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Control of Postharvest Loss of Tomato fruits Caused by *Fusarium verticilloides (Saccardo) Niremberg* with Aqueous Leaf Extracts of *Azadirachta indica. Juss* and *Vernonia amygdalina Del*

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ABSTRACT

This study was carried out to determine the effect of leaf extracts of *Azadirachta indica* and *Vernonia amygdalina* on the control of post harvest deterioration of tomato fruits caused by fungi. The extracts were applied at 5% and 10% w/v against *Fusarium verticilloides*, one of the rot causing fungi in tomato fruits, *in-vitro* in a completely randomized experimental design. The effect of the extracts on the mycelia growth diameter of the fungus was measured following two perpendicular lines passing through the centre of the culture on plates. The extracts were later applied at 10%w/v on tomato fruits inoculated with the fungus and the weight loss (a deterioration parameter) was recorded over time. The mycelia growth of the fungus was significantly ($p < 0.05$) reduced by the plant extracts. Compared with the control, the plant extracts performed better at 10% w/v than at 5% w/v. Mean weight loss of the treated fruits was significantly ($p< 0.05$) reduced throughout the period of the experiment compared with the untreated fruits and the control. Neem leaf extract was more effective. Palatability test conducted on the treated tomato fruits showed that there was no significant difference in the taste of the treated fruits and those not treated suggesting that both plant extracts can be used for the preservation of tomato fruits to prolong its shelf life.

Key words: Bitter leaf extract, *Lycopersicon esculentum*, Neem leaf extract, Palatability, Post harvest loss.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetable crops grown in Nigeria. The areas of production lie between latitudes 7.5ºN and 13ºN mostly in the North and South Western parts of the country (Denton and Swarup, 1983). Tomato has been in cultivation in Nigeria for a very long time. It is an important condiment in most diets and a very cheap source of vitamins A and C. Although a large amount of this vegetable is produced annually, its availability all year round is limited due to the attending high incidence of pest and diseases as well as poor storage facilities. Kutama *et al*. (2007) estimated a total loss in Nigeria due to these constraints of about 60%. About 21% of tomato harvested in Nigeria were lost to rot in the field and additional 20% to poor storage system, transportation and marketing (Opadokun, 1987). Microorganisms penetrate the fruits through wounds from handlers, farm equipment, wash water and soil and air borne aerosols. Decay caused by pathogens has been reported to constitute up to about 60% of losses in fresh tomatoes produced in Nigeria (Kutama *et al*., 2007).

Fresh tomato fruits are vulnerable to post-harvest losses. They are highly perishable with a short shelf-life and high susceptibility to fungal diseases. They are difficult to store for long periods without incurring losses and as the fruits ripen, they become more susceptible to microbial infections (Mujib ur Rehman *et al*, 2007; Ewekeye *et al*. 2013).

Prolonged shelf life of tomato fruits is essentially achieved by careful harvesting and handling of fruits, optimization of storage environmental conditions and the use of chemicals which are available for the control of pathogens that affect stored products. Most of the chemicals are synthetic in nature. Elucidating non-synthetic chemicals such as botanicals and other natural products in the control of post harvest losses is becoming increasingly important.

Control of post harvest losses in crops especially those caused by fungi has presented different problems which include hazardous effect of fungicides to man and the environment, development of resistance by fungi to synthetic fungicides, unaffordable cost of the chemicals to local farmers and in the recent times, the increasing demand by consumers for produce with no chemical residues (Tripathi and Dubey, 2004). It is therefore necessary to develop alternatives to synthetic chemical control to reduce

environmental risks and raise consumer's confidence.

Natural substances especially botanicals with antimicrobial properties might be potentially useful in this respect. The plant must be easy to get and widely acceptable to consumers. Leaves of Neem *(Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) plants possess some anti-microbial properties against a wide range of pathogenic bacteria and fungi (Bompeix and Cholodowski-Faivre, 2000). They are also of great therapeutic and medicinal benefits to man, hence they tend to be generally acceptable as preservatives by rural communities.

This study was therefore carried out to isolate and identify fungi associated with deterioration of tomato fruits, to test the pathogenicity of the identified fungi and to evaluate the effects of aqueous extracts of leaves of Neem *(Azadirachta indica*) and bitter leaf (*Vernonia amygdalina)* plants on the control of post harvest losses in tomato caused by the fungi *in-vitro* and *in-vivo*.

MATERIALS AND METHODS

Source of Tomato Fruits.

The tomato fruits used for this study were obtained from various food crop markets in Ilorin, Kwara state. The markets include Oko Olowo, Ipata, Oja Tuntun and Oja Oba. The fruits were purchased fresh. Rotting tomato fruits were also obtained from the same sources to serve as source of fungal inoculi.

Preparation and sterilization of culture medium.

Potato Dextrose Agar (PDA) medium was prepared by dissolving and melting 38g of the substance in 1 litre of distilled water. The mixture was then sterilized in an autoclave at 1.1kg/cm pressure and 121ºC for 1 hour.

Isolation and identification of fungi from rotten tomato fruits

Agar plate method developed by the International Seed Testing Association (ISTA, 1996) with slight modification was used to isolate the fungal organisms from the tomato fruits. The tomato fruits were first surface sterilized in 0.5% (NaOCl) solution for 30 seconds and rinsed with several changes of sterile distilled water. Small segments of tissues (2mm²) from the margin of rotted areas were cut using sterile scalpel and transferred into a previously prepared streptomycin-amended Potato Dextrose Agar (PDA) plates. The plates were incubated at 25±2^{o}C in

alternating cycles of 12hours of light and 12hours of darkness for 5-7 days and the isolated fungal colonies were purified by sub-culturing. Morphological and microscopic characteristics of the pure cultures were used for identification of the isolates following standard references (Barnett and Hunter, 1972; Kulwant *et al*., 1991; Malone and Muskett, 1997). The colony morphology used include colour of spores, presence or absence of pigmentation, elevation and nature of mycelia. Microscopic characteristics used for the identification include the type and shape of asexual and sexual spores, presence or absence of cross walls in hyphae, presence or absence of sterigmata and the sporangiophores. Confirmation of the identity of the isolates was carried out at the Pathology Laboratory of the International Institute of Tropical Agriculture, IITA, Ibadan.

Test of Pathogenicity of the Isolated Fungi

The method of Ewekeye *et. al* (2013) was used with slight modification. Mature, ripe and healthy tomatoes were surface sterilized in 0.5% NaOCl for 30 seconds and rinsed thoroughly in sterile distilled water. One side of each of the fruit in all three replicates was carefully punctured with sterile 5mm cork borer to exceed the epidermal tissue layer of the fruits. A two millimeter diameter inoculum disc of the fungal isolates to be tested was inoculated into the holes using sterile inoculating needle. The holes were later sealed with candle wax to prevent contamination by opportunist microorganisms. Un-inoculated tomato fruits (control) were also set up (Okigbo and Emeka, 2010).

All the treatments were placed separately in previously sterilized desiccators and kept at room temperature for 12 days. The rate and pattern of infection, fruit depreciation and severity were observed.

Preparation of extracts from *Azadirachta indica* and *Vernonia amygdalina* leaves.

The leaves were rinsed in sterile water and air- dried. Five hundred grams of each leaf was mixed with 500ml of sterile water in a 2 litre volumetric flask and the mixture was thoroughly homogenised for 10 minutes in a warring blender. The mixture was later sieved with sterile muslin cloth (three folds). The liquid obtained in each case was centrifuged at 5000 rpm for 30 minutes, after which the supernatants were decanted off and the sediments were collected for use (Lalitha *et. al.* 2011). Treatment solutions of 5%w/v and 10% w/v concentrations of each aqueous extract were prepared by dissolving 5g and 10g of the sediments in each case in 100 ml of the solution respectively.

Determination of *In-vitro* effects of Aqueous Extracts on the growth of Fungal Isolates.

The fungicidal property of fresh aqueous leaf extracts of *Azadirachta indica* and *Vernonia amygdalina* were evaluated *in-vitro* using a pathogenic fungus isolated from the tomato fruit, in this case, *Fusarium verticilloides*. The fungus was grown on PDA amended with the two plant extracts at both concentrations in 9cm diameter petri dishes. The PDA medium contained 10% of the plant extract amendment. The plate was then inoculated with 5mm diameter mycelia disk from 15-day-old culture of the organism. PDA medium not amended with the plant extracts served as the control while those amended with Carbendazim applied at 1g/litre served as a check. The plates were all incubated at 25 ± 2^0C under alternating cycles of 12 hours of light and darkness for 13 days. The diameter of the test organisms was measured at 5, 7, 9, 11 and 13 days after inoculation following two perpendicular lines passing through the centre of the organism. Percentage inhibition of the fungal growths was calculated from the result viz;

Percentage inhibition = dt – de / dt x 100 where dt (cm) = diameter of fungal growth in petri-dishes with un-amended medium, de (cm) = diameter of fungal growth in petri-dishes with plant extract amended medium (Wokocha and Okereke , 2005) .

Effect of the Aqueous Plant extracts on Deterioration of Treated Tomato Fruits

Healthy fruits were first surface sterilized by dipping them in 70% ethanol. The fruits were later wounded slightly on the surface with Carborundum powder. The fruits were then soaked in the plant extracts (*in-vitro* experiment previously conducted showed 10%w/v of the extracts to be more suitable) for thirty minutes. This was followed by spraying of the fruits with mycelia suspension of the test fungus prepared by comminuting ten 5mm mycelia disc from 7-day-old culture of the isolate in 100ml of sterile water. The fruits were then placed in previously sterilized desiccators and incubated at ambient temperature for ten days. Rate of deterioration and weight loss in the tomato fruits were determined. Rate of deterioration was estimated by visual observation and weight loss was measured using digital weighing balance at days 2, 4, 6, 8 and 10 after treatment application. Tomato fruits in the control were neither treated with the plant extracts nor inoculated with the pathogens. It is assumed that the fungal inoculi will accelerate metabolism and weight loss of inoculated fruits.

Palatability test of treated tomato

Twenty (20) panel members consisting of staff and students of the University of Ilorin were randomly selected for the sensory evaluation of the boiled tomato fruits that were treated. Each cooked samples was determined for colour, taste, and overall acceptability as described by Ihekoronye and Ngoddy (1985). The sample was evaluated on a 7–point hedonic scale (1 = disliked very much, 2 = disliked much, 3 = disliked moderately, 4 = neither liked nor disliked, 5 = liked moderately, 6 = liked and 7 = liked very much) in the mid morning (11.00 a.m.) in a sensory evaluation laboratory under white light. Samples were presented in 3 digits code in plates. The order of presentation of the sample to the judges was randomized. Untreated cooked tomato fruits were used as control.

Statistical Analysis

All data collected were subjected to statistical analysis using Genstat Statistical Package (Discovery Edition 3) and significant differences were separated at 5% level of probability using Least Significant Difference (LSD).

RESULTS

Isolation and identification of fungi from rotten tomato fruits

Two fungal species, *Aspergillus flavus* and *Fusarium verticilloides* were isolated from the deteriorating tomato fruits used during the experiment. The observed morphology and microscopic characteristics of the isolates as they tallied with those described in authoritative identification literatures is shown in Table1.

Pathogenicity Test

The pathogenicity tests conducted on the fungal isolates showed that only *Fusarium verticilloides* induced similar disease symptoms as seen on original rotting fruit samples used for the isolation when inoculated on healthy tomato fruits.

***In-vitro* effects of Aqueous Extracts on the growth of Fungal Isolates**

The plant extracts at both concentrations (5%w/v and 10%w/v) significantly reduced growth of the test fungus ($p< 0.05$) compared to the control. Figures 1 & 2 shows the change in the mycelia diameter of the test fungus with time after inoculation at both concentrations. There was increase in the mycelia diameter with increase in the number of days after inoculation and a decrease with increase in the concentration of the extracts. The percentage reduction in mycelia growth of the test fungus however increased with increase in the concentration of the plant extracts. At day 5 after inoculation, there was an increase in the percentage reduction of mycelia growth of the test fungus from

11.1% to 16.7% and 38.9% to 44.4% as the concentration of the extracts increased from 5%w/v to 10%w/v in bitter leaf and neem respectively. At day 7 after inoculation, the values changed from 12% to 20% and 40% to 52% accordingly as concentration changed from 5%w/v to 10%w/v of bitter leaf and neem respectively. The trend changed from day 9 after inoculation as there was no further remarkable increase in the effect of the plant extracts and even the synthetic fungicide used as a check (Table 2).

Effect of plant extracts on deterioration of treated fruits

Table 3 shows the mean weight loss of tomato fruits treated with the inoculi of the test fungus and the aqueous extracts (applied at 10%w/v). Mean weight loss of the fruits was significantly different ($p<0.05$) for the plant extracts. Mean weight loss of tomato increased with increase in the time of incubation and it was highest in untreated tomato followed by those treated with bitter leaf and then neem leaf extract compared to the control. The mean weight loss of the fruits at day 10 after treatment application was 14.7, 12.9, 19.3 and 15.2 for bitter leaf, neem, untreated and control respectively. This showed that neem leaf extract applied at 10%w/v performed better than bitter leaf extract at the same concentration in controlling post harvest deterioration of tomato caused by *Fusarium verticilloides*.

Palatability test on treated fruits

The result of sensory test conducted for the extract treated tomato fruits is given in Table 4. The lowest colour ratings of 5.9 and 6.7 were recorded from samples treated with *Azadirachta indica* and *Vernonia amygdalina* respectively. The result showed that the panelist preferred the colour of the untreated control as it appeared to be the most acceptable in terms of colour having recorded the score of 8.9. This could be due to the fact that the extracts made the color of the tomato fruits to be dull and less attractive. It is assumed that reactions between the carbonyl compounds and proteins are mainly responsible for the colour distortions. The result of the rating as regards taste indicate that lowest ratings of 6.9 and 7.6 were from the samples treated with extracts of *Azadirachta indica* and *Vernonia amygdalina* respectively. The control samples had the best rating of 8.4, although the values were not significantly different from each other. The result also showed that although there was no significant difference in the values, the control was the best in overall acceptability (9.6), followed by *Vernonia amygdalina* (8.9) and *Azadirachta indica* treated tomato fruits (7.3).

DISCUSSION

Aspergillus spp. and *Fusarium* spp. have been observed to be among the storage fungi affecting tomato fruits (Mullen, 2005). Suleiman (2011), isolated *Aspergillus viridae, Penicillium digitatum* and *Rhizopus* sp. from tomato in Kogi state and Ewekeye *et al.*, (2013) isolated *Aspergillus* spp., *Fusarium* spp. and other fungi from deteriorating fruits and vegetables in Lagos state, Nigeria. The fruits and vegetables provide food, high moisture content and low pH for the fungi. This encourages their growth and reproductive activities leading to rot thereby making them unfit for consumption. The variation in the type of organisms isolated from tomato fruits in this work and the previous work by Suleiman, (2011) might be as a result of the use of different tomato varieties and slight variation in the isolation procedures used. Other forms of post harvest damage that can result from fungal infection of produce include discoloration, loss of viability in seeds, heating and mustiness, biochemical changes and production of mycotoxins (Narong, 2003). *Aspergillus flavus* for instance produces 'aflatoxin' on many grains and oilseeds, and causes quality deterioration.

The antimicrobial substances of the plant extracts in this study appears to exert antimicrobial activity by inhibiting the growth of the fungi. *A. indica* and *Vernonia amygdalina* leaf extracts have been reported to inhibit fungal growth *in-vitro* (Davicino *et al.,* 2007; Dellavallel *et. al.,* 2011; Mondali *et. al.,* 2009; Gbadamosi *et. al.,* 2012). *Vernonia amygdalina* was found to contain secondary compounds which include tannins, saponins, cardiac glycosides and alkaloids (Anibijuwon *et. al.,* 2012) and *Azadirachta indica* was reported to contain triterpenoids e.g Nimbin, Nimbidin and Azadirachtin (Mondali *et. al.,* 2009). These compounds according to Kaufman *et. al.* (1999) are indicative of the medicinal value of the plants in which they are found. Qasem and Abu-Blam (1996) observed that differences in the effectiveness of plant extracts is usually related to differences in their active principles caused by factors such as types of extraction solvents used, method of extraction and age of the parent plants. In this study however, the difference in the effectiveness of the extracts might be related to the difference in the chemical composition of the extracts on one hand and to the difference in their concentration on the other.

The bitter taste of these plant extracts limits their use in cooking and food processing. This has also limited their use as preservative especially for food and food

products. The fact that there is no significant difference in taste between the treated and untreated tomato in this study suggests that one could treat tomato for consumption with the extracts without any fear of consumer rejection.

CONCLUSION

The use of aqueous leaf extracts of neem and bitter leaf plant for the control of post harvest losses and improvement of the shelf life of tomato should be made popular in view of their relative advantages over synthetic chemicals and their nutritional and medicinal benefits. This is however subject to determining the level of application that will be most effective and economical and yet safe for consumption.

Table 1: Morphological and Microscopic Characteristics of the Fungal Isolates

S/No	Morphological Characteristics	Microscopic Characteristics	Microorganism
1	The colony is circular and About 4.0-4.5cm in diameter. Colour is yellowish-green becoming green with age. Reverse is creamish-yellow.	Stipe is long, vesicle is dome-shaped. Metulae is small. Conidia is Globose, rough and yellowish-green.	*Aspergillus flavus*
2	The colony consists of Creamish-peach to reddish mycelium floccose. The reverse side is pale cream to salmon to violet.	There are long chains of microconidia on monophialides. Macroconidia is long slender 3-5 septate slightly curved and fusiform.	*Fusarium verticilloides*

Table 2: Effects of Plant Extracts on Percentage Reduction in Mycelia growth of *Fusarium verticilloides*

Treatments	Mean Percentage of Mycelia Diameter				
	5DAI	7DAI	9DAI	11DAI	13DAI
Neem leaf extract (5%w/v)	38.9^{bc}	40.0^{bc}	35.5^{bc}	34.3^{bc}	37.5^{bc}
Neem leaf extract (10%w/v)	44.4^{bd}	52.0^{bd}	41.9^{bd}	42.9^{bd}	40.0^{bd}
Bitter leaf extract (5%w/v)	11.1^{a}	12.0^{a}	16.1^{a}	08.6^{a}	10.0^{a}
Bitter leaf extract (10%w/v)	16.7^{b}	20.0^{b}	19.4^{b}	14.3^{b}	20.0^{b}
Carbenzimidazole	100.0^{be}	92.0^{be}	82.9^{be}	94.3^{be}	95.0^{be}
LSD	0.39	0.56	0.49	0.69	0.71

DAI= Days After Inoculation

Values are means of three replicates

Mean values followed by the same superscripts are not different significantly at $\alpha = 0.05$

Table 3: Mean Weight loss (g) of Plant Extract treated and *Fusarium verticilloides* inoculated Tomato fruits

Treatments	**Weight loss (g)**				
	2DAT	**4DAT**	**6DAT**	**8DAT**	**10DAT**
Treated with Bitter leaf	1.0^{b}	4.2^{b}	6.9^{a}	8.7^{a}	14.7^{b}
Treated with Neem	0.6^{a}	3.8^{a}	6.4^{a}	8.7^{a}	12.9^{a}
Untreated*	1.4^{bc}	4.8^{bc}	8.4^{a}	12.3^{bc}	19.3^{bd}
Control**	1.3^{bc}	4.6^{bc}	7.5^{a}	10.4^{b}	15.2^{bc}
LSD	0.23	0.35	2.92	1.25	0.53

DAT refers to Days After Treatment application

Values are means of three replicates

Mean values followed by the same superscripts are not different significantly at $\alpha = 0.05$

*Untreated did not receive plant extract treatment but was inoculated.

**Control did not receive plant extract treatment and was not inoculated.

Table 4: Mean Sensory Scores of Consumer Acceptance of Tomato treated with Extracts of *Azadirachta indica* and *Vernonia amygdalina* 12days after treatment

Sample	Colour	Taste	Overall acceptability
Vernonia amygdalina	6.7^{b}	7.6^{a}	8.9^{a}
Azadirachta indica	5.9^{b}	6.9^{a}	7.3^{a}
Control	8.9^{a}	8.4^{a}	9.6^{a}
LSD	3.4	0.7	1.1

Mean values followed by the same superscripts are not different significantly at $\alpha = 0.05$

*Control did not receive any plant extract treatment.

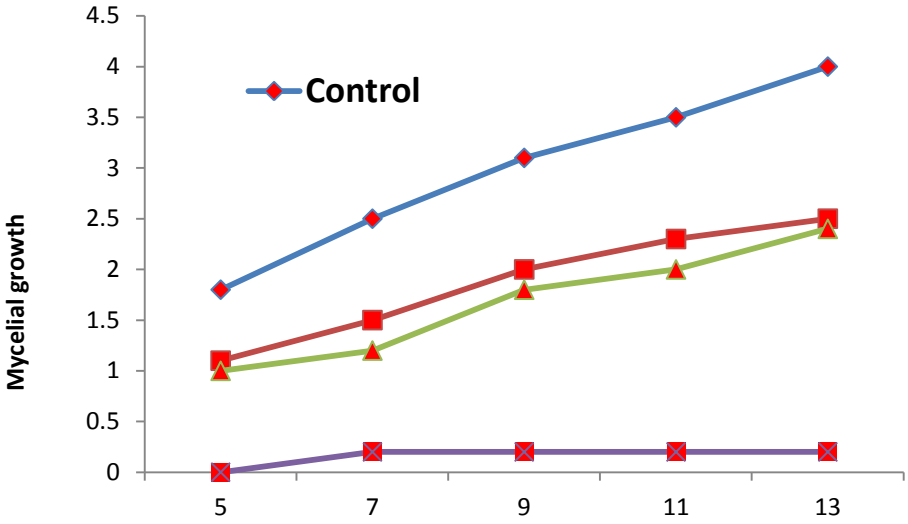

Figure 1: Response of *Fusarium verticilloides* (measured in terms of change in the diameter of growth with time of incubation) to Neem leaf extract.

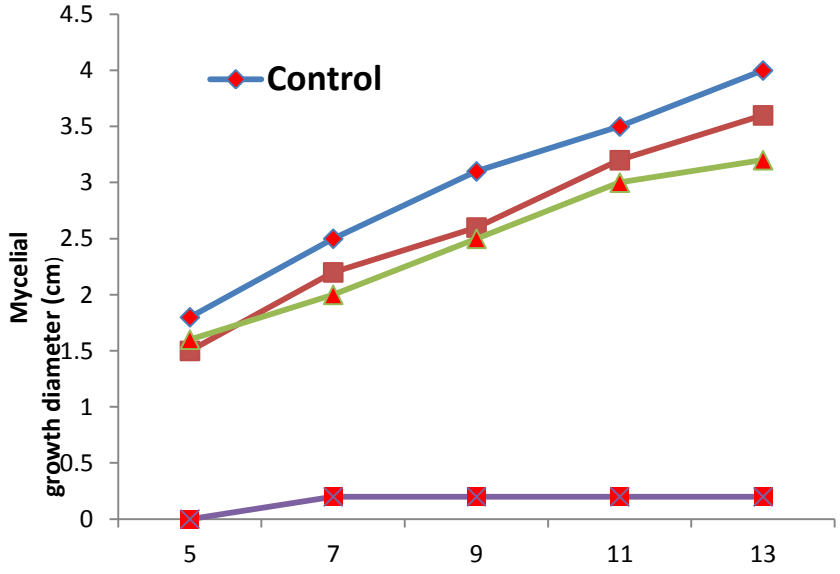

Figure 2: Response of *Fusarium verticilloides* (measured in terms of change in the diameter of growth with time of incubation) to *Vernonia* sp. leaf extract.

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