IN VITRO EFFECT OF THREE AQUEOUS PLANT EXTRACTS ON FUNGI ASSOCIATED WITH POST–HARVEST ROT OF PEPPER (*Capsicum* species L.) IN YOLA, ADAMAWA STATE, NIGERIA

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Abstract: The effect of aqueous extracts of *Azadirachta indica*, *Tridax procumbens* and *Vernonia amygdalina* on fungi causing post-harvest pepper (*Capsicum* spp. L) Fruit Rot in Yola, Nigeria was investigated. Rotted pepper fruits were randomly sampled from five markets in Yola. Fungi associated with the pepper rots were isolated using Potato Dextrose Agar (PDA) culture medium. *Aspergillus flavus*, *Aspergillus niger*, *Botrytis cinerea*, *Colletotrichum capsici* and *Phytophthora capsici* were the fungi found associated with the rots of pepper in the study area. The test plants ingredients used were extracted using sterile distilled water. 20%, 40%, 60% and 80 % concentrations of the test plants were added to PDA prior to inoculation. The effects of the extracts were proportional to concentrations and vary among the fungi. Inhibition percentage at the highest concentration (80%) revealed that *Azadirachta indica* and *Tridax procumbens* was more effective on *Phytophthora capsici* (80.93% and 71.02%, respectively), while *Vernonia amygdalina* was more effective on *Aspergillus flavus* (79.80%). Analysis of Variance (ANOVA) showed significant differences in the inhibition of mycelial growth among the extract used. Phytochemical analysis of the test plants extracts was found to contain alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, anthracenes, and steroids. The plants used were very cheap to acquire, readily available all seasons and environmentally safe. Therefore, may be considered promising and safe for protecting pepper fruits from rotting.

Keywords: Post-harvest spoilage, Fungi, Pepper fruit rot, plant extract, phytochemical analysis

Introduction

Pepper is one of the top five most important vegetable crops in Nigeria (Olowu and Onyemelukwe, 2001). Pepper consumption in Nigeria accounts for 40 percent of the total vegetable consumed per day (Grubben and Tahir, 2004). In many Nigerian diets, pepper accounts for a large source of vitamins A and C andis utilized in the dry state as spice, it contains alkaloid, which are digestive stimulant that is used in ointment treatment of arthritic and neuropathic pains (Grubben and Tahir, 2004). The major diseases of most peppers in the world are phytopathogenic fungi, bacteria, and viruses (Grubben and Tahir 2004; Melanie and Sally, 2004). Fungal diseases of pepper have been reported. Anthracnose or fruit rot caused by *Colletotrichum gloeosporioides* and to a lesser degree *Colletotrichum capsici* which may cause yield losses of up to 50% have been reported (Jeffries *et al*., 1990; Isaac, 1992). *Phytophthora* blight (crown rot or basal stem rot) caused by *Phytophthora capsici*, have also been reported (Bosland and Lindsey 1994; Ristaino and Johnson 1999). *Botrytis* fruit rot (gray mold) caused by the fungus *Botrytiscinerea* was reported as serious disease of pepper worldwide (Vagelas *et al*., 2009). Other fungi that are associated with fruit rot of pepper include *Fusarium* and *Verticillium dahliae* (Grubben and Tahir, 2004). *Aspergillus niger, Aspergillus flavus, Penicillium digitatum and Verticillium* spp. (Balogun *et al*., 2005).

The search for cultivars resistant to the major diseases and postharvest spoilage of pepper has been limited (Kiran *et al*., 2006). Awareness about the risks involved in the use of synthetic fungicides is a major concern to plant pathologist globally (Okigbo, 2009). Searching for harmless alternative method of pathogen control is necessary. Higher plants contain secondary compounds that could effectively control plant diseases, but which are yet to be exploited and used as pesticides (Kurucheve *et al*., 1997). Although there is a growing interest in the use of medicinal plants to control plant diseases, only about 20% of the plants found in the world have been subjected to pharmacological or biological test (Mothana and Lindequist, 2005).

In view of that, this study aimed at testing the efficacy of *Azadirachta indica*, *Tridax procumben*s and *Vernonia amygdalina* extracts on fungi associated with rot of pepper fruits in Yola, Adamawa State, Nigeria.

Materials and Methods

The study area

The study was conducted in Yola, Yola North Local Government Area of Adamawa State. Yola lies between latitudes 9°11′N to 9°19′N and longitudes 12°20′E to 12°30′E (UBRBDA, 1999). Yola has a tropical climate marked by dry and rainy seasons. The rainy season starts around May and ends in late October while the mean total rainfall is 1,113.3 mm (UBRBDA, 1999). Maximum temperature in Yola is about 40° C in April, while minimum temperature could be as low as 18.3° C between December and early January while relative humidity is lowest (26%) in month of January and February and increases to 58, 69, and 79% in May to July, respectively (UBRBDA, 1999). The study was conducted during the months of July, 2014 to April, 2015.

Sample collection

A total of 250 pepper fruits showing deterioration and rotting were randomly selected and purchased from different markets in Yola, Adamawa State, Nigeria. The markets include; Jimeta modern market, Jimeta shopping complex market, Phalluja market, Lake Gerewo market and Jambutu market. Fresh and healthy peppers were also purchased and packed into sterilized polythene bags and were taken to the Plant Science laboratory, Modibbo Adama University of Technology (MAUTECH), Yola for isolation and other studies.

Isolation and identification of fungi

The method of Balogun *et al*. (2005) was employed for the isolation; rotted portion of the pepper fruits were cut under aseptic conditions into small bits with the aid of scissors which was flamed over a Bunsen burner flame and dipped

inside methylated spirit (Balogun *et al*., 2005). The small bits were sterilized with 70% ethanol and placed on Petri dishes containing solidified PDA. The inoculated plates were incubated at room temperature (28 ± 2°C) in the dark for 72 h. The fungal colonies grown from the incubated plates were sub-cultured into fresh PDA medium until pure culture was obtained. Fungi were identified based on their colony characteristics in the culture media and structures under microscope with reference to the identification schemes of Snowdon (1990) and Watanabe (2002).

Pathogenicity test

To ascertain whether the fungi isolated from the rotted pepper fruits were the causal agent of the rot or not, pathogenicity test was conducted adopting the procedures as described by Balogun *et al*. (2005).Apparently healthy matured pepper fruits, that is; Bell pepper (*Tattase*), Hot pepper (*Atarubu*) and Chilli pepper (*Borkonondogo-na-mara*) were surface sterilized with 0.5% sodium hypochlorite for 5 min and then rinsed in three changes of sterile distilled water. With a 5 mm diameter flame-sterilized cork borer, cylindrical cores were removed from each fruit which were then inoculated aseptically with 5 mm diameter disc from the advancing edge of 7-days old fungal culture of any one isolate. Vaseline jelly was smeared to completely seal the surface of each of the inoculated pepper fruit to prevent external infection before incubating for 10 days. The controls were inoculated with disc of clean solidified PDA medium. Fruits were inoculated in three replicates. Rot symptoms developed with different fungal isolates were compared to the natural original rot.

Preparation of plant extracts

The following local plants: *A. indica* (Neem), *T. procumbens* (*Tridax daisy*) and *V. amygdalina* (Bitter leaf) were air-dried and grinded separately, thirty gram of each sample was added to 100 ml of distilled water in separate flasks (Zakari *et al*., 2015). This was vigorously stirred and left to stand for 24 h. The sample was filtered with a Whatman paper (No 1) and the filtrates were diluted to give 20%, 40%, 60% and 80% concentrations of the extracts (Zakari *et al*., 2015).

Evaluation of the efficacy of the test plant extracts on fungi isolates

The methods of Ijato (2011) and Zakari *et al*. (2015) were used to evaluate the efficacy of the extract on fungal growth in culture media. This was done by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The centre of the plates indicated the point of intersection of the inoculums. This was done before dispensing the PDA into the plates. The extracts were poured into the flask plugged with cotton wool and heated for about 10 minutes to avoid contamination. Exactly 2 ml of the extract of various dilution percentages were separately introduced into the Petri-dish containing 10 ml of the PDA. Each plate was inoculated with 5 mm plug of pure isolate taken from margins of actively growing culture of fungi. The plates were incubated at 25° ± 2°C. The control plates were only added with equal quantity of distilled water. Mycelial growth diameter of each isolates was measured and recorded when the growths of the isolates were completed in the control treatment. Each treatment was repeated three times. Mean radial mycelial growth of each isolate was recorded and data were transformed into inhibition percentage using the following formula.

$$\text{Inhibition percentage (\%)} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100$$

Where DC - Average Diameter of fungal spore in control.
DT - Average diameter of fungal spore with treatment.

Phytochemical analysis

The extracts were analyzed by the following procedures Harbone (1973); Sofowora (1993); Trease and Evans, (1989) to test for the presence and quantities of the alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, reducing sugars etc.

Statistical analysis

Differences between means of inhibition of radial mycelial growth were analyzed using analysis of variance (ANOVA) and means that were significant were separated using Fisher's protected LSD test ($P<0.05$) with completely randomized design (CRD).

Results and Discussion

Five pathogenic fungi were isolated from the rotted pepper fruits in Yola. The fungi were identified as *Aspergillus niger, Aspergillus flavus, Botrytiscinerea, Colletotrichum capsici, Phytophthora capsici* based on their colonial and morphological characteristics with reference to identification schemes of Snowdon (1990) and Watanabe (2002). These fungi have previously been reported as fruit rot pathogens of pepper fruits (Grubben and Tahir, 2004; Voorrips *et al*., 2004; Sharma *et al*., 2005; Pakdeevaraporn *et al*., 2005; Nduagu *et al*., 2008; Than *et al*., 2008; Ekefan *et al*., 2009; Zakari *et al*., 2015).

Aqueous extracts of the leaves of *Azadirachta indica, Tridax procumbens* and *Vernonia amygdalina* were effective against all the five fungi isolated. The effects were directly proportional to the concentration of the extracts and vary among the five fungi isolated (Tables 1 – 3). These plant extracts were previously reported to have fungicidal activity against many fungal isolates including the ones isolated in this study (Wafaa *et al*., 2007; Zakari *et al*., 2015). Winee *et al*. (2013) used *Azadirachta indica* extract against *A. niger* and were found effective. In another study, Nduagu *et al*. (2008) control *Colletotrichum capsici* using leaf extract of *Azadirachta indica* and*Vernonia amygdalina* in Makurdi, Nigeria. Sahu *et al*. (2012) also reported *Azadirachtaindica* extract reduced mycelial growth of *Phytophthora capsici in vitro* in Nigeria. Aditi*etal*. (2011) control *Aspergillus niger* and *Phytophthora capsici* using aqueous extracts of the leaves of *Azadirachta indica* in India. Varahalarao (2012) used *Tridax procumbens* to control *Aspergillus niger* and *Aspergillus flavus in vitro*.

Percentage inhibition of fungal growth revealed that, 80% of all the tested plants had higher inhibitory effect of 79.80% on *A. flavus*, 71.02% on *P. capsici* and 80.93% on *P. capsici*, respectively (Table 1 – 3). Similar observation was reported (Ijato *et al*., 2011; Zakari *et al*., 2015). While Ijato *et al*. (2011) used 80% aqueous extracts of *Vernonia amygdalina* and *Tridax procumbens* to reduced the growth of *Geotrichum candidium* to 49.20% and *Aspergillus niger* to 53.30% *in vitro* respectively, Zakari *et al*. (2015) used 80% ethanol extracts as against aqueous extracts in this study of *Vernonia amygdalina* and *Tridax procumbens* and found more effective than ones in this study. These differences could be due to differences in extraction method or differences in the medium used, as 30 g of extract per 150 ml of water was used by Ijato *et al*. (2011) and 100 g of extract per 1000 ml of water was used by Sahu *et al*. (2012) as opposed to 30 g of extract per 100 ml of water in this study.

Table 1: Percentage inhibition of fungi isolates incorporated with aqueous plant extracts of *Azadirachta indica*

Concentration	Growth Inhibition (%)				
	A. niger	*A. flavus*	*P. capsici*	*C. capsici*	*B. cinerea*
20%	23.31	23.98	40.47	23.64	21.68
40%	28.85	40.94	55.49	45.94	32.17
60%	35.97	52.05	72.25	62.83	43.43
80%	40.69	67.18	80.93	72.97	55.25
Control	0.00	0.00	0.00	0.00	0.00

Note: Data were measured in millimeters but transformed into percentage inhibition.

Table 2: Percentage inhibition of mycelia growth of fungi isolates incorporated with aqueous plant extracts of *Tridax procumbens*

Concentration	Growth Inhibition (%)				
	A. niger	*A. flavus*	*P. capsici*	*C. capsici*	*B. cinerea*
20%	33.21	27.90	44.89	34.39	20.52
40%	46.95	31.97	51.71	48.40	20.52
60%	58.40	43.02	55.11	67.51	25.26
80%	64.50	50.58	71.02	69.43	35.93
Control	0.00	0.00	0.00	0.00	0.00

Note: Data were measured in millimeters but transformed into percentage inhibition.

Table 3: Percentage inhibition of mycelia growth of fungi isolates incorporated with aqueous plant extracts of *Vernonia amygdalina*

Concentration	Growth Inhibition (%)				
	A. niger	*A. flavus*	*P. capsici*	*C. capsici*	*B. cinerea*
20%	47.73	34.00	20.00	10.19	23.12
40%	56.82	59.61	21.18	21.65	29.37
60%	66.81	65.52	52.22	33.76	39.37
80%	70.00	79.80	74.94	52.87	56.87
Control	0.00	0.00	0.00	0.00	0.00

Note: Data were measured in millimeters but transformed into percentage inhibition.

The result from Phytochemical analysis showed that the aqueous leaf extract of the test plants contains saponins, tannins, glycosides, alkaloids, terpenes, flavonoids and reducing sugars among other secondary metabolites. Alkaloids, anthracenes and steroids were not detected from the aqueous extract of *Tridax procumbens* while glycosides were not detected from the aqueous extracts of *Azadirachta indica* and *Tridax procumbens* (Table 4). This is in agreement with the report of Biu *et al.* (2009) where the aqueous extract of *Azadirachta indica*leaf contains pentosis and carbohydrates in addition to those chemical constituents detected. Quantitative analysis of the plant leaf extracts also revealed a greater proportion of Phenols, moderate concentrations of tannins, while flavonoids, alkaloids, glycosides and saponins were in low concentrations. Terpenoids and anthracenes were not quantified (Table 5). The presence of these phytochemical components may be responsible for the observed fungicidal activity of the plant extracts. This finding conforms to the report of Anyanwu and Dawet (2005) and that of Gbadamosi *et al*. (2012) where similar constituents was found to exhibits antiprotozoal, antibacterial and fungicidal activities respectively. Another phytochemical analysis of the leaf extract of *Azadirachta indica* was reported by Imran *et al.* (2010) where petroleum ether, chloroform and methanolic extracts was found to contain only glycosides, triterpenes and fatty acids in relatively higher quantities. This could explain why the low or absence of some secondary metabolites in this study as aqueous solvent was used in extraction of the plant extracts against other solvents.

Table 4: Qualitative determination of phytochemical groups of extract of test plant leaves

Phytochemical	Plant leaves		
	Azadirachta indica	*Tridax procumbens*	*Vernonia amygdalina*
Alkaloids	+	–	+
Anthracenes	+	–	+
Flavonoids	+	+	+
Glycosides	–	–	+
Phenols	+	+	+
Saponins	+	+	+
Steroids	+	–	+
Tannins	+	+	+
Terpenoids	+	+	+

+ = Present; – = Absent

Table 5: Quantitative determination of phytochemical groups of extract of test plant leaves

Phytochemical	Plant Materials		
Concentration (%)	*A. indica*	*T. procumbens*	*V. amygdalina*
Alkaloid	0.86	0.09	0.98
Anthracenes	NA	NA	NA
Flavonoids	0.43	0.56	0.68
Glycosides	NA	NA	1.18
Phenols	45.33	15.45	26.27
Saponins	0.99	1.43	1.92
Steroids	NA	NA	1.0
Tannins	34.86	10.34	20.28
Terpenoids	NA	NA	NA

NA = No Action

Conclusion

In conclusion, this study revealed that *Aspergillus niger*, *Aspergillus flavus*, *Botrytiscinerea*, *Colletotrichum capsici*, *Phytophthora capsici* were fungi associated with pepper rots in Yola. That the test plants; *Tridax procumbens*, *Azadirachta indica*, and *Vernonia amygdalina* have strong fungicidal properties against the fungal isolates from rotted pepper fruits in Yola. That the active ingredients of the test plants can be extracted even with water and yet be effective. That even at the lowest concentration (20%) of the original extracts can inhibit mycelial growth of fungi. The presence and indeed higher quantity of phenols and moderate concentrations of tannins is an indication that these two important secondary metabolites are responsible for the fungicidal activities of the extracts observed *in vitro*. These findings are very important in controlling pepper rot pathogens since unlike chemical control this would have better results as they are biologically based and environmentally safe.

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