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Effect of *Ocimum gratissimum* (L.) and *Aframomum melegueta* (K. Schum.) Extracts on the Growth of *Sclerotium rolfsii* (Sacc.)

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ABSTRACT

Southern blight disease of tomato induced by *Sclerotium rolfsii* Sacc. is known to cause about 60% reduction in tomato yield under rain fed condition. The use of resistant varieties and chemicals has not provided adequate control due to the persistent nature of the pathogen, poor adaptation of cultivars, high cost and scarcity of the chemicals. An alternative method of control was examined by evaluating the inhibitory effect of some selected spices on Southern blight disease of tomato *in vitro* and *in vivo*. Water and ethanol extraction of the seeds of *Aframomum melegueta* K. Schum. and leaves of *Ocimum gratissimum* L. was done at 1-5% v/v. *Sclerotium rolfsii* was isolated from infected plants. The *in vivo* experiment was laid out in triplicates in completely randomised design using the same extracts. The synthetic fungicide served as the control. All data collected were subjected to Analysis of Variance (ANOVA) and significant means were separated using fisher's LSD. The results showed that the spice extracts differed significantly ($p<0.05$) in their potential to inhibit the growth of *S. rolfsii*. In all, the extracts inhibition decreases with increase in concentration. Ethanol extract of the two spices at 3-5% concentration showed total inhibition (0.00 mm) on the mycelial growth. The *in vivo* experiment showed that plants treated with 5% *O. gratissimum* extract performed better in reducing Southern blight severity, gave highest fruit weight at 5% extract concentration. It was also observed. The two spice extracts are potential options for the management of *S. rolfsii* as they compared favourably with fungus force, a synthetic pesticide recommended for the control of *S. rolfsii*.

Key words: *Sclerotium rolfsii*, plant extracts, antifungal agents, botanicals, alternatives

INTRODUCTION

Sclerotium rolfsii Sacc. is an economically important soil borne fungal pathogen which causes disease on a wide range of agricultural and horticultural crops (Farr *et al*., 1989) including the diseases known as Southern blight especially on tomato. *Sclerotium rolfsii* 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 (Chet, 1975). Although no statistical data are available, the disease caused by this pathogen lead to heavy losses in vegetable crop yield especially during wet season when weather conditions are favourable for both crop production and for the growth and dissemination of the sclerotia of the pathogen (Okabe *et al*., 2000). Among over 500 plants attacked by *Sclerotium rolfsii* particularly in the tropical, sub-tropical and warm temperate areas (Okereke and Wokocha, 2007) is tomato (*Lycopersicon esculentum* Mill.) which is

an important vegetable in Nigeria for domestic purpose, accounting for about 18% of the daily consumption of vegetables which averages out at 50.6% per person (Kateria and Mittal, 1984). One medium-sized tomato fruit of about 150 g provides half the recommended dietary allowance of vitamin C for an adult (Yusuf and Okusanya, 2007). Egharevba (1991) and Yusuf and Okusanya (2007) stressed that fresh tomato fruits and other vegetables are essential components of human diet which have necessitated the increased production and commerce of commodities.

Major methods employed to manage *S. rolfsii* are fungicide applications, solarisation, use of antagonistic microorganisms, deep ploughing, crop rotation and incorporation of organic and inorganic residues (Punja, 1985). Fungicides such as Captan and Calixin have been used for seed dressing and other chemicals such as methyl bromide and Chloropicrin are used as soil fumigants to the control of this pathogen. These chemicals are environmentally hazardous, recently banned, not readily available and difficult therefore to adopt in subsistence agriculture in West Africa (Okereke and Wokocha, 2007). 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 *Sclerotium rolfsii*. The purpose of this trial was to evaluate the efficacy of *A. melegueta* and *O. gratissimum* for the *in vitro* inhibition of the *S. rolfsii*.

MATERIALS AND METHODS

Isolation of *S. rolfsii*-Infected tomato stems were obtained from the experimental plot of National Horticultural Research Institute, Ibadan Nigeria. The stems were washed with sterile distilled water, cut into 5 mm segments which were surface sterilized in 0.5% sodium hypochlorite (NaOCl) solution for 5 min and rinsed thrice in sterilized distilled water. The segments were then air dried between sterile filter paper and plated on Potato dextrose agar (DIFCO) amended with chloramphenicol (60 mg mL^{-1}). The plates were incubated at 28±2°C and examined for 5 days. Cultural and morphological identification of the organism was done with the use of microscope. The plates were sub-cultured to obtain pure cultures of isolate which were kept in slants and stored at room temperature.

Preparation of plant extracts: Dried seeds of *A. melegueta* (Alligator pepper) and fresh leaves of *O. gratissimum* (African basil) were the plant materials used. *A. melegueta* seeds were purchased from Bode market in Nigeria while *O. gratissimum* leaves were obtained from the progeny garden of the National Horticulural Research Institute (NIHORT) Ibadan, Nigeria. The plant materials were rinsed in sterile distilled water and dried at 60°C with the *O. gratissimum* leaves also rinsed as above and dried for 10 days at room temperature, after which they were milled with Marlex blender separately into powder. The powder were sieved and packed into glass bottles and sterilized in a hot air oven at 160°C for 5 h (Enikuomehin, 1995).

***In vitro* control of *S. rolfsii*:** One milligram, 2, 3, 4 and 5 g of 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 by dissolving the same quantity as described for water 100 mL absolute ethanol to obtain 1, 2, 3, 4 and 5% concentrations, respectively. Each of the extract was evaluated for antifungal properties against the fungal pathogen by agar diffusion plate method. One millilitre of each extract concentration was poured into sterile 9 cm-diameter Petri dishes, 9 mL of cooled (about 45°C) molten chloramphenicol

amended (60 mg mL^{-1}) PDA was aseptically poured into each Petri-dish and rotated gently to ensure even dispersion of extract. 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 spice extract samples. Control plates had either 1 mL of sterile distilled water of 1 mL ethanol plus 9 mL distilled water mixed with cooled molten Chloramphenicol modified PDA and inoculated as described above. There were three replicates for each plant extract 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. Data were analysed using Fisher's Least Significant Difference (Fisher's LSD) at 5%.

***In vivo* control of *S. rolfsii*:** The experiment was complete randomised design replicated three times. Tomato seedlings UC82B 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 10 kg sterilized soil. (Enikuomehin *et al*., 1998). The four weeks old seedlings were transplanted into the inoculated soil at one seedling per pot. The extracts and the synthetic fungicide were applied at the base line of the stem at the rate of 10 mL per pot. Plants were watered every other day throughout the period of the experiment. The data collected include plant height, leaf number, disease severity and fruit weight. Data were collected weekly and disease severity was rated according to the method of De Cal *et al*. (1995).

Statistical analysis: The data collected were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1984) using SAS software. Means of significant treatments were separated by Fisher's Least Significant Difference (LSD).

RESULTS AND DISCUSSION

The effect of *A. melegueta* and *O. gratissimum* extract concentrations at various time on the growth of *Sclerotium rolfsii* is shown in Table 1 and 2.

The efficacy of two spice extracts against Southern blight fungus (*S. rolfsii*) of tomato was tested *in vitro* and *in vivo*. The results showed that the two spice extracts significantly ($p<0.05$) inhibited the radial growth of the organism with inhibition varying from one extract to another. It was observed that the antifungal effectiveness of these spices in culture depends on the concentration used and the solvent of extraction. This is in agreement with the work of Udo *et al*. (2001) who reported that methanol and ethanol extracts of spices have high potency for the control of pathogenic fungi of potato and yam tubers. They also reported that the potency of aqueous extracts was low compared to the methanol and ethanol extracts. According to Zaker and Mosallanejad (2010), methanol extracts of peppermint (15%), *Lavandula* (15%), peppermint (10%) and eucalyptus (15%) demonstrated promising ability in inhibiting the mycelial growth of *Alternaria alternata* causal organism of *Alternaria* leafspot of potato. 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 *Alternaria solani* the causal organism

of early blight disease in tomato at 150 ppm and 200 p. As concentration increases it was observed that *O. gratissimum* aqueous extract gave comparable reduction in mycelial growth this is in agreement with the work of Awuah (1989) who found that extracts of *O. gratissimum* led to 24.6% reduction in radial growth of Rhizopus species and a 60% reduction of *Ustilaginoidea virens*. Ijeh *et al.* (2005) reported that aqueous and ethanolic extracts of *O. gratissimum* and *Xylopia aethiopica* showed their antimicrobial activities against five pathogenic organisms; *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Pseudomonas aeruginosa* and *Lactobacillius* specie.

Okigbo and Ogbonnanya (2006) reported that *O. gratissimum* and *A. melegueta* ethanol extracts inhibited the mycelia growth and spore germination of many rot causing microorganisms. *O. gratissimum* leaf extracts controlled spore germination and mycelia growth of *Rhizopus oryzae (*Amadioha, 2000). Enikuomehin *et al.* (1998) also proved the inhibitory effect of *O. gratissimum* leaf ash on the mycelial growth of *S. rolfsii in vitro*. Generally, radial growth increased as the hours increases. Twenty four hours after incubation, the radial growth of *S. rolfsii* on plates impregnated with water extract of *O. gratissimum* decreased with increasing extract concentration. At 120 h after incubation, plates with no extract had the highest radial growth of 4.3 mm while the least of 0.5 mm was on plates with 5% extract concentration. Plates impregnated with ethanol extracts of *O. gratissimum* had highest mycelia growth at 1% concentration and the least at 3% after 120 h of incubation. Also, at 120 h plates impregnated with water extract of *A. melegueta* had highest radial growth of 4.6 mm while the least was 2.5 mm. Highest radial growth of 4.5 mm was observed at 120 h while the least of 0 was observed for ethanol extract of *A. melegueta*. The aqueous and ethanol extracts of the two plant materials used in this trial differed significantly in their potential to inhibit the mycelium growth of *S. rolfsii*. 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 two extracts as the number of hours increased. Highest significant effect was observed at 5% for the water extracts while the least was recorded at 1%. The ethanol extracts of the two samples had significant effect on the mycelium growth. Highest inhibition was observed at 3% in the two samples with no growth on the plates.

Results of analysis of variance showed that different concentrations of *A. melegueta* and *O. gratissimum* had significant effect on plant height (6 WAT). Southern blight severity and fruit yield per plant but plant height at 8 WAT, number of leaf/plant and shoot dry weight were not significantly affected by spice extract application (Table 1). *O. gratissimum* extract at 5% concentration gave the highest plant (85.26) though this was not significantly different from *O. gratissimum* at 2% (80.83 cm) and *A. melegueta* at 1% (76.43 cm) (Table 2). At 6 WAT disease severity was significantly higher on plants treated with *A. melegueta* at 1 and 2% concentrations than those treated with *A. melegueta* at 3 and 4%, *O. gratissimum* at 1 -4% concentrations. Disease severity recorded on plants treated with *A. melegueta* extract at 5% and fungus force were significantly lower than those mentioned above while the least disease severity was observed on plants treated with *O. gratissimum* extract at 5% concentration (Table 2). At 8 WAT, disease severity observed on plants treated with *A. melegueta* extract at 1 and 2% and *O. gratissimum* at 1% concentrations was significantly higher than other treatments, this was followed by *A. melegueta* at 3, 4%, *O. gratissimum* at 2, 3 and 4% concentrations which were significantly

Table 1: Inhibition of mycelial growth of *S. rolfsii* by *O. gratissimum* extracts after 5 days incubation at 28°C

Extraction medium	Extract source	Conc. (%)	Radial growth of *S. rolfsii* at indicated extract concentrations (mm)				
			24 h	48 h	72 h	96 h	120 h
Water	*O. gratissimum*	1	0.3	1.4	2.6	2.9	3.2
		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
		0	0.6	2.0	3.3	4.1	4.3
LSD ($p<0.05$)			0.2	0.4	0.45	0.65	0.88
Ethanol	*O. gratissimum*	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
		0	0.9	2.2	2.7	3.3	3.7
LSD ($p<0.05$)			0.38	0.39	0.37	0.49	0.49

Table 2: Inhibition of mycelial growth of *S. rolfsii* by *A. melegueta* extracts after 5 days incubation at 28°C

Extraction medium	Extract source	Conc. (%)	Radial growth of *S. rolfsii* at indicated extract concentrations (mm)				
			24 h	48 h	72 h	96 h	120 h
Water	*A. melegueta*	1	0.4	1.0	2.0	2.3	2.5
		2	0.2	0.9	1.0	2.1	2.2
		3	0.2	0.7	1.6	1.7	1.9
		4	0.2	0.4	1.1	1.3	1.4
		5	0.2	0.2	0.7	0.9	1.0
		0	0.4	2.0	4.0	4.3	4.6
LSD ($p<0.05$)			0.19	0.25	0.3	0.35	0.28
Ethanol	*A. melegueta*	1	0.2	0.7	1.5	2.0	2.2
		2	0.2	0.5	0.9	1.5	1.6
		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
		0	0.9	2.1	3.6	4.2	4.5
LSD ($p<0.05$)			0.1	0.39	0.58	0.6	0.46

different from each other. Disease severity recorded on plants treated with 5% *A. melegueta* was comparable with that of the fungus force, this was lower than what was observed at lower concentrations of *A. melegueta* and *O. gratissimum* extracts. Among all the treatments, the lowest disease severity was recorded on plants treated with *O. gratissimum* extract (Table 2). Significant difference was observed in the fruit yield of plants treated with the spice extracts at different concentrations ($p<0.05$). The highest fruit yield of 154.9 g was recorded in plants treated with 5% concentration of *O. gratissimum* extracts but this was comparable with 108.9 and 116.2 g obtained from plants treated with 4% concentration of *X. aethiopica* extract and fungus force, respectively.

Maximum reduction in disease severity to (1.67) was achieved at the highest concentration (5%) of *O. gratissimum* and this was comparable to (2.3) obtained with synthetic fungus force

(Mancozeb 63 + 12.5% WP). This agrees with the work of Afroz *et al.* (2008) who affirmed that the occurrence of late blight attained an epipytotic momentum when the plants entered into their reproductive phase, that is, between 40 and 55 days after transplanting. The results obtained from this study on the effectiveness of the two spice extracts on Southern blight disease severity revealed that the extracts vary in their ability to reduce disease severity. This is in consonance with the earlier reports that many plant products contain fungitoxic constituents that have potential to control plant diseases (Amadioha and Obi, 1999; Enikuomehin, 2005).

Disease severity varied with each spice extract at different concentrations. Okigbo and Ogbonnanya (2006) had reported the antifungal effectiveness of *O. gratissimum* and *A. melegueta* *in vitro* and *in vivo* on rot pathogens of yam tubers and that the two extracts did not show significant difference in their potency against rot pathogens.

The results obtained from this study on the effectiveness of the two spice extracts on Southern blight disease severity revealed that the extracts vary in their ability to reduce disease severity. It was observed that increase in concentration of the extracts enhanced the effectiveness of individual extract to reduce disease severity. Plants treated with *O. gratissimum* extract had better growth in terms of plant height. This result agrees with the study of Okigbo and Ogbonnanya (2006) who reported that *O. gratissimum* and *A. melegueta* extracts proved effective against mycelial inhibition and spore germination of many rot causing microorganisms. Amadioha (2000) ascertained that *O. gratissimum* leaf extracts controlled spore germination and mycelial growth of *Rhizopus oryzae*. Enikuomehin (2005) also reported the inhibitory effect *O. gratissimum* leaf ash on the mycelial growth of *S. rolfsii* of wheat on agar. The antifungal effectiveness of these spice extracts in culture depends on the concentration and the solvent of extraction. Sallam (2011) concluded that extract of *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulensis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander) and *Allium sativum* (Garlic) against *Alternaria solani* at 5% concentration caused reduction in mycelial growth of *A. solani* causal organism of Early blight of tomato *in vitro* and increased the fruit yield *in vivo*. According to Abera *et al.* (2011) extracts of *Allium sativum* and *Croton macrostachyus* on *Colletotrichum kahawae* radial growth and disease development vary depending on the concentration of the extracts applied. Oyelana *et al.* (2011) reported that the leaf extracts of *Ficus* species at 75 and 100 mg mL^{-1} concentrations had profound antimicrobial properties on the fungal and bacterial pathogens of *Dioscorea rotundata*. These extracts contained alkaloids, flavonoids and cardiac glycosides and may have conferred the antimicrobial properties on this group of plants. Neem leaf extract at 15% concentration has also been reported to inhibit radial growth in *Fusarium oxysporum* f. sp. *psidii* (Derbalah *et al.*, 2011). From this trial, ethanol extracts performed better in inhibition of the mycelial growth of *S. rolfsii*. The inhibitory activities of these extracts suggest their fungitoxic ability on *S. rolfsii*. This observation agrees with the study of Iwalokun *et al.* (2003), Onwuliri and Wonang (2005), Udo *et al.* (2001), Eweis and Amber (2011), who concluded that the antifungal properties of these plants are due to their phytochemical contents and the concentration of the extracts and essential oil used. This investigation demonstrates the potential of *A. melegueta* and *O. gratissimum* as potential alternatives to synthetic chemical in the management of *Sclerotium rolfsii* which causes stem rot disease of tomato.

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