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Invitro and Invivo Efficacy of Some Plant Extracts for the Control of Tomato Fruit Rot Caused by *Aspergillus Flavus*

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***Abstract*-** Laboratory experiment was conducted in Microbiology Laboratory of School of Science and Science Technology, Abubakar Tafawa Balewa University, Bauchi, Nigeria, to determine the antifugal effect of some plant extracts at different concentration to control tomato fruit rot via both invitro and invivo approaches. The aqueous moringa, Lantana, garlic and pawpaw each with two varying concentrations (40 and 60g/l) were evaluated along with carbendazin for comparison and untreated tomatoes serving as control against *Aspergillus flavus*. The treatments were laid in a Completely Randomized Design (CRD) with three replications. The results showed a promising antifungal activity of the crude extracts of these plants against *A. flavous*. Among the various plant extracts with varying concentrations, aqueous extracts of pawpaw (40 and 60g/l) was found to have more significance (P< 0.01) inhibitory effect on radial growth of *A. flavus* both invitro (0.30 and 0.24cm and invivo 0.77 and 0.71) better than other extracts and is comparable to carbendazim. On the other hand, lantana, garlic and moringa at 40 and 60g/l reduced radial growth of *A. flavus* better than the control. This finding proved the potentiality of plant extracts for the control of post harvest and transit fungal rot of tomato fruit and could be applied to control fungal fruit rot of tomato in both storage and on transit.

***Index Terms*-** Antifungal, *Aspergilus flavus*, *Carica papaya*, radial growth, weight loss.

I. INTRODUCTION

Tomato (*Solanum lycopersicum* (L.) Karst) is a member of the family *solanaceae* which comprises short-lived perennial herbaceous plants. It is one of the most popular vegetable crops widely grow for its edible fruits, high nitritive values and also for its diversified uses (Afroz *et al.* 2008; Ewulo *et al.* 2008). It is also an important vegetable crop in Nigeria accounting for about 18% of daily consumption of vegetables which averages 50.6g per person (Kataria and Mittal, 1994). Tomatoes are grown for home consumption in the backyard of almost every homestead across Sub-Sahara Africa. They are important source of vitamins and important cash crop for both small holders and medium scale commercial farmers (Ana *et al.* 2003).

Tomato fruit is utilized by human in the preparation of soups, stews and fresh slice in salads (Wilbur, 1983). Tomato is a healthy food with low fat, cholesterol free and a good source of fibre and protein (Masyitah, 2004). It also contain abundant and also well balanced nutrition consisting of minerals such as potassium, magnesium, calcium, iron, zinc, etc and vitamin A, B, C and E (Bankole, 1996; Zhang, 1999; Ahmed and Singh, 2005). The fruit also contains plenty of antioxidant carotenoid lycopene that has recently attracted interest because of its role in preventing cancer heart disease and muscular degeration (Wener, 2008).

Despite the human need of tomato, its yield in both smallholders and medium scale commercial cropping systems are generally far below the potential of the crop. Its lower yields as a result of disease infestation as well as huge amount of post-harvest losses incured during transportation and storage by rot fungi has been source of serious concern. A number of fungal diseases in tomato fruit have been reported including Fusarium rot caused by *Fusarium oxysporum* (Mart) Sacc and *Aspergillus* rot caused by *Aspergillus niger* (Ebele, 2011) and Rhizopus rot caused by *Rhizopus stolonifer* (Snowdon, 1990). Other fungi reported to be associated with postharvest rot of tomato include *Aspeergillus flavus, Fusarium solani, Monilocaetes infuscaus, Penicillum spp, Certolystis finbriata, Diapoc batatalis* (Snowdow, 1990).

Control of tomato fruit rot has been by application of synthetic chemicals. However, these days' consumers request less use of chemicals and still want food devoid of contaminations, microbial growth, toxins as well as other qualities deteriorating factors (Lingk, 1991). Added to this, is the hazard involved in using chemical pesticides and the development of resistance to synthetic fungicide by plant pathogenic organisms, make alternative control desirable. Furthermore, synthetic fungicides are expensive and inaccessible to indigenous farmers who are the bulk producers of tomato in Nigeria (Onuegbu *et al.* 2001).

Natural plant products and their analogues have been founds as important sources of agricultural bio-pesticide which serve as antimicrobial properties of plant extracts (Cardelina, 1995 and Okigbo, 2009). Previous reports (Akpomedaye and Ejechi, 1998; Ejechi and Ilondu, 1999; Ejechi *et al.* 1999; Ijato *et al.* 2010 and Ebele, 2011) showed that spices, herbs and other plant materials possess antifungal activity. Akinsoye and Oladunmoye (2000) have reported the antifungal efficacy of stem and leaf extract of *Mirabilis jalapa* in reducing mycelial growth of four different strains of fungi. Amienyo *et al.* (2007) reported the use of indigenous plant extracts for the protection of mechanically injured sweet potato. The legendary medicinal qualities of neem

tree have been known for a long time and their aqueous leaf extracts have systemic actions (Egunjob and Onoyemi, 1981; Sownumi and Akinusi, 1983).

Investigation on the antifungal properties of *Moringa oleifera, Allium sativum, Carica papaya and Azadrachta indica* on rot fungal pathogen on post harvest tomato fruits is therefore aimed at in this finding. This is to serve as a relative alternative to the use of synthetic chemicals to extend the shelf life of tomato so as to reduce or eliminate loss due to post harvest rot caused by phytopathogens mainly fungi and the resultant economic loss to the farmers, traders and consumers.

II. MATERIALS AND METHODS

Study Area

The experiment was conducted in the microbiology laboratory of School of Science and Science Technology, Abubakar Tafawa Balewa University Bauchi in 2007. Bauchi town is located on latitude $10^0$17'N and longitude $90^0$49'E situated at (609.37m) above the sea level. Bauchi lies in Northern Guinea Savannal ecological zone of Nigeria.

Collection of Tomato Fruits

Tomato fruits with symptoms of rot were randomly collected from Muda-Lawal Market at Bauchi, Bauchi State, Nigeria. Fresh and healthy tomatoes were also collected from the market and packed into a sterile polythene bag already lined with soft paper and taken to the laboratory for further studies.

III. PREPARATION OF MEDIA

Preparation of Potato Dextrose Agar,

19.5 grams of Potato Dextrose Agar (PDA) was weighed and poured into a clean 250millilitres conical flask. 250mls of distilled water was added and stired vigorously to dissolve. Cork was inserted into the conical flask and sealed with masking tape. The content was autoclaved at 121^0C for 15minutes and allowed to cooled at room temperature.

Collection and Extraction of Plant Materials

Moringa, pawpaw, and lantana were collected from Federal College of Forestry Jos, Plateau State and fresh garlic bulbs were purchased from Muda Lawal Market, Bauchi. The plant leaves were dried for three days at room temperature (28 ± 2^0) to reduce the moisture, these dried leaves were ground using pistil and mortar into powder. The garlic bulbs were dried at room temperature (28 ± 2^0) for seven days and ground using a grinder to get garlic powder. To get concentrated solution of these plant extracts two lots (40 and 60g/l) respectively of the powdered form of the extracts were dissolved in 1 litter of distilled water separately, vigorously agitated and left for 24 hours to stud before filtration. The filtrates were used as the plant extracts in the experiment.

Isolation of Fungi from Tomato

Fruits exhibiting symptoms of spoilage were brought into the microbiology laboratory for the isolation of pathogen. Ten rotted fruits were washed with clean water and surface sterilized with 70% ethyl alcohol. A sterile scarpel was used to cut 3mm x 3mm section of tissue from the tomato moving from the healthy portion to the diseased portion where fungi is likely to be more active. Cut portion of tomato were asceptically placed on potato dextrose agar in petri plates and incubated for four (4) days at ambient temperature of 25^0C $\pm3^0$C (Banduni, 1988). The set up were observed for 7 days until the organism became fully grown. Pure culture of the isolates were obtained after series of inoculations into sterile potato dextrose agar.

Invitro Assessment of the Effect Plant Extract on *A. flavus*.

The approach of Amadioha and Obi (1990) was used to evaluate the antifungal effect of the extracts on fungal growth invitro by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicate the centre of the plates. This was done before dispensing the PDA into each of the plates. The extracts were poured into the flask, plugged with cotton and heated for about 10minutes to avoid contamination (Madari and Singh, 2005). About 2ml of the extract of various plant materials were separately introduced into petri-plates containing the media (poisoned food method) Nene and Thapilyal, 2002). A disc of 6mm diameter of the pure isolate each was placed on the extract in place with PDA at the point of intersection of the two perpendicular lines drawn at the bottom of the plate. Control plates were without any plant extract but sterile distilled water were added. After twenty four hours, the progress of the radial mycelia growth was measured and subtracted from the initial diameter and the difference were recorded and kept for later analysis.

Invivo Assessment of the Effects of Plant Extract on *Aspergillus flavus*

Fresh, healthy tomato fruits purchased from Muda-Lawal Market in Bauchi were washed with tap water, rinsed with distilled water and surface sterilized with 60% ethanol. With 6mm diameter sterile cork borer 2cm long cylinderical cores were removed from each fruit, discs of 5days old culture of the pathogen isolate were removed from the agar plates and placed in the holes on each fruit including the control. Prior to inoculation the tomato fruits were treated with various concentrations of the plant extracts with the exception of the control which were treated with distilled water only. The treatments were arranged in a completely randomized design with three replications. The radial growth of *Aspergillus spp* and weight loss of tomatos were recorded at interval of 24 hours for five (5) days.

IV. DATA COLLECTION

The data collected include the following

i. Initial weight of tomato prior to treatment.
ii. Initial cut of 0.6cm made before inoculation.
iii. Daily weight loss at the interval of 24hours for each tomato for five (5) days by subtracting the new weight from the initial weight.

Daily radial growth at the interval of 24hours for each tomato for five (5) days by subtracting the new radial increase from the initial, using a meter rule.

V. DATA ANALYSIS

The data collected were subjected to analysis of variance using spss statistical package and the means were separated using Duncan's Multiple Range Test (DMRT).

VI. RESULTS

Table 1 shows the various plant extracts, their botanical name, family they belong to and the active components contained in each plant extract that act against the pathogen.

Table 1: Plant pesticides used in the study and their active component(s).

Plant used	Part of plant used	Botanical name	Family	Active component
Lantana	Leave	*Lantana camara*	Verbenaceae	Lantadene
Moringa	Seed	*Moringa okifera*	Moringaceae	Saponins
Garlic	Bulb	*Allium sativum (L.)*	Amaryllidaceae	Diallyl sulfide Diallyl trisulfide
Pawpaw	Leaves	*Carica papaya*	Caricaceae	Purpin, Acetogenins

Source: (Dhaliwal and Ramesh, 2001)

The antifungal efficacy of crude plant extracts of pawpaw, lantana, garlic and moringa on wounded tomato fruits being inoculated with mycelial disc of the isolate of *A. flavus* was shown in table 2. All test plants significantly ($P \leq 0.01$) reduced the mycelia growth of the pathogen than the control. Pawpaw at 40 and 60g/l showed a significant ($P \leq 0.01$) reduction in the reduction of radial growth recording the lowest result of (0.30cm and 0.24cm) respectively and comparable to carbendazim which recorded (0.30cm) in the result. The result also indicated that garlic and moringa did not differed statistically in radial mycelia reduction.

The effect of concentrations of plant extracts on radial mycelia growth of *A. flavus* invivo is presented in table 3. Pawpaw at 40 and 60g/l significantly ($P \leq 0.01$) reduced the radial growth of *A. flovus* in infected tomato with lowest values (0.74 and 0.77cm) better than other plant extracts and even the fungicide (carbendazim). Moringa, lantana and garlic also inhibited the radial growth of *A. flavus* better than the control but without significant difference among their concentrations.

Similarly, significant ($P \leq 0.01$) difference was observed between different concentrations of the plant extracts on weight loss of tomato fruits infected by *A. flavus* as shown in table 4. Pawpaw at 40 and 60g/l reduced weight loss of tomato with (2.70g and 2.66g) respectively better than the concentrations of other plants. This is followed carbendazim (2.96g) and moringa (3.68g and 3.65g).

Table 2: Invitro effect of some plant extracts at different concentration on *Aspergillus flavus*

Treatments (plant used)	Concentration (g/l)	Radial growth (cm)
Moringa oleifera	40	0.57^{abc}
	60	0.78^{abc}
Lantana camara	40	1.04^{cd}
	60	0.85^{bcd}
Allium sativum	40	0.64^{abc}
	60	0.58^{abc}
Carica papaya	40	0.30^{ab}
	60	0.24^{a}
Carbendazim	2.0	0.30^{ab}
Control	0	1.38^{d}
LS		**
SE±		0.19

SE: Standard error, LS: Level of significance

Mean followed by same alphabet are not significantly different using DMRT at (P = 0.01)

Table 3: Effect of different concentrations of plant extracts on mycelia growth of *Aspergillus flavus* on infected tomato

Treatments (plant used)	Concentration (g/l)	Radial growth (cm)
Moringa oleifera	40	1.08^{abc}
	60	1.04^{abc}
Lantana camara	40	1.31^{cd}
	60	1.25^{bcd}
Allium sativum	40	1.21^{bcd}
	60	1.14^{bcd}
Carica papaya	40	0.77^{a}
	60	0.74^{a}
Carbendazim	2.0	0.94^{ab}
Control	0	1.45^{d}
LS		**
SE±		0.11

SE: Standard error, LS: Level of significance

Mean followed by same alphabet are not significantly different using DMRT at (P = 0.01)

Table 4: Effect of different concentration of plant extracts on weight loss of tomato infected by *Aspergillus flavus*

Treatments (plant used)	Concentration (g/l)	Radial growth (cm)
Moringa oleifera	40	3.68[c]
	60	3.65[c]
Lantana camara	40	4.05[de]
	60	3.91[d]
Allium sativum	40	4.31[ef]
	60	4.24[f]
Carica papaya	40	2.70[a]
	60	2.66[a]
Carbendazim	2.0	2.96[b]
Control	0	4.42[f]
LS		**
SE±		0.08

SE: Standard error, LS: Level of significance

Mean followed by same alphabet are not significantly different using DMRT at (P = 0.01)

VII. DISCUSSION

The primary reason for the application of synthetic chemicals on crops is to reduce crop loss due to plant pathogens to a level that is economically tolerable. This has reduce the crop loss, thereby increase crop production but with detrimental effects on the environmental quality and human health. This study was intended to provide some of the information required in the use of different concentrations of pawpaw, lantana, moringa and garlic extracts as an alternative to synthetic fungicides for the effective control of fruit rot. The pathogen associated with post harvest deterioration in this finding was *A. flavus*. The extracts of *Lantana camara, Carica papaya, Moringa oleifera* and *Allium sativum* are of great use in agriculture, public health, medicine, cosmetics and many more.

Investigation on the antifungal properties of *C. papaya, L. camara, M. oleifera* and *A. sativum* on the growth of isolates of *A. flavus* both in vitro and invivo showed that crude extracts of these plants posses some inhibitory components which caused significant reduction in mycelial growth of the fungus both invitro and invivo. This agrees with the results of Amadioha (1998), Owolade and Osikanlu (1999) and Adejunmo *et al.* (2000) who reported the efficacy of extracts from *C. papaya, A. ciliata, C. odirata*, among other extracts in reducing the mycelial growth of *Erysiphe cichoracearum, Collectrotrichum capsid* and *Protomycopsis phaseoli*, which compared favourably with the chemical fungicides Benlate and Ridomil. Tijjani *et al.* (2010) reported a significant inhibitory property of neem (*A. indica*) and moringa (*M. oleifera*) extracts on mycelia growth of *Rhizopus stolonifer*. Akpa *et al.* (1991) also reported a significant inhibitory property of neem (*A. indica*) extracts on mycelia growth of *Collectotrichum graminicola* just as Amuch (1989) found the extracts of *Ocimum gratissimum* to reduce the radial growth of *R. spp*. Comparing among the plant extracts in this present research, *C. papaya* was most effective in inhibiting the fungus both invitro and invivo and is comparable to the fungicide carbendazim. This was followed by *M. oleifera, A. sativum* and *L. camara*, considering their various inhibitions at the different concentrations.

The effectiveness of plants extracts defend on the nature and amount of active ingredients it contains. Increase in the concentrations of the plant seed/bulb/leaves extracts correspondingly decreased radial growth of *A. flavus* and weight loss of tomato fruit. This increase in concentrations of these extracts implied an increase in the active ingredients of the solutions which act on the fungus thereby affecting its physiological processes and consequently lowering the growth of the fungus. This study had also confirmed and established the antifungal activity of these plant crude extracts, which are interestingly systemic in action and can be used or applied as post harvest tuber treatment against fruit rot in tomato caused by *A. flavus*. This agrees with earlier reports/works of Udo *et al.* (2001) on the inhibition of growth and sporulation of fungal pathogens on *Ipomea batatas* and *Diocorea* of by garlic extracts; Okigbo and Nueka (2005) on the use of *Xylopia aethiopica* and *Zingiber officinale* to control yam tuber rot caused by *F. oxysporum, A. niger* and *A. flavus*; Amienyo *et al.* (2007) on the use of *Z. officinale, Annona muricata, Gacinia cola, Alehornea cordifolia, Allium sativum* to control wet rot on sweet potatoes caused by rot fungal pathogens; Abdul-aziz and Younes (2010) on the use of *Cinnamomum verum (Pimpinella anisum* L.) black seed (*Ngelia sativa* L.) and clove (*Syzygium aromaticum* L. Merr and Perry) against Pea (*Pisum sativum* L.) root rot fungus (*Rhizoctoni solani*); Tijjani *et al.* (2010) on the use neem and moringa seed extracts against potato wet rot caused by *R. stolonifer*; Ijato *et al.*, (2010) on the use of *A. indica* and *Chromolacna adorata* against post harvest and transit rot of tomato and Ebele (2011) on the use of *C. papaya, C. adorata* and *Acalypha ciliata* on the control of pawpaw fruit rot fungi.

In conclusion, this study had shown that the moringa seed extracts, garlic bulb extract and lantana and pawpaw leaves used, have the potentials in the protection of tomato fruit rot against rot fungus especially fruit rot caused by *A. flavus*. 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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