

## IN VITRO ACTIVITY OF SYNTHETIC FUNGICIDES AND SOYBEAN EXTRACT ON *COLLETOTRICHUM GLOEOSPORIOIDES*, CAUSAL PATHOGEN OF ANTHRACNOSE DISEASE OF CASHEW IN GHANA

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### Abstract

Anthracnose disease caused by *Colletotrichum gloeosporioides* complex, is one of the most important diseases of cashew (*Anacardium occidentale*) in Ghana and other African countries. It affects the quantity and quality of cashew nuts leading to economic loss to farmers. Chemical control using synthetic fungicides is a major approach in controlling the disease although knowledge on the efficacy of several synthetic fungicides and botanicals against the causative pathogen is limited. In this study, the bioactivity of soybean extract and nine synthetic fungicides were evaluated against five isolates of *Colletotrichum gloeosporioides* complex on amended agar plates for 3, 5 and 7 days. The fungicides tested were CUPH (77% Cupric hydroxide), MMCO (60% Copper oxide + 6% Metalaxyl-M), PYDM (7.2% Dimethomorph + 4% Pyraclostrobin), DMFL (Dimethomorph 200 g/L + Fluazinam 200 g/L), MECO (15% Metalaxyl + 35% Copper), CUHY (53.8% Copper hydroxide), CUPO (86% Cuprous oxide), CUOX (435 g/L Copper oxychloride) and FLUA (500 g/L Fluazinam). The percentage growth inhibition (PGI) of the isolates in plates amended with the soybean extract ranged from 3.73% to 81.74%, 4.46% to 80.38% and 2.53% to 76.63% after 7, 5 and 3 days of incubation respectively. With the synthetic fungicides, the PGI of the isolates ranged from 54.08% to 100%, 53.04% to 100% and 59.87% to 100% after 7, 5 and 3 days respectively. However, fungicide MMCO inhibited completely all the *C. gloeosporioides* isolates at all the incubation days. This study shows the efficacy of synthetic fungicides in the control of anthracnose disease of cashew as well as soybean extract as a potential bio-fungicide. The bioactive compounds present in the soybean extract, once identified and formulated for field application offers potential active ingredients for the control of anthracnose disease of cashew.

### Introduction

Cashew (*Anacardium occidentale* L.) is an important nut in West Africa and accounts for 45% of the world's Raw Cashew Nut (RCN) production (Dedehou et al., 2015; Monteiro et al., 2017; Oliveira, 2008). The consumption of cashew increased by 25% globally between 2011 and 2015 and it has become an important tree crop in Ghana since the launch of the Cashew Development Project (CDP) in 2002 (Ghana Business News, 2020). This has led to a considerable increase in the production of cashew nut reaching 325,407 tonnes in 2021 (Ministry of Food and Agriculture, 2022). It is an important source of essential foreign exchange for the country and has become the second major cash crop in Ghana generating \$378 million from RCN exports in 2018 (Ghana Business News, 2020). Despite the huge benefits that can be derived from cashew cultivation, factors including diseases have been reported to affect the quality and quantity of cashew nuts, therefore limiting cashew cultivation in Ghana (Ghini et al., 2011; Monteiro et al., 2017). Diseases are known to affect all parts of the cashew tree. Anthracnose, inflorescence blight, gummosis, leaf rust, leaf blight and root rot are the most important cashew diseases reported in Ghana (Amoako-Attah et al., 2021). An-

thrachnose disease caused by *Colletotrichum gloeosporioides* has been reported to cause 70-100% yield loss if not controlled (Amoako-Attah et al., 2021; Wonni et al., 2017). The disease primarily attacks the leaves, nuts, flowers and twigs of cashew trees (Freire et al., 2002; Nakpalo et al., 2017), resulting in death of inflorescence, defoliation, darkening of apples and falling of immature nuts (Amoako-Attah et al., 2021). Anthracnose disease is mostly controlled with synthetic fungicides application (Filoda, 2008; Silue et al., 2017). In Ghana fungicides containing either copper oxychloride or mancozeb as active ingredients have been used to control anthracnose disease (Amoako-Attah et al., 2021). Elsewhere, fungicides containing dithianon, azoxystrobin, benomyl and trifloxystrobin, have been used to control anthracnose (Christian, 2001; Menezes et al., 1975). There is a limited number of fungicides active ingredients available for effective control of the disease. Therefore, it is important to explore other active ingredients that can be used to control anthracnose disease. Although synthetic fungicides are effective, there are increasing worries on the long-term effect on non-target organisms, the cost of acquisition and high residues in crops (Balakumar et al., 2011; Basco et al., 2017; Sande et al., 2011). Thus,

there is a pressing need to explore more sustainable and environmentally friendly control agents such as botanicals to manage anthracnose disease. Studies have shown that plant extracts including soybean contain valuable metabolites with antifungal properties that can help in the management of plant diseases (Bukari et al., 2022; Villalobos et al., 2016; Wang et al., 2010). Soybean (*Glycine max*) has been reported to contain important phytochemicals such as alkaloids, terpenes, terpenoids, saponins, and flavonoids (Carvalho et al., 2008; Villalobos et al., 2016). Combining synthetic fungicides with extracts from plants such as soybean in an integrated disease management could reduce the frequent applications of synthetic fungicides during the peak seasons of the disease and the overall quantity of the fungicides used. Therefore, this study aimed to assess the effectiveness of soybean extract and nine synthetic fungicides namely 77% Cupric hydroxide, 60% Copper oxide + 6% Metalaxyl-M, 7.2% Dimethomorph + 4% Pyraclostrobin, Dimethomorph 200 g/L + Fluazinam 200 g/L, 15% Metalaxyl + 35% Copper, 53.8% Copper hydroxide, 86% Cuprous oxide, 435 g/L Copper oxychloride and 500 g/L Fluazinam.

## Materials and Methods

### Soybean extract

Soybean seeds were sourced from a local market in Tamale, the capital of the Northern Region of Ghana and the extract was prepared as described by Bukari et al. (2022). The seeds were washed under tap water and thoroughly rinsed with sterile distilled water (SDW). The seeds were then dried under shade for three weeks and ground into a fine powder using a blender (Binatone, BLG 45) at two burst speed for 5 minutes. Then 5 L of SDW was added to 1000 g of fine powder in a conical flask. The mixture was incubated at room temperature ( $27 \pm 2^\circ\text{C}$ ) for seven days, strained through a muslin mesh and further concentrated via rotary evaporation. A 60% (w/v) stock solution was prepared by dissolving 60 g of the fine powder in 100 ml of SDW for later use.

### Location and isolation of *C. gloeosporioides* from diseased leaves

Diseased cashew leaves showing symptoms of anthracnose were sampled from farmers' farms in the Bono East, Eastern and Volta Regions of Ghana between October and December 2022. The leaves were collected into sterile sampling bags with a tag bearing the geographical coordinates of the samples to the laboratory. The infected leaves were surface sterilized with 70% ethanol and rinsed thrice with SDW. A leaf tissue from the junction between healthy and diseased lesions was cut into 5 x 5 mm pieces, plated onto water agar (WA) and incubated at  $25^\circ\text{C}$  for 5 days. Emerging hyphal strands from the tissues were subsequently transferred onto Potato Dextrose Agar (PDA) media and incubated for seven days at  $28 \pm 2^\circ\text{C}$ . Identification of pathogens was done using cultural and morphological characteristics (Humber, 2005; Kirk et al., 2008). Five isolates of *C. gloeosporioides* showing distinct

morphological characteristics were selected for further studies. The isolates were named as Colle 1, Colle 2, Colle 3, Colle 4 and Colle 5.

### In vitro bioactivity of the soybean extract and synthetic fungicides

The antifungal activity of the soybean extract was assessed using a modified method of Nwosu and Okafor (1995). A 100 mL of molten Potato Dextrose Agar (PDA) was amended with soybean extract to various concentrations (50%, 20%, 10%, 1% and 0.1%, v/v). Also nine synthetic fungicides namely, CUPH (77% Cupric hydroxide), MMCO (60% Copper oxide + 6% Metalaxyl-M), PYDM (7.2% Dimethomorph + 4% Pyraclostrobin), DMFL (Dimethomorph 200 g/L + Fluazinam 200 g/L), MECO (15% Metalaxyl + 35% Copper), CUHY (53.8% Copper hydroxide), CUPO (86% Cuprous oxide), CUOX (435 g/L Copper oxychloride) and FLUA (500 g/L Fluazinam) were each used to amend 100 mL of PDA based on the manufacturers' recommended doses (Table 1). The amended media were poured into 90 mm diameter Petri plates under a Laminar flow chamber (Esco) and allowed to solidify. The plates were inoculated in the centre with 5 mm mycelial plug from the five selected *C. gloeosporioides* isolates. Unamended media were used as a control for comparison. The inoculated plates were incubated at  $28 \pm 2^\circ\text{C}$ . Two perpendicular measurements were made across the colony (mean diameter of growth) and growth inhibition assessed on the 3rd, 5th and 7th day post-inoculation. The Percentage Growth Inhibition (PGI) was calculated as:

$$PGI = \frac{C - T}{C} \times 100 \quad (1)$$

where C = mean diameter of growth in control plates; T = mean diameter of growth in treatment plates

**Table 1.** Active ingredients and recommended doses of tested fungicides

Fungicide	Active ingredients	Dosage (15 L)
CUPH	77% Cupric hydroxide	100 g
MMCO	60% Copper oxide + 6% Metalaxyl-M	50 g
PYDM	7.2% Dimethomorph + 4% Pyraclostrobin	50 mL
DMFL	Dimethomorph 200 g/L + Fluazinam 200 g/L	75 mL
MECO	15% Metalaxyl + 35% Copper	50 g
CUHY	53.8% Copper hydroxide	100 g
CUPO	86% Cuprous oxide	75 g
CUOX	435 g/L Copper oxychloride	100 mL
FLUA	500 g/L Fluazinam	50 mL

### Data analysis

The calculated PGI of *C. gloeosporioides* isolates on the fungicides and soybean extract was subjected to analysis of variance (ANOVA) at a 5% significance level using Genstat statistical package (11th Edition). The means of the treatment were compared using Tukey's HSD test.

**Table 2.** Locations of sampled *C. gloeosporioides*

Isolate	Location of isolates	Ecological zone	Region	Coordinates of Location
Colle 1	Prusu Nkoranza	Forest transitional	Bono East	Lat: N07° 30.588” Lon: W01° 33.931”
Colle 2	Kpando Gadza	Semi-deciduous	Volta	Lat: N07°01’38.13708” Lon: E00°21’48.48016”
Colle 3	Duabone	Forest transitional	Bono East	Lat: N07°45’4.92012” Lon: W01°42’9.75592”
Colle 4	Adunasa Nkoranza	Forest transitional	Bono East	Lat: N07°06’41.47812” Lon: W01°22’38.3818”
Colle 5	Aframase	Semi-deciduous	Eastern	Lat: N06°21’4.75812” Lon: W00°04’31.26”

## Results and Discussion

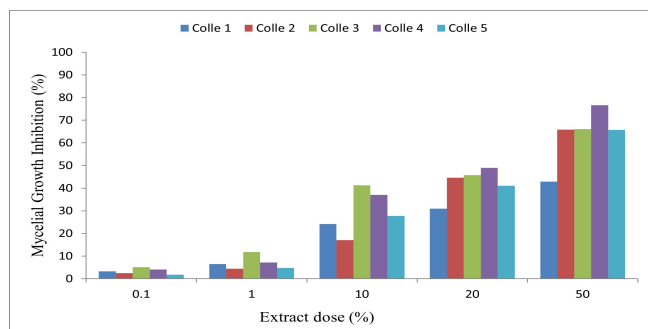
### Results

#### Location and isolation of *C. gloeosporioides* from diseased leaves

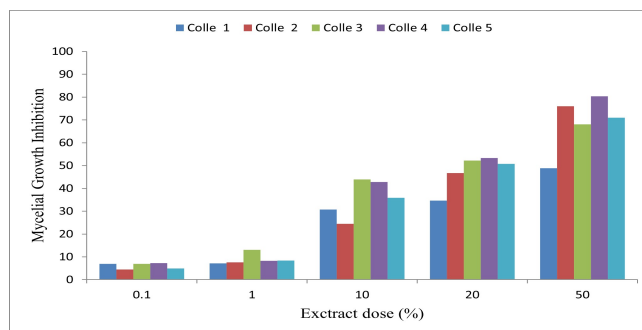
The *Colletotrichum gloeosporioides* isolates used in this study were sampled from the Forest transitional (Colle 1, 2 and 4) and Semi-deciduous ecological zones of Ghana (Colle 2 and 5) (Table 2).

#### Efficacy of soybean extract

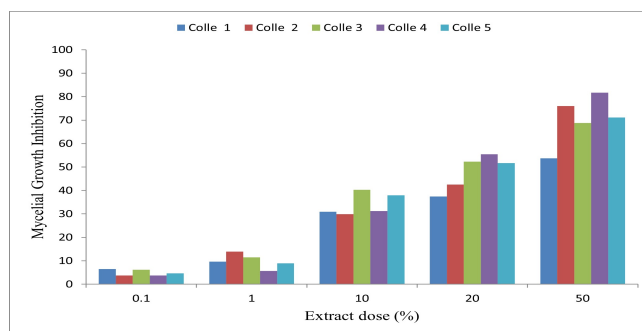
Growth inhibition of soybean extract on *C. gloeosporioides* isolates was significantly different ( $p < 0.001$ ) across all the different days of incubation. The PGI of the isolates varied from 1.83% to 76.63%, 4.46% to 80.38% and 3.73% to 81.74% after 3, 5 and 7 days of incubation respectively (Figure 1, 2, 3 and Plate 1). After incubation day 3, PGI of isolates did not differ at 0.1% concentration. The highest PGI of 76.63% was obtained by 50% concentration on Colle 4; this was not significantly different from the PGI recorded by the other isolates except for Colle 1 (Figure 1). After incubation day 5, the highest PGI of 80.38% was obtained by 50% concentration on Colle 4. This PGI was significantly higher than the PGI recorded at all the other soybean concentrations (Figure 2). During the 7th day of incubation, the highest PGI of 81.74% was obtained by 50% concentration on Colle 4 isolate and this was significantly different from the other *C. gloeosporioides* isolates (Figure 3).



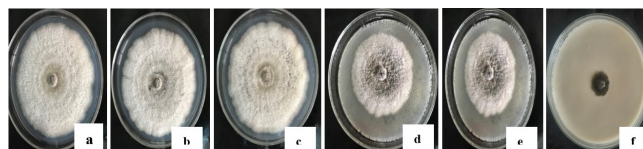
**Figure 1.** Mycelial growth inhibition of isolates of *C. gloeosporioides* on media amended with different doses of soybean extract after incubation for 3 days



**Figure 2.** Mycelial Growth Inhibition (%) of isolates of *C. gloeosporioides* on media amended with different doses of soybean extract after incubation for 5 days



**Figure 3.** Mycelial Growth Inhibition (%) of isolates of *C. gloeosporioides* on media amended with different doses of soybean extract after incubation for 7 days

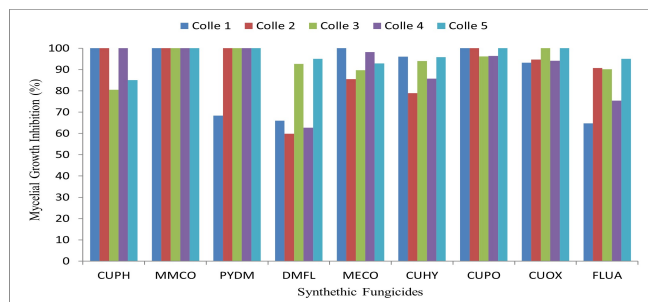


**Plate 1:** Mycelial radial growth of *C. gloeosporioides* on non-amended (control) plate (a) and 0.1% (b), 1.0% (c), 10% (d), 20% (e) and 50% (f) soybean extract - amended plates after incubation for 7 days.

**Efficacy of synthetic fungicides**

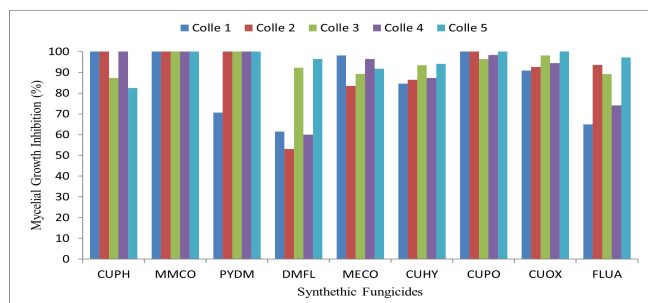
The synthetic fungicides were effective against the five selected *C. gloeosporioides* isolates. The percentage growth inhibition (PGI) of the isolates ranged from 59.87% to 100%, 53.04% to 100% and 54.08% to 100% after 3, 5 and 7 days respectively. After 3 days of incubation, CUPH-amended plates completely inhibited Colle 1, Colle 2 and Colle 4 (Figure 4). After 5 days of incubation, MMCO-amended plates attained 100% growth inhibition for all the isolates (Figure 5). PYDM-amended plates completely inhibited all the *C. gloeosporioides* isolates except Colle 1. There was complete inhibition of Colle 2 isolate on plates amended with CUPH, MMCO, PYDM and CUPO fungicides while Colle 5 was completely inhibited on plates amended with MMCO, PYDM, CUPO and CUOX fungicides (Plate 2).

After 7 days of incubation, MMCO amended plates achieved 100% growth inhibition of all the isolates (Figure 6). Also, PYDM amended plates completely inhibited all the test isolates except Colle 1. DMFL, MECO, CUHY and FLUA amended plates could not inhibit completely (100%) any of the isolates. The lowest PGI of 54.08% was recorded on DMFL-amended plates when inoculated with Colle 1.



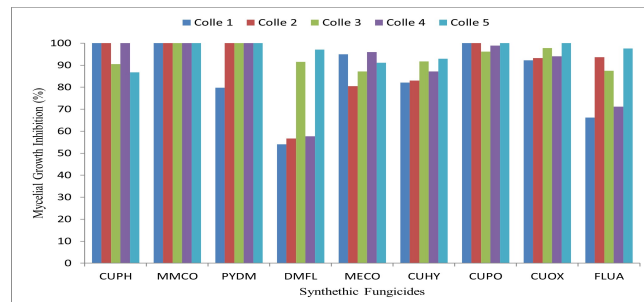
**Figure 4.** Mycelial growth inhibition of isolates of *C. gloeosporioides* on media amended with synthetic fungicides after incubation for 3 days

CUPH: 77% Cupric hydroxide; MMCO: 60% Copper oxide + 6% Metalaxyl-M; PYDM: 7.2% Dimethomorph + 4% Pyraclostrobin; DMFL: Fluazinam 200 g/L + Dimethomorph 200 g/L; MECO: 15% Metalaxyl + 35% Copper; CUHY: 53.8% Copper hydroxide; CUPO: 86% Cuprous oxide; CUOX: 435 g/L Copper oxychloride; FLUA: 500 g/L Fluazinam.



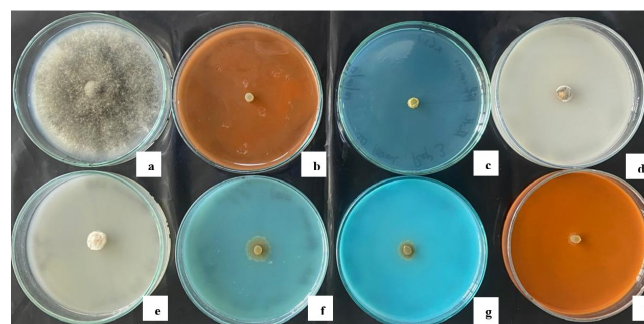
**Figure 5.** Mycelial growth inhibition of isolates of *C. gloeosporioides* on media amended with synthetic fungicides after incubation for 5 days

CUPH: 77% Cupric hydroxide; MMCO: 60% Copper oxide + 6% Metalaxyl-M; PYDM: 7.2% Dimethomorph + 4% Pyraclostrobin; DMFL: Fluazinam 200 g/L + Dimethomorph 200 g/L; MECO: 15% Metalaxyl + 35% Copper; CUHY: 53.8% Copper hydroxide; CUPO: 86% Cuprous oxide; CUOX: 435 g/L Copper oxychloride; FLUA: 500 g/L Fluazinam.



**Figure 6.** Mycelial growth inhibition of isolates of *C. gloeosporioides* on media amended with synthetic fungicides after incubation for 7 days

CUPH: 77% Cupric hydroxide; MMCO: 60% Copper oxide + 6% Metalaxyl-M; PYDM: 7.2% Dimethomorph + 4% Pyraclostrobin; DMFL: Fluazinam 200 g/L + Dimethomorph 200 g/L; MECO: 15% Metalaxyl + 35% Copper; CUHY: 53.8% Copper hydroxide; CUPO: 86% Cuprous oxide; CUOX: 435 g/L Copper oxychloride; FLUA: 500 g/L Fluazinam.



**Plate 2:** Mycelial radial growth of *C. gloeosporioides* (Colle 5) on (a) non-amended (control) plate (b) MMCO: 60% Copper oxide + 6% Metalaxyl-M (c) CUOX: 435 g/L Copper oxychloride (d) DMFL: Dimethomorph 200 g/L + Fluazinam 200 g/L (e) FLUA: 500 g/L Fluazinam (f) MECO: 15% Metalaxyl + 35% Copper (g) CUHY: 53.8% Copper hydroxide and (h) CUPO: 86% Cuprous oxide fungicide-amended plates after incubation for 7 days.

**Discussion**

Soybean extract had inhibitory effect on isolates of *C. gloeosporioides* tested, All the doses of the soybean extract inhibited the growth of *C. gloeosporioides* to some extent at the incubation days. Antimicrobial properties of plants have been widely reported and this includes the use of soybean in the control of microorganisms (Bukari et al., 2022; Ponnusha et al., 2011; Wang et al., 2010). In this study, the highest soybean extract concentration (50%) recorded the highest inhibition of

81.74% after 7 days of incubation. This result is similar to PGI of 86.8% and 80.4% obtained respectively when cocoa pathogens such as *Marasmiellus scandens* (cause of thread blight disease) and *Erythricium salmonicolor* (cause of pink disease) were grown on plates amended with 20% soybean extract and incubated for 7 days (Bukari et al., 2022). However, the PGI recorded in our study was higher than those of Igboabuchi and Llodibia (2018), who recorded PGI of 62.29% and 68.9% when ethanol extracts of soybean were tested for antimicrobial activities against *Aspergillus niger* and *Escherichia coli* respectively. Also, a lower PGI of 10.9% to 61.0% was attained when glyceollin, extracted from the seeds of soybean were tested against some isolates of *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Botrytis cinerea* (Hyo et al., 2010). The results of this present study showed that the inhibition of the isolates by the soybean extract was dose-dependent. The percentage growth inhibition increased with an increased dose of the soybean extract. Similar observations have been reported in studies by Bukari et al. (2022), Mohammadi et al. (2015) and Velazquez-Núñez et al. (2013). Soybean extracts contain antifungal compounds such as alkaloids, phenols, flavonoids and saponins (Cowan, 1999; Dahanukar et al., 2000). Wang et al. (2010) noted that isoflavones of soybean extract inhibited the synthesis of *Staphylococcus aureus* nucleic acids which results in the degradation of both their DNA and RNA. Phytochemical in soybean was reported to impede hyphal development in *Candida albicans* (Morais et al., 2013). Activities of other plant extracts such as essential oil can also be evaluated against *C. gloeosporioides* since Bukari et al. (2025) observed that essential oil of citrus were effective against *Erythricium salmonicolor* and *Marasmiellus scandens*.

The efficacy of the synthetic fungicides against isolates has been demonstrated in this study. The fungicides could inhibit the growth of isolates. MMCO completely inhibited (100%) the growth of all the five *C. gloeosporioides* isolates used. DMFL and FLUA were generally less effective compared to the other fungicides. Complete inhibition of *C. gloeosporioides*, causal pathogen of anthracnose disease of cashew using synthetic chemicals has been reported (Nakpalo et al., 2017; Patrice et al., 2021; Satapath and Beura, 2019). Growth media amended with Carbendazim, Cymoxanil + Mancozeb, Mancozeb and Prochloraz fungicides completely inhibited growth of *C. gloeosporioides* (Nakpalo et al., 2017). Also, fungicides containing Tebuconazole, Azoxystrobin + Mancozeb, Zineb and Tebuconazole + Trifloxystrobin inhibited *C. gloeosporioides* causing anthracnose disease of cashew by 94.56%, 96.17%, 95.46% and 95.06% respectively (Satapath and Beura, 2019). Patrice et al. (2021) reported that 0.5 mg/mL and 5 mg/mL of Mancozeb reduced the mycelial growth of *C. gloeosporioides* from 50% to 100%. However, some synthetic fungicides have low inhibitory effect on growth of anthracnose pathogen. Azoxystrobin and Pyraclostrobin inhibited the growth of *C. gloeosporioides* by 44.56% and 32.12% respectively (Satapath and Beura, 2019).

Integrating the synthetic fungicides and the soybean extract in the management of anthracnose disease could reduce the frequency and number of synthetic fungicides in the field.

## Conclusion

This study showed that the synthetic fungicides (CUPH, MMCO, PYDM, DMFL, MECO, CUHY, CUPO, CUOX and FLUA) are very effective in the control of *C. gloeosporioides*, the causal pathogen of anthracnose disease of cashew in Ghana. Also, soybean extract showed promising results in the inhibition of growth of the pathogen. Further studies on the use of synthetic fungicides and soybean extract in an alternating spraying regime should be done to reduce the quantity and frequency of synthetic fungicides needed to manage the disease in the field.

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