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Effect of *Ocimum gratissimum* and *Cymbopogon citratus* Extracts on *Sclerotium rolfsii* Sacc of Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) cultivation is severely affected by Sclerotium wilt caused by *Sclerotium rolfsii* Sacc. Chemical control has been a major strategy for its control. However, hazards associated with the use of chemicals have necessitated the search for alternatives, particularly among botanicals. An alternative method of control was examined by evaluating the inhibitory effect of two spices (*Ocimum gratissimum* and *Cymbopogon citratus*) on the growth of *S. rolfsii in vitro* and its subsequent development on tomato plants *in vivo.* FunguForce® (Mancozeb 63% + Carbendazim 12.5 WP), a synthetic pesticide and sterile distilled water served as controls. The spices were extracted in water and ethanol and tested against the growth of *S. rolfsii* at six concentrations (0, 1, 2, 3, 4 and 5%, v/v). The highest inhibition of mycelial growth was recorded with 3% of the ethanolic extract of *O. gratissimum* in which no growth was recorded. The lowest inhibition (3.7 mm) was recorded with the control (0%). Generally better inhibition was possible with the ethanolic extract than with the aqueous extract. In pot experiments, the two plants reduced disease severity to 1.7 and 2.0, respectively at 5% and these values were comparable to 2.3 obtained with FunguForce® and significantly lower to what was recorded in untreated plants. However, plants treated with *O. gratissimum* extract at 5% and FunguForce® at 2.5 kg/ha gave significantly comparable plant height, number of leaves and fruit weight per plant. The plants in the control pot recorded the highest disease severity (4.7) and sclerotial rhizosphere population (82 Kg^{-1} soil). The two spice extracts are potential options for the management of *S. rolfsii* on tomato.

Keywords: antagonistic, antifungal agents, botanicals, mycelia, plant extracts, solvents, subsistence
Abbreviations: PDA, potato dextrose agar; **WAT**, weeks after transplanting

INTRODUCTION

Sclerotium rolfsii Sacc is an economically important soil-borne fungal pathogen that causes southern blight disease characterized by the wilting and yellowing of leaves on a wide range of agricultural and horticultural crops. Studies have shown an increase in the incidence of southern blight in tomato plants. During colonization of host tissue, it produces a considerable mass of mycelia on the plant's surface leading to tissue decay and subsequent production of sclerotia. *S. rolfsii* is known to produce sclerotial exudates which are thought to help the fungus survive (Leopold *et al.* 2011). It forms brownish sclerotia that can survive in soil for long periods, frequently tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Ilan 1975). In Benin, losses caused by *S. rolfsii* varied between 35 and 90% (Sikirou *et al*. 2010) while in Morocco, the fungus has caused yield losses of up to 50% on sugarcane (*Saccharum officinarum* L.) and 60 to 80% on sunflower (Achbani and Tourvielle de Labrouhe 2000; Sikirou *et al.* 2010). The disease caused by this pathogen leads to heavy yield loss in vegetable crops especially during wet season when weather conditions are favourable for crop production and likewise for the growth and dissemination of the pathogen (Okabe *et al*. 2000). Tomato is an important vegetable in Nigeria, it accounts for about 18% of the daily consumption of vegetables which averages out at 50.6% per person (Kataria and Mittal 1984). Tomato is an excellent source of lycopene, and numerous studies have confirmed that people who consume increased amounts of tomato products experience marked reduction in cancer risk (Giovaanucci *et al*. 1995). Because of the health benefits associated with tomato in the diet, it is ranked as the 16th among all fruits and vegetables as a source of vitamins A and 13th in vitamin C. Also, it has been ranked as the single most important fruit or vegetable of western diet in terms of overall source of vitamins and minerals (Jones 1999).

However, among over 500 plants attacked by *S. rolfsii*, particularly in tropical, sub-tropical and warm temperate regions, is tomato (Okereke and Wokocha 2007).

Major methods employed to manage *S. rolfsii* are fungicide applications, solarization, use of antagonistic micro-organisms, deep ploughing, crop rotation and incorporation of organic and inorganic residues (Punja 1986). Fungicides such as Captan® and Calixin® have been used for seed dressing and other chemicals such as methyl bromide and Chloropicrin as soil fumigants for the control of this pathogen. Moreover, these chemicals are environmentally hazardous and therefore difficult to adopt in subsistence agriculture in West Africa (Okereke and Wokocha 2007). There is therefore the need to search for the use of environmentally friendly and readily available alternatives such as plant extracts for the control of *S. rolfsii*. In view of this, this trial was carried out to evaluate the efficacy of *Ocimum gratissimum* L. and *Cymbopogon citratus* Stapf. on the growth of *S. rolfsii in-vitro* and *in-vivo*.

MATERIALS AND METHODS

Isolation of *S. rolfsii*

Infected tomato stems were obtained from the experimental plot of National Horticultural Research Institute, Ibadan. The stems were washed with sterile distilled water, cut into 5-mm segments which

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were surface sterilized in 0.5% sodium hypochlorite solution (Sigma Aldrich Product) for 2 min and rinsed thrice in sterilized distilled water. The segments were then air dried between sterile Whatman No. 1 filter paper and plated on potato dextrose agar (PDA) product of DIFCO amended with chloramphenicol (Sigma Aldrich, St. Louis, USA) (60 mg/ml). The plates were incubated at 28 ± 2°C and examined for 5 days. Isolate was identified based cultural characteristics and with the help of identification scheme of Bernet and Hunter (1972). The plates were sub-cultured to obtain pure cultures of isolate.

Preparation of plant extracts

Two years old fresh leaves of local *O. gratissimum* (African basil) and *C. citratus* (lemon grass) were obtained from the National Horticultural research Institute (NIHORT) Ibadan. The plant materials were rinsed and dried for 10 days at room temperature 28 ± 2°C, after which they were milled separately into powder with Marlex blender. The powder were packed into glass bottles and sterilized in a hot air oven at 160°C for 5 h (Enikuomehin 2005).

In-vitro control of *Sclerotium rolfsii*

1, 2, 3, 4 and 5 g each sterilized sample were suspended in 100 ml of sterilized distilled water to obtain concentrations of 1, 2, 3, 4 and 5%, respectively. Each mixture was agitated manually to obtain even particle distribution. Solution of ethanol extracts of the same plant materials were made as described above by dissolving 1, 2, 3, 4 and 5 ml of each sample in 100 ml of absolute ethanol (product of BDH Chemicals) to obtain 1, 2, 3, 4, and 5%, respectively (Adekunle *et al*. 2009). 1 ml of each concentration was poured into sterile 9-cm diameter Petri dishes, 9 ml of cooled (about 45°C) molten chloramphenicol (obtained from Danax Pharmaceuticals Ibadan, Nigeria) amended (60 mg/ml) PDA was aseptically poured into each Petri-dish and rotated gently to ensure even dispersion of powder. A 6 mm mycelial disc obtained from the margin of a 5-day-old culture of *S. rolfsii* was placed at the centre of each Petri-dish containing each plant samples. Control plates had either 1ml of sterile distilled water of 1 ml ethanol plus 9 ml distilled water mixed with cooled (45°C) molten chloramphenicol-modified PDA and inoculated as described above. There were three replicates for each plant concentration. Ethanol extracts of the samples were also prepared as above. All plates were incubated at 28 ± 2°C for 5 days. Measurement was taken as the mean growth along the two axes on two pre-drawn perpendicular lines on the reverse side of plates.

In-vivo control of *S. rolfsii* by spice extracts

The experiment was complete randomised design replicated three times. Tomato *Lycopersicon esculentum* Mill. (cv. 'UC82B') was obtained from Premier Seed Co., Ibadan, Nigeria. Seedlings were raised in steam sterilized soil for four weeks in the nursery. *S. rolfsii* inoculum was prepared by adding 10 sclerotia of *S. rolfsii* to 100 g of moist autoclaved wheat seeds in 500-ml conical flasks. Incubation was done for two weeks at 28°C. Soil infestation was carried out after watering the soil for one week by mixing 10 g of inoculum in 10kg sterilized soil (Enikuomehin *et al*. 1998). The four-weeks-old seedlings were transplanted into the inoculated soil at one seedling/pot. The extracts and the synthetic fungicide were applied at the base line of the stem at the rate of 10 ml/pot. Plants were watered every other day throughout the period of the experiment. The data collected include plant height, leaf number, and disease severity fruit weight and rhizosphere population of sclerotia/Kg soil. Data were collected weekly and disease severity was rated according to De Cal *et al*. (1995) as follows: 1 - (all leaves green), 2 - (25-49%) lower leaves yellow, 3 - (50-74%) lower leaves dead and some upper leaves yellow, 4 - (75-99%) lower leaves dead and some upper leaves wilted, 5 - (100%) dead plant. The data collected were subjected to analysis of variance (ANOVA) using SAS software and significant means were separated using Least Significant difference (LSD).

RESULTS AND DISCUSSION

The effect of *O. gratissimum* extracted with water on the growth of *S. rolfsii* as shown in **Table 1** indicates that radial growth increased as period of observation progressed at all concentrations but as concentration increased, radial growth as *S. rolfsii* decreased right from 24 to 120 h. When ethanol was used as medium of extraction, radial growth also increased with increase in hours of observation with no radial growth at 24 h (**Table 2**). The radial growth also decreased as concentration increased up to 2% after which there was no radial growth (**Table 2**). **Table 3** shows that as the period of observation increased so also the radial growth increased but the radial growth decreased as concentration increased except at 24 and 96 h where radial growth increase with increase in concentration from 0 to 1% but later decreased as the concentration increased from 1 to 5%. No radial growth was observed from 24 to 72 h when ethanol was used as extracting medium but radial growth increased as the hour increased from 92 to 120 h (**Table 4**). As it was under aque-

Table 1 Inhibition of mycelial growth of *Sclerotium rolfsii* by *Ocimum gratissimum* water extract after 120 h incubation at 28°C.

Concentration (%)	Radial growth of *S. rolfsii* at indicated extract concentration (mm)				
	24 h	48 h	72 h	96 h	120 h
0	0.6	2.0	3.3	4.1	4.3
1	0.3	1.4	2.6	2.9	3.0
2	0.2	1.2	1.7	2.1	1.7
3	0.2	0.6	1.3	1.5	1.6
4	0.1	0.4	0.7	0.9	1.0
5	0.1	0.2	0.4	0.5	0.5
LSD ($P < 0.05$)	0.2	0.4	0.45	0.65	0.88

Table 2 Inhibition of mycelial growth of *Sclerotium rolfsii* by *Ocimum gratissimum* ethanol extract after 120 h incubation at 28°C.

Concentration (%)	Radial growth of *S. rolfsii* at indicated extract concentration (mm)				
	24 h	48 h	72 h	96 h	120 h
0	0.9	2.2	2.7	3.3	3.7
1	0.0	0.9	1.6	2.0	2.3
2	0.0	0.5	0.8	1.2	1.4
3	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0
LSD ($P < 0.05$)	0.38	0.39	0.37	0.49	0.49

Table 3 Inhibition of mycelial growth of *Sclerotium rolfsii* by *Cymbopogon citratus* water extract after 120 h incubation at 28°C.

Concentration (%)	Radial growth of *S. rolfsii* at indicated extract concentration (mm)				
	24 h	48 h	72 h	96 h	120 h
0	0.6	2.0	3.3	4.1	4.3
1	0.8	1.2	1.7	2.6	4.1
2	0.5	1.1	1.8	2.5	3.7
3	0.3	0.7	1.5	2.0	3.2
4	0.1	0.5	1.5	1.9	2.7
5	0.0	0.5	1.1	1.8	2.0
LSD ($P < 0.05$)	0.19	0.25	0.3	0.35	0.28

Table 4 Inhibition of mycelial growth of *Sclerotium rolfsii* by *Cymbopogon citratus* ethanol extract after 120 h incubation at 28°C.

Concentration (%)	Radial growth of *S. rolfsii* at indicated extract concentration (mm)				
	24 h	48 h	72 h	96 h	120 h
0	0.9	2.2	2.7	3.3	3.7
1	0.0	0.0	0.0	0.9	2.7
2	0.0	0.0	0.0	0.9	2.0
3	0.0	0.0	0.0	0.8	1.7
4	0.0	0.0	0.0	0.4	1.2
5	0.0	0.0	0.0	0.3	1.0
LSD ($P < 0.05$)	0.1	0.39	0.58	0.6	0.46

Table 5 Effect of aqueous spice extracts on Southern blight severity and growth of tomato grown in *Sclerotium rolfsii*-infested soil at 8WAT.

Treatment	Concentration (%)	Blight severity	Plant height (cm)	Leaf number	Fruit weight (g)	Rhizosphere sclerotial population (Kg^{-1} soil)
Ocimum gratissimum	1	3.7	95.1	48	78.8	43
	2	3.0	96.3	48	76.9	42
	3	3.0	92.2	42	48.0	33
	4	3.0	89.8	43	62.3	28
	5	1.7	97.2	68	154.9	23
Cymbopogon citratus	1	3.7	93.5	22	18.9	68
	2	3.3	84.7	21	7.3	67
	3	3.0	99.1	27	24.6	61
	4	2.7	87.8	26	22.3	57
	5	2.0	88.9	33	25.9	53
* FunguForce®	-	2.3	92.3	60	116.2	31
Control	-	4.7	66.2	14	5.9	82
SE ±	-	0.5	7.7	11.0	50.0	3.5

* FunguForce® (a.i. 63% Mancozeb); SE; Standard Error ; WAT; week(s) after transplanting

ous extract so it was under ethanol extract where radial growth deceased with increase in concentration (**Table 4**).

Mycelial reduction was enhanced with 5% extract. Highest mycelial reduction (2.0 mm) was observed at 5% for the *C. citratus* water extracts while the least (4.1 mm) was recorded at 1%. This is in agreement with the works of Abd-El-Khair and Haggag (2007) and Praveen *et al*. (2009) who concluded that higher concentration 50 µg/ml of 20 essential oils of aromatic plants induced maximun inhibition in *S. rolfsii* and *Rhizoctonia bataticola*. Tobacco and neem extracts have also been reported to possess antifungal properties as complete inhibition of mycelial growth was recorded at 60% on *Aspergillus viridae* and *Penicillium digitatum* cultures (Suleimam 2011). Nashwa (2011) concluded that the extract of *Ocimum basilicum* (sweat basil), *Azadirachta indica* (neem), *Eucalyptus chamadulonsis* (eucalyptus), *Datura stramonium* (jimsonweed), *Nerium oleander* (oleander) and *Allium sativum* (garlic) against *Alternaria solani* at 5% reduced the mycelial growth of *A. solani*, the causal organism of Early blight of tomato *in vitro* and increased the fruit yield *in-vivo.*

The inhibitory effect of the two spices was enhanced when extracted with ethanol. There were no growths recorded for the *C. citratus* ethanol extracts until 96 h. However, at 120 h, the highest inhibitory effect (1.0 mm) was recorded at 5% while the least (2.7 mm) effect was recorded at 1%. For the two extracts, reduction in disease severity to (1.7) was achieved at 5% and this was statistically comparable to 2.3 obtained with synthetic FunguForce® (Mancozeb 63% + 12.5% WP) (**Table 5**). This is in consonance with the report of Okigbo (2009) in which plant products from neem, *Nicotiana tabacum* (tobacco) and *Aloe vera* (aloe) at 10-40% aqueous extract contained compounds that were fungitoxic to *Fusarium oxysporium*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicilium oxalicum*) (dry rot-causing fungi) in yam (*Dioscorea rotundata*).

Plants treated with *O. gratissimum* extract had better growth in terms of plant height and number of leaves. It was also observed that increase in concentration of the extract did not give significant increase in plant height. Moreover, number of leaves varied significantly among the extracts. Plants treated with *O. gratissimum* were found to have more leaves which were comparable with the number of leaves on plants treated with synthetic fungicide and control. This result again agrees with report of Enikuomehin (1998) in which it was observed that an increase in concentration (10 and 15%) of 11 ash samples from the organs of nine tropical plants inhibited mycelial growth and sclerotial germination of a Nigerian isolate of *S. rolfsii* on wheat (*Triticum aestivum*) in the root rhizosphere; this might be due to the toxicity of the extracts which inhibit the sclerotial population in the root rhizosphere. This agrees with the work of Blum and Rodriguez-Kabana (2003) who reported that aqueous leaf extracts of kudzu (*Pueraria lobata*), velvetbean (*Mucuna deeringiana*) and pine-bark (*Pinus elliottii* and *Pinus taeda*) at 50 and 100 g/Kg, which inhibited sclerotial production of *S. rolfsii* in *Glycine max* (soy bean).

The results obtained in this study agree with those of Amadioha (2000) who ascertained that *O. gratissimum* leaf extracts controlled spore germination and mycelial growth of *Rhizopus oryzae*. Derbalah *et al*. (2011) also reported the antifungal activity of crude extracts of seven plant species (*Cassia senna*, *Caesalpinia gilliesii*, *Thespesia populnea* var. *acutiloba*, *Chrysanthemum frutescens*, *Euonymus japonicus*, *Bauhinia purpurea* and *Cassia fistula*) against *A. solani*, the causal organism of early blight disease in tomato, at 150 and 200 ppm. According to Babu *et al*. (2008), plant extracts and plant essential oils are effective antimicrobial agents against soil-borne fungi and do not produce any residual effects, are non-pollutive, cost effect, non-hazardous, easily available and do not disturb the ecological balance.

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 mycelial growth of *S. rolfsii*. Effectiveness of aqueous extract of *C. citratus* has been reported by Somda *et al*. (2007) and Suleiman *et al.* (2008) on *Fusarium* species.

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