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Fungicidal efficacy of crude extracts from some indigenous plants against fungal pathogens associated with tomato (*Solanum lycopersicum* L.) fruit rots

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Abstract
The research work was conducted in the biology laboratory of federal college of forestry Jos (latitude 7º11'N and longitude 7º38'E) in 2016. The objectives of the study were to determine the occurrence of rot fungi in Jos north and Bassa local government areas of Plateau state, to assess the fungicidal effect of crude extracts from some selected indigenous plants on two most prevalent rot fungi and to assess the best concentration of the extracts that is comparable to synthetic fungicide. The treatments were four varying concentrations (75g/l, 100g/l, 150g/l and 200 g/l) for each plant, standard fungicide (Carbendazim at 2g/l), and control laid in a Completely Randomized Design (CRD) with three replications. In general, *Azadirachta indica* gave the best reduction for radial mycelial growth (1.65 cm) on *Aspergillus niger* and (1.50 cm) on *Rhizopus stolonifer* compared to the controls (7.67 cm) for *Aspergillus niger* and (8.40 cm) for *Rhizopus stolonifer*

Keywords: Antifungal, *Aspergillus Niger*, *in vitro*, in vivo, plant exracts, *Rhizopus stolonifer*

Introduction
Tomato (*Solanum lycopersicum* Syn *Lycopersicon esculentum* Mill.) is an herbaceous plant that belongs to the family *Solanaceae*, a nightshade family that is widely cultivated for its fruits throughout the humid and subtropics (Dania and Okeye, 2017) [11]. Tomato originated from the coastal strip of South America (Agrios, 2005; Wani, 2011) [2, 39] and highlands of Peru is believed to be the centre of diversity for tomato and the herbaceous green plant with small green fruits are the progenitors of tomato based on genetic evidence (Etebu *et al*., 2013) [15]. It was introduced into West Africa by Portuguese traders and freed slaves from West Indies (Tindall, 1983) [36]. Tomato has a high consumers acceptance and demand due to its sufficient and well balanced nutrition containing minerals like potassium, zinc, magnesium e.t.c (Osemwegie *et al*., 2010) [24] and rich in vitamins A, B, C and E (John *et al*., 2010; Uke and Chiejina, 2012) [19, 38] which help to minimize the possibility of prostate and breast cancer (Giovannucci, 1999) [18]. Additionally, tomato fruit contains lycopene (red pigment) an excellent antioxidant that has attracted interest recently because of its activity against radicals that cause aging or muscular degradation, cancer and heart diseases (Uke and Chiejina, 2012; Etebu *et al*., 2013; Borghesi *et al*., 2016) [38, 15, 8]. Nigeria is the second largest producer of tomato in Africa (Erinle, 1989) [14] where a total area of one million hectares is used for tomato cultivation every year (Celis *et al*., 2014) [9]. Tomato is the second most highly consumed vegetable crop globally after potato, and it is cultivated in almost every region of the world (FAOSTAT, 2013; Pirondi *et al*., 2017) [17, 26]. Tomato is widely grown as an important commercial as well as vegetable crop in the world (Celis *et al*., 2014; Borghesi *et al*., 2016) [9, 8]. The world leading producer is China with 50,000,000 million tons, followed by India with 17,500,000 million tons, then United States of America with 13,206,950 million tons, then Turkey with 11,350,000 million tons and Egypt with 8,625,219 million tons in that order (FAO, 2012) [16].

The plant is highly adaptable to diverse environmental conditions, making it possible for its cultivation all year round in almost all parts of the world (Shenge *et al*., 2017) [30]. Farmers at all levels of production including small scale, medium and large commercial farmers were attracted by tomato because of its quick maturity and high yielding potentials. Moreover, the crop has some peculiar economic importance that includes domestic trades, exports and development of local food and agro-allied industries (Borghesi *et al*., 2016) [8].

Despite the nutritional values of tomato in human diet, the yield potentials of the crop is affected by the activities of plant pathogens (fungi, bacteria, viruses e.t.c) that often cause the crop failure and have been worldwide (Zebena *et al*., 2014; Sundin *et al*., 2016) [32, 40]. Tomato is susceptible to many destructive plant pathogens, especially more prone to postharvest fungal pathogens (Taskeen-Un-Nisa *et al*., 2011) [20]. Postharvest fruit rot diseases caused by fungal pathogens are the major share of crop losses in yield and quality in the field as well as in the storage and transport on a global scale (Saxena, 2016; Sundin *et al*., 2016) [29, 32]. Agrios (2005) [2] estimated an overall worldwide loss of 30-40% due to postharvest diseases of tomato. Wet rot disease of the fruit is one of the major postharvest rot diseases caused by *Aspergillus niger, Rhizopus stolonifer* and other rot causing fungal pathogens. The pathogens may initiate the infection in the green fruit but remain latent or quiescent till ripening of the fruits. During ripening the lesion starts to develop very fast and may cause total ripe fruit decay in the field and during post-harvest stages of storage and transport. The high moisture content, nutrient composition and pH of the ripe fruits support the growth of the fungal pathogens thereby causing rots and fruit contaminations by mycotoxins production (Amadi *et al*., 2014; Choudhury *et al*., 2017) [3, 10].
Drawbacks associated the with available control methods that affect their potency in disease management necessitate the increased interest in developing further alternative control methods, particularly those that are eco-friendly, biodegradable, feasible to the farmers, non-toxic to human and animals, specific in their action and have a broad spectrum of antimicrobial activity (Abhishek *et al*., 2013) [1].
The plants world is rich store house for natural phytochemicals which could be exploited for use as biopesticides (Satish *et al*., 2007; Pereira *et al*., 2015) [28, 25]. For example medicinal plants like *Jatropha curcas, Lantana camara* and *Azadirachta indica* extracts containing a quantum of crucial phytochemicals rich in phenolic compounds, flavonoids, tannins, saponins and alkaloids are now emerging as secure, safer and more compatible way to manage plant pathogens. All parts of these plants including flowers, roots, bark, leaves, stem, seeds and essential oils have antimicrobial qualities and/or properties and are therefore used for medicinal and other purposes (Anwar *et al*., 2007; Dwivedi and Enepsa, 2012) [5, 12]. The choice and use of these potential plants for the control of postharvest rots of tomato and other perishables is due to: (1) their safety for human consumption, (2) non environmental pollution. (3) More acceptable to the local farmers because they are indigenous (4) their biological activity and dispersion in harvested tissue (5) our ability to develop formulations that allow the delivery of non-toxic concentrations but at the same time interfere with fungal development (Bhattacharjee and Dey, 2014; Sogvar *et al*., 2016) [6, 31]. Plant extracts are the most pressing sources of natural active compounds and can be screened from local traditional plants (Mari *et al*., 2016) [31]. These plant compounds have been extracted using different solvents and are shown to acquire antimicrobial, antifungal, antibacterial properties against various pathogens (Bhattacharjee and Dey, 2014) [6].
The objectives of the present research were to determine the determine the occurrence of rot fungi in Jos north and Bassa local government areas of Plateau state, to assess the fungicidal effect of crude extracts from some selected indigenous plants Jatropha (*Jatropha curcas*), Lantana (*Lantana camara*) and neem (*Azadirachta indica*) on two most prevalent rot fungi and to assess the best concentration of the extracts that can be used in place of synthetic fungicide

Materials and methods

Study side

The research work was carried out at the biology laboratory of federal college of forestry Jos, plateau state Nigeria at a temperature of 26 ± 30ºC. The collage lies in the northern guinea savannah and is located at latitude 7º11'N and longitude 7º38'E with an altitude of 1250 m above sea level. The climate of the area ranges between (146-1480) mm and daily temperature ranges between 10ºC to 32ºC minimum and maximum respectively.

Tomato fruits

Tomato fruits at 80% maturity were purchased from central markets In Bassa and Jos North local areas of Plateau state, Nigeria. The fruits were then surface sterilized with sodium hypochlorite 1 % (v/v) rinsed three times in distilled water, dried on paper towel at 28±2˚C and used in the study.

Isolation of the Pathogens

Potato dextrose agar (PDA, Difco™, Becton, USA) was prepared 39 g/L, autoclaved at 121˚C for 15 min and used for the isolation of the fungal pathogens from infected tomato fruits. Tissue fragments (approximately 5 mm ×5 mm) were taken from the margin of the diseased fruits, sterilized with 1% NaOCl for 1 min, washed three times with distilled water and then plated on PDA medium amended with 1mg.ml^{-1} streptomycin to suppress bacterial growth and incubated at 28±2°C under normal fluorescent light for 5 days. Repeated subcultures were done until pure cultures of *Aspergillus niger* and *Rhizopus stolonifer* were obtained. The pure cultures of *Aspergillus niger* and *Rhizopus stolonifer* were maintained on PDA slants and stored for further studies. All steps were performed in laminar flow to avoid contaminations. The identity of the fungal pathogens was confirmed based on the colony morphology and spore characters.

Pathogenicity assay

To confirm the ability of isolated pathogens to cause wet rot disease in healthy tomato fruits pathogenicity assay was carried out as a confirmatory test. The tomato fruits surface sterilized with 1% sodium hypochlorite solution were used for the assay. Then the method of Okigbo and Ikediugwu (2000) [23] was employed, in which holes were made in the tomato fruits with the aid of a flamed 6 mm cork borer, and aseptically inoculated with a disc of 7 day-old cultures of *Aspergillus niger* and *Rhizopus stolonifer* isolates. The inoculated fruits were labeled accordingly and incubated at room temperature. They were observed for symptoms of wet rot like colour change, softening, characteristic foul odour, etc. A control experiment was also set up by removing the core in the fruit without introducing any organism in it except replacement with 6mm sterile PDA. Fungal isolates which caused clearly visible wet rot were considered pathogenic compared to the control. The pathogens were re-isolated, compared with original isolates and those with high pathogenicity were used as test organisms for treatment with the plant extracts.

Preparation of Plant Extracts

Extracts were obtained from leaves of Jatropha, Lantana and Neem collected from the worker's nursery federal college of

forestry Jos (W.N.F.C.F.J.) Plateau State. This extraction was performed with water as the solvent by dissolving 75, 100, 150 and 200 g of dried leaves of Jatropha (*Jatropha curcas*), Lantana (*Lantana camara*) and neem (*Azadirachta indica*) separately in 1 litre of sterilized distilled water in 2 litre Erienmeyer flasks, vigorously shaken and left for 24 h at 28±2°C. After 24 h, the mixture was filtered with two layered Muslin's cheese cloth. The obtained filtrate was then centrifuge at 6000 rpm for 15 min (Avanti J-26 XPI centrifuge, Beekman Coulter, USA). The supernatant collected was filtered again with a Whatman's No. 1 filter paper (ALBERT^{R}). The crude extracts were filtered with a sterilized 0.22 µm syringe filter (Sartouris® Syringe filters) to remove unwanted material.

***In vitro* assessment of plant extracts**

Antifungal efficacy of the crude extracts from different plants against *Aspergillus niger* and *Rhizopus stolonifer* was done using the poisoned food technique (Choudhury *et al.,* 2017) [10] at four different concentrations: 75g/l, 100g/l, 150g/l and 200 g/l mixed with plant extracts as well as Carbendazim (2g/l) and sterile distilled water as check. The mixture was gently swirled to obtain efficient miscibility of the agar and the extracts. Five-day old fungal cultures of *Aspergillus niger* and *Rhizopus stolonifer* isolates were aseptically punched with a sterile cork borer of 6 mm (0.6 cm) diameter and the fungal discs were placed at the Centre of PDA gelled plates. Perpendicular lines were drawn at the bottom of each plate and the point of intersection was taken as the Centre of the plate to ease precise measurement. The plates were incubated at 28±2°C until the control plates were filled. The diameter of the colony was recorded on daily basis by taking the measurement of the 2 paradoxical circumference of the colony growth along the lines drawn at the bottom of each plate from four replicates for each fungus. Daily radial growth was taken by subtracting the new radial increase from the initial, using a meter ruler and the difference was recorded for analysis.

In vivo assessment of plant extracts

For the in vivo bioassay of the plant extracts, the tomato fruits surface sterilized with sodium hypochlorite 1 % (v/v) were used. Cylindrical cores were made with flamed cork borer 6 mm (0.6 cm) in each healthy tomato fruit. Then, one set of fruits were treated first with plant extracts by dipping method (preventive) and another sets of fruits were first inoculated with 0.6 cm mycelial plug of the test fungal pathogens and dipped in plant extracts (curative). A standard fungicide Carbendazim 2 g/l and a control were used to compare with the treatments. The daily difference in radial mycelial growth was obtained as in the *in vitro* tests.

Experimental design and Statistical analysis

All experiments (*in vitro* and in vivo) were arranged in completely randomized design (CRD) and data were subjected to analysis of variance (ANOVA) using SPSS software (Version 23.0) and Means with significant difference were separated with Duncan's New Multiple Range Test (DNMRT).

Results

Pathogenicity studies revealed that the fungal isolates of *Aspergillus niger and Rhizopus stolonifer* tested were pathogenic to inoculated tomato considered in this study. After 10 days of inoculation, un-inoculated (control) tomato fruits were symptomless for wet rot while inoculated tomatoes were observed with wet rot symptoms. Fungal isolates (*Aspergillus niger and Rhizopus stolonifer*) were re-isolated from the infected plants not from the control to confirm Koch's postulates (Koch, 1882; Roberts and Boothroyd, 1972) [20, 27].

Brief description of the test plants, their local names, their common names, their scientific names, family they belong to and their parts used in the extraction of the crude extracts for fungicidal evaluation in the experiments were presented in Table 1. Table 2 shows the percentage prevalent of rot fungi of tomato in Jos north and Bassa Local Government areas of Plateau state, *Aspergillus niger* and *Rhizopus stolonifer* are found to be the two most prevalent pathogens than others with 40% observed in *Aspergillus niger* under Bassa Local Government area and 35% in *Rhizopus stolonifer* under Jos north area all in Plateau state.

Table 1 Brief description of the test plants

Local name	Common name	Scientific name	Family	Part used
Binidazugu	Jatropha	*Jatropha curcas*	Eupharbiaceae	Leaf
Kashinkuda	Lantana	*Lantana camara*	Verbenaceae	Leaf
Dogon yaro	Neem	*Azadirachta indica*	Meliaceae	Leaf

Table 2 Percentage prevalence of some fungal pathogens in some Local Government area of Plateau state

Pathogens	Percentage prevalence	
	Bassa (%)	Jos North (%)
Aspergillus niger	40	30
Penicillium	15	5
Microsporumaudoulnill	20	15
Pseudalleacheriaboydill	0	15
Rhizopus stolonifer	25	35

The results of antifungal efficacy of *Jatropha curcas*, *Lantana camara* and *Azadirachta indica* were presented in Table 3, which showed that for the management of *Aspergillus niger and Rhizopus stolonifer* on tomato fruits, *Azadirachta indica* gave the best reduction for radial mycelial growth for *Aspergillus niger* (1.50 cm) and *Rhizopus stolonifer* (1.65 cm) followed by *Jatropha curcas* and *Lantana camara* (3.67 cm) for *Aspergillus niger* and for *Rhizopus stolonifer*, *Jatropha curcas* recorded (3.67 cm) and *Lantana camara* (3.58 cm) compared to the control that recorded (7.67 cm) for *Aspergillus niger* and (8.40 cm) for *Rhizopus stolonifer* at $P<0.05$ and $P<0.01$. The inhibitory activity of the plant extracts may be due to direct toxic effect on the pathogens (Bhutia *et al.,* 2015; Chowdhury *et al.,* 2017) [7, 10]. The antifungal activities of the plant extracts may also be due to the presence of secondary plant metabolites like terpinoides, phenols, flavonoides, alkaloids that was earlier reported by Mohamed and EI-Hadidy (2008) [22]. The outcome of this research corroborates with earlier reports indicating the fungicidal properties of natural plant products and their potential to control plant diseases (Tunwari and Nahunnaro, 2014) [37]. The results obtained in Table 4 indicated that with an increase in the concentration of plant extracts, radial growth of *Aspergillus niger and Rhizopus stolonifer* decreases compared to the controls. The effectiveness of *Azadirachta indica* (1.33 cm) at 200 g/l is comparable to standard fungicide (Carbendazim) (1.13 cm) at 2 g/l under *Aspergillus niger* and under *Rhizopus stolonifer* 1.07 cm was observed at

200 g/l which is similarly comparable to standard fungicide (Carbendazim) (0.97 cm) at 2 g/l. It was also observed that there is no statistical difference (P<0.01) among the concentrations at 100g/l and 150 g/l for all the test plants considered under *Aspergillus niger* and among the concentrations at 100g/l and 150 g/l for *Jatropha curcas* and *Lantana camara* under *Rhizopus stolonifer* compared to the highest radial growth (8.20 cm) observed in the control under *Rhizopus stolonifer*. Earlier reports that are in consistent with the results of the present study are that of Tijjani *et al.* (2014) [34] and Chowdhury *et al.* (2017) [10] which indicated that with increase in concentration of plant extracts implied an increase in the active ingredients of the crude extracts which act on the test pathogens thereby affecting its physiological processes, lowering the growth of the pathogens.

Table 3 In-vitro effect of plant extracts on radial mycelial growth of *Aspergillus niger* and *Rhizopus stolonifer*

Treatment	Radial growth (cm)	
	Aspergillus	Rhizopus
Jatropha curcas	3.67^{b}	3.67^{b}
Lantana camara	3.67^{b}	3.58^{bc}
Azadirachta indica	1.65^{c}	1.50^{c}
Carbendazim	1.00^{d}	0.67^{d}
Control	7.67^{a}	8.40^{a}
Level of Significance	**	*
SE±	0.93	1.32

Means with different superscripts in the same Colum are significantly different.
** = Significant at 1%
** = Significant at 0.05%
SE = Standard error

Table 4 Effect of different concentration of some plant extracts on the radial growth (cm) of *Aspergillus niger* and *Rhizopus stolonifer* In-vitro

Treatment	Concentration (g/l)	Radial growth (cm)	
		Aspergillus	Rhizopus
Jatropha curcas	75	4.32^{b}	5.33^{b}
	100	3.33^{c}	3.67^{cd}
	150	3.33^{c}	3.67^{cd}
	200	2.67^{cd}	3.33^{cd}
Lantana camara	75	4.33^{b}	4.00^{c}
	100	3.00^{c}	3.17^{cde}
	150	3.00^{c}	3.00^{cde}
	200	2.27^{cd}	2.67^{de}
Azadirachta indica	75	3.00^{c}	4.00^{c}
	100	3.00^{c}	3.00^{bc}
	150	2.32^{cd}	2.67^{de}
	200	1.33^{d}	1.07^{e}
Carbendazim	2g/l	1.13^{d}	0.97^{ef}
Control	0	7.67^{a}	8.22^{a}
Level of Significance		**	*
SE±		0.93	1.32

Means with different superscripts in the same Colum are significantly different.
** = Significant at 1%
** = Significant at 0.05%
SE = Standard error

Table 5 and 6 presented the antifungal efficacy of the plant extracts with respect to different concentrations and methods of application as either preventive or curative. It was generally observed that *Azadirachta indica* leaves extracts significantly (P<0.01) reduces radial mycelial growth of *Aspergillus niger* (1.72 cm) and *Rhizopus stolonifer* (1.20 cm) under preventive methods of application and is comparable to Carbendazim 1.33 cm and 1.07 cm for *Aspergillus niger* and *Rhizopus stolonifer* respectively. *Azadirachta indica* gave better radial mycelial growth on *Rhizopus stolonifer* (1.07 cm) under preventive control method (Table 5). It was also observed that application of *Azadirachta indica* leaves extracts at 200g/l gave the best reduction for radial mycelial growth on *Aspergillus niger* (1.00 cm) under preventive control method and is comparable to standard fungicide (Carbendazim) (0.99 cm). Application of plant extracts at varying concentrations reduced wet rot disease under preventive method of control better than under curative method of control. This is probably as a result of the microbes being killed on exposure to a higher concentrations of these plant extracts when the inoculum was introduced on the treated parts of the tomato (i.e under preventive) which inhibit their ability to establish nutritional relationship (infection) that will subsequently enable the pathogen to get nourishment or nutrient required for its growth and development. This is in agreement with the reports of Tijjani *et al.* (2010) [35] on the use of *Moringa oleifera* and Neem seed extracts to control wet rot disease on irish potato caused by *Rhizopus stolonifer* and the report of Amienyo *et al.* (2007) [4] on the use of *Zingiber officinale*, *Annona muricata*, *Gacinia cola*, *Alchornea cordifolia* and *Allium sativum* to control wet rot on sweet potatoes caused rot fungal pathogens.

Table 5 Effect of different plant extracts and method of control on radial growth of *Aspergillus niger* and *Rhizopus stolonifer* in-vivo

Treatment	Radial growth (cm)			
	Aspergillus		Rhizopus	
	Curative	Preventive	Curative	Preventive
Jatropha curcas	3.58^{b}	4.17^{b}	3.08^{b}	1.67^{b}
Lantana camara	3.33^{bc}	3.67^{c}	3.00^{bc}	1.67^{b}
Azadirachta indica	2.42^{d}	1.72^{d}	2.67^{d}	1.20^{c}
Carbendazim	1.67^{e}	1.33^{de}	1.33^{e}	1.07^{c}
Control	8.00^{a}	8.00^{a}	7.83^{a}	7.33^{a}
Level of Significance	**	**	**	**
SE±	0.68	0.80	0.74	0.55

Means with different superscripts in the same Colum are significantly different.
** = Significant at 1%
** = Significant at 0.05%
SE = Standard error

Table 6 In-vivo effect of different concentration of plant extracts and method of control on radial growth of *Aspergillus niger* and *Rhizopus stolonifer*

Treatment	Concentration (g/l)	Radial growth (cm)			
		Aspergillus		Rhizopus	
		Curative	Preventive	Curative	Preventive
Jatropha curcas	75	6.00^{bc}	4.27^{b}	3.67^{b}	2.67^{b}
	100	4.33^{e}	3.33^{c}	3.00^{bc}	2.00^{bc}
	150	3.67^{f}	3.00^{cd}	3.00^{bc}	1.67^{d}
	200	3.33^{fg}	2.30^{ef}	2.67^{bcd}	1.55^{de}
Lantana camara	75	6.67^{b}	3.57^{c}	3.33^{bc}	2.33^{bc}
	100	6.33^{bc}	3.33^{c}	3.00^{bc}	2.04^{bcd}
	150	6.33^{bc}	3.00^{cd}	3.00^{bc}	1.67^{d}
	200	3.67^{f}	2.67^{e}	$2..47^{bcd}$	1.33^{e}
Azadirachta indica	75	5.67^{d}	2.67^{e}	2.45^{bcd}	2.33^{bc}
	100	4.67^{e}	2.33^{ef}	2.34^{bcd}	1.33^{e}
	150	3.67^{f}	2.33^{ef}	2.34^{bcd}	1.33^{e}
	200	2.00^{h}	1.33^{g}	1.52^{e}	1.00^{f}
Carbendazim	2g/l	1.76^{h}	1.33^{g}	1.33^{e}	0.90^{f}
Control	0	7.67^{a}	7.67^{a}	8.33^{a}	7.33^{a}
Level of Significance		**	**	**	**
SE±		0.60	0.81	0.38	0.60

Means with different superscripts in the same Colum are significantly different.
** = Significant at 1%
** = Significant at 0.05%
SE = Standard error

Conclusion

Based on the present study, it was discovered that there was significant difference in the potency of the extracts against the test pathogens at the *in vitro* and in vivo levels compared to their controls. The variation in the activity of the extracts at both levels of tests was due to possible toxic effect of the quantum phytochemicals present in the extracts. Generally, the results showed that the botanicals possess antifungal activity and have the potentials for exploitation and utilization as bio-control agents in the fight against wet rot of tomato. Therefore, due to the fact that chemical control of disease is environmentally hazardous and very expensive, this inexpensive, non-hazardous and biodegrable plant material could be used as an alternative way of reducing and controlling rot disease by farmers to increase tomato production in many developing countries, where tomato is common vegetable crop.

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