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# Evaluation of spices extracts for antifungal properties

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## ABSTRACT

*Pepper cultivation is severely affected by anthracnose caused by Colletotrichum capsici. Conventional control of the diseases has been by the use of synthetic chemicals which are not readily available to farmers, expensive and environmentally hazardous. However, hazards associated with the use of chemicals have necessitated the search for alternatives particularly in botanicals. Two solvents (ethanol and Omi-ogi (supernatant solution of fermented maize slurry) were used to extract the active ingredients from dried parts of 5 spices (Ocimum gratissimum, Cymbopogon citratus, Allium sativum, Xylopia aethiopica, Aframomum melegueta) at 2.5, 5, 7.5 and 10% concentrations respectively. Generally, mycelia growth decreased with increase in each of the spices concentration. Also all the tested spices extract reduced significantly the mycelial growth of C. capsici. The most potent spice extract was O. gratissimum recording a growth of 0.9 mm compared to 4.4 mm at 10%. The same trend was recorded with supernatant of fermented maize extract where extract of O. gratissimum recorded significantly the least mycelial growth. Generally better antifungal activities were recorded with ethanol extracts compared to extracts from supernatant of fermented maize slurry.*

**Key words:** Alternatives, antimicrobial, ethnobotanical, infection, pathogen.

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## INTRODUCTION

The most destructive disease of pepper anthracnose is caused by *Colletotrichum capsici* (Sydow) Butler and Bisby (Ascomycota: Phyllachorales) [5]. It is well known for infection on leaves, stems, mummification of unripe green pepper fruits, pre-mature fruit drop and fruit rot [2, 16]. It also has been reported that, pre and post-harvest fruit losses of up to 50% was caused by this fungi [8]. It is characterised by very dark, sunken lesions, containing spores [13]. The disease appears as small circular spots that coalesce to form large elliptical spots on fruits and leaves. Under severe conditions, defoliation of affected plants occur [15] and causes losses by pre- and post-emergence damping off, leaf spots, pre-mature fruit drop, mummification of unripe green pepper fruits and fruit rots [2]. In Nigeria, losses of about 95% have been recorded in farmers' fields with several farmers abandoning pepper production as a result of the disease [5]. Between

50 - 100% fruit loss has been reported in India, North America and tropical Africa due to anthracnose infection [5, 17]. Generally, *Colletotrichum* diseases can be controlled by a wide range of chemicals such as copper compounds, dithiocarbamates, benzimidazole and trizole compounds however, the high cost of these chemicals forbids their use by ordinary farmers. Furthermore, continuous use of these chemicals may pose ecological problems. There is therefore the need to search for the use of cheaper environmentally friendly and readily available alternatives such as plant extracts for the control of this pathogen This, in turn, necessitates the search for alternatives in plant products, many of which have been reported to be effective in the control of several plant diseases (11,19]. The conventional method to extract plant materials is to use methanol, ethanol, acetone and so on as extracting solvents, but the the ethnobotanical approach like the use of "Omidun/ekan-ogi"(the water derived from three days fermented milled maize), as extracting solvent has received less attention. The type of solvents and methods of preparation affect antimicrobial activity of plants [9, 1]. The purpose of this trial was to evaluate the efficacy of the five spice plants for the in -vitro inhibition of the *Colletotrichum capsici*.

## MATERIALS AND METHODS

**Pathogen isolation:** Bell pepper fruits with anthracnose lesions were collected from pepper experimental field of the National Horticutural Research Institute, Ibadan. Sections of 3-5 mm were cut from the margin of the infected lesions and sterilized for one minute in 1.0% sodium hypochlorite solution and rinsed in three changes of sterile distilled water (SDW). The sterile pieces were blotted dry using sterile filter papers and placed on Potato Dextrose Agar (PDA) in 9cm Petri dishes. The dishes were incubated at 30 ± 2°C for 7 days after which cultures with salmon-pink sporulation typical of *Colletotrichum* spp. were subcultured to obtain pure cultures. Culture identification was confirmed by microscopic examination and comparison with reference cultures [6].

**Plant materials:** Leaves of *Ocimum gratissimum* (L.) and *Cymbopogon citratus* (Stapf), were collected from the progeny garden of Spices Programme of National Horticultural Research Institute (NIHORT), Ibadan while *Allium sativum* (L.) bulb, *Xylopia aethiopica* (Dun.), *Aframomum melegueta* (L) seeds were purchased from Bode market in Ibadan. The spices materials were rinsed in three changes of sterile distilled water and oven dried at 60$^{o}$C for 10days,after which they were milled separately into powder with mechanical grinder (Marlex). The powder were sieved and packed into glass bottles and sterilized in a hot air oven at 160$^{o}$C for 5hours [10].

***In Vitro* Study**

Crude extraction of the ground leaves was carried out by adding two solvents (ethanol and supernatant solution of fermented maize slurry ('Omi-ogi')) separately to extract the active ingredients from dried parts of the 5 spices (*Ocimum gratissimum, Cymbopogon citratus, Allium sativum, Xylopia aethiopica, Aframomum melegueta)*. Concentrations tested were 2.5, 5, 7.5 and 10% concentrations respectively lower concentrations had been tested before with no antifungal effects. Evaluation of the spice extracts against the mycelial growth of *C. capsici* was carried out *in vitro* using pour plate technique. Crude plant extracts were obtained by separately weighing 2.5g, 5g, 7.5g and10g of each grounded spice material into 97.5ml, 95ml, 92.5ml and 90ml ethanol and 'Omi-ogi'. Each mixture was agitated manually to obtain even particle distribution to give 2.5, 5, 7.5 and 10% w/v respectively in a 250ml conical flask. The extracts were filtered through double-layered cheese cloth.. One millilitre of each concentration was poured into each sterile 9cm-diameter Petri dishes. 9ml of cooled (about 45$^{0}$C) molten chloramphenicol amended

(60µg/ml) Potato Dextrose Agar was aseptically poured into each Petri-dish and rotated gently to ensure even dispersion of extracts. All plates were left to stand overnight and then inoculated at the centre with 5mm diameter mycelia plug of *C.capsici culture* obtained from the edge of 5-day –old culture. Three replicate plates for each extract concentration were inoculated. Control treatments consisted of plates containing either 1 ml ethanol (absolute ethanol) and 10 ml sterile molten PDA or 1 ml of 'Omi-ogi'in 9 ml molten PDA, All plates were incubated at 28°C and the diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri plate 5 days after inoculation. The experiment was laid out in three replicate in completely randomized design. Data on mycelia radial growth was taken and data was analysed using Fisher's least significant difference.

## RESULTS AND DICUSSION

The spice extracts differed significantly in their potential to inhibit the growth of *C. capsici* Though, non of the aqueous extracts had total inhibition on the growth of the pathogen at ($P<0.05$). However, concentration had significant effect on the mycelium growth which decreases with increasing concentrations for the five spice extracts .Highest significant effect was observed at 10% for the five solvent while the least was recorded at 2.5% irrespective of the solvent of extraction. The ethanol extract of the tested spices differed significantly in their ability to reduce mycelia growth of *C.capsici* (Table 1).

**Table 1: Inhibition of mycelial growth of *Collectotrichum capsici* by ethanol extracts of some spices after 5 days incubation at 28°C**

| | Concentration (%) | | | |
|---|---|---|---|---|
| Treatments | 2.5% | 5% | 7.5% | 10% |
| *A. melegueta* | 2.6 | 1.8 | 1.3 | 1.2 |
| *C. citratus* | 2.7 | 2.3 | 1.7 | 1.4 |
| *X. aethiopica* | 2.3 | 1.6 | 1.3 | 1.1 |
| *A. sativum* | 3.8 | 3.2 | 2.5 | 2.3 |
| *O. gratissimum* | 2.0 | 1.4 | 1.0 | 0.9 |
| Control (ethanol) | 4.4 | 4.4 | 4.4 | 4.4 |
| LSD | 0.33 | 0.33 | 0.3 | 0.25 |

Means in the same column followed by the same letter were not significantly different ($P<0.05$)
Generally, mycelial growth decreased with increase in each of the spices concentration. Also all the tested spices extract reduced significantly the mycelia growth of *C. capsici*. However, at all tested concentration *A. sativum* had the least inhibition on the mycelial growth. At 7.5% and 10% concentrations, *X. aethiopica* ethanol extract had significantly lower inhibitory activity than *O. gratissimum*. There were no significant difference in mycelial inhibition by *A. melegueta* and X. *aethiopica* ethanol extract at 7.5% and 10% respectively. The highest reduction (0.9mm) in mycelial growth of ethanol extract was recorded with *O. gratissimum* extract. Except at 2.5% concentration, *C. citratus* and *A. melegueta* supernatant maize slurry extracts were not significantly different in their inhibitory activities. Similarly, supernatant maize slurry extracts of *X. aethipica* and *O. gratissimum* did not show significant difference in their inhibitory activity on the mycelial growth of *C. capsici* at 7.5% and 10% concentrations respectively (Table 2). The highest mycelial reduction (1.7mm) with supernatant maize slurry extracts was observed with *X. aethiopica* and *O. gratissimum* extracts.

Means in the same column followed by the same letter were not significantly different ($P<0.05$)
At all tested concentrations there were no significance difference in inhibitory activity of supernatant of fermented maize extract of *C. citratus* and *A.melegueta.*Also, at 5%, 7.5% and

10% no significance difference was observed with, *X.aethiopica* and *O.gratissimum* extracts The most potent spice extract was *O. gratissimum* recording a growth of 0.9 mm compared to 4.4 mm at 10%. Generally better antifungal activities were recorded with ethanol extracts compared to extracts from supernatant solution of fermented maize (Tables 1 and 2).

**Table 2: Inhibition of mycelial growth of *Collectotrichum capsici* by fermented maize supernatant extracts of some spices after 5-day incubation at 28$^{o}$C**

| | Concentration % | | | |
|---|---|---|---|---|
| Treatment | 2.5% | 5% | 7.5% | 10% |
| *A. melegueta* | 3.7 | 3.3 | 2.6 | 2.4 |
| *C. citratus* | 3.5 | 3.2 | 2.7 | 2.5 |
| *X. aethiopica* | 2.9 | 2.5 | 2.2 | 1.7 |
| *A. sativum* | 4.1 | 3.8 | 2.7 | 2.3 |
| *O. gratissimum* | 3.2 | 2.7 | 2.2 | 1.7 |
| Control('Omi-Ogi') | 4.4 | 4.4 | 4.4 | 4.4 |
| LSD | 0.2 | 0.2 | 0.4 | 0.2 |

The result obtained in this study agrees with the work of [18] who reported that *O. gratissimium* and *A. melegueta* extracts proved effective against mycelia inhibition and spore germination of many rot causing microorganisms They also concluded that ethanol extracts were most effective followed by cold-water and hot water extracts. [4] ascertained that *O. gratissimum* leaf extracts controlled spore germination and mycelia growth of *Rhizopus oryzae*. [10] also reported the inhibitory effect *O. gratissimum* leaf ash on the mycelia growth of S. *rolfsii* of wheat on agar. The most potent spice extract was *O. gratissimum*, [22] observed that eugenol was the principal component of *O. gratissimum* oil which gives the plant its antimicrobial property. This compound was found to exert a pronounced inhibitory effect on *Alternaria alternata, Colletotrichum capsici* and *Sclerotium rolfsii*. This result also supports the observation of other investigators [23, 12] who found that garlic (*A. sativum*) extract was effective in controlling anthracnose pathogen *Colletotrichum* spp. Fungitoxic activity observed with *C .citratus* extracts in this study may be attributed to the presence of 75-85% aldehydes, much of which is citral [21], which probably makes the extract fungitoxic. [3] has demonstrated the fungitoxic activity of seed extract of *X. aethiopia* against the anthraconose fungus (*Collectotrichum lindemuthianum*) of cowpea. [7] reported the activity of *X. aethiopica* extract attributing its antimicrobial effect to the presence of xylopic acid and two other diterpenes. The antifungal effectiveness of these spice extracts in culture depends on the concentration and the solvent of extraction. From this trial, ethanol extracts performed better in inhibition of the mycelia growth of *C.capsici*. The inhibitory activities of these extracts suggest their fungitoxic ability.This observation agrees with the work of [14, 20] who concluded that the antifungal properties of plants are due to their phytochemical contents and the concentration of the extract used.

## CONCLUSION

This investigation demonstrates the potential of the *C.citratus, A.melegueta, X.aethiopica, A.sativum* and *O.gratissimum* as potential alternatives to synthetic chemical in the management of *Colletotrichum capsici* which causes anthracnose disease of pepper. However, there is need for *in-vivo* trial to further establish the fungicidal potential of these spice materials as alternatives to conventional fungicides in the management of *Colletothricum capsici*.

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