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Fungicidal Effects of O*cimum gratissium* and V*ernonia amygdalina* on Fungi Associated with Rhizosphere and Rhizoplane of *Capsicum annuum* L.

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ABSTRACT

This research work was carried out to test the antifungal effects of leaf extract of *Ocimum gratissimum* and *Vernonia amygdalina* on fungi associated with rhizoplane and rhizosphere of *Capsicum annuum*. Soil samples were collected from the rhizoplane and rhizosphere of *Capsicum annuum* at Gerewu, Ilorin, Kwara State. The soil samples were analyzed using serial dilution and spread plate methods. The leaf extract of each of the test plant was prepared using ethanol as the extractant. The antifungal potential of leaf extract of *Ocimum gratissimum* and *Vernonia amygdalina* were tested on the isolated fungi using poisoned food method. From the rhizosphere, four fungi were isolated viz: *Aspergillus niger, A. oryzae, Paecilomyces varioti and Saccharomyces cerevisae*. Only *Aspergillus niger*, *A. clavatus*, and *Paecilomyces varioti* were isolated from the rhizoplane. Comparatively, the antifungal potential of *Ocimum gratissimum* in the inhibition of mycelial growth of the isolated fungi was observed to be higher than that of *Vernonia amygdalina* .

Key words: Fungi, *Ocimum gratissimum,* Rhizoplane, Rhizosphere*, Vernonia amygdalina*

INTRODUCTION

Capsicum annuum (Chili peppers) is one of the important food crops in Nigeria. It belongs to Family Solanaceae along with *Solanum lycopersicum, Solanum tuberosum* and *Solanum melongena*. *C. annuum* is widely used as spice in the world (Mohammed *et al*., 2013) and it is known with high medicinal and nutritional value. The species are rich in Vitamin A which is responsible for red colour of the mature fruits. *C. annuum* and *C. frutescens* are important raw materials in food manufacturing industries for seasoning of processed foods and in the preparation of curry powder (Tindal, 1986). The wet season crops are usually attacked by pests and diseases as a result of poor management practices leading to reduced production (Jaliya and Sani, 2006).

Most of the fungi causing diseases in *Capsicum spp* are usually soilborne residing at either the rhizosphere or rhizoplane of the plant and they are responsible for diseases such as *Phytophthora* blight caused by the fungus, *Phytophthora capsici* while *Rhizoctonia solani*, *Pythium spp* and *Fusarium spp*. are responsible for damping off and root rot diseases of the plant. The seedling may fail to emerge or there may be sudden drooping of the seedling (damping off) and the seedlings may appear stunted, root rot/ collar rot (Black *et al*., 2013). Other diseases are *Verticillium* wilt, gray mold, gray leaf spot and *Fusarium* wilt caused by *Verticillium albo-atrum* and *V. dahlia, Botrytis cinerea, Stemphylium solani* and *Fusarium oxysporum* f. sp. *capsici* respectfully (Black *et al,* 2013). The most frequent fungi in the rhizosphere of the

Capsicum spp are *Rhizopus stolonifer, Aspergillus niger* and *Mucor mucedo* (Ajokpaniovo and Oyeyiola, 2011).

This study evaluated the antifungal effects of the extracts from the leaves of *Ocimum gratissimum* and *Vernonia amygdalina* on fungi associated with rhizoplane and rhizosphere of *Capsicum annuum*.

MATERIALS AND METHODS

Collection of soil Samples

Soil samples were collected from a farm at Gerewu in Ilorin, Kwara State using the method of Dongmo and Oyeyiola (2009). The rhizosphere soil samples were collected by carefully uprooting the plants and the soil adhering to the roots were gently shaken into sterile polythene bags. The bags were tied and labeled. The roots of the plants were aseptically cut into small fragments. Each unit of the fragments with adhering soil was put into sterile polythene bag, tied and labeled as rhizoplane sample. For the collection of non-rhizosphere soil (Control), the spots of collection were dug 5cm -10cm deep with a sterile hand trowel. The samples were kept in sterile polythene bags, tied and labeled.

Determination of Soil pH and Moisture Content (%)

The pH of the soil sample was determined according to the method described by Oyeyiola (2009). This was done by dissolving 2g each of soil sample in 25ml of distilled water. The suspension was stirred together for about 20 minutes to homogenize the mixture .The pH meter was standardized with buffers before been used for measurement. The pH of the suspension was then determined using a pH meter (Searchtech Instrument PHS -3c Ph meter).

The percentage moisture was calculated using the formula below:

$$\% \text{ moisture content} = \frac{M_1 - M_2}{M_1 - M} \times 100$$

Where M = Weight of the crucible in gm

M_1 = Weight of crucible with sample before drying in gm

M_2 = Weight of crucible with sample after drying in gm

Isolation and Identification of Fungi from the Soil Samples

Two methods were used to isolate fungi from the soil samples namely, soil dilution plate method of Waksman (1922) and soil sprinkle plates of Kostadinova, *et al*. (2009). In the soil dilution plate method, 1g of soil was suspended in 9ml of sterile distilled water which gave a dilution of 1:10 from which serial dilutions of 1:100, 1:1000, 1:10000 and 1:100000 were made. One ml of aliquot suspension from each dilution was poured in sterilized petri plates containing Potato Dextrose Agar (PDA) culture medium. Three replicates were prepared for each sample. The plates were incubated at 27±2°C for 5 days and examined thereafter for fungal growth. In soil sprinkle method, the soil samples were mixed thoroughly. Two grams of each sample was sprinkled on the surface of the petri dishes containing PDA culture medium. In the culture medium, 30mg/L of streptomycin was added to suppress the growth of bacteria. The plates were incubated at room temperature and following fungal growth, plates were subcultured to obtain pure culture of the resulting fungal isolates.

Identification of the isolates was also done based on their colonial morphology and microscopic examination. Slides were prepared from the pure culture of each isolate and these were observed under light microscope for identification following the method of Campell and Stewart (1980) and Barnett and Hunter (2010).

Preparation of Leaf Extracts of *Vernonia amygdalina* and *Ocimum gratissimum*

Fresh leaves of *Vernonia amygdalina* and *Ocimum gratissimum* were collected from Oja Oba market, Ilorin, Kwara State and authenticated in the Herbarium unit of Plant Biology Department, University of Ilorin. The extracts were prepared following the method of Pawar (2011) with slight modifications. The leaf samples were weighed and air-dried at room temperature for 2 weeks. After drying and pulverizing, 10 gram of powder from each of the plant was soaked in 100ml ethanol (1:10 dilution) for 48hrs. The crude preparation was placed on a shaker at room temperature for 1 hour and then centrifuge at 11000rpm for 20minutes.The plant extracts was filtered using Whatman filter paper, the ethanol in the extracts was then evaporated using water bath at 65^{o}C.

Antifungal Sensitivity Testing of the Leaf Extracts

Determination of the inhibitory effects of *Vernonia amygdalina* and *Ocimum gratissimum* extracts on fungal growth was based on the method described by Okigbo and Nmeka (2005). Four equal sections on each petri dish were created by drawing two perpendicular lines at the bottom of the plate, the point of intersection indicating the centre of the plate. PDA was then dispensed into each of the plates. Two milliliters each of extracts of *V. amygdalina* and *O. gratissimum* were separately introduced into petri dishes containing PDA and then allowed to solidify (Poisoned food method). In control experiment, 2ml of sterile distilled water was used to amend PDA. A fungal disc (4 mm diameter) of the pure culture of each isolate was then inoculated at the centre of the plates containing the solidified Agar-Extract Complex and control. Three replicates were made. Inoculated plates were incubated at room temperature and growth measured

along the perpendicular lines. The mycelial growth was monitored and recorded every other day.

Statistical Analysis of Data

All data were analyzed using statistical software called Statistical Package for Social Science (SPSS). The means were separated by Duncan's Multiple Range Test (DMRT).

RESULTS

Percentage Moisture Content and pH Of Soil Samples

The result obtained from the soil moisture content analysis revealed that the control soil had the highest moisture content (11.68%) while the rhizoplane had the lowest moisture content (8.08%).The pH range was 7.6-7.9 as shown in Table 1.

Isolation of Fungi from Rhizoplane and Rhizosphere

Aspergillus niger, Paecilomyces varioti, A. oryzae, and *Saccharomyces cerevisae* were isolated from the rhizosphere. Three fungi, *Aspergillus niger, Aspergillus clavatus* and *Paecilomyces varioti* were isolated from the rhizoplane. Only *A. clavatus, A. niger* and *Saccharomyces cerevisae* were isolated from the control. *A. niger* was found in all the soil zones, while *A. oryzae,*and *Saccharomyces cerevisae* were conspicuously absent from the rhizoplane but present in the rhizosphere (Table 2).

Effects of leaf extracts on mycelial growth of Isolated Fungi

The results revealed that*Ocimum gratissimu m* displayed the highest level of inhibition of growth of *Saccharomyces cerevisae* and this was significantly different from the effects of *Vernonia amygdalina* throughout the experimental period on the same test organism (Table 3). Mycelial growth of *A. niger* treated with *V. amygdalina* on Day 6 was 25.33mm which was lower and significantly different from that of control

(47.33mm) (Table 4). At Days 4 and 6, the level of inhibition of mycelial growth of *Aspergillus clavatus* by leaf extracts of *O. gratissimum* and *V. amygdalina* were not significantly different from each other but were significantly different from the control as shown in Table 5. Similarly, the antifungal potential of *O. gratissimum* against the mycelial growth of both *A. oryzae* and *Paecilomyces varioti* was more noticeable than the control (Tables 6 and 7 respectfully).

DISCUSSION

The moisture content of the soil samples was in line with the optimum water requirement for *Capsicum annuum,* and the pH values of the soil samples spotted within the alkaline pH and ranged from 7.6 to 7.9. These agreed with the results of Andrew (1984). The isolation results revealed that *Aspergillus niger, A.oryzae, Paecilomyces varioti* , and *Saccharomyces cerevisae* are associated with the rhizosphere of *Capsicum annuum* whereas *Aspergillus clavatus* was conspicuously absent in rhizophere but present in rhizoplane. The presence of *Aspergillus niger* and *Saccharomyces cerevisae* was supported by the work of Sule and Oyeyiola (2012).

According to literatures, the aforementioned fungi played a detrimental role directly or indirectly to farmers and consumers. *Aspergillus* species are known to produce mycotoxins which are hazardous to human and animal health (WHO, 2004). Also, according to Bose *et al*. (2002) post-harvest rots of *Capsicum annuum* have been found to be associated mostly with *Aspergillus* species and *Paecilomyces varioti* which caused severe reduction on the yield of *Capsicum annuum* which might have infected the plant right from the field.

Researchers had reiterated the potential benefits of using higher plants in the control

of these fungi so as to curb the menace of using synthetic fungicides. The antifungal ability of *V. amygdalina* was reported by Nduagu *et al.* (2008) that the extracts from the plant inhibited the growth of *Colletotrichum capsici* (Synd) isolated from pepper. The leaf extract of the plant inhibited the mycelial and conidial growth of *Colletotrichum gloeosporioides* in rubber tree (Ogbebor *et al.,* 2007). Also, the aqueous extract of *V. amygdalina* leaf hindered the survival of seed borne fungi such as *Fusarium moniliforme*, *Botryodiplodia theobromae*, *A. niger* and *A. flavus* both in *in vitro* and *in vivo* (Nwachukwu and Umechuruba, 2001). The fungitoxic property of Vernonia species is scientifically attributed to important phytochemical compounds such as alkaloids, tannins, saponins, anthraquinones and flavonoids (Ogundare *et al.,* 2006).

The results of this study also agreed with previous reports on antifungal ability of *Ocimum amygdalina*. According to Morales and Simon (1996), *Ocimum* extracts are used in traditional medicine and had been shown to contain biologically active constituents that are insecticidal, nematicidal, fungicidal and generally antimicrobial. Also report by Nguefack *et al*. (2004) showed that the leaf extract of *O. gratissimum* exhibit high antifungal activities against *Fusarium moniliforme,* and *Aspergillus* spp.

The ethanoic leaf extracts of the plant caused mycelial reduction of the most commonly occurring fungal pathogen inducing rot in yam tubers such as fungi include *Aspergillus flavus, A. niger, Fusarium oxysporium, Rhizopus stolonifer, Penicillium chrysogenum* and *Botryodiplodia theobromae* (Okigbo and Ogbonnaya, 2006). The antifungal potential of *O. gratissimum* may be a predictable consequence of its high content in thymol, a phenolic compound (Koba *et al*., 2009).

Conclusion

This findings revealed that the role of botanicals as fungicidal agents is currently gaining attention with a view of averting the hazardous effects and cost of synthetic fungicides. The plant materials are biodegradable and eco-friendly. Both *Ocimum gratissimum* and *Vernonia amygdalina* are options in inhibiting the growth of fungi with *O. gratissimum* possessing higher efficacy.

Table 1: Moisture Content and pH of Soil Samples

Soil types	% Moisture content	pH
Rhizosphere	10.41	7.6
Rhizoplane	8.08	7.8
Control	11.68	7.9

Table 2: Occurrence of Fungi in Soil Samples

Soil types	*Aspergillus oryzae*	*Aspergillus clavatus*	*Aspergillus niger*	*Paecilomyces Varioti*	*Saccharomyces Cerevisae*
Rhizoplane soil	-	+	+	+	-
Rhizosphere soil	+	-	+	+	+
Control soil	-	+	+	-	+

Present = + Absent = -

Table 3: Effect of leaf extracts on the growth of *Saccharomyces cerevisae*

Treatments	Day 2	Day 4	Day 6
Ocimum gratissimum	4.66^{c}	5.00^{c}	5.33^{c}
Vernonia amygdalina	$I5.00^{b}$	26.00^{b}	29.66^{b}
Control	29.33^{a}	41.66^{a}	53.66^{a}

Values bearing the same letter(s) along the same column are not significantly different at $p \leq 0.05$.

Table 4: Effect of leaf extracts on the mycelial growth of *Aspergillus niger*

Treatments	Day 2	Day 4	Day 6
Ocimum gratissimum	4.67[b]	16.00[c]	16.33[c]
Vernonia amygdalina	7.00[b]	22.33[b]	25.33[b]
Control	28.00[a]	40.67[a]	47.33[a]

Values bearing the same letter(s) along the same column are not significantly different at $p \leq 0.05$.

Table 5: Effect of leaf extracts on the mycelial growth of *Aspergillus clavatus*

Treatments	Day 2	Day 4	Day 6
Ocimum gratissimum	3.67[c]	9.00[b]	9.00[b]
Vernonia amygdalina	6.67[b]	8.33[b]	9.67[b]
Control	17.00[a]	32.33[a]	40.00[a]

Values bearing the same letter(s) along the same column are not significantly different at $p \leq 0.05$.

Table 6: Effect of leaf extracts on the mycelial growth of *Aspergillus oryzae*

Treatments	Day 2	Day 4	Day 6
Ocimum gratissimum	3.00[b]	11.67[b]	12.00[c]
Vernonia amygdalina	4.33[b]	14.00[b]	20.00[b]
Control	14.67[a]	30.67[a]	63.00[a]

Values bearing the same letter(s) along the same column are not significantly different at $p \leq 0.05$.

Table 7: Effect of leaf extracts on the mycelial growth of *Paecilomyces varioti*

Treatments	Day 2	Day 4	Day 6
Ocimum gratissimum	4.67[a]	12.33[a]	12.67[b]
Vernonia amygdalina	14.00[b]	17.67[b]	29.67[c]
Control	7.33[a]	15.00[ab]	25.67[a]

Values bearing the same letter(s) along the same column are not significantly different at $p \leq 0.05$.

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