

Fungicidal effect of three plants extracts in control of four phytopathogenic fungi of tomato (*Lycopersicum esculentum* L.) fruit rot.

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Abstract— Fungicidal effect of leaf aqueous extracts of *Azadirachta indica*, *Tithonia diversifolia* and *Chromolaena odorata* were determined on rot causing fungi. In the study, the phytopathogenic fungi isolated from the infected tomato fruit parts and identified based on morphological and cultural characters were: *Aspergillus niger* Van Tiegh, *Fusarium oxysporum* Schlecht, *Geotrichum candidum* Link and *Rhizopus stolonifer* Ehrenb. ex. Fr. as confirmed by pathogenicity tests. Leaf aqueous extracts of different concentrations (20, 40, 80, 60 and 100 % w/v) of *A. indica*, *T. diversifolia* and *C. odorata* were added to growth media prior to inoculation. All aqueous extracts of the tested plants significantly ($p < 0.05$) reduced mycelial growth of the fungal pathogens and this effect gradually increased with increasing concentration. Fungicidal activity was strongly exhibited by *A. indica* extract at 100% w/v against all the pathogenic fungi. In the case of *T. diversifolia* extracts inhibitory effects at 20, 40, 60, 80 and 100% w/v were greater than those of *C. odorata* on *A. niger*, *F. oxysporum* and *G. candidum* while for *R. stolonifer* inhibition, *C. odorata* produced the highest in the all five concentrations than *T. diversifolia* extracts. It could be emphatically concluded that the tested plant extracts can effectively control rot causing fungi disease of tomato. This makes them potential biocide in diseases management in that they are cheap and environmentally safe as they showed fungicidal and fungitoxic ability.

Key words— Aqueous extracts, Fungicidal effect, Inhibitory effects, Phytopathogenic.

I. INTRODUCTION

Tomato (*Lycopersicum esculentum* L.) is the second most important vegetable crop in the world because of its special nutritive value. The edible fruit of the tomato plant has a series of usages in different forms. The crop is nutritious and contain high amount of dietary source of vitamins A, B, C, E and nicotinic acid (Kanneh *et al.*, 2015; Godia, 2014). In Sierra Leone and other parts of the world, it is consumed as fresh fruit, salads, soup and stew

and often used in other dishes (Osei *et al.*, 2014). Its cultivation provides source of employment to many and continue to play a key horticultural role in the sub-region in terms of reducing poverty and food insecurity (Osei *et al.*, 2014). Recently, according to Kanneh *et al.* (2015), the Global production of fresh fruit tomato is about 100 million tons cultivated on 3.7 million hectares.

In Sierra Leone as well as other countries in Africa, the average yield on farm is between 7.5-10t/ha (Godia, 2014) which is far below the potential yield of the crop 45-50 M t/ha. Tomato production is seriously affected by over 200 diseases caused by pathogenic fungi, bacteria, viruses and nematodes (Abad Z. G. and Abad J. A., 1997; Koch *et al.* 1992; Lukyanenko, 1991; Moriones and Navas-Castillo, 2000). Major fungal diseases affecting tomato production are late blight, early blight, septoria leaf spot, fusarium wilt, and verticillium wilt, corky root rot, damping-off, leaf mould and powdery mildew (Panthee and Chen, 2010).

Most farmers control these diseases using fungicides. However, the negative environmental impacts, mammalian toxicity and high costs are making their use unattractive thereby searching for alternatives such as natural plant-based chemicals (Asawalam, 2006). Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan, 1991). These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (Das *et al.*, 2010). Many research workers have tried to find out safe and economical control of plant diseases by using extracts of different plant parts (Hasan *et al.*, 2005; Bdllya and Alkali, 2008). Hence the objective of the study was to determine to efficacy of aqueous leaf extracts for controlling some important postharvest tomato fruit rot diseases in *in-vitro*.

II. MATERIALS AND METHODS

2.1 Experimental Site and Source of Materials

Experiments were carried in Department of Crop Protection, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta. The tomato fruits with symptoms of rot were randomly collected from two different markets in Ogun State, Nigeria. The markets are Osele main market, Oeda Local government and Kuto main market, Abeokuta South.

2.2 Preparation of culture media

Potato Dextrose Agar (PDA) (BAM Media M127) was prepared by dissolving 39 grams in 1 litre Erlenmeyer flask and then made up to 1 litre using sterile distilled water. The medium was autoclaved at 121 °C for 15 minutes at 15 lb. The sterilized medium was allowed to cool to 45°C, before supplemented with streptomycin sulphate (3 grams) and aseptically dispensed into sterilized 9 cm diameter glass Petri dishes.

2.3 Isolation and Identification of Fungal Pathogens

Diseased tomato fruits were randomly collected from the two different Markets. The Chiejina (2008) isolation method was used. Thin sections (2 mm diameter) were cut from the Periphery of diseased tomato fruits and surface sterilized in 0.1% mercuric chloride for 2-3 min, after which they were rinsed in three changes of sterile distilled water. The sections were plated in water agar and mycelium was transferred into clean PDA plates.

The plates were incubated at room temperature ($27 \pm 2^\circ\text{C}$) for 6-7 days. Subcultures were made aseptically from the plates into similar clean PDA plates and were incubated under similar conditions until pure cultures were obtained. The identification of the isolated fungi was done macroscopically and microscopically. Macroscopic identification was based on observed culture growth patterns and mycelial colour. Small portions of the fungal cultures were teased and mounted in lactophenol in cotton blue on clean slides, covered with clean cover slips and then viewed microscopically. Fungal identification was confirmed with the aid of books by Barnett and Hunter (1999), Alexopoulos *et al.* (2002), Agrios (2005) and Ellis *et al.* (2007)

2.4 Pathogenicity Test

Each of the fungal isolates obtained from the diseased tomato fruits were tested for their ability to cause the same disease condition previously observed in healthy tomato fruits. Healthy tomato fruits were washed in sterile distilled water and surface sterilized by dipping into 0.1% mercuric chloride and with the aid of a sterile cork borer, cylindrical cores were removed from each of the tomato fruits. Pure cultures of the isolates of the isolates were

introduced into the cores and the cores were replaced and sealed with sterile petroleum jelly. The fruits were kept at room temperature for 7-10 days. On establishment of disease condition, inocula were taken from the infected tomato fruits and cultured. The organisms were re-isolated and identified as previously isolated organisms. This was taken as evidence that they incited the disease.

2.6 Sources of plant materials and Preparation of Extracts.

Leaves of three plant species namely *Azadirachta indica* (A. Juss) (Neem), *Tithonia diversifolia* (Hemsley) A. Gray (Mexican sunflower) and *Chromolaena odorata* (Linn) were used in the experiment. These were obtained within the premises of the Federal University of Agriculture, Abeokuta. Fresh leaves of *A. indica*, *T. diversifolia* and *C. odorata* were washed in tap water then surfaced-sterilized with (1% NaOCl for 5min and rinsed in five changes of sterile distilled water) and air dried at ($28 \pm 2^\circ\text{C}$) for 1h. 20grams, 40grams, 60grams, 80grams and 100 grams of each plant material were grounded using sterilized Brabantia 5-speed blender (Model BBEK 1051) in 100 ml distilled water, and then filtered through a Whatman® No. 9 filter paper separately into a 250 ml Erlenmeyer flask to produce 20 %, 40 %, 80%, 60% and 100% extract concentrations.

2.7 Effect of plant extracts on mycelial growth inhibition of fungal pathogens.

Extract-media mixtures were prepared by mixing 1 ml extract with 9 ml molten PDA prior to solidification for each extract concentration. Media amended with mycelial disc of a 5- day-old cultures of each fungus were placed in the centre of the petri dishes. The control plates consisted of PDA mixed with 1 ml sterile distilled water. Benlate a standard fungicide, at a concentration of 20 mg/ml was used to assess the efficacy of the plant extracts Owolade and Oskkanlu (1999) modified method. All treatments were in three replicates and incubated at $28 \pm 2^\circ\text{C}$. Radial growth in treatments and control were measured at 24 h interval for seven days. This was expressed as the mean growth along two axes on two pre-draw perpendicular lines on the reverse side of each plate. The percentage inhibition of mycelial growth by each extract was computed using formula.

$$I = 100 \times (C - T) / C$$

Where;

I = percentage inhibition of mycelial growth
 C = mycelial growth of fungus in control plate
 T = mycelial growth of fungus in the treatment
 (Sobia *et al.*, 2011)

III. STATISTICAL ANALYSIS

Data were subjected to analysis of variance (ANOVA) and significant means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

IV. RESULTS

4.1 Inhibition of mycelial growth of *Aspergillus niger* by three aqueous plant extracts

The aqueous extracts of the tested plants reduced mycelial growth and inhibition of *A. niger*.

However, the inhibitory effect of the plant extracts and benlate solution on the mycelial growth *A. niger* was significantly different ($P < 0.05$) at all the various concentrations. *A. indica* exerted the highest inhibitory effect of (92.0 %) at 100 % (w/v) followed by *T. diversifolia* and *C. odorata* at the same concentration. Fungitoxicity of tested plant extracts against *A. niger* increased as the concentration increased (Table 1).

Table.1: Inhibition of mycelial growth of *Aspergillus niger* by three aqueous plant extracts

Plant Extracts	<i>Azadirachta indica</i>		<i>Tithonia diversifolia</i>		<i>Chromolaena odorata</i>	
	Concentration % (w/v)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)
100	0.90	92.0	1.44	80.5	2.00	75.7
80	2.04	75.5	2.20	70.5	3.34	64.5
60	3.67	60.2	2.52	67.4	3.95	56.5
40	4.70	54.5	3.65	50.9	5.01	45.9
20	6.98	40.7	3.98	44.8	6.83	25.6
Benlate	0.00	100.0	0.00	100.00	1.00	88.9
Contol	9.00	-	8.10	-	9.09	-
LSD (0.05)	0.840	20.25	1.022	8.08	0.840	10.45

4.2 Inhibition mycelial growth of *Fusarium oxysporum* with three aqueous plant extracts

The effect of aqueous tested plant extracts mycelial growth *F. oxysporum* revealed that three extracts produced significant ($P < 0.05$) levels of inhibition of mycelial growth of *F. oxysporum* at various

concentrations. The highest inhibitory of mycelial growth was manifested by *A. indica* (87.4%) at 100 % (w/v) concentration while the least (15.5%) inhibition was recorded for *A. indica* at the concentration of 20 % (w/v). However, benlate solution was superior in mycelial growth inhibition (Table 2)

Table.2: Inhibition mycelial growth of *Fusarium oxysporum* with three aqueous plant extracts.

Plant Extracts	<i>Azadirachta indica</i>		<i>Tithonia diversifolia</i>		<i>Chromolaena odorata</i>	
	Concentration % (w/v)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)
100	0.73	87.4	2.00	76.8	3.33	62.7
80	1.11	80.8	2.77	68.5	5.66	40.0
60	3.14	45.0	3.07	64.6	6.34	35.4
40	4.04	30.1	3.40	60.3	6.45	31.0
20	4.87	15.5	3.47	53.8	7.68	25.2
Benlate	0.00	100.0	1.00	89.4	1.02	70.0
Contol	5.70	-	5.01	-	8.85	-
LSD(0.05)	0.440	7.70	0.77	9.13	1.882	24.10

4.3 Inhibition mycelial growth of *Geotrichum candidium* with three aqueous plant extracts.

The three plant extracts showed relatively high fungitoxic effect at 100 % (w/v) concentration on mycelial growth inhibition of *G. candidium*. At 80 % (w/v) concentration,

A. indica induced the highest (70.6 %) mycelial growth reduction compared to *T. diversifolia* and *C.odorata*. However, benlate solution was high in exerting mycelial growth reduction. (Table 3)

Table.3: Inhibition mycelial growth of *Geotrichum candidum* with three aqueous plant extracts.

Plant Extracts Concentration % (w/v)	<i>Azadirachta indica</i>		<i>Tithonia diversifolia</i>		<i>Chromolaena odorata</i>	
	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)
100	1.13	79.8	1.50	65.5	1.52	60.9
80	4.52	70.6	2.01	54.0	2.05	50.5
60	2.20	62.5	2.54	45.1	2.50	40.0
40	2.80	50.0	2.82	32.5	2.88	31.9
20	4.40	30.4	3.25	24.7	3.30	19.8
Benlate	1.12	81.6	1.14	60.0	1.00	55.4
Contol	8.01	-	6.01	-	7.09	-
LSD(0.05)	1.73	16.05	0.82	23.82	1.20	21.5

Inhibition mycelial growth of *Rhizopus stolonifer* with three aqueous plant extracts.

Result showed that the tested plant extracts utilized produced significant levels of inhibition of mycelial growth of *R. stolonifer* at various concentrations.

However, the highest inhibition was exerted by *A. indica* at 100 % (w/v) while the least inhibition (15.9 %) was exhibited by *T. diversifolia* at 20 % (w/v). As the concentration increased mycelial inhibition growth increased (Table 4)

Table.4: Inhibition mycelial growth of *Rhizopus stolonifer* with three aqueous plant extracts.

Plant Extracts Concentration %(w/v)	<i>Azadirachta indica</i>		<i>Tithonia diversifolia</i>		<i>Chromolaena odorata</i>	
	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)
100	1.50	64.7	1.42	60.5	1.55	63.6
80	2.07	51.2	2.07	49.2	2.10	51.8
60	2.67	39.7	2.17	35.4	2.18	45.5
40	3.27	30.5	2.67	22.8	2.60	30.4
20	3.87	22.8	2.91	15.9	2.89	22.8
Benlate	1.01	48.2	1.12	44.8	1.20	50.3
Contol	4.27	-	5.89	-	6.98	-
LSD(0.05)	0.74	18.50	0.64	17.01	0.86	18.09

IV. DISCUSSION

The mycelial growth inhibition and of the pathogens by the leaf aqueous extracts of *A. indica*, *T. diversifolia*, and *C. odorata* investigated in this study indicated that, antifungal activity showed by the tested plant extracts had inhibitory effects on the growth of *A. niger*, *F. oxysporum*, *G. candidum* and *R. stolonifer*. These results further revealed that antifungal activities of the extracts were enhanced by increasing the concentration from 20 to 100 % (w/v); hence the inhibition activities of the extracts were concentration dependent. This is in agreement with the report of Ilondu (2012) and Chiejina and Ukeh (2013) who indicated that increase in the antifungal activities had corresponding increase in concentration of plant extracts. *A. indica* exhibited high fungitoxic effect in inhibiting mycelial growth reduction against the four pathogen fungi.

The antifungal activity of *A. indica* conforms to the results of (Ogbebor and Adekunle 2005; Conventry and Allan 2001) that this extract is very effective in inhibiting

the growth of *F. moniliforme*, *A. flavus* and *A. niger*. Fungitoxic properties of *A. indica* could be attributed to the presence of saponin and alkaloid, chemical components which has been identified as antifungal agents in the plant (Kumar *et al.* 2008). The fungicidal effects of plant extracts on different pathogens of crop plants have been widely reported (Amadioha and Obi 1999; Okigbo and Ogbonnaya, 2006; Olufolaji, 1999 and Onifade, 2002).

However, the differences in the efficacy of the extracts could be attributed to the differences their active ingredients (Onifade, 2002; Okigbo *et al.* 2009). Major compounds of plant extracts are phenols, flavonoids, alkaloids, quinones, saponines, tannins and sterols (Halama and Van Haluwin, 2004) and their fungicidal or fungistatic properties against various plant pathogens have been established (Scheuerella and Mahaffee, 2002). These products might either have direct inhibitory effects on pathogens, exhibiting fungicidal or fungistatic properties. They could help in the establishment of

favorable conditions for antagonistic microbes (Scheuerella and Mahaffee, 2002). Benlate solution at 20mg/ml was found to be more effective than aqueous plant extracts in inhibiting the mycelial growth of the pathogens. This may be as result of the refined nature of benlate and its active ingredients being more concentrated than of the plant extracts.

V. CONCLUSION

This study demonstrated that *A. indica*, *T. diversifolia*, and *C. odorata* should be used as a potential biocide in plant disease management, as they showed fungicidal and fungitoxic ability (at 100% w/v). The utilization of plant extracts to control disease in vegetable field minimizes or eliminates the risks and hazards of toxic fungicides, especially on freshly consumed vegetables. It is anticipated that further research into these extracts would identify the active compounds responsible for their fungicidal activity.

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