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Research Article

Survival of Mushrooms and Termites Upon Pesticide Exposure in the Cocoa Agro-ecosystem

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ABSTRACT

Pesticides have become integral parts of cocoa cultivation for the management of insect pests and fungal pathogens which cause significant damage to the crop. However, continuous pesticide usage in the cocoa agro-ecosystem is of concern due to perceived adverse effects on non-target organisms. In this study, mushrooms and associated termites were used to elucidate the possible effect of fungicides and insecticides on non-target organisms in the cocoa agro-ecosystem. The vegetative phase of *Pleurotus sajor-caju* (Oyster mushroom), *Volvariella volvacea* (Paddy straw mushroom), *Termitomyces globulus*, and *Termitomyces robustus* (Termite mushrooms) were subjected to concentrations of commercially formulated fungicides (metalaxyl 12 % + copper (I) oxide 60 % and cupric hydroxide 77 %) and insecticides (imidacloprid 20 % and bifenthrin 2.7 %) to observe their growth rates on Potato Dextrose Agar (PDA). Worker termites, *Macrotermes bellicosus*, were also exposed to the pesticides in Petri dishes for 24 h to observe their mortality. The manufacturer's recommended concentration of 245 ppm for bifenthrin completely inhibited mycelial growth of all the mushrooms and caused 100% mortality of termites. At 0.0245 ppm, the insecticide caused 60% mortality of termites, but it had no inhibitory effect on the mushrooms. Except for *P. sajor-caju*, mycelial growth of all the other mushrooms was completely inhibited by metalaxyl + copper (I) oxide at the manufacturer's recommended concentration of 2400 ppm. However, mycelial growth rate of the mushrooms at 0.24 ppm of the fungicide was similar to the control plates. Although the recommended concentrations of the pesticides inhibited mushroom activity under controlled conditions, mushroom survival in the cocoa agro-ecosystem amidst pesticides could be due to diluted pesticide concentrations that result in the soil after application which is usually directed at the pods, trunk, and foliage.

Keywords: Agro-ecosystem, Cocoa, Fungicides, Insecticides, Mushrooms, Termites

Introduction

Mushrooms are fruiting bodies of macrofungi, usually produced above the surface of substrates [1]. They grow on diverse substrates, including deadwood, leaf litter and soil [2]. Mushrooms play significant ecological and agricultural roles in forests and other terrestrial ecosystems by providing food sources for wildlife, forming mycorrhizal associations with plants, and decomposing dead organic matter [3]. Economically, they are used for food, industrial purposes, and medicine [4]. Commonly consumed in Africa and Southeast Asia are mushrooms in the genus *Termitomyces* [5-9]. They are probably the most preferred mushrooms due to their health benefits. Fresh extract powder or paste of fruiting bodies of *Termitomyces globulus* is used for wound healing, whereas the syrup of *Termitomyces robustus* is used as a remedy for constipation and indolence [10]. According to a survey by Apetorgbor *et al.* [11], the consumption of some

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edible mushrooms with medicinal and nutraceutical properties lowers blood pressure, and they serve as a blood tonic for children suffering from kwashiorkor. Such haematinic properties have also been observed in other mushroom species, such as *Pleurotus tuber-regium* [12]. Unlike *P. sajor-caju* (Oyster mushroom) and *Volvariella volvacea* (Paddy straw mushroom), which can be cultivated on agricultural wastes and industrial by-products, the formation of fruiting bodies by *Termitomyces* species is aided by termites in the subfamily Macrotermitinae [13] and hence, the name termite mushrooms. The mushroom exists in a symbiotic relationship with the termites, and this makes it difficult for domestication under laboratory conditions. Generally, termites are described as ecosystem engineers due to their activities in the soil which result in the availability of resources for other organisms [14]. However, they attack and destroy cocoa trees [15, 16].

Cocoa is an important cash crop in the tropics, and its cultivation usually involves the conversion of forests into agro-forests [17-19] which provide forest-like habitats for the survival of plants, animals and microorganisms interacting in an ecosystem. In the cocoa agro-ecosystem are, edible mushrooms collected, sold, and consumed by the rural population [20]. Conservation of the cocoa agro-ecosystem requires planting trees [21-23], which may attract pests and pathogens to destroy the crop [24]. Notable among diseases of cocoa are black pod, witches' broom, vascular streak dieback and monilia pod rot, which, together with the cocoa swollen shoot virus, account for 40 % of global annual crop yield losses [25]. Insect pests contribute significantly to worldwide yield losses of cocoa [26-28], and in Ghana, mirid damage is estimated to reach 30-40 % [29, 30].

Though there are many methods of managing insect pests [31] and pathogens [32] in cocoa, pesticides have become the preferred option among farmers who consider them a quick solution to the problems of insect pests and diseases. In this regard, a number of insecticides and fungicides are available to farmers for use in the cocoa agro-ecosystem. Before using bifenthrin, imidacloprid and thiamethoxam for the management of mirids, many insecticides were used but banned due to their adverse effects on the crop and the environment [30, 33, 34,]. These insecticides formed the basis for preparing other insecticide cocktails against mirids [31]. However, neonicotinoids, including imidacloprid and thiamethoxam, are currently banned in the European Union (EU) because of the threat they pose to honeybees and other pollinators [35]. Effective management of *Phytophthora*, the cause of black pod disease, depends on copper-based fungicides [32]. *Phytophthora megakarya*, which is noted for causing the severe form of black pod disease, is effectively managed by combining copper and metalaxyl or its isomer, metalaxyl-M fungicides [36, 37].

The aim of using pesticides in the cocoa agro-ecosystem is to boost cocoa yield by killing pests and pathogens that destroy the crop. Unfortunately, only 10% of the pesticides reach the target pests, while the remaining contaminates the environment [38, 39, 40]. These pesticides, including cypermethrin, copper and glyphosate, have been reported to have adverse effects on beneficial insects and earthworms [41, 42]. Although fungicides are known to reduce the population size of soil microflora [43], Kwodaga *et al.* [44] found no adverse effects of copper and metalaxyl-based fungicides on mycoflora in cocoa soils. Even so, there is the perception that pesticides used in the cocoa agro-ecosystem negatively impact beneficial organisms, including edible mushrooms and associated termites. This perception was worth investigating, especially when residues of copper have been detected in cocoa soils [45]. This study aimed to investigate the effect of pesticides used against cocoa pests on mushrooms (*Termitomyces globulus*, *Termitomyces robustus*, *P. sajor-caju* and *V. volvacea*) and termites (*Macrotermes bellicosus*).

Material and Methods

Pesticides

Two commercially formulated fungicides and insecticides commonly used to control black pod disease and mirids on cocoa in Ghana were selected for this study. The fungicides containing metalaxyl 12 % + copper (I) oxide 60 % and cupric hydroxide 77 % as active ingredients were used according to the manufacturer's recommended rates of 50 (2400 ppm) and 100 g (5130 ppm) in 15 L of water, respectively. The active ingredients of the insecticides were imidacloprid 20 % and bifenthrin 2.7 %. They were respectively used at rates of 30 and 100 mL in 11 L of water as recommended by the manufacturers, thus 545 and 245 ppm. The pesticides were further tested at lower rates of 10^{-1} to 10^{-4} folds of their recommended

rates corresponding to 240, 24, 2.4 and 0.24 ppm for metalaxyl + copper (I) oxide; 513, 51.3, 5.13 and 0.513 ppm for cupric hydroxide; 54.5, 5.45, 0.545 and 0.0545 ppm for imidacloprid; 24.5, 2.45, 0.245 and 0.0245 ppm for bifenthrin.

Collection of mushrooms

Termitomyces globulus and *T. robustus* were collected from the floor of a cocoa farm (Plot D20) at the Cocoa Research Institute of Ghana (CRIG), New Tafo-Akim in March 2020 when few drops of rain were experienced after a long drought. This plot is routinely sprayed with fungicides and insecticides for the control of black pod disease and mirids, respectively. *P. sajor-caju* was collected from a local mushroom farm at New Tafo-Akim whereas *V. volvacea* was harvested from a dead oil palm tree at Old Tafo-Akim, all in the Abuakwa North Municipality of the Eastern Region of Ghana. Both mushrooms were collected in June 2020. The mushrooms were identified based on macro-morphological characteristics such as shape, colour and position of cap, stipe, gills, volva, and annulus [46, 47]. They were photographed and harvested into sterile transparent ziplock bags for culturing on agar media in the laboratory.

Culturing of mushroom

The isolation technique described in Tudses [48] was used to obtain cultures of the mushrooms on Potato Dextrose Agar (PDA) (Oxoid, UK). Small pieces (2×2 mm^2) of inner tissues were removed from the caps with sterile sharp blades and placed on PDA amended with chloramphenicol at 100 $mg.L^{-1}$ to inhibit bacterial growth. The plates were incubated in the dark at 25°C for 5 days for the mycelial growth of *P. sajor-caju* and *V. volvacea.* Plates of *T. globulus* and *T. robustus* were incubated for 10 days. The cultures were purified through several transfers on PDA plates at 25°C. Colony morphology was recorded as growth pattern and growth diameter was measured at right angles to each other after 5 days of incubation for *P. sajor-caju* and *V. volvacea* and 21 days for *Termitomyces*. The growth diameter was converted to radial growth by dividing it by two. The cultures were stored in sterile distilled water at 25°C until needed.

Effect of pesticides on vegetative growth of mushrooms

Poisoned agar technique was used to evaluate the effect of the pesticides on the vegetative growth of the mushrooms [49]. The pesticide concentrations as stated above were prepared in 100 mL of molten PDA (Oxoid, UK) at 50°C and poured into Petri dishes in a laminar flow cabinet (ESCO, Heal Force Bio-Meditech Holdings Limited, China). The plates were allowed to set and left overnight in the cabinet for a sterility test. The centre of the plates was inoculated with 5 mm mycelial disc plugs taken from the periphery of 5-day-old cultures of *P. sajor-caju* and *V. volvacea* whereas 21 days old cultures were used for *T. globulus* and *T. robustus*. Non-amended PDA plates were similarly inoculated to serve as control. There were 3 replicated plates per treatment for each mushroom and they were incubated in the dark at 25°C. Growth diameter was measured at right angles to each other 5 days after incubation of *P. sajor-caju* and *V. volvacea*. Diameter growth for *T. globulus* and *T. robustus* was recorded after 21 days of incubation. Radial growths of the mushrooms were calculated by dividing diameter growths by two.

Pesticide toxicity on termite

The test termites were collected from experimental plots of CRIG at New Tafo-Akim. The test concentrations were obtained by diluting the pesticides with distilled water. Filter paper was moistened with 1 ml of pesticide concentration and used to line the base of a Petri dish. Ten worker termites (*Macrotermes bellicosus*) were placed on the filter paper. The setup was allowed to stand for 24 h to observe termite mortality. This was replicated thrice for each pesticide concentration and the controls. Distilled water was used for the controls. The test was done under the following laboratory conditions; a temperature of 25-29°C, relative humidity of 75-86%, and a photoperiod of 12 L: 12 D. The number of dead termites in each treatment was counted and converted to percentage mortality.

Data analysis

Each experiment was repeated twice and homogeneity of variances for datasets was confirmed by F-tests. Data was pooled together for transformation. Data for mycelial growth rate of mushrooms was square root transformed. Arcsine square root transformation was performed on insect mortality data. Effect of different concentrations of pesticides on mycelial growth rate of

mushrooms and percentage mortality of termites was statistically analysed by one-way analysis of variance (ANOVA) using GenStat (11th edition, VSN International Ltd.) at 5% probability level.

Results and Discussion

Mushrooms in the cocoa agro-ecosystem

The collection of *T. globulus* and *T. robustus* from the floor of a cocoa farm indicates the conduciveness of agro-ecosystem as a habitat for the survival of edible mushrooms [20]. The floor was the best place to find the T*ermitomyces* because soil covered with rich tree leaf litter presents a good substrate for their growth [50]. The collection of *T. globulus* and *T. robustus* at the onset of the rainy season in March confirms their period (February – April) of occurrence in Ghana [11]. In Côte d'Ivoire, fruiting bodies of *Termitomyces* are usually observed in the rainy season [8]. Fructification of *Termitomyces* is often associated with termites in the subfamily Macrotermitinae [9, 13, 51] as was evidenced in the collection of *T. globulus* where the worker termites, *M. bellicosus*, emerged from the holes. *Macrotermes bellicosus* is among a group of termites reported to be associated with cocoa farms in Ghana [52]. However, none of the cocoa trees was attacked by termites in the cocoa farm where *T. globulus* and *T. robustus* were collected.

The limited period of survey for mushrooms in cocoa farms might have prevented the collection of *Lentinus squarrosulus* and *V. volvacea* which have been reported to grow in cocoa plantations in Cameroon [20]. To increase the number of edible mushrooms used in this study, *P. sajor-caju* and *V. volvacea* were added even though they were not collected from the cocoa agro-ecosystem. These mushrooms were considered because of their availability and consumption in Ghana. *Pleurotus sajor-caju* was collected from prepared sawdust bags whereas *V. volvacea* was harvested from a decaying oil palm tree. They are the most preferred mushrooms in Ghana [7, 53] which grow on wood logs, forest floors, and decaying oil palm [11] but *Pleurotus* species are domestically cultivated on a wide range of substrates including sawdust [7, 54].

In addition to edible mushrooms in cocoa, agro-ecosystems are pathogenic ones. Those that are pathogenic to cocoa are mostly not edible. For instance, *Armillaria mellea* causes root rot in cocoa [55] and its consumption causes stomachache [56]. *Moniliophthora perniciosa,* the mushroom that causes witches' broom disease of cocoa, is a major problem in Brazil [57]. In Ghana, *Marasmiellus scandens* has been identified to cause thread blight disease [58]. The destructive nature of these pathogens requires a combination of phytosanitary practices and fungicides for effective management. Amoako-Attah et al. [59] recommended copper and metalaxyl-based fungicides for the management of *M. scandens*. These fungicides are also effective against *Phytophthora*, the pathogen of black pod disease. However, these fungicides are perceived to have a negative effect on beneficial fungi including edible mushrooms in the cocoa agro-ecosystem. This perception was investigated by growing the vegetative phase of *T. globulus*, *T. robustus, P. sajor-caju,* and *V. volvacea* on PDA amended with the manufacturers' recommended and lower concentrations of metalaxyl + copper (I) oxide and cupric hydroxide fungicides. The study was expanded to include imidacloprid and bifenthrin insecticides used for the control of mirids on cocoa.

Macro-morphological characteristics of mushrooms

Macro-morphological characteristics of *P. sajor-caju*, *V. volvacea*, *T. globulus*, and *T. robustus* are summarized in Table 1. Typical of *P. sajor-caju* was the grey to a cream oyster or sea seep-shaped cap (Figure 1a). Under the cap were crowded, decurrent, soft, and white gills. The solid stipe lacked annulus and it attached to the cap at its margin. There were tiny white mycelial threads at the base of the stipe without volva. On the contrary, *V. volvacea* produced a brown volva at the base of the stipe. It had long, thick, and numerous mycelial threads which supported its growth on a dead oil palm tree. The off-white solid stipe without annulus was attached to the centre of a pinkish-brown umbonate-shaped cap (Figure 1b) with crowded, free, and pinkish-brown gills underneath. On the white solid stipe of *T. globulus* was a large, white pendant annulus. The stipe was attached to the centre of a white umbonate cap with brown colouration at the centre (Figure 1c). The stipe tapered at the soil level, and it was deeply rooted. Worker termites, *M. bellicosus*, emerged from the holes of the mushroom when uprooted. The mycelial thread of *T. robustus* was relatively short. It had a white scaled stipe, and it was attached to the centre of a tawny brown and flat cap

Table 1. Macro-morphological characteristics of mushroom

Mushroom	Cap	Gills	Stipe ring (Annulus)	Stipe	Cup (Volva)	Mycelial threads
P. sajor-caju	Grey to cream and oyster or sea seep-shaped	Crowded, decurrent, soft and white	Absent	White and attached at cap margin	Absent	White and tiny
V. volvacea	Pinkish-brown and umbonate with cracks	Crowded, free and pinkish-brown	Absent	Off-white	Brown	Long, thick and numer-ous
T. globulus	White umbo-nate cap with brown colour-ation at the cen-tre	Crowded, free and white	White pen-dent	White and it tapers at the soil level	Absent	Long and deeply rooted
T. robustus	Tawny brown and flat with cracks	Crowded, free and white	Absent	White with scales	Absent	Short and tiny

(a) (b) (c) (d)

Figure 1. (a) *P. sajor-caju* on sawdust (b) *V. volvacea* on dead oil palm tree (c) *T. globulus* on the floor in a cocoa farm (d) *T. robustus* on the floor in a cocoa farm.

with cracks (Figure 1d). Under the cap were crowded, free, and white gills.

Mushroom cultures

Table 2 summarizes the colony characteristics and growth rate of *P. sajor-caju, V. volvacea*, *T. globulus*, and *T. robustus*. The culture of *P. sajor-caju* produced white felty, circular, and flat colonies with an entire margin on PDA (Figure 2a). The colonies of *V. volvacea* were cottony and creamy white in appearance (Figure 2b). The growths of cultures of *P. sajor-caju* and *V. volvacea* were rapid, attaining radial growths of 42.9 ± 0.1 and 44.5 ± 0.5 mm respectively, on PDA after 5 days of incubation at 25°C. On the other hand, cultures of *T. globulus* and *T. robustus* were slow in growth, attaining radial growths of 15.5±1.0 and 16.9±1.1 mm respectively, after 21 days of incubation. Colonies of *T. globulus* were whitish-brown and umbonate in appearance with an undulating margin (Figure 2c), whereas *T. robustus* produced whitish-brown colonies on PDA (Figure 2d) after 21 days of incubation at 25°C.

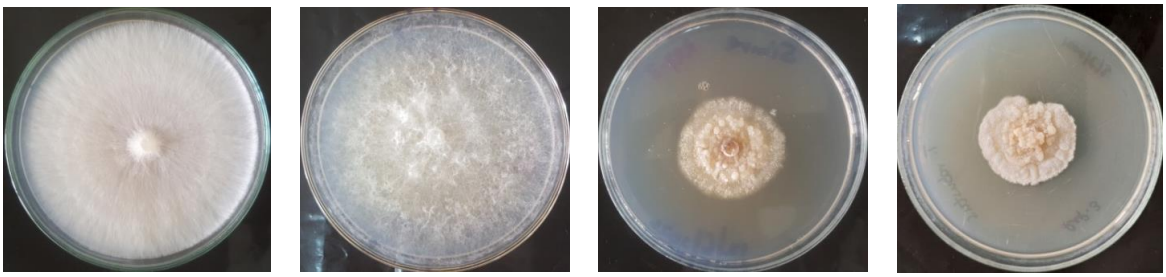
Effect of pesticides on vegetative growth of mushrooms

There were significant ($p < .001$) differences in radial growths of the mushrooms when they were grown on only PDA or when grown on PDA amended with different concentrations of the pesticides. Mycelial growths of the mushrooms were reduced at the manufacturer's recommended concentration of the pesticides, but they increased as concentrations of the pesticides reduced. Bifenthrin completely inhibited mycelial growths of all the mushrooms at the manufacturer's recommended concentration of 245 ppm for the control of mirids on cocoa. At 10-fold reduced concentration of 24.5 ppm, mycelial growths ranging from 3.0 mm for *P. sajor-caju* and *T. robustus* to 4.3 mm for *V. volvacea* were recorded. There was a gradual increase in the growths of *V. volvacea* and *T. globulus* as the concentration of bifenthrin reduced (Figure 3). Unlike bifenthrin, imidacloprid did not completely inhibit mycelial growths of the mushrooms but significantly ($p < .001$) reduced them at the manufacturer's concentration of 545

Table 2. Colony characteristics and radial growth of *Pleurotus sajor-caju*, *Volvariella volvacea*, *Termitomyces globulus* and *Termitomyces robustus* on Potato Dextrose Agar at 25˚C

Mushroom	#Doi	Form	Elevation	Margin	Texture	Colour	Reverse	*Radial growth (mm)
P. sajor-caju	5	Circular	Flat	Entire	Felty	White	White	42.9±0.1
V. volvacea	5	Filamentous	Raised	Filiform	Cottony	Creamy white	Creamy white	44.5±0.5
T. globulus	21	Irregular	Umbonate	Undulate	Compact	Whitish-brown	Brownish-white	15.5±1.0
T. robustus	21	Irregular	Umbonate	Undulate	Compact	Brownish-white	Brown	16.9±1.1

#Doi: Number of days of incubation period
*Each value is a mean of 3 replicated plates.



(a) (b) (c) (d)

Figure 2. Cultures of (a) *Pleurotus sajor-caju* (5 days old) (b) *V. volvacea* (5 days old) (c) *T. globulus* (21 days old) and (d) *T. robustus* (21 days old) on Potato Dextrose Agar at 25˚C.

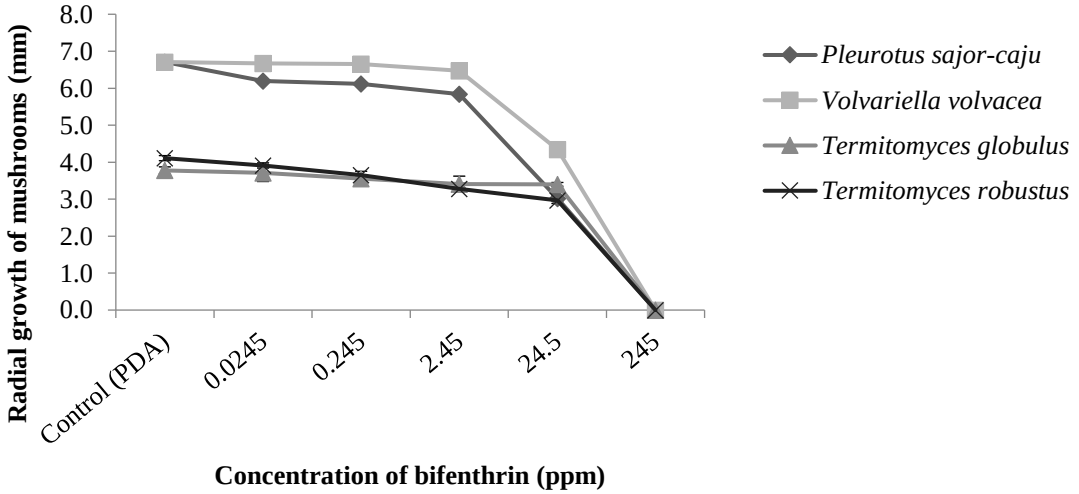


Figure 3. Effect of different concentrations of bifenthrin on radial growth of *P. sajor-caju*, *V. volvacea*, *T. globulus* and *Termitomyces robustus* on Potato Dextrose Agar. Square root transformed data and analyzed by one-way ANOVA at 5% probability level. Least significant difference (Lsd) = 0.5. Bars represent standard error of means.

ppm (Figure 4). Mycelial growth of the *Termitomyces* was completely inhibited by cupric hydroxide at the manufacturer's recommended concentration of 5130 ppm and its 10-fold reduction (Figure 5). Except *P. sajor-caju*, mycelial growth of the other mushrooms was completely inhibited at

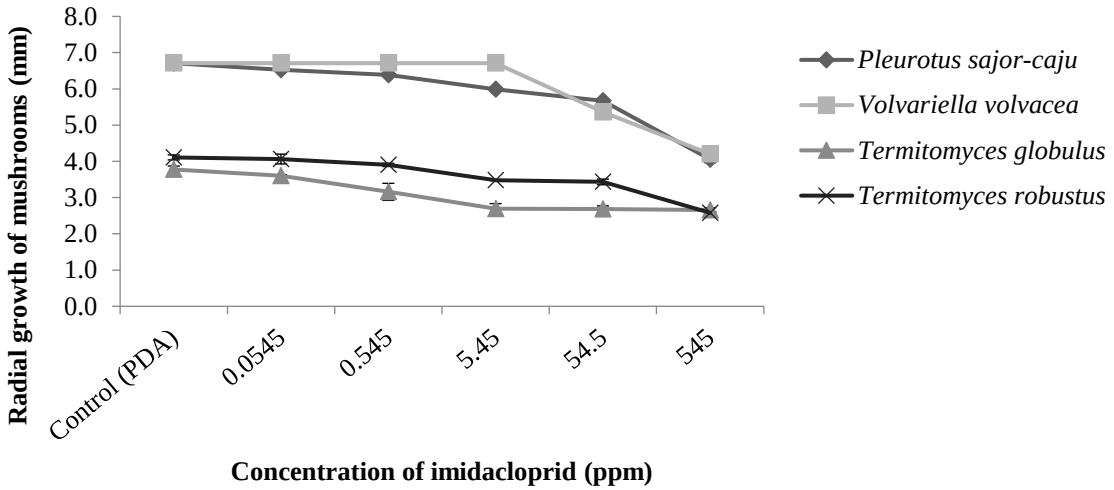


Figure 4. Effect of different concentrations of imidacloprid on radial growth of *P. sajor-caju, V. volvacea, T. globulus* and *T. robustus* on Potato Dextrose Agar. Square root transformed data and analyzed by one-way ANOVA at 5% probability level. Least significant difference (Lsd) = 0.5. Bars represent standard error of means.

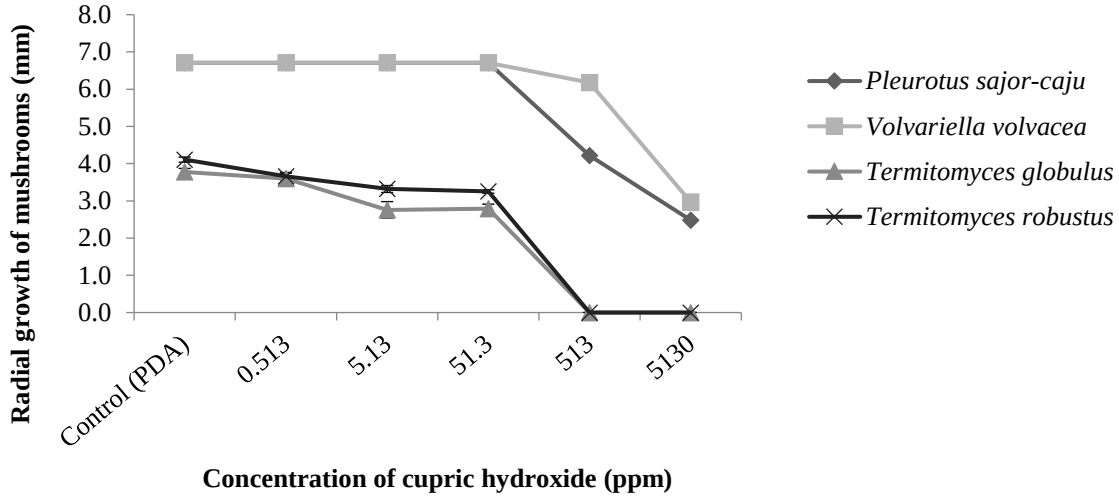


Figure 5. Effect of different concentrations of cupric hydroxide on radial growth of *P. sajor-caju, V. volvacea, T. globulus* and *T. robustus* on Potato Dextrose Agar. Square root transformed data and analyzed by one-way ANOVA at 5% probability level. Least significant difference (Lsd) = 0.4. Bars represent standard error of means.

the manufacturer's recommended concentration of 2400 ppm for metalaxyl + copper (I) oxide. However, radial growth of the mushrooms increased drastically at 10-fold reduced concentration of 240 ppm below the recommended concentration (Figure 6).

The effect of bifenthrin and imidacloprid on vegetative growth of these four test mushrooms demonstrated in this study is the first time in Ghana. The inhibitory effect of the insecticides on vegetative growth of the mushrooms is not surprising. This is because of the known toxicity of some insecticides to mushrooms. Sharma and Dewangen [60] found cartap hydrochloride to be toxic to *Agaricus bisporus* by inhibiting mycelial growth of the mushroom on PDA. Copper is a broad-spectrum fungicide and hence, its effect on the macro-fungi is possible. According to Das [61], mycelial growth of macro-fungi may be inhibited by heavy metals such as copper at low to medium concentrations, but death of mushrooms may occur at higher concentrations. This was evi-

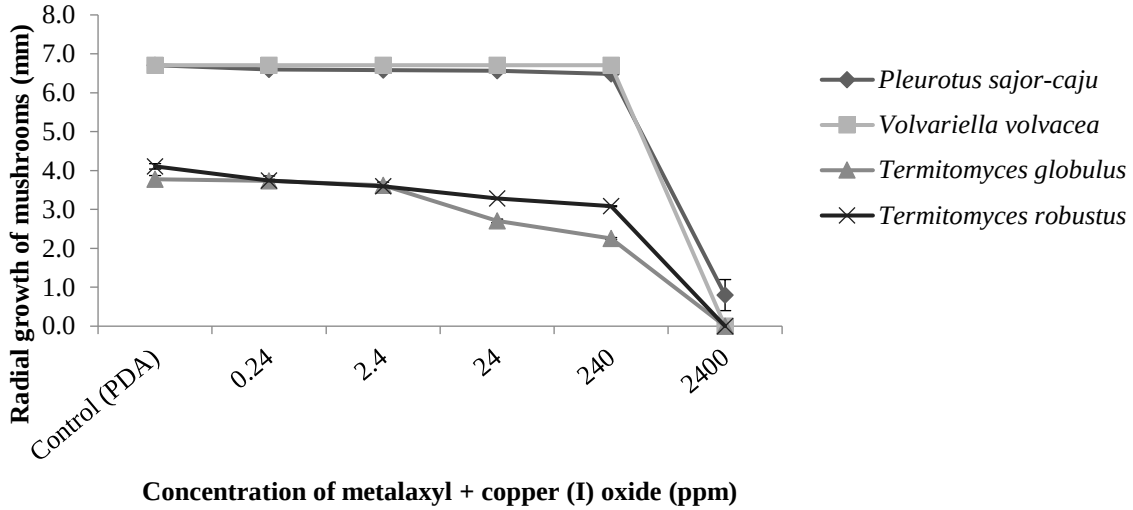


Figure 6. Effect of different concentrations of metalaxyl + copper (I) oxide on radial growth of *P. sajor-caju, V. volvacea, T. globulus* and *T. robustus* on Potato Dextrose Agar. Square root transformed data and analyzed by one-way ANOVA at 5% probability level. Least significant difference (Lsd) = 0.5. Bars represent standard error of means.

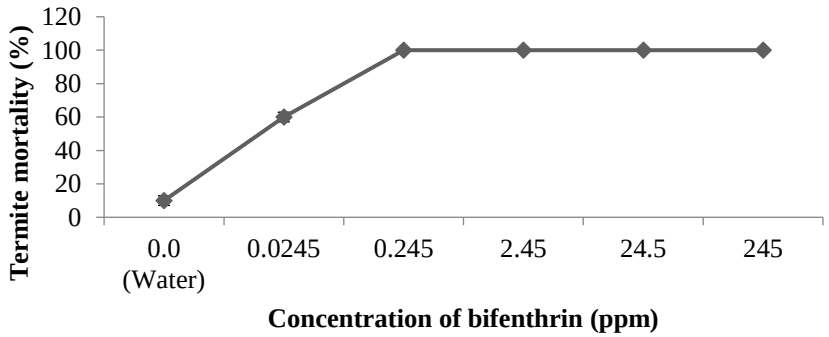


Figure 7. Effect of different concentrations of bifenthrin on mortality of *M. bellicosus.* One-way ANOVA at 5% probability level. Least significant difference (Lsd) = 10.27. Bars represent standard error of means.

dent in this study where mycelial growth of *T. globulus* and *T. robustus* were completely inhibited at the highest concentration of cupric hydroxide. Vegetative growth of the *Termitomyces* was mainly inhibited by the pesticides due to their slow growth. Radial growths of 15.5 and 16.9 mm were respectively recorded for *T. globulus* and *T. robustus* on PDA after 21 days of incubation. These growths were comparatively slower than 42.9 and 44.5 mm which were respectively recorded for *P. sajor-caju* and *V. volvacea* after 5 days of incubation. The synergistic effect of metalaxyl and copper was demonstrated in the complete inhibition of mycelial growth of *V. volvacea, T. globulus* and *T. robustus* at the manufacturer's concentration. However, lower concentrations of the fungicide did not significantly ($p > 0.05$) have inhibitory effect on radial growth of the mushrooms. This suggests that the pesticides may not have a direct effect on mushrooms in the cocoa agro-ecosystem. This is because of the method of pesticide application which is directed at tree trunks and cocoa canopies where pests and pathogens attack the crop. The pesticides are washed down the cocoa trees during rainfall and hence diluted before reaching the soil where edible mushrooms thrive. Detectable residues of bifenthrin in cocoa soils range from <0.01 – 0.03 ppm [62]. Kwodaga et al. [63] found residues of copper in cocoa soil to range from 14.90 – 27.50 ppm. Residues of bifenthrin in cocoa soils are less than or equal to 0.0245 ppm, where radial growth of *V. volvacea, T. globulus*

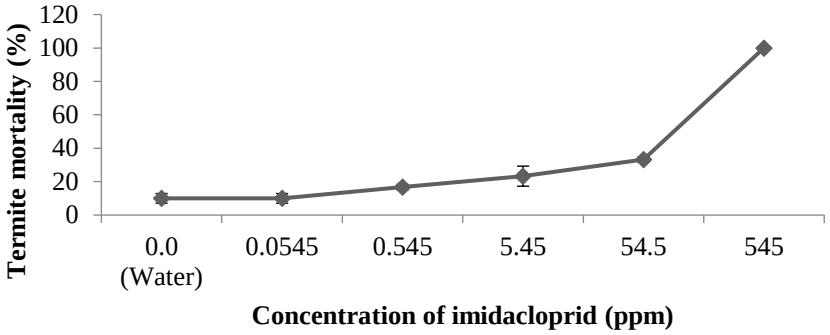


Figure 8. Effect of different concentrations of imidacloprid on mortality of *M. bellicosus.* One-way ANOVA at 5% probability level. Least significant difference (Lsd) = 19.22. Bars represent standard error of means.

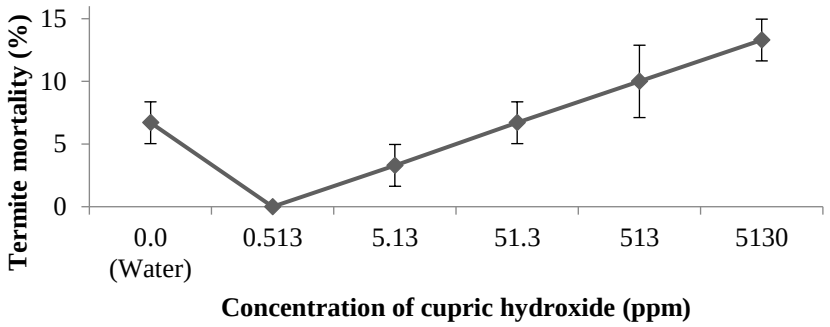


Figure 9. Effect of different concentrations of cupric hydroxide on mortality of *M. bellicosus.* One-way ANOVA at 5% probability level. Least significant difference (Lsd) = 11.09. Bars represent standard error of means.

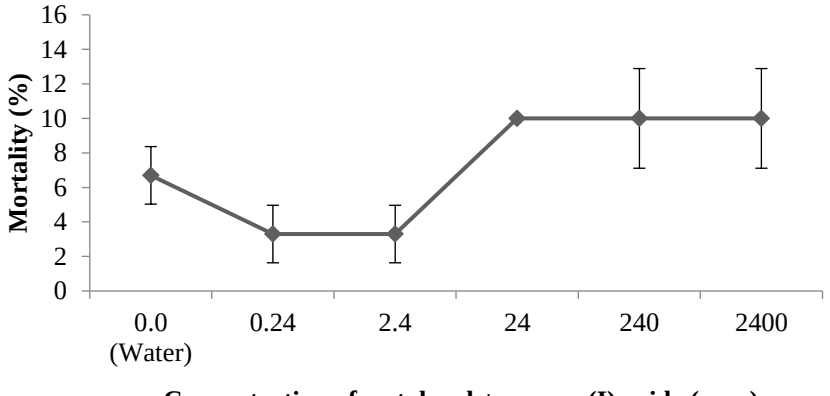


Figure 10. Effect of different concentrations of metalaxyl + copper (I) oxide on mortality of *M. bellicosus.* One-way ANOVA at 5% probability level. Least significant difference (Lsd) = 12.58. Bars represent standard error of means.

and *T. robustus* on bifenthrin-amended PDA plates were similar to the control plates. The concentration of 51.3 ppm for cupric hydroxide, at which the radial growth of *P. sajor-caju* and *V. volvacea* was similar to the control plates, is more than the residues of copper in cocoa soils. This indicates that lower concentrations of the pesticides in cocoa soils have no effect on vegetative growth of mushrooms and hence their survival in the cocoa agro-ecosystem.

Effect of pesticides on termite

The insecticides caused a mortality of 60 to 100% (bifenthrin) and 10 to 100 % (imidacloprid) on the termites after 24 h while cupric hydroxide and metalaxyl + copper (I) oxide caused a termite mortality of 0 to 13.3% and 3.3 to 10%, respectively. Higher termite mortality (100%) was recorded at the manufacturer's recommended concentration of 245 ppm for bifenthrin and its 1000-fold reduced concentration of 0.245 ppm (Figure 7). Similarly, the manufacturer's recommended concentration of 545 ppm for imidacloprid caused 100% mortality but significantly (<.001) declined at reducing concentrations of the insecticide (Figure 8). Cupric hydroxide caused 13.3% mortality at the manufacturer's recommended concentration of 5130 ppm (Figure 9) whereas metalaxyl + copper (I) oxide caused 10% mortality at 2400 ppm (Figure 10).

Bifenthrin and imidacloprid, commonly used in the cocoa ecosystem for the control of mirids, were also effective against termites by causing 100% mortality at the manufacturers' recommended concentrations. The recommended insecticide for termite control in cocoa is fipronil [34]. However, it was not included in this study because of its limited use in the cocoa ecosystem compared to the test insecticides. Efficacies of bifenthrin and imidacloprid confirm previous reports [64-66] but lower imidacloprid concentrations of 54.5 ppm and lower were less lethal to the termites. This suggests that the less detectable residues of the pesticides [62] in cocoa soils do not affect the activity of termites in the cocoa agro-ecosystem.

Conclusion

The possible effect of pesticides on mushrooms and associated termites in the cocoa agro-ecosystem was reported in this study. Of the four pesticides used, bifenthrin and metalaxyl + copper (I) oxide had inhibitory effects on mycelial growth of mushrooms (*P. sajor-caju*, *V. volvacea*, *T. globulus* and *T. robustus*) at the manufacturers' recommended concentrations for the control of mirids and *Phytophthora* on cocoa. Also, bifenthrin caused high mortality of termites (*M. bellicosus*). However, lower concentrations of the pesticides did not have any effect on the vegetative growth of the mushrooms and termites, hence, the existence of mushrooms in the cocoa agro-ecosystem amidst pesticide usage. For continuous existence of mushrooms and other beneficial or non-target organisms in the cocoa agro-ecosystem, strict compliance to good agricultural practices (GAP) with respect to pesticide usage should be adhered to.

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