordia) formed. The spiked or inoculated bags with *T. harzianum* reduced pinheads formation at each flushing period (Fig. 1). At the 3rd flushing the uninoculated bags (control) survived performed best (Fig. 1) producing more pinheads even though in the production of pinheads was also reduced such as in bag 5 during the 1st flush and repeated in the 2nd flush (Fig. 1) which is indicative of resident fungi in the sterilised bag.

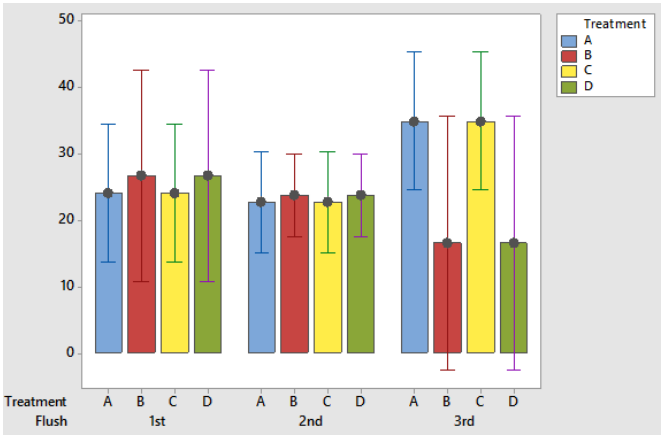

Figure 1: Total number of pinheads formed on uncontaminated and contaminated bags per flush

Key:
A = Uncontaminated 'wawa' sawdust spawn bag (control),
B = 'Wawa' sawdust spawn bag inoculated with *T. harzianum* (mushroom pathogen)
C = Uncontaminated growth substrate,
D = **T. harzianum** contaminated growth substrate

Fruiting bodies formation

The compost bags in each group of inoculated and uninoculated samples behaved independently in the 1st, 2nd and 3rd flushes in terms of the number of successful fruiting bodies formed (Fig. 2).

The second and third flushes also produced successful mature fruiting bodies but the adverse effect of spiking the bags with *T. harzianum* was evident in the 3rd flush with drastically reduced successful fruiting bodies (Fig. 2). In the third flush, the control bags without spiking with *T. harzianum* produced substantial fruiting bodies while only two bags (Bags 2 and 3) of the inoculated compost formed fruiting bodies. Bags 1, 4, 5 inoculated with *T. harzianum* produced moribund (non-viable) fruiting bodies. Interestingly the control bags also reduced successful fruit body formation (Plates 1 and 2).

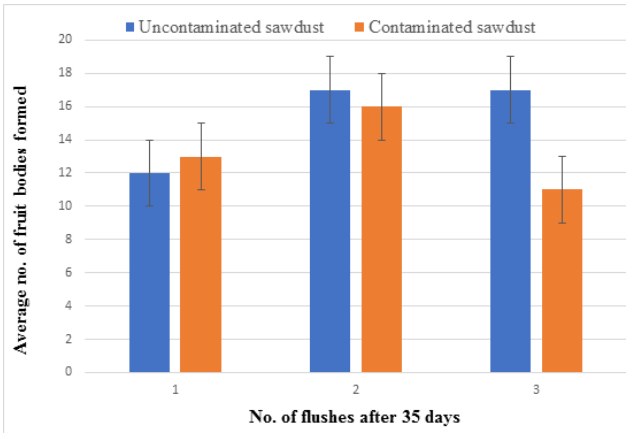

Figure 2: Average number of fruiting bodies formed per bag on uncontaminated and contaminated 'wawa sawdust (inoculated with *T. harzianum*) after 35 days of cropping

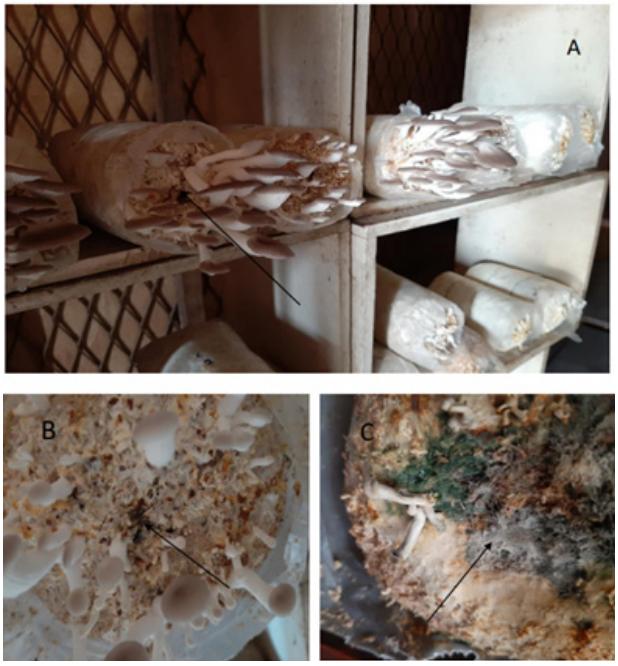

Plate 1: Substrate bags contaminated with *Trichoderma harzianum*, note the progression and appearance of the green mould fungus on the growth substrate (A to C). (Mag X0.05)

Plate 2: Appearance of matured fruit bodies of *P. ostreatus* EM-1 grown on uncontaminated 'wawa' substrate bags (Mag X0.05)

Percentage Pin Conversion to Fruiting Bodies

The bags again behaved independently. In the first flush conversion from pinheads to fruiting bodies varied from nil to 54.4 % in the uninoculated bags and nil to 35.3% (Fig 3) in the

bags spiked with *T. harzianum*. In the second flush, conversion from pinhead to fruiting in the control varied from nil to 84.7% while in the inoculated bags percentage conversion from pinheads to fruiting bodies varied from nil to 85.7% (Fig. 3). The 3rd flush produced nil to 73.9% pinhead conversion to fruiting the control as compared to nil to 73.3% in the inoculated bags (Bags 2 and 3). It was evident that contrary to expectation the uninoculated control bags also showed declining percentage conversion of pinheads to fruiting bodies (Fig. 3). This could also be partly attributed to aerospores of the green mould pathogen finding it way onto the uninoculated bags.

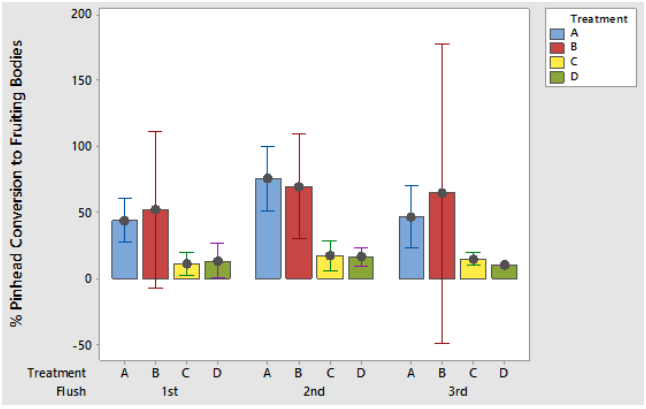

Figure 3: Percentage (%) conversion of pinheads to fruiting bodies on uncontaminated and contaminated bags per flush

Key:

A = Uncontaminated 'wawa' control sawdust spawn bag,

B = 'Wawa' sawdust spawn bag inoculated with *T. harzianum* (mushroom pathogen)

C = Uncontaminated growth substrate,

D = *T. harzianum* contaminated growth substrate

Weight of fruiting bodies at different flushing times and the attendant total yield and Biological Efficiency

The bags again behaved differently and independently. In the first flush, weight of fruiting bodies in the *T. harzianum* inoculated bags compared favourably with the uninoculated (Fig 4). The second flush was adversely affected in all instances in the inoculated and uninoculated bags (Fig 4). The third flush produced some fruiting bodies with weight ranging for 19.7g to 54.0 g in the control as compared to nil to 13.6 g in the bag spiked with *T. harzianum* spores.

Table 1 summarises the results of the yield/flush cycle (1-3), total yield and the Biological Efficiency. Inoculation or spiking the compost with *T. harzianum* reduced the yield of the 1st, 2nd and 3rd flushes by 67.4, 68.5 and 73.6% respectively. Subsequently, the total yield was reduced by 52.3% and Biological Efficiency of the substrates declined by 52.8% (Table 1).

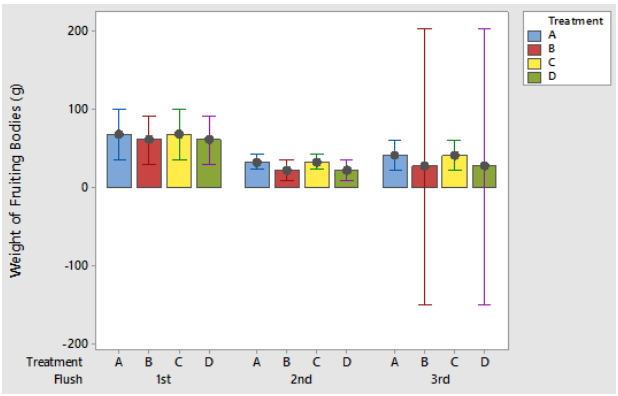

Figure 4: Comparison of weight of fruiting bodies formed on uninoculated 'wawa sawdust and *Trichoderma* inoculated 'wawa sawdust bags after three flushes for 35 days

A = Uncontaminated wawa sawdust spawn bag,

B = Wawa sawdust spawn bag inoculated with *T. harzianum* mushroom pathogen

C = Uncontaminated growth substrate,

D = *T. harzianum* contaminated growth substrate

Substrate type	Yield/Flush (g)			Total Yield (g)	Biology Efficiency (%)
	1st	2nd	3rd		
uninoculated	271.48	97.08	208.29	576.85	164.91
wawa sawdust spiked with *T. harzianum* spores	182.89	66.50	54.98	304.37	87.02

Table 1: Total Yield and Biological Efficiency (BE) of *P. ostreatus* Strain EMI per flush growing on sawdust with or without artificial spiking with spores of *Trichoderma harzianum* at 28°C for 35 days.

Influence of the inoculation of the compost with or without *Trichoderma harzianum* on the morphometric characters the stipe, and cap (pileus) of the oyster mushroom (*P. ostreatus* EM-1).

Pathogenicity of *T. harzianum* against growth and yield of *P. ostreatus* will be shown not only in the formation of fewer pinheads, conversion of pinheads into fruitbodies, weight of fruitbodies, yield and Biological Efficiency but will also lead to diminutive fruitbodies reflected in shorter stipes, stipe diameter and dimensions of cap (pileus). Figures 5a, b & c show that the bags showed the same trend in the reduction of the stipe length in all the three flushes of the fruiting bodies. These changes were more accentuated in bags inoculated with *T. harzianum*. Apart from bags 2 and 3 inoculated with *T. harzianum* none of the remaining bags 1, 4 and 5 formed pinheads to produce viable fruiting body at the 3rd flush (Figs. 5a). The development of the cap diameter during the first, second and the third flushes is shown in Table 2. The bags 1, 4 and 5 inoculated with *T. harzianum* did not form pinheads let alone form viable fruit bodies at the 3rd Flush (Fig. 5a) to record any cap dimensions.

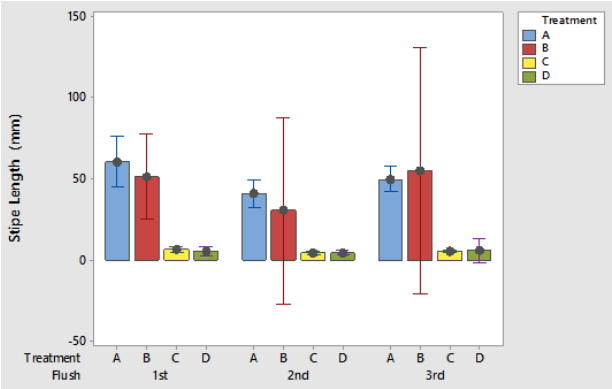

Figure 5a: Influence of inoculated 'wawa' compost with *T. harzianum* and uninoculated 'wawa' compost on stipe, and cap parameters of *P. ostreatus* EM-1

Key:
A = Uncontaminated 'wawa' control sawdust spawn bag,
B = 'Wawa' sawdust spawn bag inoculated with *T. harzianum* (mushroom pathogen)
C = Uncontaminated growth substrate,
D = *T. harzianum* contaminated growth substrate

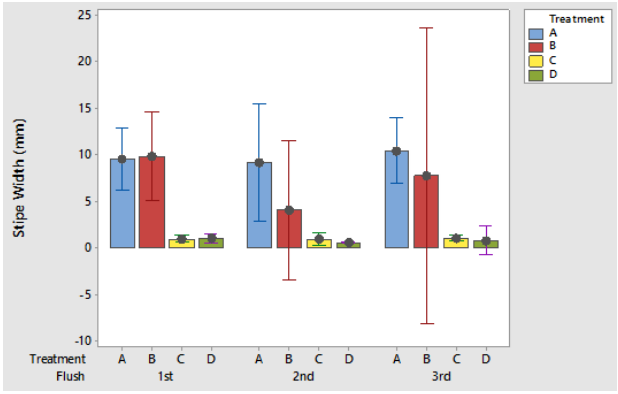

Figure 5b: Influence of inoculation of 'wawa' compost with or without *T. harzianum* the morphometric characters stipe, and cap parameters of oyster mushroom (*P. ostreatus* EM-1)

Key:
A = Uncontaminated 'wawa' control sawdust spawn bag,
B = 'Wawa' sawdust spawn bag inoculated with *T. harzianum* (mushroom pathogen)
C = Uncontaminated growth substrate,
D = *T. harzianum* contaminated growth substrate

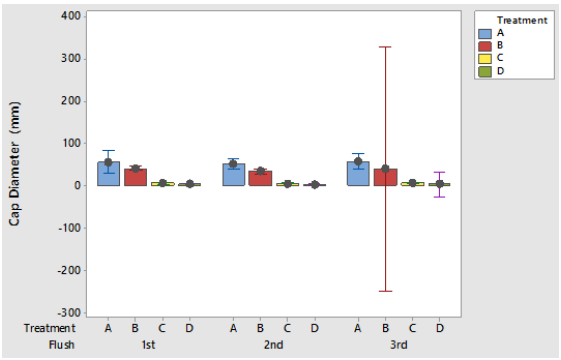

Figure 5c: Effect of the inoculation of the compost bags with or without *T. harzianum* on cap diameter of *P. ostreatus* EM-1

Key
A = Uninoculated 'wawa' sawdust spawn bag,
B = Wawa sawdust spawn bag inoculated with *T. harzianum* mushroom pathogen
C = Uncontaminated growth substrate,
D = *T. harzianum* contaminated growth substrate

Sample compost	Replicates of Bags	Diameter of pileus/cap (mm) after 35 days		
		1st Flush	2nd Flush	3rd Flush
Wawa sawdust (uninoculated control)	1	75.0 ± 0.0	50.0 ± 7.0	39.5 ± 10.0
	2	60.5 ± 3.0	48.0 ± 4.0	80.5 ± 10.0
	3	34.0 ± 6.0	-	57.0 ± 10.0
	4	-	57.0 ± 3.0	51.5 ± 11.0
	5	55.0 ± 13.0	-	61.0 ± 2.0
Wawa sawdust spiked with spores of *T. harzianum*	1	-	36.0 ± 13.0	-
	2	39.5 ± 6.0	-	17.0 ± 4.0
	3	42.5 ± 3.0	31.5 ± 5.0	62.5 ± 3.0
	4	-	35.5 ± 13.0	-
	5	42.0 ± 0.0	-	-

Table 2: Comparative cap diameters of fruiting bodies of *P. ostreatus* EM-1 growing in different wawa sawdust bags either uninoculated (control) or inoculated with spores of *T. harzianum* and assessed during the indicated flush periods for 5 weeks (35 days) at 28°C

Analyses of mineral elements and heavy metals content of the fruiting bodies of *P. ostreatus* EM-1 cultivated on either wawa sawdust with or without artificial spiking with spores of *T. harzianum*

The mineral content of fruiting bodies of *P. ostreatus* cultivated on contaminated sawdust were almost same as the control uninoculated samples. All the samples contained Na, Zn Ca, Cu, Fe, K, Mg, P. The only difference was that the fruiting bodies grown on contaminated sawdust inoculated with *T. harzianum* did not contain Na, and Zn (Table 3). There were no statistical differences ($p \geq 0.05$) in the amounts detected in the control and the inoculated substrates except Ca and Fe.

Substrate Treatment	Mineral Content (mg/kg)							
	Ca	Cu	Fe	K	Mg	Na	P	Zn
Non spiked 'wawa' sawdust (uninoculated control	4.43	0.04	1.13	16.66	3.56	0.01	12.40	0.03
'wawa' sawdust spiked with *T. harzianum*	0.99	0.04	0.22	16.00	2.61	0.0	12.80	0.0

P value = 0.415206

Table 3: Comparative Total mineral and heavy Metals contents of the fruiting bodies of *P. ostreatus* EM-1 grown on either 'wawa' sawdust with or without artificial spiking with spores of *T. harzianum* at 28°C for 35 days

Comparative proximate analyses and energy (kcal/100g) of the fruiting bodies of *P. ostreatus* EM-1 cultivated on either wawa sawdust with or without artificial spiking with spores of *T. harzianum*

Table 4 summarizes results obtained. There was no significant change ($p \geq 0.05$) in the dry matter content of the control samples and the fruiting bodies obtained from the contaminated wawa substrate. On the contrary, total ash value nearly doubled from 8.78% to 16.33% in the samples harvested from the contaminated substrate (Table 4). Moisture, fat, Crude fibre, Crude Protein, Carbohydrate decreased by 3.2 - 10% in the contaminate samples while that was a near 20% decrease in energy from 33.49.7 kcal / 100g in the uncontaminated control to 273.10 Kcal / 100g in the substrate spiked with *T. harzianum* (Table 4). The effect of the potential pathogen *Trichoderma harzianum* is illustrated in Plates 1 and 2.

Substrate type	Proximate parameter							
	Dry matter (%)	Moisture	Fat (%)	Crude fibre (%)	Crude protein (%)	Total ash (%)	Carbohydrate	Energy (Kcal/100g)
'wawa' sawdust uninoculated (control)	52.5 ± 0.01	3.08 ± 0.02	1.19 ± 0.0	91.13 ± 0.0	15.90 ± 0.0	8.87 ± 0.0	72.86 ± 0.01	334.97 ± 0.03
'wawa' sawdust spiked with *T harzianum*	52.56 ± 0.00	12.45 ± 0.00	0.75 ± 0.0	83.67 ± 0.0	14.35 ± 0.01	16.33 ± 0.0	67.81 ± 0.02	273.10 ± 0.04

P value = 0.432457

Table 4: Comparative Proximate Analyses of the fruiting body of *P. ostreatus* EM-1 grown on native wawa sawdust with or without artificial spiking with spores of *T. harzianum* at 28°C for 35 days

Discussion

In this present study, *T. harzianum*, the most prevalent pathogen isolated from the mushroom composts used by Adalete (2020) and also found by Wiafe-Kwagyan et al. (2015) to be pathogenic *in vitro to Pleurotus ostreatus* and *P. eous* was used to test in vivo the influence of this fungus on the developmental stages of the fruiting body of *P. ostreatus* and the proximate and elemental composition. The identity of *T. harzianum* confirmed by molecular sequencing technique and the pathogen is akin what has been described by Cailleux et al. (1978) and Singh et al. (2020) to have caused much crop losses to mushroom farmers worldwide. The pathogen presumably antagonises the mushroom by the phenomenon of antibiosis. Antibiosis is a well-known biological interaction where chemical antagonism between two or more organism is detrimental to at least one of them or there is antagonistic association between an organism and the metabolic substance produced inhibits growth and sporulation of one which may be lethal.

There are recorded instances where steam sterilization was ineffective and some pathogens and competitor fungi can reappear especially when grains used for spawn preparation, or the compost is not properly sterilized as prescribed, and this became a source of contaminant survival. During incubation of already spawned bags, the temperature of the compost may increase to about 30°C due to mycelial metabolic activity of *P. ostreatus*. In this situation, where the pH of the substrate is acidic to neutral (pH 5.0 - 7.0) and relative humidity may rise to ERH 80% it may cause residual green moulds to begin de novo growth to proliferate the compost bags (Belletini et al., 2017). Alkalisation of the growth substrate by the application

of calcium carbonate (lime, $CaCO_3$) has been used in instance to increase pH of the compost to pH 8 - 9. This effectively slows down the proliferation of the green mould fungus *T. harzianum* (Komon-Żelazowska et al., 2007). However, where pasteurisation of substrate has been ineffective (such as in this study), the green mould takes a heavy toll of the culture to affect crop yield.

Kortei et al. (2018) cultivated *P. ostreatus* on composted "wawa" (*Triplochiton scleroxylon*) sawdust subjected to physical pre-treatment technique of moist heat sterilized at either 95 - 100°C for 2.5 hours or gamma irradiation with a Co60 source (5 - 82 KGy). Sorghum grains for spawns were also steamed in a similar fashion and irradiated with the same doses (5 - 32KGy). Gamma irradiation was shown to be a food substitute and more efficient for sterilizing composted sawdust for mushroom cultivation and yield on the Ghanaian tropic condition. However, in this present study, composted sawdust from the farms inoculated with spawn had been and steam-treated and were used to ascertain the pathogenic effect of *T. harzianum* on the growth and yield of *P. ostreatus*. It was noted that all the bags used for the experiment were purchased from the same source.

Figures 1 - 5 summarises results of the assessment of some developmental parameters of the fruiting bodies in the control (uninoculated) and bags spiked with *T. harzianum* spores. The results showed that each group of (5) inoculated and (5) uninoculated for substrate bags behaved differently in terms of all the criteria for yield. Spiking with spores of *T. harzianum* reduced pinheads formation (Fig. 1), number of fruiting bodies (Fig. 4), stipe length (Fig. 5), and cap diameter (Table 4) in all the three flushes of the mushroom. Therefore, pathogenicity against *P. ostreatus* was shown by the formation of fewer pinheads, lower conversion of pinheads into fruiting bodies, weight of fruiting bodies, yield and biological efficiency but also led to production of diminutive fruiting bodies reflected in shorter stipe length, stipe diameter, and dimensions of the pileus (cap) (Table 4, Fig. 5a and b).

At each harvest of yield (1st, 2nd and 3rd) the yield of the inoculated bags was reduced by 67.4, 68.5 and 73.6% respectively (Table 1). Subsequently the total yield was reduced by more than one-half (52.3%) and the Biological Efficiency of the substrate was also reduced by more than half (52.8%) (Table 1). There was another important observation. The green mould also attacked the uninoculated (control) bags indicative of insufficient pasteurisation of both the spawn (sorghum seeds) inoculated with the mycelium of the mushroom and the wawa sawdust substrate. Alkalization and pasteurisation of growth substrate and the grains used in the making of the spam at 60°C for 10 hours has been known to inhibit growth *Trichoderma* species but this seems to have been insufficiently applied in this present instance (Komon-Zelazowska et al., 2007).

Chukwurah et al. (2013) and Kortei et al. (2018) and Wiafe-Kwagyan et al. (2022) have shown that correlations provide the possibility of to use only cap diameter and stipe lengths to predict the biological efficiency and also use this parameter for growing and pricing of mushrooms earmarked for the consumer market. Results from this study with infection by *T. harzianum* produced diminutive fruiting bodies, stipe length, cap diameter, lowering of biological efficiency etc. will make the produce downgraded on the market. In most cases, there can be a complete crop failure depending on the stage of infection, mycological quality of comport and environmental conditions (Sharma et al., 2007). Fungi resident in the compost and spawn which is insufficiently sterilized can transfer pathogenic species such as *T. harzianum* to the developing fruiting bodies. Kortei (2015) reported that 64% mushroom farmers of *Pleurotus ostreatus* in Ghana were dissatisfied with the sterilization process made using steam oil drum method as currently obtains by the suppliers of the compost and spawn.

Determination of the nutrient profile of fruiting bodies grown in wawa sawdust either spiked (contaminated) with *T. harzianum* or uninfected showed that the wawa sawdust grown without *T. harzianum* contamination produced fruit bodies of *P. ostreatus* varying concentrations of Ca, Cu, Fe, K, Mg, Na, P and Zn (Table 2). On the other hand, the substrate spiked with *T. harzianum* produced fruiting body with Ca, Cu, Fe, K, Mg and P. Neither sodium, nor Zn were detected. Furthermore, Cu, Na and P concentration did not change. The essential mineral elements required for growth of Fungi can be divided into two categories. Macronutrients (K, P, Mg, N, S, Ca) and micronutrients (Fe, Cu, Mn, Zn Mo). It implies that *T. harzianum* did not utilize Na and Zn, K, and Cu (Table 2) but utilized Ca Fe, Mg albeit in small quantities. The biochemical basis for this preference was not investigated.

However, the nutritional requirements of an organism can reflect the availability of the nutrients in its environment (Carlile et al., 2005). *T. harzianum* utilized Calcium (Ca) an enzyme activator often found in membranes, Iron (Fe) important in the cytochromes, haem apoenzymes and Magnesium (Mg) an enzyme activator required in ATP metabolism (Kendrick, 2000).

Comparative proximate analyses of the mushrooms harvested from the substrate spiked (Contaminated) with *T. harzianum* or uncontaminated (control) showed a reduction in fat, crude fibre, crude protein, and carbohydrates (Table 3) of the contaminated substrate. Presumably metabolic activity increased and resulted in doubling of total ash in the spiked bags (16.33%) as compared to 8.87% in the control (Table 3) with attendant marginal increase in moisture content.

Conclusion and Recommendation

This study has provided novel information on the mycobiota of substrates ('wawa' sawdust) used in six mushroom farms in the Greater Accra Region and one farm in the Central Region of Ghana. Information on the aeromycoflora in the mushroom houses is also provided for the first time (Adalete, 2020). The 20 mycoflora profile was common in all the farms and was predominated by members of the *Aspergillus* species followed by *Penicillium* and *Saccharomyces*. Each farm had a

unique profile of fungi but shared many fungi in common. The mycological load of the comports used in the different farms varied from 2.28 to 5.24 log10 CFU/g sample.

Trichoderma harzianum was one of the most frequently occurring species in all the substrates and fruit bodies as well as the aeromycoflora followed *Saccharomyces*. The identity of this pathogen was confirmed by molecular sequencing technique and was ubiquitous on all the farms and has previously been showed to be pathogen of *Pleurotus ostreatus* in *vitro* and *in vivo* (Wiafe-Kwagyan et al. 2015).

Pathogenicity of *T. harzianum* against *Pleurotus ostreatus* in vivo was tested by inoculating one batch of five (5) compost bags with the spores of the potential pathogen and the other five (5) remained uninoculated. Pathogenicity against *P. ostreatus* was shown by the formation of fewer pinheads of the developing fruiting bodies, lower conversion of pinheads into mature fruiting bodies, reduced weight of fruiting bodies. The yield of the inoculated bags were reduced by 26.7 - 67.4% (depending of the fruiting cycle). Subsequently the total yield was reduced by 52.3% and the Biological Efficiency of the substrate was also reduced by 52.8% (Table 4). Surprisingly, the green mould also infected the sterilized uninoculated control bags indicative of insufficient pasteurisation of both the Spawn (Sorghum seeds inoculated with the mycelium of the mushroom) as well as the sawdust substrate. The fruiting bodies produced in the infected bags were diminutive in size (the length, diameter of stipe and cap diameter). It was clear that fungi resident in the compost and spawn which were insufficiently sterilized could transfer pathogenic *T. harzianum* to the developing fruiting bodies of *P. ostreatus*. This constituted a substantial loss of revenue to the entrepreneur farmers. There was another quality loss; There was a reduction in fat, crude fibre, crude protein and carbohydrates of the resulting fruit bodies of the inoculated bags although total ash content of the spiked bags with *T. harzianum* was doubled (16.33%) as compared to the uncontaminated (8.87%). Although Ca, Cu, Fe, K, Mg, Na, P and Zn were detected in the control fruitbodies, substrates spiked with *T. harzianum* lacked Na, and Zn. However, Cu, Na and P concentrations did not change. These findings underscore report of Cailleux and Diop (1978) who showed that *T. harzianum* reduces the nutritional quality of harvested oyster mushrooms.

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