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SOIL & CROP SCIENCES | RESEARCH ARTICLE

In vitro activity of soybean extract on cocoa disease pathogens (*Erythricium salmonicolor* and *Marasmiellus scandens*) and termite pest (*Microtermes subhyalinus*)

Yahaya Bukari¹, Silas W. Avicor², Godfred Awudzi², Michael K. Ainooson^{3*}, Eric K. Asare¹ and Ishmael Amoako-Attah¹

Abstract: This study evaluated the antifungal and insecticidal properties of soybean extract on *Marasmiellus scandens* (causal pathogen of white thread blight disease on cocoa), *Erythricium salmonicolor* (causal pathogen of pink disease of cocoa) and termite (*Microtermes subhyalinus*). Agar plate test was used to determine the inhibitory activity of the extract on mycelial growth of the pathogens while a contact toxicity bioassay was conducted on the termites. Extract of the soybean reduced mycelial growth in a dose-dependent pattern, with 20% of the extract attaining the highest inhibition. Percentage inhibition of 80.4 and 86.8 were recorded for *E. salmonicolor* and *M. scandens*, respectively after 7 days of incubation. The IC₅₀ values ranged between 0.64–2.44% and 0.12–0.45% for *E. salmonicolor* and *M. scandens* respectively. However, activity of the extract on the termite was low, with an LC₅₀ of 15.42% after 24 hours of exposure. The effect of the extract on the fungal pathogens indicates that it could be further explored towards the development of a plant-based control method against white thread blight and pink diseases of cocoa in Ghana.

Subjects: Agriculture; Agriculture and Food; Plant Pathology; Entomology

Keywords: Antifungal activity; inhibition; soybean; cocoa disease pathogens; termites

1. Introduction

Ghana's economy, over decades, has been largely supported by cocoa, the most important cash crop in the country (McKay & Aryeetey, 2004). The country continues to be the world's second largest producer and exporter of cocoa. Insect pests and diseases have been identified as key factors limiting cocoa production worldwide. Diseases such as *Phytophthora* pod rot (black pod), witches broom, swollen shoot virus, vascular streak dieback, and monilia pod rot have been known to cause 40% of annual global loss of cocoa (Flood et al., 2004). Of these diseases, black pod, caused by *Phytophthora megakarya* and *Phytophthora palmivora*, is considered as the most economically important fungal disease of cocoa in Ghana (Akrofi et al., 2003; Dakwa, 1987; Opoku et al., 2000). Yield loss due to black pod disease could reach 100% without proper control measures (Dakwa, 1987). Apart from black pod, thread blight and pink diseases are emerging as serious diseases of cocoa in Ghana (Akrofi et al., 2014; Amoako-Attah et al., 2016).

Erythricium salmonicolor (Berk. & Broome), the causal pathogen of pink disease of cocoa, is widely distributed and has been reported to attack many plant species worldwide (Smith, 1985).

Plants such as coffee, tea, cashew (Hilton, 1958; Seth et al., 1978), and citrus (Pradhanang, 1994) have been reported as economic host plants of the pathogen. Pink disease is widespread in Ghana (Akrofi et al., 2008) and cocoa yield loss of 60–100% on experimental plots at Bunso in the Eastern region of Ghana has been reported (Opoku et al., 2001). The pathogen girdles around stems and branches, affecting the flow of water and nutrients leading to branch and stem die-back especially in the distal parts of the tree. White thread blight disease, caused by *Marasmiellus scandens* (Masse) Dennis & Reid is widely distributed and has been found on important economic plants such as apple and tea (Adedeji, 2006). Plants such as plantain, banana and coconut which are found in cocoa farms are also susceptible to the disease (Benchimol et al., 2001). It is an important disease of cocoa in Ghana and has been reported in all cocoa regions among which the Ashanti Region recorded the highest severity of 13.8% (Amoako-Attah et al., 2016). The disease is recognized by thick creamy-white mycelial strands which spread mostly beneath branches and leaves leading to eventual death of whole branches or trees (Opoku et al., 2007). High disease incidence and severity is usually recorded in farms with low levels of sanitation (Amoako-Attah et al., 2016).

Pruning branches and leaves infected with pink and white thread blight diseases and supplementing with fungicide application help in the control of these diseases (Akrofi et al., 2014; Amoako-Attah et al., 2016).

Cocoa is also attacked by a diverse range of insect pests (Awudzi et al., 2019; Avicor et al., 2022) and these are major determinants of productivity. Besides the hemipterans such as mirids, stink bugs, coreid bugs and mealybugs, termites are also important pests of cocoa (Acknor, 1997; Ambele et al., 2018; Awudzi et al., 2019; Sylvain et al., 2015) and other crops (Rouland-Lefèvre, 2011), although known for their roles in ecosystem engineering (Evans et al., 2011; Jouquet et al., 2011; Pringle et al., 2010). During a 3-year study in Ghana, Acknor (1997) reported *Microtermes subhyalinus* as a pest of cocoa and among the most common termite species in cocoa farms. *Microtermes subhyalinus* and other *Microtermes* species are nearly ubiquitous in different habitats and cropping systems (Mora et al., 1996; Schyra & Korb, 2019; Usher, 1975) including cocoa (Kissi et al., 2022; Ollo et al., 2022; Sylvain et al., 2015, 2019). Termite attack can cause substantial damage and yield loss. According to Mora et al. (1996), *M. subhyalinus* infestation of sugarcane resulted in an annual yield loss of 10% in Central African Republic. In cocoa, the activity of termites is most important in the dry season as they chew roots of the crop at establishment (Awudzi et al., 2019). Their presence is usually seen by the presence of tunnels or runways on the stems of infested plants. This damage makes establishment difficult, especially in termite-endemic areas. It also makes crop establishment very expensive because of the need for refilling.

The main method used by farmers in controlling cocoa pests such as insect pests and diseases is the use of synthetic pesticides. Application of synthetic pesticides remains expensive and there is the risk of environmental pollution, development of resistance and high residues in crops (Balakumar et al., 2011; Sande et al., 2011). It is therefore necessary to search for safer and effective alternatives which will not have adverse effects on the environment and reduce health risk to farmers. Studies have shown that plant extracts including soybean (*Glycine max*) contain vital secondary metabolites which provide protective effects against fungal and insect attack (Igboabuchi & Llodibia, 2018; Kikuta, 2020; Villalobos et al., 2016). Soybean is rich in protein and contains several secondary metabolites such as isoflavones, phenolic acids, alkaloids and saponins (Lisanti & Arwin, 2019; Villalobos et al., 2016). Aqueous phenolic extract from soy flour possessed antimicrobial activity against several medically important microbes such as *Listeria monocytogenes*, *Listeria innocua*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Yersinia enterocolitica*, *Escherichia coli* and *Salmonella enterica* and moderate activity on 5 yeast species; *Torulaspota* spp, *Cryptococcus* spp, *Rhodotorula* spp, *Aureobasidium pullulans* and *Debaryomyces* spp (Villalobos et al., 2016). Soy flour and soybean extract containing the major soybean isoflavone, genistein, adversely affected the survivorship of *Tribolium castaneum* (Kikuta, 2020) and reduced larval and pupal weight of *Anticarsia gemmatilis* (Piubelli et al., 2005), respectively. Several authors have also reported on the inhibition of fungi (*Candida albicans* and *Aspergillus niger*) and bacteria (*S. aureus* and *L. monocytogenes*) growth by

soybean extracts (Hosseini Chaleshtori et al., 2017; Igboabuchi & Llodibia, 2018; Wang et al., 2010). Although several studies indicate the activity of soybean extracts against insects and microbes, knowledge of their activity on cocoa pests for potential integration into pest management strategies for cocoa production is scant. Therefore, this study assessed the *in vitro* fungicidal and insecticidal activity of soybean extract in controlling the causative pathogens of white thread blight and pink diseases of cocoa as well as the termite *Microtermes subhyalinus*.

2. Material and methods

2.1. Soybean extract

Soybean seeds were obtained from Tamale, the capital city of the Northern region of Ghana and the crude aqueous extract was obtained following the description in Gagman et al. (2022) with some modifications. The seeds were washed first with tap water and then distilled water before being shade-dried for 3 weeks. They were then pulverised into powder. Water (5 L) was added to the powdered seeds (1 kg) and incubated at room temperature for 1 week. This was then filtered with a muslin mesh and concentrated to drying. The concentrated powder (60 g) was dissolved in 100 ml of water to obtain a stock solution of 60% v/v. Water was used for the extraction because it is a non-toxic and cheap solvent (Cater et al., 1974; González & Muñoz, 2017). It is also abundant and has a wider application (Cater et al., 1974; González & Muñoz, 2017) and water-based extracts can easily be prepared by resource-poor farmers.

2.2. Isolation of pathogens

Isolation techniques described in Amoako-Attah et al. (2020) were used to obtain pure cultures of *Marasmiellus scandens* and *Erythricium salmonicolor* from cocoa trees infected with white thread blight and pink diseases, respectively. Pink pustular growth forms and hyphal strands from infected branches and leaves were directly plated on water agar and subsequently cultured on Potato Dextrose Agar (PDA) media and incubated at 28°C for seven days. Sub-culturing was done until pure cultures of *M. scandens* and *E. salmonicolor* were obtained. Three isolates each of *M. scandens* (MS 1, MS 2, MS 3) and *E. salmonicolor* (ES 1, ES 2, ES 3) were used in all experiments. Pure cultures were identified microscopically using morphological characteristics as described by Humber (2005) and Kirk et al. (2008). *Erythricium salmonicolor* isolates on agar plates were pinkish and later turned creamy while *M. scandens* isolates formed white thread-like mycelia with feathering margins (Akrofi et al., 2014; Amoako-Attah et al., 2016). The isolates have previously been differentiated using molecular techniques (Amoako-Attah et al., 2020; Kwarteng et al., 2018), coded and added to the pathogen library at the Cocoa Research Institute of Ghana.

3. In vitro antifungal activity

The antifungal activity of the soybean extract was performed using the agar plate test as described by Nwosu and Okafor (1995). Molten PDA was amended with different concentrations (20, 10, 1, 0.1, 0.01 and 0.001% v/v) of the soybean extract to a volume of 100 ml and poured into Petri dishes to set overnight. The plates were inoculated with 5 mm disc plugs taken from the edge of pure cultures of *M. scandens* and *E. salmonicolor*. Non-amended PDA plates inoculated with the test pathogens were included as controls. There were three replicated plates for each treatment per pathogen and incubated at 28 ± 2°C for 7 days. Colony diameters were recorded each day and percentage inhibition of radial growth was calculated as:

$$PIRG = \frac{C - T}{C} \times 100$$

where PIRG = Percentage Inhibition of Radial Growth; C = average radial growth in control plates; T = average radial growth in test plates

4. Termiticidal activity

Termites (*Microtermes subhyalinus*) collected from experimental plots of the Cocoa Research Institute of Ghana (CRIG) at New Tafo-Akim were used for the test. Concentrations (50%, 40%,

30%, 20% and 10% v/v) of the soybean extract were prepared with distilled water and used for the residual toxicity test (Ackonor & Adu-Acheampong, 2007) as follows. Whatman No. 10 filter paper was used to line the base of a Petri dish and 1 ml of the extract concentration was used to moisten it. Ten worker termites were placed on the filter paper in each Petri dish and the mortality of termites after 24 hours was noted. Distilled water was used to moisten filter paper for the control. Five replicates each were done for the extract concentrations and the control. The experiment was conducted at a temperature of $25 \pm 4^\circ\text{C}$, relative humidity of $80 \pm 7\%$ and a photoperiod of 12 h: 12 h Light: Dark.

5. Data analysis

The PIRG of each fungus in response to the extract concentrations was computed. These were used to obtain the Minimum Inhibition Concentration (MIC) and then used in probit analysis to obtain the concentration inhibiting 50% of radial growth (IC_{50}), slope, intercept, standard error and 95% confidence interval (CI) with IC_{50} values with overlapping 95% CIs not considered as significantly different. Analysis of variance (ANOVA) was also performed on the PIRG data using Genstat 10th edition and at a significance level of 5%, Tukey post hoc test was done. The number of dead termites compared to the total number of test termites was used to compute the percent mortality per concentration. Mortality data was arcsine-transformed and used for ANOVA with Tukey post hoc test done at 5% significance level. The mortalities for the various concentrations were analysed using probit to obtain the median lethal concentration (LC_{50}) and associated parameters as indicated for the IC_{50} . Probit analysis was done using the QCal software by Lozano-Fuentes et al. (2012).

6. Results

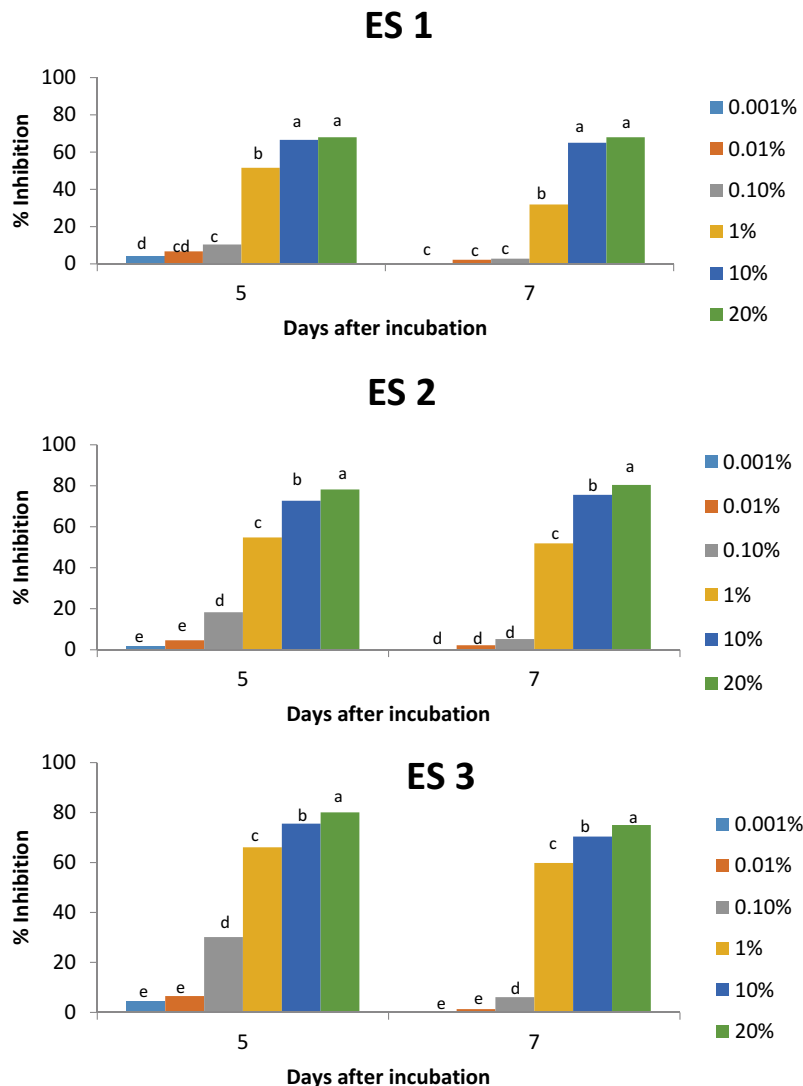
6.1. In vitro antifungal activity

The PIRGs of *E. salmonicolor* and *M. scandens* induced by the soybean extract are presented in Figures 1 and 2, respectively. Generally, the highest concentration (20%) of the soybean extract highly inhibited all the isolates of *E. salmonicolor* and *M. scandens*. The 20% concentration of the extract inhibited ES 2 by 80.4% after 7 days of incubation and this percentage inhibition (PI) was the highest among the isolates of *E. salmonicolor* (Figure 1). Generally ES 1 had the lowest PI among the isolates of *E. salmonicolor* across all the tested concentrations of the soybean extract. There was no significant difference in PI of isolate ES 1 between 20% and 10% extract concentrations after 5 and 7 days of incubation. However, there was a significant difference between the PI of both concentrations after 5 and 7 days of incubation for the other isolates of *E. salmonicolor* (ES 2 and ES 3). The highest PI of 86.8% was recorded at 20% concentration of the soybean extract after 7 days of incubation on plates inoculated with MS 1. This value was however not significantly different from the PI obtained at 20% concentration for the other *M. scandens* isolates after 7 days of incubation. There was no significant difference between the PI by the 10% and 20% soybean extract for all the isolates of *M. scandens* after 5 and 7 days of incubation except for MS 2 where there was a significant difference in PI between the 10% and 20% concentrations after 5 days of incubation.

Figures 3 and 4 show the effect of the soybean extract on *E. salmonicolor* and *M. scandens* after 5 and 7 days of incubation. Higher inhibitions of the isolates were obtained as the concentration of the soybean extract increased.

The *E. salmonicolor* isolates had higher IC_{50} values than the isolates of *M. scandens* after 5 days of incubation. The IC_{50} of *E. salmonicolor* was from 0.6428 to 2.4370 %v/v while that of *M. scandens* was 0.1193 to 0.4500 %v/v (Table 1). The *E. salmonicolor* isolates had significantly different IC_{50} values. Two isolates (MS 2 and MS 3) of *M. scandens* had IC_{50} values that were not significantly different from each other but they were significantly different from MS 1. However, MS 3 and ES 3 had IC_{50} values that were not significantly different (Table 1).

Figure 1. Effect of different concentrations of soybean extract on the mycelial growth of *E. salmonicolor* (ES) at 5 and 7 days of incubation ($28 \pm 2^\circ\text{C}$). Values of percentage inhibition bearing the same letter within the same incubation day are not significantly different at $p < 0.05$ using Tukey's post hoc test.



After 7 days of incubation, ES 2 and ES 3 had the same IC_{50} value and were significantly different from all the other fungal isolates while MS 2 and MS 3 also had similar IC_{50} values and were significantly different from the other fungal isolates (Table 2).

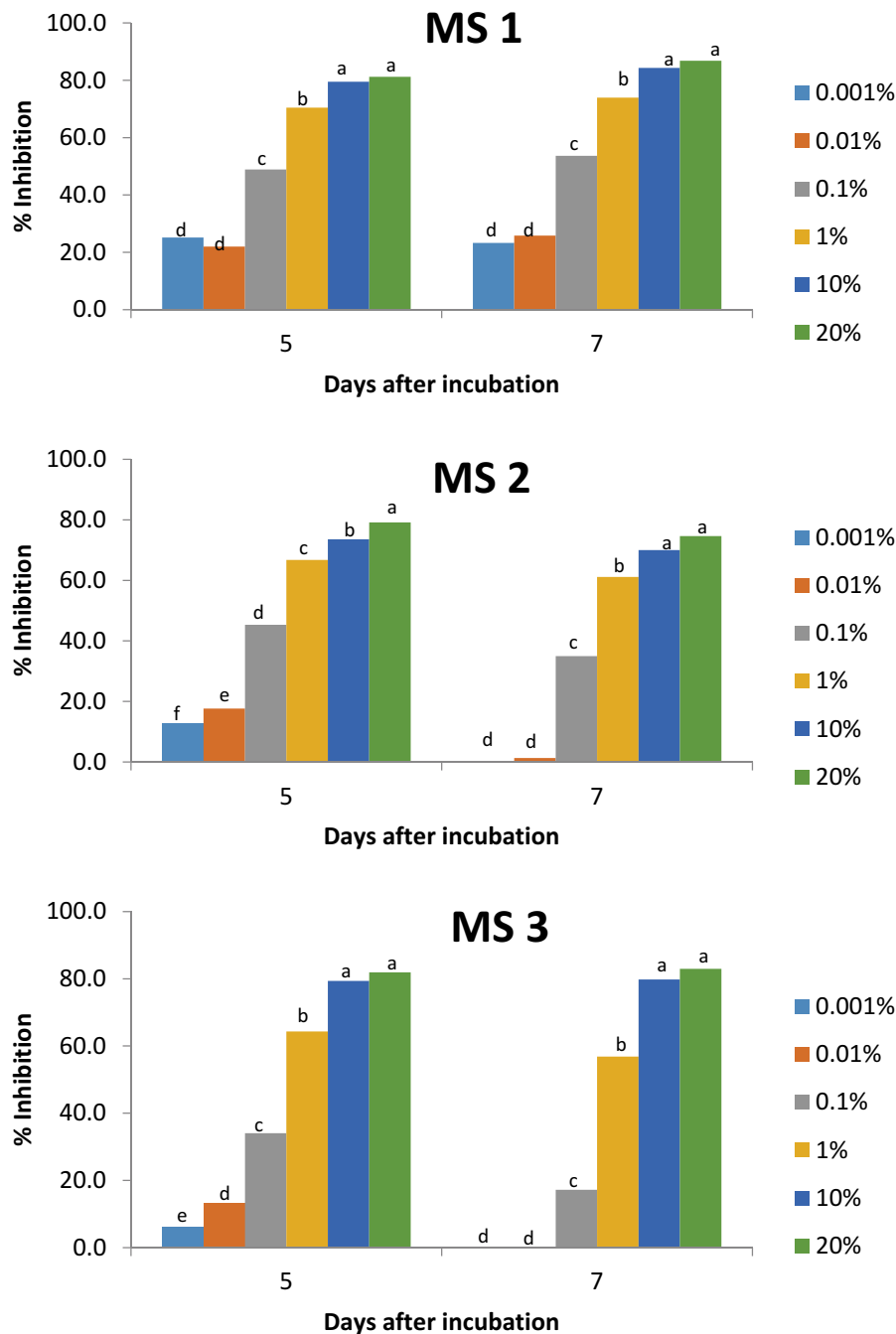
7. Termiticidal activity

Termite mortality due to the various extract concentrations was significantly different except mortality caused by 30% and 40% extract concentrations and also the 30% and 20% extract concentrations. The control treatment recorded 2% termite mortality. The binary logistic regression of the effect of the soybean extract on termite workers indicates an increase in activity with increase in extract concentration and significantly different LC_{50} values among the termite species (Figure 5, Table 3).

8. Discussion

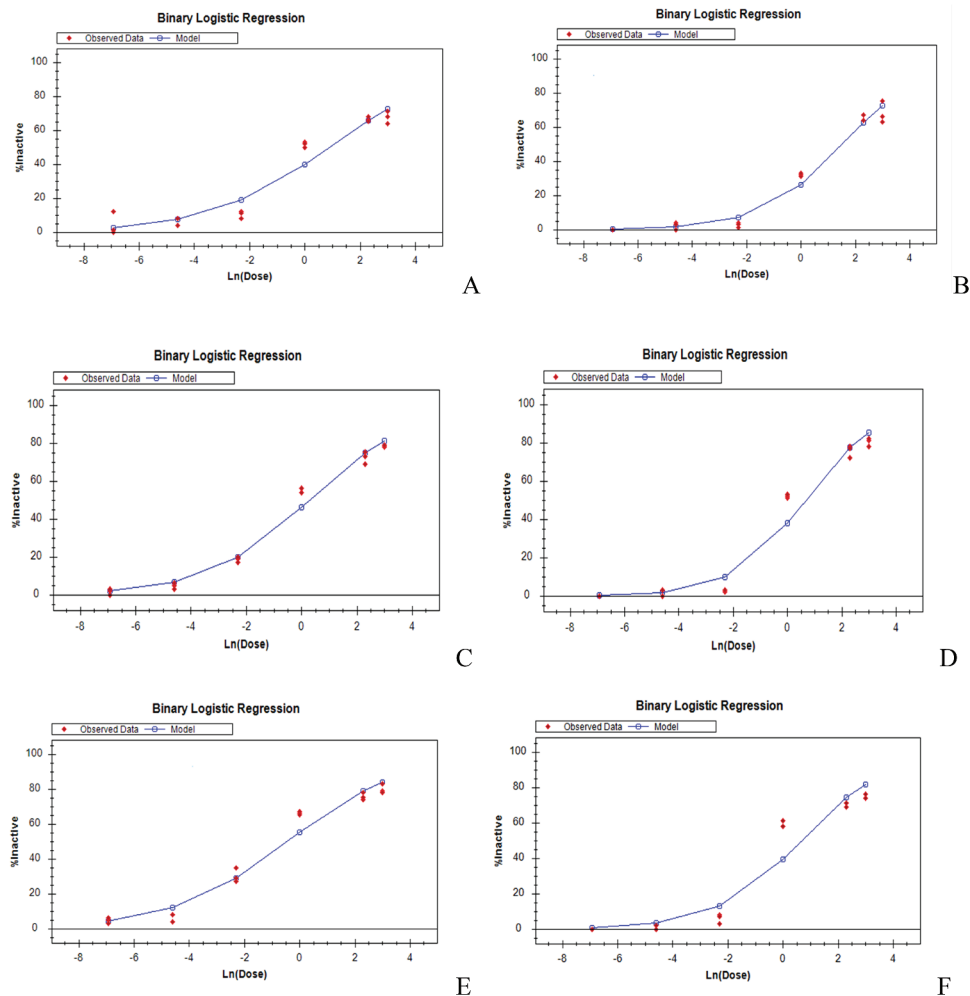
The bioactivity of soybean extract was evaluated against isolates of *E. salmonicolor* and *M. scandens*. The results show that the soybean extract had inhibitory effect on the pathogens in culture. Various studies on the antimicrobial properties of soybean have been reported (Ponnusha et al., 2011; Wang et al., 2010) with different inhibition activities. In our study, the highest inhibition of *E. salmonicolor* and *M. scandens* by the soybean extract was 80.4% and 86.8%, respectively at the highest concentration of 20% after seven days of incubation. The inhibition

Figure 2. Effect of different concentrations of soybean extract on the mycelial growth of *Marasmiellus scandens* (MS) isolates at 5 and 7 days of incubation (28 ± 2°C). Values of percentage inhibition bearing the same letter within the same incubation day are not significantly different at $p < 0.05$ using Tukey's post hoc test.



percentage values obtained at both 10% and 20% were higher than those obtained by Igboabuchi and Llodibia (2018) who tested the inhibitory activity of 15% ethanol extracts of soybean (seed and leaf) on various bacteria and fungi. The percentage inhibition of the seed extract against *A. niger* and *E. coli* was 62.2% and 68.9%. However the leaf extract inhibited *A. niger* at 49.4% and *E. coli* at 53.9%. Percentage inhibition of 10.9 to 61.0% were recorded when gycellins extracted from soybean seeds were tested for their antifungal activities against *Fusarium oxysporum*, *Phytophthora capsici*, *Sclerotinia sclerotiorum* and *Botrytis cinera* (Hyo et al., 2010). Nevertheless, complete inhibition of microbial growth using plant extracts have been reported such as the study of Tao et al. (2014) on *Penicillium italicum* and *Penicillium digitatum* using essential oil of *Citrus reticulata* at 0.00025% and 0.004%, respectively.

Figure 3. Binary logistic model of soybean extract on the mycelial growth of isolates of *Erythricium salmonicolor* (ES) after different incubation days. A: ES 1 after 5 days of incubation B: ES 1 after 7 days of incubation C: ES 2 after 5 days of incubation D: ES 2 after 7 days of incubation E: ES 3 after 5 days of incubation F: ES 3 after 7 days of incubation.

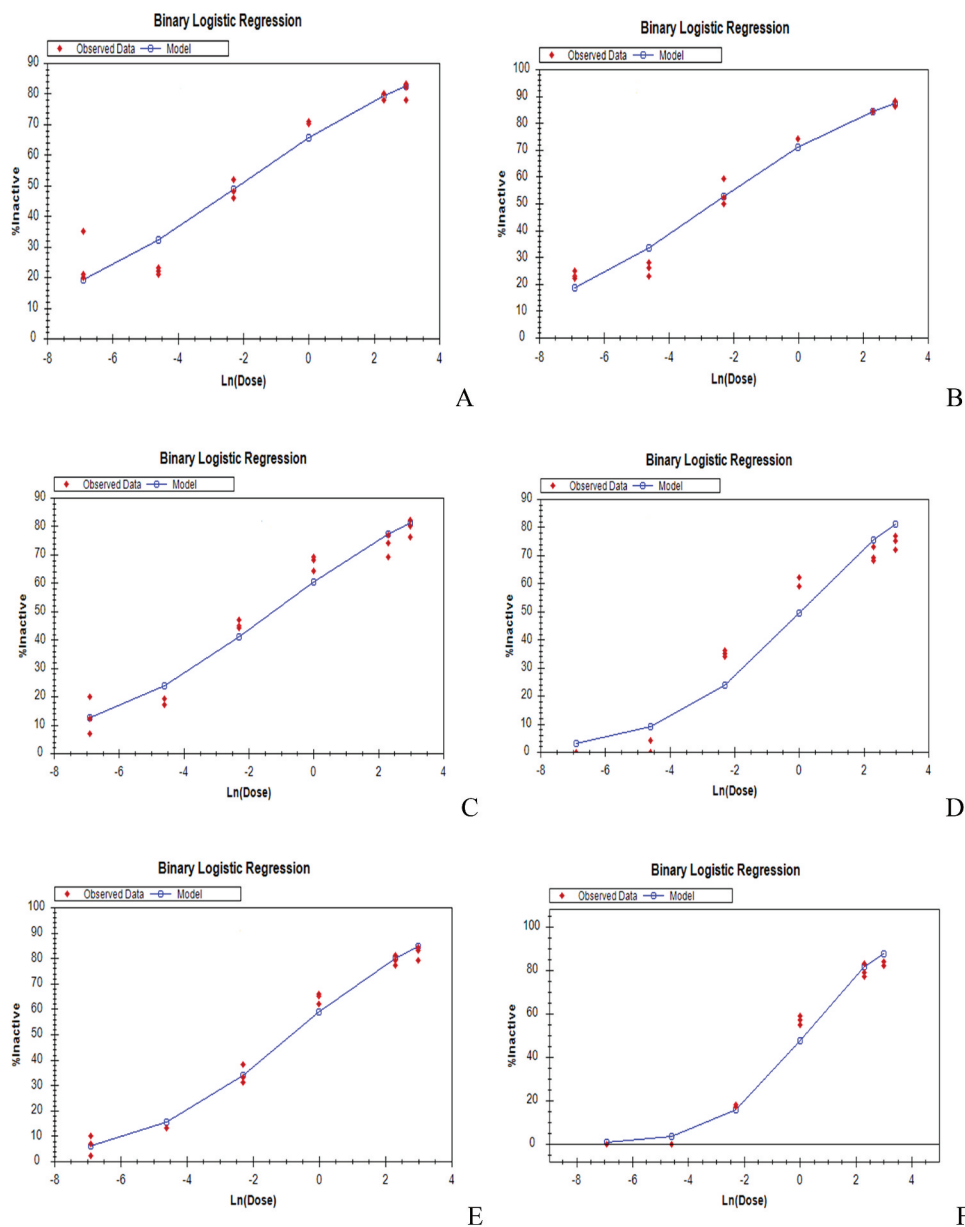


Generally, the mycelial inhibition of the test pathogens increased when the concentration of the soybean extract was increased from 0.001% to 20.0% (v/v). This trend is consistent with reports of antifungal activities of plant extracts including soybean (Mohammadi et al., 2015; Velazquez-Nunez et al., 2013). Studies by Hosseini Chaleshtori et al. (2017) indicated an increase in the diameter of the zone of inhibition of *L. monocytogenes* from 11.78 to 22.16 mm and 10.21 to 20.01 mm when the concentration of methanolic extracts of 2 soybean varieties were increased from 25 to 100 mg/ml.

All the test isolates of *E. salmonicolor* obtained MICs of 0.01% while *M. scandens* 1, *M. scandens* 2 and *M. scandens* 3 obtained MICs of <0.001, 0.01 and 0.1%, respectively after seven days of incubation. The MIC values obtained in this present study are similar to the MIC (0.01%) obtained when essential oil of soybean seeds was tested against *B. subtilis* and *E. coli* (Ghahari et al., 2017). Studies by Hyo et al. (2010) reported MIC values ranging from 0.0025% to 0.0075% when glyceollins isolated from soybean seeds were tested for their antifungal activities against *F. oxysporum*, *P. capsici*, *S. sclerotiorum* and *B. cinera*. MIC of 0.013% was also reported when isolates of *P. digitatum* were inhibited in medium amended with Night shade crude extract (Kanan & AL-Najar, 2008). However, Sobowale et al. (2013) and Rahman et al. (2016) obtained higher MIC values when extracts of *Calotropis procera* and *Cassia alata* were tested against *A. niger* and *Penicillium marneffeii*, respectively.

The IC₅₀ values obtained for isolates of *E. salmonicolor* ranged from 0.6428 to 2.437% while values for *M. scandens* ranged from 0.1193 to 0.45%. Lower values of IC₅₀ obtained for *M. scandens* indicate that they are more sensitive to the soybean extracts than isolates of *E. salmonicolor*.

Figure 4. Binary logistic model of soybean extract on the mycelial growth of isolates of *Marasmiellus scandens* (MS) after different incubation days. A: MS 1 after 5 days of incubation B: MS 1 after 7 days of incubation C: MS 2 after 5 days of incubation D: MS 2 after 7 days of incubation E: MS 3 after 5 days of incubation F: MS 3 after 7 days of incubation.



Rahman et al. (2016) obtained IC_{50} values of 2.8% when methanolic extracts of *P. sarmentosum* were tested against *Pseudomonas fuscovaginae*. However, lower values of IC_{50} (0.0168–0.0185%) were recorded when Bello et al. (2019) evaluated the antimicrobial activities of hexane extract of *Balanite aegyptiaca* and *Tapinanthus preussi* against *Candida albicans*.

Antimicrobial activities of plant extracts have been attributed to the presence of bioactive compounds in the extracts (Burt, 2004; Chah et al., 2006; Daferera et al., 2000). Many studies have attributed the antimicrobial properties of soybean extract to the presence of phytochemicals such as phenols, saponins, flavonoids and alkaloids (Cowan, 1999; Dahanukar et al., 2000; Villalobos et al., 2016). Igboabuchi and Llodibia (2018) attributed the inhibition of *S. aureus*, *E. coli* and *A. niger* by the ethanolic extract of the leaves and seeds of soybean to the presence of phytochemicals such as phenols and flavonoids in the extracts. Similarly the inhibition of nucleic acid synthesis in *S. aureus* by soybean isoflavones has been reported by Wang et al. (2010). The inhibition of *E. salmonicolor* and *M. scandens* by soybean extract in this study could be attributed to the presence of phytochemicals in

Table 1. Inhibition activity of soybean extract on isolates of *Erythricium salmonicolor* and *Marasmiellus scandens* after 5 days of incubation

Fungi	IC ₅₀ (%v/v) [95% CI]	Slope ± SE	Intercept ± SE
<i>Erythricium salmonicolor</i> 1 (ES1)	2.4370 ^a [1.8691–3.1772]	0.4574 ± 0.0227	0.4074 ± 0.0607
<i>Erythricium salmonicolor</i> 2 (ES2)	1.3406 ^b [1.0647–1.6881]	0.5398 ± 0.0247	0.1583 ± 0.634
<i>Erythricium salmonicolor</i> 3 (ES3)	0.6428 ^c [0.5013–0.8241]	0.4796 ± 0.0216	0.2120 ± 0.0618
<i>Marasmiellus scandens</i> 1 (MS1)	0.1193 ^d [0.0838–0.1699]	0.3015 ± 0.0163	0.6409 ± 0.595
<i>Marasmiellus scandens</i> 2 (MS2)	0.2929 ^e [0.2141–0.4008]	0.3442 ± 0.0173	0.4226 ± 0.586
<i>Marasmiellus scandens</i> 3 (MS3)	0.4500 ^{ce} [0.3468–0.5841]	0.446 ± 0.0202	0.3563 ± 0.0615

CI: Confidence Interval. IC: Inhibition Concentration. SE: Standard Error. IC₅₀ values with the same alphabet in the same column are not significantly different at p < 0.05.

Table 2. Inhibition activity of soybean extract on isolates of *Erythricium salmonicolor* and *Marasmiellus scandens* after 7 days of incubation

Fungi	IC ₅₀ (%v/v) [95% CI]	Slope ± SE	Intercept ± SE
<i>Erythricium salmonicolor</i> 1 (ES1)	4.6941 ^a [3.8393–5.7391]	0.6760 ± 0.0350	1.0453 ± 0.0816
<i>Erythricium salmonicolor</i> 2 (ES2)	1.9425 ^b [1.6104–2.3432]	0.7517 ± 0.0350	0.4991 ± 0.0763
<i>Erythricium salmonicolor</i> 3 (ES3)	1.9425 ^b [1.5812–2.3864]	0.6433 ± 0.0299	0.4272 ± 0.0698
<i>Marasmiellus scandens</i> 1 (MS1)	0.0735 ^c [0.05295–0.1019]	0.3418 ± 0.0172	0.8925 ± 0.0643
<i>Marasmiellus scandens</i> 2 (MS2)	1.0459 ^d [0.8195–1.3350]	0.945 ± 0.0226	0.0222 ± 0.0615
<i>Marasmiellus scandens</i> 3 (MS3)	1.1590 ^d [0.9494–1.4148]	0.6856 ± 0.0305	0.1012 ± 0.0701

CI: Confidence Interval. IC: Inhibition Concentration. SE: Standard Error. IC₅₀ values with the same alphabet in the same column are not significantly different at p < 0.05.

the extract. Bioactive compounds in plants inhibit the growth of microorganisms by the denaturing of vital enzymes, alteration of cell wall and degradation of fungal hyphae (Hylgaard et al., 2012; Nychas, 1995; Tian et al., 2011). Manso et al. (2013) also reported the disintegration of conidia and mycelia of *A. flavus* in a medium amended with cinnamon essential oil. Soybean toxin, an antifungal protein isolated from soybean has also been reported to inhibit hyphal development and block nutrient uptake systems in *Candida albicans* (Morais et al., 2013).

Botanical extracts can cause toxic effects on pests and as such have been investigated as potential pesticidal agents (Pavela, 2015; Žabka et al., 2021). In comparison with the findings of plant extracts on diverse insects of medical and agricultural importance in other studies (Pavela, 2015; Avicor et al., 2021), the extract in this study was less toxic to the test termite species. A study of 41 plant extracts (essential oils) for repellency effect against 3 mosquito species showed that soybean extract was among the most ineffective (Amer & Mehlhorn, 2006a). However, it had variable repellency on *Culex quinquefasciatus* (100%), *Anopheles stephensi* (76.2%) and *Aedes*

Figure 5. Binary logistic model of soybean extract on *Microtermes subhyalinus* after exposure for 24 hours.

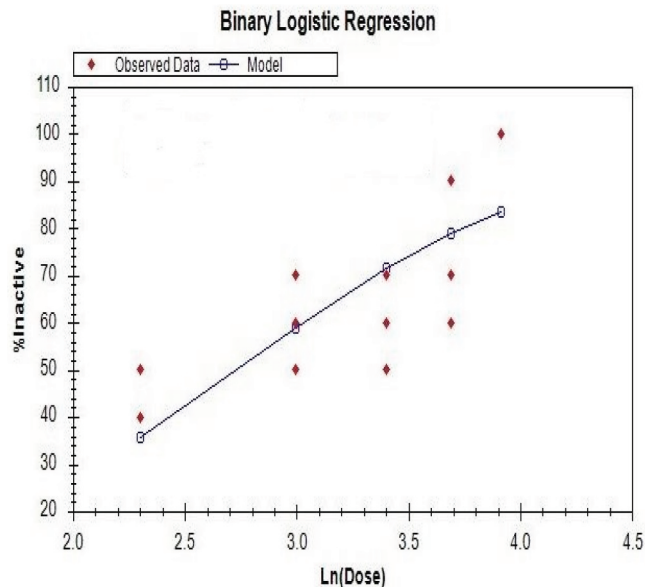


Table 3. Mortality effect of soybean extract on *Microtermes subhyalinus* after 24 hours of exposure

Conc (% v/v)	*Mortality (%)	LC ₅₀ (%) [95% CI]	Slope ± SE	Intercept ± SE
50	100.00 ± 0.00 ^e	15.418 [14.219–16.718]	1.3747 ± 0.0801	-3.7606 ± 0.2575
40	70.00 ± 5.48 ^d			
30	60.00 ± 4.47 ^{cd}			
20	56.00 ± 4.00 ^c			
10	42.00 ± 2.00 ^b			
0	2.00 ± 2.00 ^a			

Conc: Concentration. CI: Confidence Interval. LC: Lethal Concentration. SE: Standard Error. *: Untransformed data. Mortality values with the same alphabet are not significantly different at $p < 0.05$ using Tukey post hoc test after arcsine-transformation.

aegypti (54%). It (at 50 ppm) was also among the least effective in terms of mortality, causing no mortality in *Ae. aegypti* larvae after 24 hours (Amer and Mehlhorn, 2006b). The high LC₅₀ of the soybean extract on *Microtermes subhyalinus* indicates the relative insensitivity of the extract on the insect. This is similar to the reported low efficacy of soybean extract on mosquitoes (Amer & Mehlhorn, 2006ab). Naturally, the concentration of the phytochemicals in soybean extract may be less toxic to termites in terms of inducing high mortality. However, as observed in other studies (Kikuta, 2020; Amer & Mehlhorn, 2006a; Piubelli et al., 2005), it possesses other anti-insect properties such as repellent and anti-developmental effects which can contribute to suppress insect pests. Hence, the need for further investigations on the effect of the extract on the behaviour and developmental stages of the pest. Termites are polyphagous and feed on several plant species. Hence, they could have adapted to some of the phytochemicals in plant extracts. As such, the low toxicity of the soybean extract could also be due to the ability of the termites to resist or tolerate biologically active compounds in the extract through several strategies including physiological and metabolic means. Although not observed in this study, extracts from some plants have been shown to be effective termiticides (Elsayed, 2011) on some *Amitermes* and *Microtermes* species. Studies by Elango et al. (2012) and Bakaruddin and Ab Majid (2019) also indicate the efficacy of

some plant extracts on termites species like *Coptotermes formosanus*, *Coptotermes gestroi* and *Globitermes sulphureus*. It is therefore important to continue the search for effective termiticides from plants to be integrated into a sustainable and environmentally-friendly crop production system.

9. Conclusion

Antifungal effect of soybean extract against *M. scandens* and *E. salmonicolor* has been demonstrated in this study. Inhibition of 80.45 and 86.8% were attained by 20% concentration of the extract against *E. salmonicolor* and *M. scandens*, respectively after 7 days of incubation. The soybean extract was however ineffective against the termite *Microtermes subhyalinus*. The bioactive compounds in the soybean extract are yet to be isolated and tested against the pathogens under laboratory and field conditions. This could be part of an Integrated Pest Management (IPM) of these important diseases of cocoa in Ghana.

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Data availability

Data used in support of the publication of this manuscript is available upon request from the corresponding author

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