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Effect of some selected plant extracts on *Aspergillus flavus,* a causal agent of fruit rot disease of tomato (*Solanum lycopersicum*) in Bauchi State

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Abstract

Experiment was conducted in microbiology laboratory of School of Science and Science Technology, Abubakar Tafawa Balewa University, Bauchi, in 2007. The objective of the study was to determine the antifungal effect of different concentrations of some selected plant extracts (Neem seed, Moringa seed, Garlic bulb and Emulsified neem seed oil each with five concentrations of 10, 20, 30, 40 and 50g/0.25L of water and 1:1, 1:5, 1:15 and 1:20 mother extract: water for emulsified neem seed oil) on tomato and a control (untreated tomato) constituted the treatment in this study. The treatments were laid in a Completely Randomized Design (CRD) with three replications. The results obtained showed significant difference ($P \leq 0.01$) on radial mycelia growth and weight loss of tomato when the aqueous plant extracts where used. Garlic bulb, emulsified neem seed oil and aqueous moringa seed extracts exhibited the highest control of the pathogen than aqueous neem extract. Also from the result obtained, it is evident that the plant extracts possessed antifungal properties which if fully exploited could be used in integrated pest management and post-harvest treatment for tomato before storage and on transit as control measures for *Aspergillus flavus* .

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Introduction

Tomato (*Solanum lycopersicum*) 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-saharai 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. It also contain abundant and also well balanced nutrition consisting of minerals such as potassium, magnessium, calcium, iron, zinc, etc and vitamin A, B, C and E (Bankole, 1996; 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 nuscular 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 incurred 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). Oher 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 quality 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 (Okigbo, 2009; Cardelina, 1995). Previous reports (Ebele, 2011; Ijato *et al.,* 2010; Akpomedaye and Ejechi, 1998; Ejechi *et al.,* 1999; Ejechi and Ilondu, 1999) show 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. Gurama *et-al.* (2013) reported the use of compost extracts of plant origin against *Fusarium oxysporum* on tomato wilt disease. Also Zhang *et-al.* (1998) reported the use of ccompost extract of plant origin as they induced systemic resistance acquired in cucumber and Arabidopsis.The legendary medicinal qualities of neem tree have known for a long time and their acqueous leaf extracts have systemic actions (Egunjob and Onoyemi, 1981).

The antifungal properties of *Moringa oleifera, Allium sativum, Carica papaya and Azadrachta indica* on rot fungal pathogen on post harvest tomato fruits are 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

resultanteconomic loss to the farmers, traders and consumers.

The objectives of the study were:

1) to evaluate the efficacy of different plant extracts (Neem, Moringa and Garlic) on *Aspergillus flavus*.

2) to assess the best concentration that can be used as alternative to synthetic fungicide for postharvest control of rot fungal pathogen (*Aspergillus flavus*).

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°17'N and longitude 90°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.

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°C for 15minutes and allowed to cooled at room temperature.

Collection and extraction of plant material

Preparation of Mother Extract

50 grams of sodium hydroxide (NaOH) was weighted and poured into 100mls of distilled water. 20mls of the solution was added to 60mls of neem seed oil (NSO) to form mother extract (M.E). The M.E. was mixed separately with water in the proportion of 1:01, 1:05, 1:10, 1:15, 1:20 mls of M.E and water respectively.

Preparation of Neem Extract

Dried neem (*Azadirachta indica*) seeds collected and the shells cracked using pesitle and mortar to separate the shell from the kernels. The kernels were grounded into paste using mortar and pestle. Ten, 20, 30, 40, and 50g of the paste was weighed and 250ml of water added stirred for 10 minutes and allowed to stand for 24hours before filtering with muslin cloth.

Preparation of Moringa Extract

Dried Moringa (*Moringa oleifera*) seeds where weighed into 10, 20, 30, 40 and 50g respectively grounded into paste using mortar and pestle. each quantity was dissolved into 25mls of water, and then they were filtered after 24hours using muslin cloth.

Preparation of Garlic Extracts

Garlicc (*Allium sativum*) bulb were collected and weighed in 10, 20, 30, 40 and 50g respectively. The shells were separated from the bulbs and pounded into paste using mortar and pestle. Each quantity was dissolved into 250mls of water and filtered after 24hours using muslin cloth.

Isolation of Spoilage Fungal from rotten Tomato Fruits

Pieces of rotten tomato were washed in a running tap and these were cut from the periphery of a rotten tomato. These were surface sterile with 60% ethanol for just 1 minute and dropped on sterile soft paper and cultured out on potato dextrose agar already prepared. The petri-dishes were incubated at room temperature (27 $\pm$ 2°C) for five days and observed for fungal growth. Fungal pathogen associated was re-cultured to obtain pure culture and the pure isolates and stored in slant for further use.

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 3mm 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 growths of *Aspergillus flavus* and weight loss of tomatoes were recorded at interval of 24 hours for five (5) days.

Data collection

The data collected include the following

1. Initial weight of tomato prior to treatment.
2. Initial cut of 2cm made before inoculation.
3. Daily weight loss at the interval of 24hours for each tomato for five (5) days by subtracting the new weight from the initial weight.
4. 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.

Data analysis

The data collected from the above experiment were subjected to analysis using analysis of variance. Means were separated using least significance difference.

Results

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

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

Plant used	Part of plant used	Botanical name	Family	Biologically active component
Neem	Seed	*Azadrachta indica*	Meliaceae	Azadrachitin limonoid
Moringa	Seed	*Moringa okifera*	Moringaceae	Benzyl-isothiocyanate
Garlic	Bulb	*Allium sativum (L.)*	Amaryllidaceae	Dially sulfide Dially trisulfide
Pawpaw	Leaves	*Carica papaya*	*Caricaceae*	papain

Source: (Dhaliwal and Ramesh, 2001).

Table 2. Effect of different plant extract on radial mycelial growth of *Aspergillus* fruit rot of tomato.

Plant extracts	Radial growth
Control	2.5
E.N.S.O	1.7
A.N.S.E.	2.0
A.M.S.E	1.8
A.G.B.E	1.8
Mean	1.78
SE±	0.06
L.S	**
L.S.D	0.2

Key: E.N.S.O = Emulsified neem seed oil; A.N.S.E = Aqueous neem seed extract; A.M.S.E = aqueous moringa seed extract; A.G.B.E = Aqueous garlic bulb extract; SE± = Standard error; LS = Level of significance; ME = mother extract; LSD = Least significant differences.

Pathogenecity of the wounded fruits being inoculated with mycelial discs of the isolates of *Aspersillus flavus* showed that all the plant extracts significantly ($P \leq 0.01$) reduced the mycelial growth of the pathogen than the control (Table 2). Among the plant extracts, E.N.S.O, A.G.B.E and A.M.S.E significantly slowed down the mycelial growth of the pathogen better than A.N.S.E. However, the three plants extracts namely

E.N.S.O (1.7), A.M.S.E (1.8) and A.G.B.E (1.8) did not differ with each other in the reduction of mycelial growth of the pathogen.

Table 3 showed the effect of different concentrations of plant extracts on radial mycelial growth of Aspergillus fruit rot. Increasing concentration of plant extract significantly reduced the mycelial growth of the pathogen. Application of 10g/250mls of distilled water of A.N.S.E., A.M.S.E and A.G.B.E respectively did not differ significantly in the reduction of mycelial growth of the pathogen when compared with that of the control. However, application of the concentrations (20g, 30g, 40g and 50g/250mls of water) of A.N.S.E, A.M.S.E. and A.G.B.E and all the five concentration used in E.N.S.O significantly reduced the mycelial growth of fruit rot pathogen than the control A.N.S.E., A.M.S.E and A.G.B.E at 50g/250mls control the pathogen better than any other concentration with 1.5cm, 1.5cm and 1.6cm radial mycelial growth recorded in that order.

Table 3. Effect of different concentrations of plant extract on radial growth of *Asperjillus flavus* on tomato fruit.

Plant extracts	Concentration(g/l)	Radial growth(cm)
Control	0	25
E.N.S.O (ME:water)	1:1	1.9
	1:5	1.6
	1:10	1.8
	1:15	1.8
	1:20	1.8
A.N.S.E (g)	10	2.5
	20	2.0
	30	2.0
	40	1.9
	50	1.5
A.M.S.E (g)	10	2.3
	20	1.7
	30	1.7
	40	1.6
	50	1.5
A.G.B.E (g)	10	2.1
	20	1.8
	30	1.8
	40	1.8
	50	1.6
Mean		1.8
SE±		0.14
LS		**
L.S.D		0.4

Key: E.N.S.O = Emulsified neem seed oil; A.N.S.E = Aqueous neem seed extract; A.M.S.E = aqueous moringa seed extract; A.G.B.E = Aqueous garlic bulb extract; SE± = Standard error; LS = Level of significance; ME = mother extract; LSD = Least significant differences.

Effect of Plant Extracts on Weight Loss due to Aspergillus Fruit Rot

The effect of plant extracts on weight loss due to *Aspergillus* fruit rot on tomato was presented in table 4. The result shows a significant ($P \leq 0.01$) reduction in weight loss when plant extracts were applied on infected tomato. Weight loss was found to be significantly lower in tomato treated with E.N.S.O, A.G.B.E and A.M.S.E than tomato treated with A.N.S.E and the control.

The result in table 5 shows the effect of different concentrations of plant extracts on weight loss of tomato infected with *flavus*. Treatment of tomato fruit with 1:5 and 1:10 E.N.S.O, 20, 30, 40 and 50g/250mls of A.M.S.E and A.G.B.E respectively

significantly (P≤0.01) reduced weight loss better than all concentrations in A.N.S.E and 1:1 E.N.S.O, 10g/250mls of A.M.S.E and A.G.B.E. Highest reduction in weight loss was recorded with application of 50 gm /0.25L of AGBE (15.1 g), followed by 1.20 (ME. Water application of ENSO (15.4g) and 50g/0.25L application of AMSE (15.6g) respectively.)

Table 4. Effect of the plant extract on weight loss of tomato fruits infected with rot pathogen.

Plant extracts	Weight Loss (g)
Control	22.2
E.N.S.O	19.5
A.N.S.E	20.9
A.M.S.E	18.2
A.G.B.E	17.8
Mean	17.8
SE±	0.73
LS	**
LSD	2.2

KEY: E.N.S.O = Emulsified Neem seed oil, A.N.S.E= Aqueous Neem Seed extract, A.M.S.E=Aqueous Moringa seed extract; A.G.B.E= Aqueous Garlic bulb extract; SE_+ = Standard erros LS = Level of significance; ME= mother extract; LSD = Least significant differences.

Discussion

The microbe associated with the post harvest deterioration in this finding was *Aspergillus flavus*. All the test plants: *Azadracta indica, Moringa oleifera, Carica papaya* and *Allium sativum* had inhibitory effect on post harvest rot pathogen of tomatoes in acqueous and emulsified neem seed oil concentrations. Extracts of *A. indica, M. oleifera, C. papaya* and *A. safivum* have been reported to have antimicrobial properties. The derivatives of *Azadrachta indica, Moringa oleifera* and *C. papaya* are of great use in agriculture, public health, medicine, cosmetics and many more.

Table 5. Effect of the different concentration of plant extract on weight loss of tomato fruits infected with *Aspergillus* fruit rot.

Plant Extract	Concentration	Weight Loss (g)
Control	0	25.0
E.N.S.O(ME: water)	1:1	24.0
	1:5	21.0
	1:10	20.1
	1:15	18.0
	1:20	15.4
A.N.S.E (g)	10	22.2
	20	22.3
	30	21.0
	40	20.0
	50	20.1
A.M.S.E(g)	10	22.8
	20	19.1
	30	19.0
	40	16.6
	50	15.6
A.G.B.E (g)	10	22.3
	20	18.5
	30	16.8
	40	16.6
	50	15.1
Mean		18.5
SE±		1.65
LS		**
LSD		5.0

Investigation on the antifungal properties of *A. indica, M. oleifera, C. papaya* and *A. sativum* on the growth of isolates of *Aspergillus flavus* invivo shows that these plants' crude extract posses some inhibitory components (Table 1) which cause significant reduction in mycelial growth of the fungus (Table 2). Akpa *et al.,* (1991) reported a significant inhibitory property of neem (*A. indica*) extracts on mycelial growth of *Colletotrichum graminicola*; Amuchi (1999) found that the extracts of *Ocimum gratissium* reduced the radial growth of *Rhizopus spp*, just as Ebele (2011) found tha the extracts of *C. papaya* reduced the radial mycelial growth of *Aspergillus niger, Fusarium solani* and *Botryodipdia theobromae.*

The effectiveness of plants extracts depend on the nature and amount of biologically 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. Increasing 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. The optimum concentration for the control of *A. flavus* as revealed by this study is 1:15 and 1:20 ME/water of E.N.S.O and 40 and 50g/250mls of A.G.B.E and A.M.S.E. 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 neem and moringa seed extracts, garlic bulb extract and emulsified neem seed oil used, have the potentials in the protection of tomato fruit rot against rot fungi 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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