

## Management of Anthracnose Disease on Mango Fruit with Aqueous Extracts of Nut Grass (*Cyperus rotundus* L.) and Coat Button (*Tridax procumbens* L.)

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### Abstract

Anthracnose disease caused by *Colletotrichum gloeosporioides* is a serious pre- and post-harvest disease of mango. At the post-harvest stage, anthracnose infects mango fruits, thereby reducing the quality and quantity of the mango fruits; usually resulting in economic losses. This study examined the effect of aqueous extracts of *Cyperus rotundus* and *Tridax procumbens* on the anthracnose disease of mango fruits. Aqueous extracts of *T. procumbens* and *C. rotundus* were each prepared by soaking 100 g of powdered plant materials in 1000 ml of distilled water (100% concentration) and serially diluted to 50 % of the concentration. Three wounded mango fruits each treated with 50 and 100 % extract concentrations of *T. procumbens* and *C. rotundus*, were inoculated with spore suspension of *C. gloeosporioides* and used for the study. Six treatments comprising four aqueous extracts namely, 50 % *T. procumbens*, 50 % *C. rotundus*, 100 % *T. procumbens*, and 100 % *C. rotundus*, and two controls, namely PDA amended with sterile distilled water (negative) and PDA amended with Mancozeb 640g/kg + Metalaxyl 80g/kg WP (positive) were evaluated in a completely randomised designed experiment replicated three times. Compared to the negative control, the different concentrations of the plant extracts significantly reduced mycelia growth of *C. gloeosporioides* and lesion diameter of anthracnose disease on the mango fruits. . Except for the positive control, which inhibited mycelia growth of *C. gloeosporioides* by 100 %, all the plant extracts inhibited growth by about 50 %. In conclusion, the aqueous extracts of *T. procumbens* and *C. rotundus* exhibited antifungal activity against mycelia growth of *C. gloeosporioides* and reduced anthracnose infection. Hence, combining these botanical extracts with the synthetic fungicides used in this study could help mango producers and exporters reduce the cost of managing anthracnose disease on mango fruits; further studies would be needed to explore this.

**Keywords:** Anthracnose; *Colletotrichum gloeosporioides*; Mango fruit; phytochemicals and plant extract

## Gestion de l'antracnose sur les fruits de manguier à l'aide d'extraits aqueux d'agrumes (*Cyperus rotundus* L.) et de boutons de coton (*Tridax procumbens* L.).

### Résumé

L'antracnose causée par *Colletotrichum gloeosporioides* est une grave maladie de la mangue avant et après la récolte. Au stade post-récolte, l'antracnose infecte les fruits de la mangue, réduisant ainsi la qualité et la quantité des fruits, ce qui entraîne généralement des pertes économiques. Cette étude a examiné l'effet des extraits aqueux de *Cyperus rotundus* et de *Tridax procumbens* sur l'antracnose des mangues. Des extraits aqueux de *T. procumbens* et de *C. rotundus* ont été préparés en trempant 100 g de matériel végétal en poudre dans 1000 ml d'eau distillée (concentration de 100 %) et dilués en série jusqu'à 50 % de la concentration. Trois fruits de mangue blessés, traités chacun avec des concentrations de 50 et 100 % d'extraits de *T. procumbens* et de *C. rotundus*, ont été inoculés avec une suspension de spores de *C. gloeosporioides* et utilisés pour l'étude. Six traitements comprenant quatre extraits aqueux, à savoir 50 % de *T. procumbens*, 50 % de *C. rotundus*, 100 % de *T. procumbens* et 100 % de *C. rotundus*, et deux contrôles, à savoir PDA amendé avec de l'eau distillée stérile (négatif) et PDA amendé avec Mancozeb 640g/kg + Metalaxyl 80g/kg WP (positif) ont été évalués dans le cadre d'une expérience complètement randomisée répétée trois fois. Par rapport au contrôle négatif, les différentes concentrations des extraits de plantes ont réduit de manière significative la croissance des mycéliums de *C. gloeosporioides* et le diamètre des lésions de la maladie de l'antracnose sur les fruits de manguiers. A l'exception du contrôle positif, qui a inhibé la croissance du mycélium de *C. gloeosporioides* de 100 %, tous les extraits de plantes ont inhibé la croissance d'environ 50 %. En conclusion, les extraits aqueux de *T. procumbens* et de *C. rotundus* ont montré une activité antifongique contre la croissance du mycélium de *C. gloeosporioides* et ont réduit l'infection par l'antracnose. Par conséquent, la combinaison de ces extraits botaniques avec les fongicides synthétiques utilisés dans cette étude pourrait aider les producteurs et les exportateurs de mangues à réduire les coûts de gestion de la maladie de l'antracnose sur les fruits de la mangue; d'autres études seraient nécessaires pour explorer ce point.

**Mots clés:** Anthracnose; *Colletotrichum gloeosporioides*; fruit de mangue; composés phytochimiques et extraits de plantes.

### Introduction

Mango (*Mangifera indica* L) is a significant fruit crop in most tropical parts of the world, with industrialised nations consuming the bulk of it (Abera *et al.*, 2016). *Mangifera indica* is referred to as the "king of fruits" because of its variety of flavours, high nutritional and medicinal value (Sankaran *et al.*, 2021).

Despite the importance of mango, several challenges, including the anthracnose disease

caused by *Colletotrichum gloeosporioides*, confront its production at the pre- and post-harvest stages of the crop. The anthracnose disease is a major post-harvest disease of mango, which reduces the quality and quantity of mango fruits, resulting in huge economic losses to mango farmers and traders (Maqsood *et al.*, 2014). In unripe mango fruits, anthracnose infection is latent, with symptom development occurring in ripened fruits (Nelson, 2008). On a ripe mango fruit, anthracnose symptoms appear as sunken

dark-brown to black decay spots, which coalesce as the infection progresses and gradually penetrate into the fruit, resulting in fruit rot (Nelson, 2008).

Managing mango fruit anthracnose is necessary to maintain the quality and enhance the shelf life of the fruits; thereby reducing losses resulting from the disease. Several methods, such as cultural, biological, chemical and cultivating mango varieties resistant to anthracnose are adopted in managing the disease (Kankam *et al.*, 2022). Synthetic fungicides are presently the most common method used in managing the anthracnose disease of *M. indica*, before and after harvest (Ruffo Roberto *et al.*, 2019). Concerns about human health and environmental threats from chemical control, as well as the formation of new pathogen races, have shifted research to other environmental friendly approaches to managing mango anthracnose disease (Ratnadass *et al.*, 2012).

According to Ranjitha *et al.* (2019), plant extracts are a safer alternative to chemical fungicides for disease management since they are environmentally friendly and safe. Plant extracts can potentially replace synthetic fungicides and significantly preserve foodstuffs against fungal infection (Ranjitha *et al.*, 2019). The phytochemicals contained in botanical fungicides play a key role in their fungitoxicity in plant disease management (Deresa & Diriba, 2023). The mode of action of phytochemicals such as alkaloid, flavonoid, saponin, tannin and triterpenes as fungicides include causing leakage of fungal cellular content by destroying plasma membrane, inhibition of cutinases and laccases production and also ergosterol synthesis which makes these phytochemicals toxic to phytopathogenic fungi (Nmom & Ajuru, 2020; Adamczyk *et al.*, 2023).

This study, therefore, seeks to determine the phytochemical constituents of aqueous extracts of nut grass (*Cyperus rotundus*) and coat button (*Tridax procumbens*). The study also examined the fungicidal effects of *C. rotundus* and *T. procumbens* on the mycelia growth of *C. gloeosporioides*, as alternatives in managing anthracnose on mango fruits.

## Materials and methods

### Study area

The research was carried out in the Microbiology Laboratory of the University for Development Studies, Nyankpala Campus. The campus is located in the Tolon District (9° 15' and 10° 02' North and 0° 53' and 1° 25' West), about 20 km South-West of Tamale, Northern region, Ghana. The mean annual rainfall ranges between 950 to 1,200 mm. Day and night mean temperatures range between 33 to 39 °C and 20 to 26 °C, respectively. The soil type is generally sandy loam, and the vegetation is Guinea Savanna.

### Materials for study

Kent, a mango variety highly susceptible to anthracnose disease, was used for the study. Mango fruits with typical anthracnose symptoms were randomly sampled from mango sellers in the Tamale market, Northern Region, Ghana. The mango fruits were stored in envelopes, labelled, and then transported to the laboratory.

### Preparation of plant extracts

Shoots of nut grass (*Cyperus rotundus*) and coat buttons (*Tridax procumbens*) were collected from the Kpachi Community Agricultural Fields in the Tolon district.

The Abd-EL-Khair & Haggag (2007) method was employed in the preparation of the plant extracts for the study. The shoots of *C. rotundus* and *T. procumbens* were thoroughly washed with distilled water, dried in the shade at air temperature for two weeks, chopped and

milled into powder. The milled samples were then sieved through a sieving mesh (with a 2 mm hole size) to obtain a fine powder. A 100 g of the powdered samples of *C. rotundus* and *T. procumbens* was each added separately to 1000 ml of sterile distilled water and stirred. The mixture was kept at room temperature for 24 h and filtered through a sterilised white cotton cloth. The filtrate served as the stock solution (i.e., 100 % concentration) of the aqueous extracts of the plant materials for

serial dilution to 50% concentration.

#### Phytochemical constituent

The assessment of phytochemical constituents of *C. rotundus* and *T. procumbens* was carried out using protocols in Table 1.

#### Media preparation

Potato Dextrose Agar (PDA) media was prepared according to the manufacturer's

Table 1. Methods used in determining phytochemical constituents in the plant extracts

| Phytochemical | Method of determination                                                                                                                                                                                       | References                                                                                                        |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Alkaloid      | Three drops of Hager's reagent were added to 2 ml of the plant extract. A yellow precipitation formation indicated the presence of alkaloids.                                                                 | Tiwari <i>et al.</i> , 2011                                                                                       |
| Flavonoid     | To 2 ml of plant extracts, two drops of sodium hydroxide was added. A yellow colour formation which became colourless upon the addition of few drops of dilute HCl indicated the presence of flavonoids.      | Egbuna <i>et al.</i> , 2018<br>Tiwari <i>et al.</i> , 2011<br>Trease & Evans, 1989<br>Tiwari <i>et al.</i> , 2011 |
| Saponin       | Two 2 ml of water was added to 0.5 g of the plant extract and then shaken vigorously. The formation of foam which persisted for 10 minutes indicated saponin presence.                                        | Tiwari <i>et al.</i> , 2011                                                                                       |
| Tannin        | To 5 ml of the plant extract, 1 % gelatin solution containing sodium chloride was added. A white precipitate formation indicated the presence of tannins.                                                     | Trease & Evans, 1989                                                                                              |
| Triterpene    | Chloroform solution was added to the plant extract. To this, a few drops of concentrated HCl was added and then shaken. The presence of triterpenes was indicated by the formation of a golden yellow colour. | Tiwari <i>et al.</i> , 2011                                                                                       |

(Oxoid, UK) instructions. 39 g of PDA powder was weighed into a conical flask and suspended in 1000 ml of distilled water. 150 mg of amoxicillin was added to the suspension to suppress bacteria growth. The suspension was then stirred with a glass rod to obtain a homogenous mixture. The mixture was then autoclaved at 121 °C and 1.06 kg/cm<sup>2</sup> pressure for 15 min.

#### Isolation and identification of *C. gloeosporioides*

Mango fruits with anthracnose symptoms were washed under running tap water. Mango slices (1 cm fragments) consisting of healthy and anthracnose diseased portions were obtained from the periphery of lesions on fruits. The slices were sterilised in 70 % ethanol for 2 min and then rinsed with sterilised distilled water. The mango slices were then blotted with sterilised paper tissues to rid them of surface water. These were then inoculated on solidified PDA contained in Petri dishes and incubated at room temperature for 7 days. Fungi, which grew in the culture were sub-cultured until pure cultures were obtained. Slides of 5-day old pure culture were developed and examined under a compound microscope (Leica DME, Leica Microsystems, Shanghai, China). The *C. gloeosporioides* isolate was identified based on their culture and morphological characteristics comparing them with those documented by Ajay Kumar (2014).

#### Pathogenicity test

Healthy mango fruits were washed under running tap water, surface sterilised in 70 % alcohol for 3 minutes and then washed three times with sterilised distilled water. The fruits were inoculated with spore suspension of *C. gloeosporioides* prepared following the procedure of Onyeani (2017). The pathogen was re-isolated from fruits that showed symptoms of anthracnose after 7 days of incubation and its culture characteristics

compared to the one used to inoculate the mango fruits.

#### *In vitro* evaluation of the botanicals

The conventional food poisoning method was used to determine the effect of 50 and 100 % aqueous extract concentrations of *C. rotundus* and *T. procumbens* on *C. gloeosporioides* mycelia growth. Five millilitres of plant extract was poured into a Petri dish, after which 20 ml of molten PDA was added. The mixture was swirled to uniformly mix the content and then allowed to solidify. At the bottom of the Petri dish, two parallel lines were drawn along the plate's circumference. A 5 mm mycelial disc of *C. gloeosporioides*, cut with a sterile 5 mm cork borer, was placed at the central point of the two lines. The inoculated Petri dishes were incubated for 5 days at 37 ± 2 °C under 12 h light and 12 h darkness cycle. Two controls, namely, a negative control which contained PDA amended with sterile distilled water and a positive control PDA amended with synthetic fungicide (i.e., Mancozeb 640g/kg + Metalaxyl 80g/kg WP) prepared at the rate of 2g/L of water in accordance with the manufacturer's recommendation and used for the study. The treatments were laid in a completely randomized designed (CRD) experiment replicated three times. Mycelia growth of *C. gloeosporioides* was measured daily along the radii of the lines drawn at the bottom of the Petri dish.

The percentage inhibition of mycelium was calculated using the formula described by Jamil & Ashraf (2020):

$$I = \frac{C - T}{C} \times 100 \%$$

Where,

I = percentage inhibition of mycelium

C = growth of mycelium in control

T = growth of mycelium in treatment

### Assessment of the effect of the plant extracts on mango fruit anthracnose

The effect of the plant extracts on the mango fruit anthracnose disease was assessed using the method of Meng *et al.* (2015). Healthy mango fruits were washed with sterile distilled water, surface sterilised with 70 % alcohol for 3 minutes, rinsed in sterile distilled water and dried with blotter paper. The fruits were then rinsed in sterile distilled water and blotted dry. Three fruits per treatment were then soaked separately in the 50 and 100 % extract concentrations of *C. rotundus* and *T. procumbens* for 30 mins, after which they were removed and air dried in a laminar flow cabinet. A wound was created on the mango fruits using a sterile pin and then inoculated with 10 µl spore suspension of *C. gloeosporioides*. The mango fruits were then kept in a humid chamber and incubated for 14 days under 12 h light and 12 h darkness cycle. The positive and negative controls used in the *in vitro* study served as control treatments for this study too. Each treatment was laid in a CRD replicated three times. The diameter of the anthracnose lesions developed at the end of the incubation period was measured using a ruler.

### Data analysis

Data obtained were subjected to analysis of variance (ANOVA) using GENSTAT statistical package (GENSTAT, 12<sup>th</sup> Edition). Duncan's Multiple Range Test was used to separate means at 5 % probability.

## Results

### Phytochemical constituents

The phytochemicals: alkaloids, flavonoids, saponin, tannins and triterpenes were present in the aqueous extracts of *C. rotundus* and *T. procumbens* (Table 2). The aqueous extracts of *C. rotundus* and *T. procumbens* contained alkanoids and tannins (Table 2). While flavonoids and triterpenes were present in only the aqueous extract of *C. rotundus*, saponin was present only in the aqueous extract of *T. procumbens* (Table 2).

### Isolation of fungal pathogen and pathogenicity test

The fungus *Colletotrichum gloeosporioides* was isolated from the mango fruits with anthracnose symptoms. The pathogenicity test carried out fulfilled Koch's postulates, suggesting that the *C. gloeosporioides* was the causal agent of the mango fruit anthracnose disease.

### Effect of the plant extracts on the mycelial growth of *C. gloeosporioides*

The positive control (synthetic fungicide) totally inhibited mycelial growth of *C. gloeosporioides* (Table 3). *C. gloeosporioides* growth in the negative control treatment was significantly ( $P \leq 0.05$ ) higher than the 50 and 100 % aqueous extract concentrations of *C. rotundus* and *T. procumbens* (Table 3). Among the plant extract treatments, the highest percentage of *C. gloeosporioides* mycelia growth inhibition (57 %) was

Table 2. Phytochemical constituents of aqueous extracts of *C. rotundus* and *T. procumbens*

| Plant extract        | Photochemical constituent |           |         |        |             |
|----------------------|---------------------------|-----------|---------|--------|-------------|
|                      | Alkaloid                  | Flavonoid | Saponin | Tannin | Triterpenes |
| <i>C. rotundus</i>   | +                         | +         | -       | +      | +           |
| <i>T. procumbens</i> | +                         | -         | +       | +      | -           |

+ = present; - = absent

Table 3. Effect of plant extracts on mycelia growth of *C. gloeosporioides*

| Parameters       | Mycelia growth (cm) (Mean±s.e) | % growth inhibition |
|------------------|--------------------------------|---------------------|
| Positive control | 0.00±0.00 <sup>a</sup>         | 100.00              |
| Negative control | 1.17±0.21 <sup>c</sup>         | -                   |
| CP50             | 0.57±0.15 <sup>b</sup>         | 51.28               |
| CP100            | 0.50±0.10 <sup>b</sup>         | 57.26               |
| TP50             | 0.63±0.06 <sup>b</sup>         | 46.15               |
| TP100            | 0.53±0.06 <sup>b</sup>         | 54.70               |
| P-value          | <0.001                         | -                   |

Different lowercase letters in the column show significant differences in mycelia growth.

Positive control = PDA amended with Mancozeb 640g/kg + Metalaxyl 80g/kg WP; Negative control = PDA amended with sterile distilled water; CP 50 and CP 100 = Aqueous extract of *Cyperous rotundatus* at 50% and 100% concentrations, respectively; TP 50 and TP 100 = Aqueous extract of *Tridax procumbens* at 50% and 100% concentration

recorded for the 100 % *C. rotundus* whereas inhibition of *C. gloeosporioides* mycelia growth was lowest (46%) for 50 % *T. procumbens* (Table 3).

#### Effect of plant extracts on anthracnose lesion on mango fruits

Compared to the negative control, the different concentrations of the plant extracts significantly reduced the lesion diameter of anthracnose on the mango fruits (Figure 1). The lesion diameter of anthracnose on the mango fruits did not vary between the positive control and 100 % *C. rotundus* and 100 % *T. procumbens*. The lesion diameter of anthracnose on the mango fruits did not also vary between 50 % *C. rotundus* and 50 % *T. procumbens*. However, the lesion diameter on the mango fruits treated with 50 % *C. rotundus* and 50 % *T. procumbens* was significantly higher than those treated with

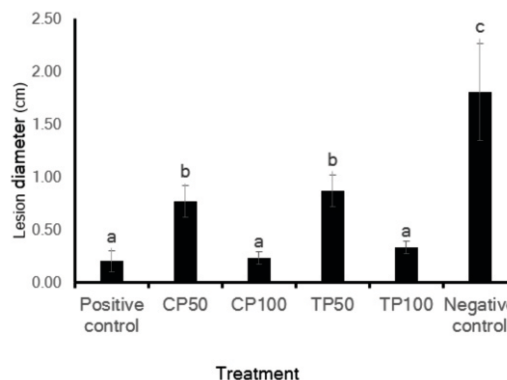


Figure 1. Effect of plant extracts on the lesion diameter of anthracnose of mango fruits.

Different lowercase letters in the column show significant differences in mycelia growth. Bars indicate standard errors of means. Positive control = PDA amended with Mancozeb 640g/kg + Metalaxyl 80g/kg WP; Negative control = PDA amended with sterile distilled water; CP 50 and CP 100 = Aqueous extract of *Cyperous rotundatus* at 50% and 100% concentration, respectively; TP 50 and TP 100 = Aqueous extract of *Tridax procumbens* at 50% and 100% concentrations.

positive control, 100 % *C. rotundus* and 100 % *T. procumbens* (Figure 1).

#### Discussion

Anthracnose is a serious post-harvest disease of mango, which reduces the quality and quantity of infected mango fruits. Anthracnose decreases the market value of infected mango fruits, causing economic losses. Several challenges are associated with synthetic fungicide usage in managing plant diseases because they have the potential to pollute the environment and could be harmful to humans and other non-targeted organisms (Ankit *et al.*, 2020). Others have suggested plant extracts as an alternative to managing mango fruit anthracnose disease because they are less harmful to the environment and humans (Ranjitha *et al.*, 2019). Many plant and plant products have been reported as having antimicrobial activities against plant

pathogenic fungi (Samarrai & Khaleel, 2020), suggesting their potential use in managing anthracnose and other plant diseases. The exploitation of the antifungal activity of *C. rotundus* and *T. procumbens* against *C. gloeosporioides* causing mango fruit anthracnose is necessary since it will establish their antifungal properties and also widen the scope of plant extracts that can be employed in the management of the disease. The aqueous extracts of *C. rotundus* and *T. procumbens* used in this study exhibited antifungal properties which could be exploited for the management of mango fruit anthracnose disease.

We detected alkaloid, flavonoid, tannin and triterpenes in the aqueous extracts of *C. rotundus*, which agreed with the findings of Ngoc & Minh (2021), who also detected alkaloid, flavonoid, phenols, tannin and triterpenes in aqueous extracts of *C. rotundus*. Also, the presence of alkaloid, saponin and tannin in *T. procumbens* supports the findings of Kuldeep and Pathak (2013) and Babayi *et al.* (2018) which indicated the presence of alkaloid, phenolic group, saponin, tannin and terpene in aqueous extracts of *T. procumbens*. Phytochemicals are responsible for the antifungal properties of botanical fungicides and may have a lytic or inhibitory effect on fungal pathogens, thereby playing a key role in plant disease management (Zaker, 2016; Deresa & Diriba, 2023).

The significantly higher mycelia growth of *C. gloeosporioides* recorded for the negative control compared to the aqueous extracts of *C. rotundus* and *T. procumbens* suggested that these extracts contained antifungal properties which inhibited the mycelia growth of *C. gloeosporioides*. The findings of this study are consistent with those of Kwodaga *et al.* (2019) and Kagale *et al.* (2004), who also reported the inhibition of mycelia growth of

*C. gloeosporioides* by plant extracts containing antifungal properties.

Differences in mycelia growth of *C. gloeosporioides* between the synthetic fungicide (Mancozeb 640g/kg + Metalaxyl 80g/kg WP) and the aqueous concentrations of *C. rotundus* and *T. procumbens* suggested that the effectiveness of the synthetic fungicide in inhibiting mycelia growth of *C. gloeosporioides* than the aqueous plant extracts. Despite the effectiveness of the synthetic fungicide in inhibiting mycelia growth of *C. gloeosporioides* relative to the aqueous plant extracts, concerns regarding the effects of chemical control on human health, the environment, as well as the formation of new pathogen races, have been raised, necessitating the need to explore these plant extracts that are more environmentally friendly and safer in managing plant diseases (Ratnadass *et al.*, 2012).

The significant reduction in anthracnose lesion developed on the mango fruits by aqueous extracts of the various concentrations of the plant extracts (*C. rotundus* and *T. procumbens*) when compared with the negative control suggested their potential use as botanical fungicides in the management of mango fruit anthracnose. This supports the findings of Kagale *et al.* (2004) that many plant products contain antifungal substances and properties which can be exploited for disease management in plants. The observed reduction in lesion diameter of anthracnose on the mango fruits when higher concentrations of aqueous extracts were applied (Figure 1) suggested that the efficacy of the antifungal activities of plant extracts is concentration dependent (Kwodaga *et al.*, 2019; Kalidindi *et al.*, 2015).

## Conclusion

The aqueous extracts of *C. rotundus* and *T.*

*procumbens* inhibited the mycelia growth of *C. gloeosporioides* when compared to the negative control. The aqueous extracts of *C. rotundus* and *T. procumbens* reduced the diameter of anthracnose lesions on the mango fruits, which suggested their antifungal activity against mango fruit anthracnose infection. Combining these botanical extracts with the synthetic fungicides used in this study could help mango producers and exporters effectively manage anthracnose disease on mango fruits; further studies would be needed to explore this.

### Conflict of Interest

The authors have not declared any conflict of interests

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