*Moor Journal of Agricultural Research 19, 45 - 50, 2018*

# Identification and *in vitro* control of spot disease of sweet orange fruit caused by *Colletotrichum gleoesporiodes*

**Oyedeji, E. O[1] and Aminu–Taiwo B. R[2].**
[1] *Citrus Research Programme, National Horticultural Research Institute, Ibadan.*
[2] *Spices Research Programme, National Horticultural Research Institute, Ibadan.*
**Corresponding author: ennyhorlar@yahoo.com, +2348038254136*

## Abstract
Citrus is an important cash crop which contributes significantly to the income of millions of smallholder farmers in Nigeria as the fruits are grown largely for local consumption. *Citrus* production is limited by low fruit yield and fruit blemishes caused by diseases and pests. *Fruit spot* is a disease of great economic importance to citrus production in Nigeria and constitutes a threat to citrus cultivation. The causal pathogen of citrus fruit spot disease was isolated from infected fruits obtained from National Horticultural Research Institute (NIHORT) orchard, Ibadan and from farmers' fields. Efficacy of three fungicides namely Z-force (a.i 80% mancozeb), Forcelet (a.i 50% carbendazim) and Funguforce (63% mancozeb + 12.5% carbendazim) at a single dose were tested. Of the four pathogens isolated from diseased fruits, *Colletotrichum gloeosporioides* had the highest frequency of occurrence (80%) and a pathogenicity test revealed this specie as the causal pathogen of citrus fruit spot. All the fungicides tested had total inhibition (100%) on the mycelial growth of the fruit spot pathogen *in vitro* at the concentrations that were used.

**Keywords**: *Colletotrichum gloeosporioides;* Fungicides; Fruit spots; *In vitro,* Sweet orange

## Introduction

Introduction

Citrus species are widely grown in Nigeria and many other tropical and subtropical regions (Guo and Deng, 2001). They are major sources of vitamins especially vitamin C, sufficient amounts of folacin, calcium, potassium, thiamine, niacin and magnesium (Angew, 2007). They also contain natural antioxidants and other bioactive components such as carotenoids and flavonoids that prevent cancer and degenerative diseases (Ejaz *et al*., 2006). They are low in calories and cholesterol but rich in the dietary fibre pectin which is beneficial to persons with obesity. The global production of the crop is 122.3 million tons (FAOSTAT 2011) and the sweet orange (*Citrus sinensis* (L.) Osbeck is ranked first among all fruit crops. In Nigeria, the total area dedicated to citrus production is estimated to be 743,000 hectares with a production output of 3.500,000 metric tons in 2011 and this placed the country in the ninth position of top citrus-producing countries of the world (FAOSTAT, 2011). However, despite the high ranking, Nigeria is not among the world's leading fresh citrus exporters even though large expanses of land area are dedicated to its production. Most of the citrus fruits produced in the country are consumed locally (Opeke, 2005). Fruit spot caused by *Colletotrichum gleoesporiodes* is among the most common and most damaging diseases of tropical fruit crops. It can severely limit fruit production and has been reported in several countries (Alfieri *et al*., 1994). It is a major pre- and post-harvest disease of citrus fruits. It causes anthracnose on fruits and it is also responsible for fruit set disease, post bloom and fruit drop in citrus. It has a wide host range and can also infect fruits at any stage of the development (Snowdon, 1990). Infection on young fruit causes rotting or the formation of spots which later coalesce to form a hardened plug. Losses of up to 37% have been reported in avocado fruits (Fitzell 1987).

Infection on sweet orange fruits begin as small, brown spots that quickly enlarge and become black circular hard spots which coalesce until the entire fruit surface is affected. The effect the disease has on the physical appearance of citrus fruits makes them less desirable to the end users. As such, economic loss is incurred as the fruits are marketed at a reduced price. Urgent attention is therefore required to initiate control measures in order to reduce economic loss. The objective of this study was to identify the causal pathogen(s) of citrus fruit spot and also to evaluate the efficacies of some fungicides in the control of the pathogen *in vitro*.

## Materials and Methods

### Isolation of pathogens

Infected fruits of sweet orange (*C. sinensis)* were obtained from farmers' fields and the orchard of National Horticultural Research Institute, Ibadan. Fruits were surface-sterilised with 2% sodium hypochlorite for 1 minute, rinsed in five changes of sterile distilled water and wiped dry with sterile cotton wool. The edge of the infected part of the orange fruits was cut into small pieces (3mm diameter) and placed on chloramphenicol-modified (6mg/ml) Potato Dextrose Agar (PDA) in 90mm Petri dishes. The inoculated plates were incubated at room temperature (28 ± 2$^{O}$C) for 5 days. Subculturing was done to obtain pure cultures. Isolates were identified using the identification guide by Barnnet and Hunter (1998). The frequency of occurrence of each fungal isolate was determined by counting the number of times each fungus was encountered on each plate. The percentage frequency of occurrence was calculated according to the methods of Ebele (2011).

$$\text{Percentage frequency} = \frac{\text{Number of times a fungus was encountered} \times 100}{\text{Total fungal isolations}}$$

### Pathogenicity test

Healthy fruits of *C. sinensis* at the same stage of ripening were used. The fruits were surface-sterilised with 70% ethanol, rinsed in five changes of sterile distilled water and blotted dry with sterile cotton wool. The fruits were sprayed with spore suspension ($10^6$/ml) of the isolates and incubated for 7 days at room temperature. To fulfil koch's postulates, re-isolation of the pathogenic fungi was done and cultures were compared with those of the original isolates.

### Evaluation of fungicides against fruit rot pathogen

Efficacies of three fungicides namely: Z-force (a.i 80% mancozeb), Forcelet (a.i 50% Carbendazim) and Funguforce (63% mancozeb + 12.5% carbendazim) were tested (Table 1). Fungicides were suspended in sterile water according to manufacturers' instructions at the following rates (Z-force 2kg/100L of water), Forcelet (1.5kg/100L) and Funguforce (2.5kg/100L). Approximately 0.1ml was dispensed in 9cm diameter petri dishes with sterile needle and syringe after which 10mls of Potato Dextrose Agar (PDA) was dispensed into the petri dishes, gently swirled and allowed to solidify. Petri dishes of PDA without fungicides served as control. After

**Table 1: List of fungicides with their description**

| Product/ trade name | Active ingredient (a.i) | Formulation | Rate (kg/ ha) |
|---|---|---|---|
| Z-force | 80% active mancozeb | 80wp | 2.0kg |
| Forcelet | 50% carbendazim | 50wp | 0.5 – 1.5kg |
| Funguforce | 63% mancozeb and 12.5% carbendazim | | 2.5kg |

solidifying, each dish was inoculated with a 5mm diameter disc obtained from an actively growing margin of the fungal colony. Plates were arranged in a Completely Randomised Design and each treatment was replicated 5 times. The petri dishes were incubated at room temperature (28 ± 2$^{O}$C) and radial mycelial growth was measured daily until the control treatment was fully covered with the mycelial growth of the fungus. Radial growth was measured along two axes on pre-drawn perpendicular lines on the reverse side of the plate. Fungitoxicity was expressed as percentage inhibition of mycelial growth using the formula adapted from Awuah (1989);

$$Mp = \frac{M_1 - M_2}{M_1} \times 100$$

Where Mp = percentage inhibition of mycelia growth

$M_1$ = mycelia growth in control plate

$M_2$ = mycelia growth in treatment plate

Data were subjected to analysis of variance and means were separated using Duncan Multiple Range Test (DMRT).

**Result and Discussion**

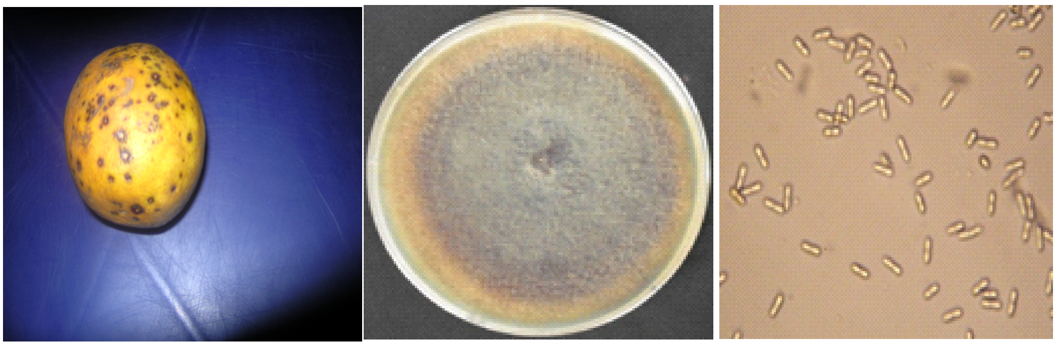
Four pathogens were isolated from diseased fruits. These were *Colletotrichum gloeosporioides*, *Aspergillus niger*, *Fusarium* sp., and *Macrophomina phaseolina*. *C. gloeosporioides* had the highest frequency of occurrence of 20 and percentage occurrence of (80%) followed by *A. niger* with frequency of occurrence of 4 and percentage of occurrence of (16%) while *Fusarium* sp and *M. phaseolina* had the least frequencies of occurrence of 1 and 4 percentage of occurrence respectively (Table 2).

Pathogenicity test conducted with the two most frequently occurring fungal pathogens from symptomatic orange fruits indicated that only *C. gloeosporioides* reproduced symptoms typical of the original symptoms observed on symptomatic fruits (Plate 1a). On Potato dextrose agar, *C. gloeosporioides* had colonies that were whitish to dark grey with thick to sparse lawns of aerial mycelium (Plate 1b). Conidia were observed to be hyaline; single celled and cylindrical with obtuse ends (Plate 1c). Based on these features, the fungus was therefore identified to be *C. gloeosporioides* (Cannon *et al.*, 2008). Isolation of *C. gloeosporioides, M. phaseolina* and *A. niger* in this study was in agreement with the observations of Rawal (1998) and Sangchote (1991) who implicated these three fungal species to be the fungi responsible for postharvest diseases of mango fruits. *C. gloeosporioides has been reported* as the causal agent of anthracnose of mango by other workers (Than *et al.*, 2008; Wharton and Dieguez-Uribeondo, 2004). *C. gloeosporioides* has been identified as the causal pathogen of leaf spot of *Erythrina indica* var. *picta* in

**Table 2: Occurrence of *C. gloeosporiodes* and associated organisms on infected fruits.**

| Pathogen | Sample size | Frequency of occurrence | % occurrence |
|---|---|---|---|
| *C. gleoesporoides* | 25 | 20 | 80 |
| *A. niger* | 25 | 4 | 16 |
| *Fusarium spp.* | 25 | 1 | 4 |
| *M, phaseolina* | 25 | 1 | 4 |

Roraima (Haifeld-vieira and Nachet 2010). All the fungicides used in this study affected total mycelial growth inhibition of *C. gloeosporioides. At the recommended rate,* the radial growth of *C. gloeosporioides wa*s significantly inhibited by 100% in all the treated plates compared to the control plate (Table 3, Plate 2). This was in line with the findings of Pandey and Pandey (2001) who reported that fungal diseases of Brinjal caused by species of *Fusarium* were best controlled by the use of Mancozeb. In similar findings, applications of mancozeb against papaya anthracnose caused by *C. gloeosporioides* have resulted in low disease incidence (Solano and Arauz 1995). Mancozeb and Benlate were also found more effective in controlling Colletotrichum crown rot than Captan and Thiram (Legard, 2000). The efficacy of Carbendazim in this study corroborates the findings of (Ebenezar and Alice, 1996) in that anthracnose and fruit rot disease of chillis caused by C*olletotrichum capsici* was effectively controlled by the use of Carbendazim and Mancozeb.



a b c

**Plate 1: a-c**
(a) Infected sweet orange fruit
(b) 7-day old culture of *C. gloeoesporiodes* on PDA
(c) Spores of *C. gloeoesporiodes* under microscope

**Table 3: *In vitro* inhibitory effect of fungicides on mycelial growth of *C. gleoesporiodes***

| Fungicide | Radial growth (cm) | % inhibition |
|---|---|---|
| Z-force | 0.00a | 100 |
| Forcelet | 0.00a | 100 |
| Funguforce | 0.00a | 100 |
| Control | 8.70b | 0 |

Values are obtained from means of 5 replicates per treatment. Means with the same alphabets in the same column are not significantly different (P= 0.05) Duncan's multiple range test.

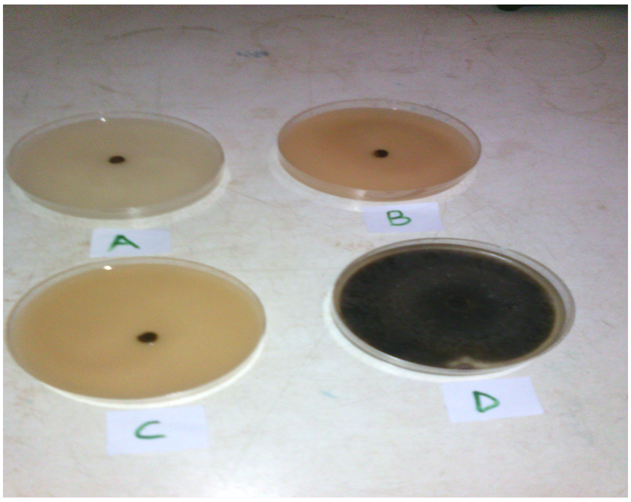



**Plate 2:** Effect of different fungicides on *Colletotrichum gleoesporiodes* :A : Forcelet
B: Z-force C: Funguforce D: Control without fungicide

## Conclusion

The result from the pathogenicity test revealed that *C. gloeosporioides* was a major causal pathogen of citrus fruit spot disease. It was also evident that all the fungicides tested had total inhibition (100%) on the mycelial growth of *C. gloeosporioides* at the recommended rates of application. However, further studies should be carried out to test the efficacies of the fungicides *in vivo*.

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