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Sensitivity of *Colletotrichum gleosporioides* (Mango anthracnose) to copper-1-oxide metalaxyl fungicide and ridomil gold in Ado Ekiti, Southwestern Nigeria

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Abstract
The yield and quality of mango production in the tropics have been considerably poor due to the problem caused by *Colletotrichum gleosporioides* (mango anthracnose). Control of the disease relies on the use of chemical fungicide which has an adverse effect on soil and the environment in addition to the development of resistance by pathogens. Based on this, laboratory studies were conducted to determine the baseline sensitivity of *C. gleosporioides* to varying levels of copper 1 oxide metalaxyl and ridomil gold. The fungicide was mixed with Standard Dextrose Agar (SDA) at five concentrations (0.6, 1.3, 1.9, 2.6 and 3.3 g/litre). The effect of the mixture was observed on the growth, mycelia inhibition and conidia germination of the fungus *in-vitro*. Control plates were those without fungicide mixture. Data collected were analyzed and means were separated by the least significant difference. Results from the study showed statistically significant differences (p<0.05) in growth rate, mycelia inhibition and condition of the fungus. Inhibition of growth was directly proportional to the concentrations of the fungicide used in the study. At 3.3 g/litre of the fungicide, complete inhibition (100%) was observed while at 0.6 -2.6 g/litre, inhibition was between 70-77%. Growth of the fungus was faster on SDA attaining 13.4 mm/day when there was no fungicide (control). However, at different concentrations of the fungicide (0.6-3.3) growth of the fungus reduced from 13.4 mm/day to values between 3.1-4.0 mm/day. There was 73-100% inhibition of conidia germination irrespective of the concentrations of the fungicide used in the experiment. The study concluded that *C. gleosporioides* responds to the varying levels of the fungicide and the optimum value of 3.3 g/litre is the most effective in the management of the disease.

Keywords: *C. gleosporioides*, Mango anthracnose, Copper-1-Oxide Metalaxyl, Radial growth

1. Introduction
Mango, (*Mangifera indica* L.) belongs to the Family Anacardiaceae, Order Sapindales and Class Magnoliopsida (Bally, 2006) [2]. It is basically grown in tropical and subtropical regions of the world (Diedhiou *et al.*, 2007) [5]. With about 790, 200 tonnes of mangoes produced yearly, Nigeria covers about 3% of the world production, presenting it as the largest producing country in West Africa and it is closely followed by Kenya, Egypt and Madagascar (FAOSTAT, 2020) [10]. Because of the high percentage in mango production in the Guinea and Sudan savanna zones of Nigeria, high credibility is accorded to these zones especially Benue State being the largest producer of all (Olaniyan, 2004) [13]. With Nigeria's position in the world population, many of the fruits produced are locally consumed in its fresh state with little or none left for export (FAOSTAT, 2021) [10]. Hence, there is a need for increased production to pave the way for exportation which is capable of increasing the gross domestic product (GDP) of the country.

Mango undergoes a variety of physicochemical alterations that are influenced by external factors, resulting in the fruit perish-ability, microorganism contamination, post-harvest management, and storage practices all of which tend to reduce the quantity and quality of mango and a times result in yield losses between 50 - 100% (Wei *et al.*, 2021) [19]. Several pests and diseases were reported on various mango cultivars, which are major production limitations inflicting significant losses by damaging mostly the leaves and fruits. Some of the fungal diseases include Powdery mildew (*Oidium Mangifera B.*), Sooty mould (*Copnodium Mangifera*) and Stem-end rot (*Lasiodiplodia theobromae*).

Anthracnose is a fungal disease known to be one of the most important post-harvest diseases which is caused by the fungus *Colletotrichum gleosporioides* as the asexual stage and *Glomerella cingulata* as the sexual stage (Siddiqui and Ali, 2014) [17]. Its symptoms develop during the pre-and post-harvest stages, causing major input and output losses. (Akem, 2006) [1]. Mode of dispersal occurs by airflow, rain splash and heavy dew.

At pre-harvest stage, symptoms of anthracnose are evident on leaves, flowers, fruits and branches causing losses of about 50-100% (Chowdhury and Rahim, 2009) [4]. Post-harvest symptoms occur on mature fruit as the pathogen penetrates through the cuticle and stays inactive until the fruit ripening reducing fruit quality.

Mango quality can be preserved by applying post-harvest pre-treatments and other disease management techniques. Such techniques include cultural, chemical and biological control methods (Hoque, *et al*., 2018) [11]. Farm maintenance (collecting and burning of decayed fruits and leaves), packing of individual fruits in sacks and disposing of unripe, damaged, injured and infected fruits are all examples of cultural management (Siddiq, *et al*., 2017) [16-17].

Chemicals such as benomyl, mancozeb and copper have been used against fungal infections in time past. (Ray *et al*., 2009) [14]. Pathogen resistance to chemical fungicides necessitate further study considering the uncontrollable spread of disease even with the use of fungicides resulting in increased incidence and severity of disease (Malik *et al*., 2003) [12]. Apart from this, the nature of susceptibility might vary even in the same fungal species. Fungi may develop new pathways to replace those blocked by fungicides and the secretion of chemical components may overpower the blocked biological route. Arising from the above, this study was carried out to examine the effectiveness of Tandem® and Ridomil Gold® fungicides on mango anthracnose disease and hence, its level of sensitivity to the fungicides.

2. Materials and Methods

2.1 Location of experiments

Laboratory studies were conducted in the Department of Crop, Horticulture and Landscape Design at the Faculty of Agricultural Sciences, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria (Latitude 7.7136 ºN and Longitude 5.2467 ºE).

2.2 Source of plant material (Leaf) and fungicide

Mango leaves showing distinct symptoms of the disease (*Colletotrichum gleosporioides*) were collected from the mango field at Ekiti State University Teaching and Research Farm (T & R Farm), Ado Ekiti, Nigeria (Latitude 7.7136 ºN and Longitude 5.2467 ºE). The synthetic fungicides Tandem® containing 65% Copper (1) Oxide in 12% Metalaxyl a wettable powder (WP) (plate 1) and Ridomil Gold® (plate 2) containing Metalaxyl and Mancozeb as water dispersible Granules (WG) were purchased from Agro-stores in Ado Ekiti, Nigeria.

Plate 1: Tandem fungicide used in the experiment

Plate 2: Ridomil Gold fungicide used in the experiment

2.3 Preparation of Sodium Hypochlorite Solution for Sterilization

To prepare the hypochlorite solution, 1 ml of hypochlorite with 500 ml of sterile distilled water was mixed together in a beaker, and then it was transferred to a neat bottle to avoid contamination. All these were used for surface and plant sample sterilization to reduce microbial load. Glass wares such as McCarthy bottles, flasks and beakers were washed and rinsed in sterile distilled water and inverted to drain on a sterile surface.

2.4 Preparation media and inoculation of *C Gleosporioides*

Sabouraud Dextrose Agar (SDA) was used as the culture medium. Mango leaves showing symptoms of the disease were collected, surface sterilized by washing in detergent water, sprayed with 99.9% ethanol and cut into pieces (1-2 cm) with the use of a scalpel after immersing in 0.2% sodium hypochlorite for 2 minutes. The surface of the chamber used was also sterilized with 99.9% ethanol. The Sabouraud Dextrose Agar (SDA) was prepared by dissolving 6.5 g SDA into 100 ml of distilled water and autoclave for 40 - 50 minutes. After cooling to 50 - 55 ºC,

the media was amended with 1 ml of chloramphenicol to suppress bacteria growth. The SDA amended with chloramphenicol were poured around Bunsen flame into sterile disposable 9 cm Petri dishes and allowed to solidify for 10-15 minutes.

2.5 Isolation of C. *gleosporioides*

The Mango leaf showing symptoms of the disease was sterilized firstly by washing it in detergent water then immersed in hypochlorite solution and finally rinsed in two changes of sterile distilled water. The leaves were placed on filter paper and left to dry before spraying with 99.9% ethanol. The lesions were extracted in tiny pieces (1 - 2 cm²) from the mango leaves using a scalpel around Bunsen flame, two leaves cutting per Petri dishes containing solidified SDA earlier prepared were placed at the centre of the plates. The Petri dishes were taped around and left to incubate for 4-5 days.

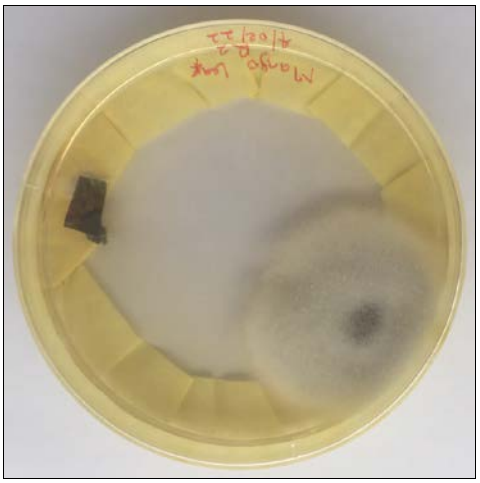

Plate 3: Sub-cultured growth of mango lesion on SDA

2.6 Subculture of *C. Gleosporioides* isolates

After 5 days incubation period, single spores of pure colonies of fungus that appeared (plate 3) were picked and isolated around a bunsen flame with a scalpel sterilized with 99.9% ethanol to obtain a pure culture. The sub-cultured pathogens in Petri dishes containing SDA were taped round with parafilm supplemented with chloramphenicol aseptically. Each colony was replicated twice.

2.7 Evaluation of base-line sensitivity to different fungicides

2.7.1 Preparation of media for copper (1) oxide 60% + Metalaxyl 12% WP (Tandem®)

A standard SDA of 65g was mixed with 1000 ml of distilled water in a beaker. The agar was amended with different concentrations of SDA Tandem® fungicide (60% wettable powder WP, active ingredient; copper (1) oxide + Metalaxyl 12%). Five concentrations were obtained as follows; Control (SDA), 0.09g, 0.19g, 0.29g, 0.39g and 0.49g. The beaker containing the different concentrations were covered, sealed at the tip and finally autoclave for about 50 minutes. McCarthy bottles containing distilled water was also autoclave alongside the different concentrations of Tandem®. After cooling off, around Bunsen flame, the different concentrations were aseptically poured into 10 Petri dishes. The control plate did not contain any fungicide.

2.7.2 Preparation of conidia suspension

Sporulation was prepared by removing an actively growing cell (plate 4) from the isolated fungus into McCarthy bottles using a scalpel (plate 5). This mixture was agitated using a vortex mixer to completely dissolve it. A sterilized inoculating loop was used to inoculate the Tandem SDA plates with an agar plug of each of the isolates at the centre for each of the different concentrations and replicated three times. The plates were sealed, left to solidify and labelled accordingly. Mycelia growth of the colony was measured daily for eleven days along two orthogonal axes which were marked (plate 6).

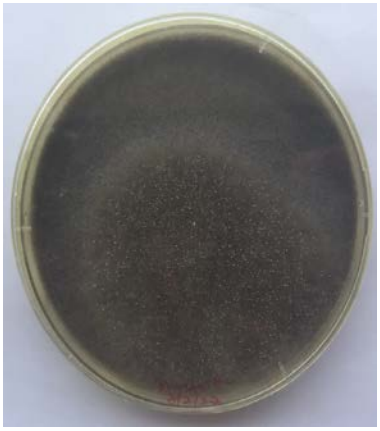

Plate 4: Sporulation was prepared by removing an actively growing cell

Plate 5: Isolated fungus into McCarthy bottles using a scalpel

Plate 6: Mycelia growth of the colony was measured daily for eleven days along two orthogonal axes which were marked

3. Results and Discussion

3.1 Results

Figure 1 shows the rate of inhibition of the growth of *Colletotrichum gloeosporioides* to varying levels of copper-1-oxide metalaxyl fungicide. Results from the study show that inhibition of the growth of the fungus was directly proportional to the concentration of the fungicide at lower concentrations of 0.6-2.6 g/litre. Inhibition was between 70-77%. However, as the concentration of the fungicide increased from 2.6 g/litre to 3.3 g/litre, inhibition was 100%.

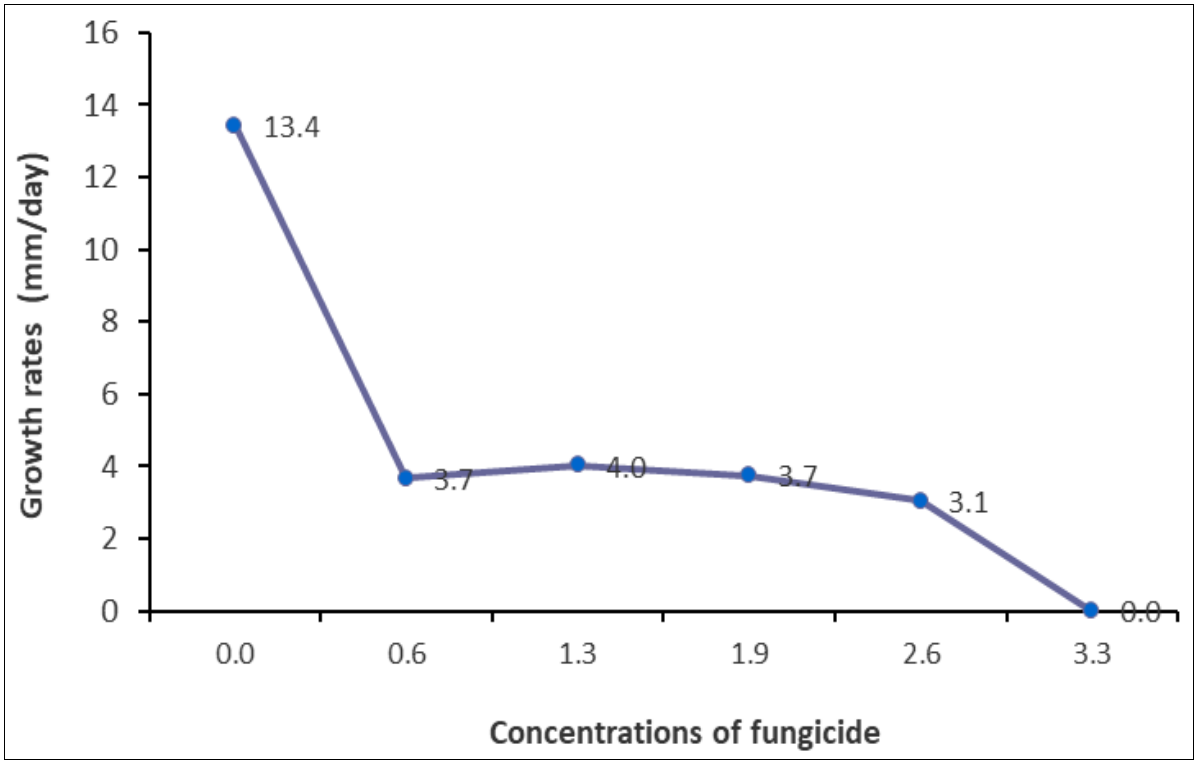

Fig 1: Growth rates of *Colletotrichum gloeosporioides* under different concentrations of Copper-1-Oxide + Metalaxyl fungicide in SDA growth media

Figure 2 shows the growth rates of *Colletotrichum gloeosporioides* amended with different concentrations of copper-1-oxide metalaxyl. Results from the study show that the growth of the fungus was faster on SDA attaining 13.4 mm/day when there was no amendment (control) in which case water was freely available. However, as different concentrations of the fungicide (0.6-2.6 g/litre) copper-1-oxide metalaxyl was added, growth reduced from 13.4 mm/day to values between 3.1 and 4.0 mm/day. At 3.3 g/litre of copper-1-oxide metalaxyl which is the highest concentration used in the experiment, growth of the fungus was completely inhibited.

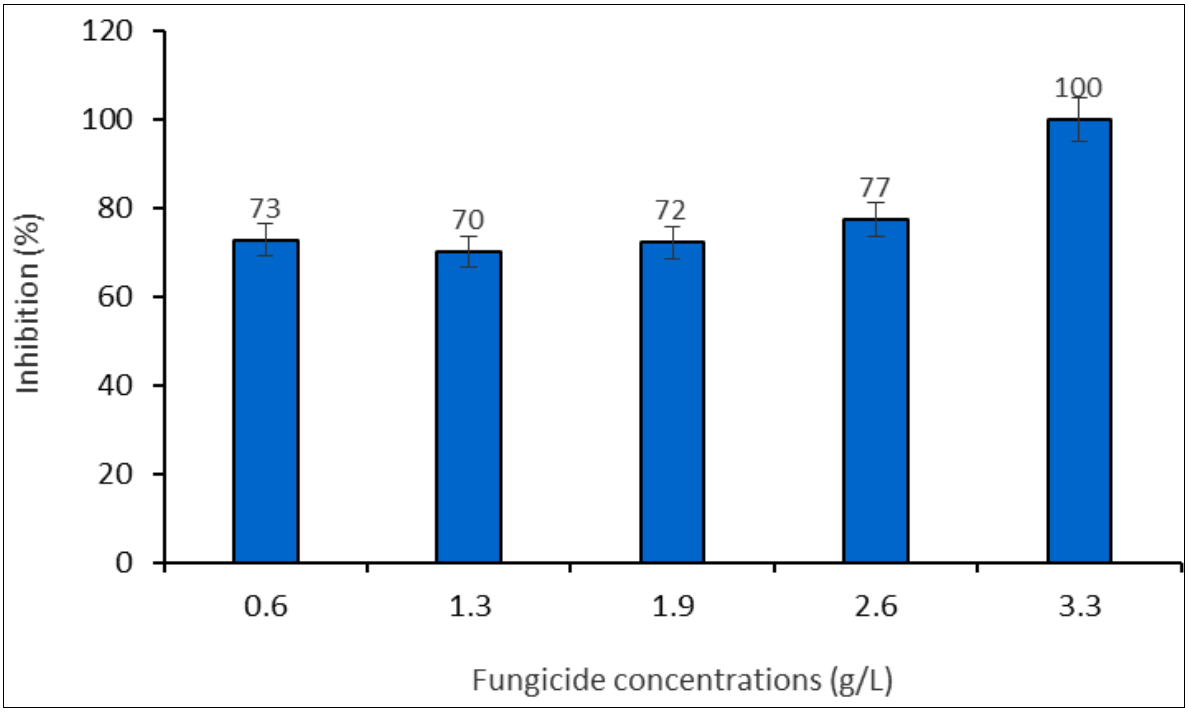

Fig 2: Percentage inhibition of vegetative growth of *C. gloeosporioides* by Copper-1-Oxide + Metalaxyl

Figure 3 shows the effect of the different concentrations of the copper-1-oxide metalaxyl on conidia germination of the *Colletotrichum gloeosporioides*. Results from the figure show that there was 73-100% inhibition of conidia germination irrespective of concentrations. At lower concentrations with 0.6 g/litre of copper-1-oxide metalaxyl, conidia germination was 73% but at 3.3 g/litre of copper-1-oxide metalaxyl, conidia germination was 100%.

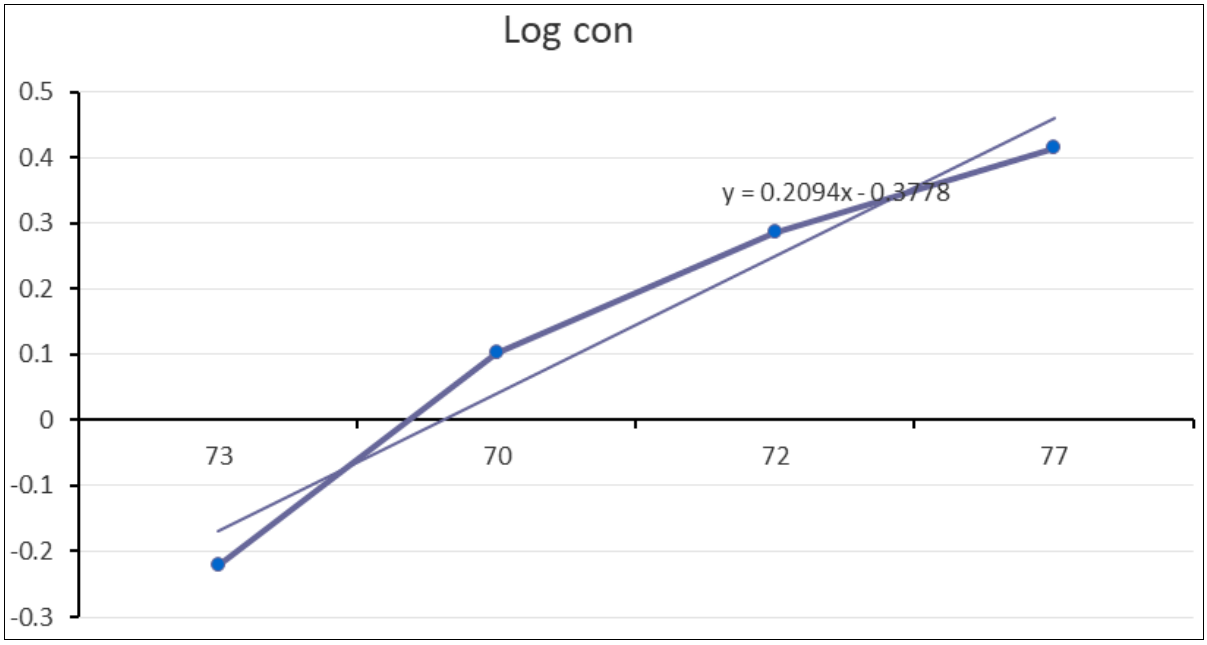

Fig 3: Effect of the different concentrations of the copper-1-oxide metalaxyl on conidia germination of the *Colletotrichum gleosporioides*

Figure 4 shows the effect of Ridomil Gold at five concentrations (0.9 g, 1.1 g, 1.3 g, 1.5 g, 1.7 g) on the growth of *C. gloeosporioides*. The result shows that the fungicide inhibits the growth of the pathogen at all the tested concentrations.

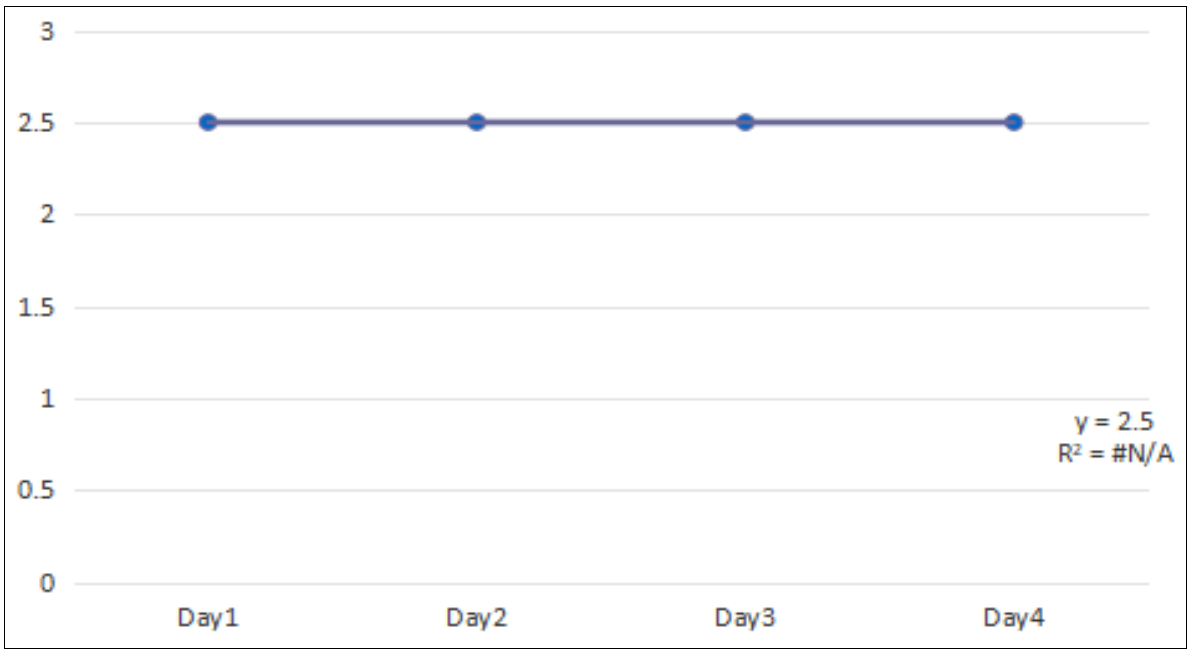

Fig 4: Effect of Ridomil Gold on Growth of *C. gloeosporioides*

Discussion

The quality and quantity of mango produced in the tropics have been considerably low due to the problem caused by *Colletotrichum gleosporioides* (mango anthracnose). The disease is capable of causing significant yield loss between 50-100% when not controlled. In this study, the effect of the pathogen to varying levels of copper 1 oxide metalaxyl was examined on mycelia growth inhibition and conidia germination of the fungus when the Standard dextrose agar was modified with fungicide. The result from the study shows that there was 75-100% inhibition of conidia germination irrespective of concentration. Conidia germination was directly proportional to the concentrations of the fungicide used in the study. At 3.3 g/litre of copper-1-oxide metalaxyl used in the study, conidia germination was 100%. It has been established that when mycelia growth is inhibited, it is a form of stress to which fungus would respond to by producing large number of conidia which is responsible for the result obtained in this study (Falade, 2022) [9]. The result of this study agreed with the work of Enyiukwu and who evaluated the effects of *Piper guinensense* seeds, *Carica papaya* roots and seeds on *in vitro* control of *Colletotrichum destructivum* O Gara, the Incitant cowpea anthracnose at five concentrations. The study concluded that all the extracts inhibited conidia

germination and inhibition was concentration dependent which is in agreement with the present study.

In this study, the growth of the pathogen was examined on SDA by comparing the varying level of the fungicide mixture with that of the control in which no fungicide was added. Results showed that growth of *C. gleosporioides was* faster in the control plate attaining 13.4 mm/day. However, on mixing of 0.6-2.6 g/litre of the fungicide with the SDA, growth reduced from 13.4 mm/day to values between 3.1 - 4.0 mm/day. This is so because water plays a significant role in the growth of microorganisms, water was readily available in the control plates compared to the other ones hence, the increased growth observed in the control. However, the water activity of the media after modifying it with different concentrations of the fungicide was not assessed in the current study. Water activity has been shown to affect the growth and condition of phytopathogenic fungi in different studies (Borisade & Magan, 2014) [3]. Falade (2018) [8] reported that water activity significantly affects the growth of *Colletotrichum lindemathianum* (cowpea anthracnose), the study concluded that the growth of the fungus was faster on PDA when water was freely available and decreased with water stress which is in line with the current study. Similarly, Roberto *et al*., (2004) [15] reported that water activity significantly modified the growth and sporulation of wild and genetically engineered *Aspergillus Niger* strains under different temperature conditions. The study shows that maximum growth rates were observed under moderate water activity which is in line with the current study.

In this study, Ridomil Gold was modified with SDA at five concentrations (0.9 g, 1.1 g, 1.3 g, 1.5 g, 1.7 g.) to examine it effects on the growth of *C. gloeosporioides*. The result from the study shows that the growth of the pathogen was completely inhibited at all the five concentrations suggesting the fact that a small concentration as low as (0.9 g,) will completely inhibit the growth of the pathogen. This will help researchers to reduce the cost of fungicides and possibly avoid wastage.

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