

## Biological Control of Some Fungal Pathogens of Tomato (*Solanum lycopersicum* Mill.) Using Ethanolic Leaf Extracts of Plants

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**Abstract:** Tomato is one of the world's most important vegetables. However, global cultivation of tomatoes has been greatly hindered by several biotic and abiotic factors. Chief amongst biotic factors limiting global annual tomato production are fungal pathogens. Ethanolic leaf extracts of *Azadirachta indica*, *Jatropha curcas*, and *Tridax procumbens* were evaluated *in vitro* for biological control activity against some fungal pathogens of tomato namely; *Colletotrichum gloeosporioides*, *Pythium sp.*, and *Fusarium oxysporum*. Varying concentrations of crude ethanolic leaf extracts (0%, 10%, 50%, and 100%) were tested by using poison food technique. aseptically mixed with molten potato dextrose agar (PDA) and allowed to solidify. mycelia plugs (7 mm diameter) obtained from 5 days old cultures of *C. gloeosporioides*, *Pythium sp.*, and *F. oxysporum* were inoculated on solidified PDA-leaf extract mixture and incubated at 28°C. Fungal colony diameters were measured daily for a duration of seven days. Colonial growth of *C. gloeosporioides* was inhibited by all concentrations of ethanolic leaf extracts of *Azadirachta indica* and 10% concentration of leaf extracts of *J. curcas*. Colony diameters of *Pythium sp.* and *F. oxysporum* were not inhibited by any of the leaf extracts. Results of phytochemical screening of ethanolic leaf extracts of *A. indica*, *J. curcas*, and *T. procumbens* indicated the absence of phlobatannins only in *A. indica*, while all plant extracts tested positive for the presence of terpenes, tannins, alkaloids, flavonoids and saponins. Hence, we conclude that ethanolic leaf extracts of *A. indica* could serve as potent biofungicides in the control of plant diseases caused by *Colletotrichum sp.*

**Keywords:** Antimicrobial activity, Plant Protection, Chemotherapeutants, *Azadirachta indica*, *Jatropha curcas*, *Tridax procumbens*, *Colletotrichum gloeosporioides*, *Pythium sp.*, and *Fusarium oxysporum*.

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## 1. Introduction

Tomato (*Solanum lycopersicum* Mill.) is one of the most important vegetable crops grown worldwide. In Nigeria, it accounts for about 18% of daily consumption of vegetables which averages 50.6g per person. This accounts for the country's record as the 16<sup>th</sup> largest tomato producing nation in the world (FAO, 2010). Nigeria also boasts of the largest area (541,800Ha) cultivated for fresh tomato in Africa (FAO, 2014). In 2010 alone, the production of tomatoes in Nigeria reached 1.8 million metric tonnes, accounting for about 68.4% of West Africa, 10.8% of Africa's total output and 1.28% of global output (FAO, 2010). Currently, the demand for tomato and its by products in Nigeria far outweighs its supply (Ugonna et al., 2015).

The high cost of critical farming inputs such as chemical pesticides as well as the challenges of pre- and post- harvest diseases have been identified as major constraints to tomato cultivation in Nigeria

(Ebele, 2011; Terna et al., 2016; Ugonna et al., 2015). In the Guinea Savannah Belt of Nigeria, significant tomato yield losses have been attributed majorly due to fungal pathogens belonging to the genus *Sclerotium*, *Colletotrichum*, *Fusarium*, *Alternaria*, and *Pythium* (Terna, 2017).

Owing to the residual toxic effect of persistent overuse of pesticides as well as their nature and undesirable non-target effect on terrestrial and aquatic ecosystems (Bernhardt et al., 2017; Carvalho, 2017; Chagnon et al., 2015; Ccancapa et al., 2016; Hashmi, 2016; Mitchell et al., 2017; Munns et al., 2016), and the application of plant extracts as well as plant metabolites for plant disease management has become an important viable, sustainable and environmentally friendly component of integrated disease management (Harish et al., 2008; Stavi et al., 2016; Zhang et al., 2016).

Leaf extracts of different plants have significant antimicrobial potential (Shabana et al., 2017), and can

be exploited in agriculture for biological disease management (Gurjar et al., 2012). Antimicrobial, antiviral and antioxidant activities of plant extracts are attributed to the presence of saponins, flavonoids, ketones, and aldehydes (Galeane et al., 2017; Subapriya and Nagini, 2005). More than 280 phytochemicals are reported to have fungicidal properties. Among these phytochemicals, phenolic compounds, terpenoids, alkaloids and ketones are among the top mentioned (Boulogne et al., 2012).

The reported study was aimed at evaluating the potentials of leaf extracts of *A. indica*, *J. curcas* and *T. procumbens* in the biological control of some common fungal pathogens of field grown tomatoes, namely; *C. gloeosporioides*, *Pythium sp.*, and *F. oxysporum*. The study seeks to provide a useful and sustainable alternative to the often expensive and non-eco-friendly synthetic fungicides in the management of fungal diseases of tomato in the study area.

## 2. Materials and Methods

### 2.1. Source and Identification of Plant Materials

Leaves of *A. indica*, *J. curcas* and *T. procumbens* were collected within the environs of Federal University Lafia and identified at the Botany Laboratory of Federal University Lafia, Nigeria.

### 2.2. Source of Fungal Pathogens

Cultures of fungal pathogens of Tomato used in the study were donated by the Plant Pathology Unit of the Department of Botany, Federal University Lafia, Nigeria. The isolates were originally obtained from diseased tomato plants, their pathogenicity confirmed on healthy tomato plants and their pure cultures preserved on agar slants for further research. The identities of the collected fungi were confirmed via cultural and microscopic observation in line with identification keys of Samson et al. (2010).

### 2.3. Preparation of Plant Materials

Plant samples were prepared using the methods of Arikpo et al. (2013) with slight modifications. Fresh leaves of *A. indica*, *J. curcas* and *T. procumbens* were thoroughly washed with sterile distilled water and allowed to air dry at 28°C for two hours. The samples were further dried in an oven at 50°C for 30 minutes and pounded with mortar and pestle.

### 2.4. Preparation of Crude Extracts

Crude extracts of the three plant samples were prepared using the methods of Fatope et al. (1999). This involved soaking 50g of the powdered extract in 200ml of 95% ethanol for 72 hours at ambient temperature (28°C ± 2) and evaporation of the filtrate

to eliminate the solvent using water bath at 60°C for 24 hours. The residues were stored in reagent bottles in the refrigerator at 12°C until when used.

### 2.5 Preparation of Extracts Concentrations

The extracts were reconstituted in sterile distilled water to obtain various concentrations namely; 10, 50, and 100%.

### 2.6 Experimental Design and Data Analysis

The experiment was set up using the completely randomized design (CRD), comprising a total of 36 treatments and 3 replications in a 3×3×4 layout. The experimental treatments were; plant extracts (3), test fungi (3) and extract concentrations (4), making a total of 108 experimental units. Data obtained from the treatments were subjected to analysis of variance (ANOVA) at 5% level of probability, using SPSS software version 17. Significant differences were analysed using the Duncan Multiple Range Test (DMRT).

### 2.7 Effect of Plant Extracts on Pathogen Growth

Effect of plant extracts on pathogens' growth was determined using the method of Emoghene et al. (1998). One millilitre (1ml) from each of the concentrations of leaf extracts (10, 50 and 100 percent) was dispensed separately into 8.5-cm-diameter petri dishes and agitated gently with 15ml of sterile molten PDA to form potato dextrose leaf extract agar (PDLEA). The medium was allowed to solidify and inoculated centrally with 7mm diameter of mycelia plugs of test fungi obtained from 5 days old cultures, using a sterile cork borer. Test fungi growing on PDA plates inoculated with sterile water served as control. All cultures were incubated at 28°C and fungal colony diameters measured daily for 7 days. Percentage inhibition was determined as reported by Whipps (1987), as follows:

$$\text{Percentage inhibition} = \frac{R1-R2}{R1} \times 100 \quad [1]$$

Where, R1 = Radial diameter of fungus in control plates (PDA + Water); R2 = Radial diameter of fungus in the presence of leaf extracts (PDA + Leaf extracts).

### 2.8. Phytochemical Screening

The methods of Evans (2002); Sofowora (2008) and African Pharmacopoeia (1989) were used as follows:

#### 2.8.1. Test for Saponins

**Front Test:** An equal volume of ethanol (95%) was added to 1ml of the leaf extract. The mixture was boiled, filtered and 2.5ml of the filtrate was added to

10ml of distilled water in a test tube. The test-tube was stoppered and shaken vigorously for about 30 seconds and allowed to stand for over 30 minutes. Persistent honey-comb froth was indicative of the presence of saponins.

**Fehling's Solution Test:** To 1ml of extract was added an equal amount of Fehling's solution A and B. The presence of a bluish-green precipitate was indicative of the presence of saponins.

#### 2.8.2. Tannins Determination

About 2g of each plant extracts were separately stirred with 10ml of distilled water on a hot plate. The mixture was filtered and the resulting filtrate was added to 1ml Ferric Chloride solution. The presence of a blue or green color was indicative of the presence of tannins (Sofowora, 2008).

#### 2.8.3. Flavonoids Determination

Five millilitres (5ml) of leaf extracts was completely de-tanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered and the filtrate was used for the following tests:

**Lead Acetate Test:** To 5ml of the de-tanned extract was added 10% lead acetate solution. The presence of a colored precipitate was indicative of the presence of flavonoids.

**Sodium Hydroxide Test:** About 5ml of 10% sodium hydroxide were added to an equal volume of the de-tanned leaf extracts. The presence of a yellow solution was indicative of the presence of flavonoids (Sofowora, 2008).

#### 2.8.4. Terpenes Determination

One millilitre (1ml) of leaf extracts were separately stirred in 10ml of 95% ethanol, filtered and the filtrate evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and then filtered. The filtrate was mixed with 1ml of acetic anhydride, followed by the addition of 1ml of concentrated sulphuric acid down the wall of the test-tube to form a lower layer. The formation of reddish-violet coloration at the junction of the two liquids and a green color in the chloroform layer was indicative of the presence of terpenes (Evans, 2002).

#### 2.8.5. Sterols Determination (Salkowski's test)

One millilitre (1ml) of leaf extracts was stirred in 10ml of 95% ethanol, filtered and the filtrate evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and filtered. The filtrate was mixed with 2ml of concentrated sulphuric

acid such that the acid formed a lower layer. The presence of a reddish-brown coloration at the interface was indicative of the presence of sterols.

#### 2.8.6. Alkaloids Determination

Leaf extracts were prepared by macerating 3gm of the powdered sample in 50ml methanol. The extract was evaporated to dryness and 0.5gm of the residue mixed with 10ml of 1% aqueous hydrochloric acid on water bath. 1ml each of the filtrates was treated with a few drops of Mayer's reagent, Wagner's reagent and Dragendorff's reagent. Turbidity of precipitates was indicative of the presence of alkaloids (Evans, 2002).

#### 2.8.7. Test for Anthraquinone Derivatives

Ten millilitre (10ml) of chloroform were added to 1ml of leaf extracts in a dry test tube and allowed to stand for 5minutes. The extract was filtered and an equal volume of ammonia solution added to the filtrate and shaken. The appearance of a bright pink colouration in the upper aqueous layer was indicative of the presence of free anthraquinones (African Pharmacopoeia, 1989).

#### 2.8.8. Phlobatannins Determination

One millilitre (1ml) of plant extracts was stirred with 10ml of distilled water on a hot plate. The mixture was filtered and 1ml of the resulting filtrate boiled with an equal volume of 1% HCL. The presence of a reddish precipitate was indicative of the presence of phlobatannins (African Pharmacopoeia, 1989).

### 3. Results

Response of selected fungal pathogens of tomato to radial growth inhibition by ethanolic leaf extracts of *J. curcas*, *A. indica* and *T. procumbens* (Table 1) indicates that inhibitory activity was observed in only leaf extracts of *J. curcas*, *A. indica* on and *C. gloeosporioides*. Ethanolic leaf extracts (100%) of *A. indica* produced the highest radial growth inhibition of *C. gloeosporioides* (27.27%). Inhibition of Colony growth diameter of *C. gloeosporioides* by ethanolic leaf extracts of *Jatropha curcas* (7.71%) was only observed when 10% concentration of leaf extracts were inoculated. Differences in mean radial growth inhibition of test organisms were significant ( $P < 0.05$ ). Results of phytochemical screening of ethanolic leaf extracts of *A. indica*, *J. curcas*, and *T. procumbens* (Table 2) revealed the presence of phytochemicals such as saponins, tannins, flavonoids, alkaloids, terpenes and sterols in leaf extracts of *A. indica*, while anthraquinones and phlobatannins were absent.

**Table 1: Inhibitory Activity of Ethanolic Leaf Extracts of *Jatropha curcas*, *Azadirachta indica* and *Tridax procumbens* on Some Common Fungal Pathogens of Tomato (6 Days after extracts inoculation).**

Leaf extracts	Concentration (%)	% Radial growth inhibition		
		<i>C. gloeosporioides</i>	<i>F. oxysporum</i>	<i>Pythium sp.</i>
<i>J. curcas</i>	10	7.71ab	0.00	0.00
	50	0.00a	0.00	0.00
	100	0.00a	0.00	0.00
<i>A. indica</i>	10	12.40b	0.00	0.00
	50	11.30ab	0.00	0.00
	100	27.27c	0.00	0.00
<i>T. procumbens</i>	10	0.00a	0.00	0.00
	50	0.00a	0.00	0.00
	100	0.00a	0.00	0.00

Means followed by different letter within same column are significantly different (P<0.05).

In *T. procumbens*, saponins, tannins, flavonoids, alkaloids, terpenes, sterols and phlobatannins were present while anthraquinones were absent. Metabolites found in *J. curcas* included saponins, tannins, flavonoids, alkaloids, terpenes and phlobatannins while anthraquinones and sterols were absent.

#### 4. Discussion

Results presented here highlight that ethanolic leaf extracts of *A. indica* were most effective against *C. gloeosporioides*, and all tested concentrations of ethanolic leaf extracts of *A. indica* reduced radial growth of *C. gloeosporioides*. In a similar study by Shrivastava and Swarnkar (2014), it was also reported that all concentrations of ethanolic neem leaf extracts inhibited growth of *Aspergillus flavus*, *Alternaria solani*, and *Cladosporium sp.* Akpa et al. (1991) also reported a significant inhibitory property of neem extracts on mycelial growth of *Colletotrichum graminicola*. The inhibitory activity of leaf extracts of *A. indica* on radial growth of *C. gloeosporioides* observed in the study could be attributed to the presence of biologically active anti-fungal compounds in the extracts as evidenced by its wide usage in traditional medicine in different parts of the world (Benelli et al., 2017; Gao et al. 2017; Gupta et al., 2017; Heyman et al., 2017; Kamte et al., 2017; Maan et al., 2017; Paul et al., 2011; Ramos et al.,

2007). This is also supported by the work of Kumar et al. (2010) who reported anti-fungal activity of biologically active compounds isolated from the neem plant.

Phytochemicals detected in ethanolic leaf extracts of the neem plant in the reported study included; Saponins, tannins, flavonoids, alkaloids, terpenes and steroids. Prashanth and Krishnaiah (2014) also reported the presence of flavonoids, alkaloids, and absence of glycosides in neem leaf extracts. Antimicrobial activities of flavonoids, quinones, phenols, tannins, terpenoids and alkaloids, were also previously reported by Tiwari et al. (2011).

Antifungal activity of ethanolic extracts of *J. curcas* had been previously attributed to its broad spectrum of antifungal activity by Mbakwem-Aniebo et al. (2012).

#### 5. Conclusion

The study revealed *in vitro* inhibitory potentials of ethanolic leaf extracts of *A. indica* and *J. curcas* against colony growth of a plant pathogenic strain of *C. gloeosporioides*. Further studies could consider field evaluation of biological control potentials of crude extracts as well as isolated active ingredients of *A. indica* and *J. curcas* in the control of crop diseases caused by *Colletotrichum* species.

**Table 2: Phytochemical Composition of Ethanolic Leaf Extracts of Screened Plants**

Leaf extracts	Phytochemicals							
	Sap	Tan	Fla	Alk	Terp	Ster	Anthra	Phlob
<i>A. indica</i>	+	+	+	+	+	+	-	-
<i>T. procumbens</i>	+	+	+	+	+	+	-	+
<i>J. curcas</i>	+	+	+	+	+	-	-	+

Sap = Saponins, Tan = Tannins, Flav = Flavonoids, Alk = Alkaloids, Terp = Terpenes, St = Sterols, Anthra = Anthraquinones, Phlob = Phlobatannins; + = Present; - = Absent.

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**Conflict of Interest:** The authors declare that they have no competing interests.

**Author Contribution:** T.P.T. designed the experiment, conducted the statistical analyses and drafted the manuscript. A.S. completed all experiments.

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