



ANTIFUNGAL ACTIVITY OF COPPER SULPHATE AGAINST COLLETOTRICHUM GLOESPORIODES

Oziengbe E.O

Department of Basic Sciences, Benson Idahosa University, Benin City, Nigeria

Osazee J.O

Department of Botany and Biotechnology, University of Benin

ABSTRACT

*Anthracnose, caused by the fungus *Colletotrichum gloeosporioides* is the most important post harvest disease of mango. The effect of various concentrations of copper sulphate on the mycelium growth and conidia germination of *Colletotrichum gloeosporioides* the causal agent of anthracnose disease of mango fruits was studied under in vitro conditions. Copper sulphate at 0.8mg/l gave significantly reduction of *C. gloeosporioides* growth and conidia germination by 78.2% and 66.3% respectively while 0.6mg/l of copper sulphate gave 51.6% and 46.7% for growth and conidia reduction respectively.*

Key Words: Mango, Copper sulphate, *Colletotrichum gloeosporioides*, Mycelia growth.

INTRODUCTION

Mango (*Mangifera indica*) is considered one of the most popular fruits ([Food Agriculture Organization., 2005](#)). Mango fruits are sensitivity to decay; low temperature and general fruit perish ability due to the rapid ripening and softening which limits the storage, handling and transport potential.

Anthracnose is one of several fruit diseases that affect pre- and post-harvest quality of mango ([Ploetz, 2003](#)). It affects leaves, flowers and fruit, and inoculum is present year-round throughout the canopy. The disease occurs as quiescent infectionson immature fruit and the damage it incites is moreimportant in the postharvest period ([Muirhead and Gratitude, 1986](#); [Dodd et al., 1997](#)). Anthracnose is caused by two related species of fungi. *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*) which is responsible for most anthracnose situations ([Dodd et al., 1997](#)), and *C. acutatum* (teleomorph: *G. acutata*) which plays a minor role in a few locations ([Fitzell, 1979](#); [Ploetz and Prakash, 1997](#); [Tarnowski and Ploetz, 2008](#))

Spores (conidia) of the pathogen are dispersed passively by splashing rain or irrigation water causing infection and pathogen develop on immature fruits and young tissues, these spores germinate and penetrate through the cuticle and epidermis to ramify through the tissues. Symptoms include small black spots and or larger black lesions on the surface of the skin. The lesions may coalesce and penetrate deep into the fruit resulting in extensive fruit rot.

Several post harvest control methods are employed in reducing anthracnose fruit rot including copper fungicides. These copper fungicide formulations are readily available and can effectively kill fungi and bacteria. Copper sulfate (also called bluestone) was one of the original form of copper used as a fungicide (Ellis and Bradley, 1992). There are minor differences among the different copper formulations (CuO , CuCl_2 , $\text{CuCl}_2 \cdot 3\text{Cu}(\text{OH})_2$ e.t.c). Bordeaux mixture combines copper sulfate with lime (calcium hydroxide) and has been used successfully for more than 150 years on fruits, vegetables, and ornamentals (Johnson and Hofman, 2009). The objective of this study was to investigate the antifungal activity of copper sulphate against *Colletotrichum gloeosporioides* growth and spore germination.

METHODS

Isolation of the Pathogens

The pathogen *C. gloeosporioides* was isolated from infected mangos (*Mangifera indica*). Diseased tissue was obtained from the outer margin of lesions, washed, dipped for 1 minute in 70% ethanol, re-washed with sterile distilled water and plated on Potato Dextrose Agar (PDA). Growing edges of the mycelia emerging from the tissue were sub-cultured to obtain pure cultures.

Effect of Different Copper Sulphate Concentrations on Linear Growth of *C. Gloeosporioides*

The Potato Dextrose Agar (PDA) used for assay was amended with anhydrous copper sulphate at varying concentrations (0, 0.2, 0.4, 0.6 and 0.8 mg/l). Three plates as replicates were used for each treatment while 10mm of the inoculum (*C. gloeosporioides* of the same physiological age) was placed centrally on the petridishes. The average linear growth of fungi tested was calculated.

Effect of Different Copper Sulphate Concentrations on Spore Germination of *C. Gloeosporioides*

Conidia of 10 days old *C. gloeosporioides* cultures were harvested in sterilized water containing 0.1% (Tween 80), aliquots of spore suspension (10^6 spore/ml) were inserted into plates containing different concentrations of copper sulphate i.e., 0, 0.2, 0.4, 0.6 and 0.8 mg/l and then PDA medium were poured into the plates. Three plates as replicates were used for each treatment. Inoculated plates were incubated at 22-25°C for 48hr. Spore germination was determined microscopically and the percent of germinated spores was calculated.

Analysis of Data

Data were analysed using the SPSS software. Where there was significant difference, Least Significance difference (LSD) & Duncan multiple range test were used to ascertain the difference at $\alpha=0.05$ (95% confidence limit)

RESULTS AND DISCUSSION

Copper sulphate in 4 concentrations i.e, 0.2, 0.4, 0.6 and 0.8 mg/l were tested for their inhibitory effect on *C. gloeosporioides* linear growth (Table 1). Results indicate that all copper sulphate concentrations reduced linear growth of tested fungus. 0.8m g/l of copper sulphate gave a 78.2% linear growth reduction, 51.6% linear growth reduction at 0.6mg/l and 28.5 % linear growth reduction at 0.4mg/l as compared with control treatment. Meanwhile, concentration of 0.2m g/l of copper sulphate was less effective giving a 16.8% reduction as compared with the control.

Table- 1. Effect of copper sulphate on linear growth of *C.gloeosporioides*

Copper sulphate concentrations (mg/l)	<i>C. gloeosporioides</i>	
	Linear growth (mm)	Reduction (%)
0.2	77.3b	16.8
0.4	66.5c	28.5
0.6	45.0d	51.6
0.8.	20.3e	78.2
0 (control)	93.0a	-----

Values in the same column with different alphabet are significant ($P<0.05$).

Results in Table (2) indicate that conidia germination of *C. gloeosporioides* was inhibited by all tested copper sulphate concentrations. The inhibitory effect was observed to be increasing with increased copper sulphate concentrations. The most effective concentration was 0.8mg/l, where an inhibition of 66.3% was observed. Meanwhile, it was 46.7% inhibition at 0.6mg/l, 44.4% inhibition at 0.4mg/l and 30.6% inhibition at 0.2mg/l copper sulphate.

Table -2. Effect of copper sulphate on conidia germination of *C.gloeosporioides*

Copper sulphate concentrations (mg/l)	<i>C. gloeosporioides</i>	
	Conidia Germination (%)	Reduction (%)
0.2	62.4b	30.6
0.4	50.0c	44.4
0.6	47.9c	46.7
0.8.	30.3a	66.3
0 (control)	90.0	----

Values in the same column with different alphabet are significant ($P<0.05$).

Anthracoze is one of several fruit diseases that affect pre- and post-harvest quality (Ploetz, 2003). Copper sulphate demonstrated good antimicrobial activity against *C.gloeosporioides* isolated from mangoes in this study. A steady decreased in both linear growth and conidia germination was

observed with increasing concentration. Copper and copper compounds have been shown to effectively kill a wide range of yeast and fungi such as *Aspergillus carbonarius*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, *Trichoderma viride*, e.t.c (Borkow and Gabbay, 2009) proving their indispensability in agriculture world over. Thus, the importance of the findings of this work. Similar findings on the effect of copper sulfate on *Colletotrichum gloeosporioides* have been reported by (Everett and Timudo-Torrevilla, 2007) where they observed inhibition of 50% spore germination of *Colletotrichum gloeosporioides* and other fungal pathogens of fruits at concentrations ranging from 0.1- 141µg/ml.

The antifungal effect of copper sulphate on conidia and linear growth could be attributed to copper ions which can catalyze the production of highly hydroxyl radicals, with subsequent damage to lipids, proteins, DNA and other biomolecules. Extensive copper-induced disruption of membrane integrity which inevitably leads to loss of cell viability (Kumbhar *et al.*, 1991). Different copper formulations have been shown to inhibit spore germination of *Colletotrichum gloeosporioides* to varying concentration (Borkow and Gabbay, 2009).

In conclusion, the safety of copper to humans and its potent biocidal properties allow the use of copper and copper compounds in many applications. The present study shows that copper sulphate could directly inhibit the growth of *Colletotrichum gloeosporioides* in vitro and potentially induce defense reactions in mango fruit.

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