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Evaluation of Stem Bark Extracts of *Azadiratcha indica* (A. Juss) and *Vernonia amygdalina* (Del.) for the Management of *Aspergillus flavus* on Tomato

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Authors' contributions

This article is a collaborative work between both authors. Author CE designed, supervised the work and wrote the manuscript. Author OME conducted the experiment. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigated the antifungal effects of three concentrations of the stem bark extract of neem (*Azadiratcha indica* A. Juss) and bitter leaf (*Vernonia amygdalina* Del.) on *Aspergillus flavus* causal agent of rot in tomato fruits. Five, ten and fifteen percent concentration of the plant extracts were prepared by weighing 25 g, 50 g and 75 g and infusing each in 500 ml sterile cold water for 48 hours (weight by volume w/v). Potato dextrose agar (PDA) media was amended with the plant extracts and inoculated at the centre with 1 mm diameter mycelia discs taken from a advancing edges of a pure culture of seven days old *A. flavus.* Healthy tomato fruits were dipped in 5% w/v, 10% w/v and 15% w/v concentrations of the stem bark extracts for five minutes and the tomato fruits inoculated with 7 day old pure cultures of *A. flavus*. All the plant extracts tested at all concentrations significantly reduced the mycelia growth of *A. flavus* compared with the control. The tomato fruits treated with 5% w/v of *A. indica* had the lowest firmness decrease of 1.67 and was considered marketable. Tomato fruits treated with 15% w/v of *A. indica* had significantly (P≤ 0.05)

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lower weight loss of 20.30% compared with the control (61.73%). The study suggests that *V. amygdalina* can be used for the preservation of tomato fruits while *A. indica* can be used to improve tomato fruit quality in storage.

Keywords: Tomato; stem bark; extracts; management; Aspergillus flavus.

1. INTRODUCTION

Tomato (*Solanum lycopersicum L.*) is one of the most widely cultivated and extensively consumed horticultural crops globally [1]. Tomato rank second to potatoes in global production of all horticultural produce [2]. Tomato can be eaten in various ways. It can be eaten raw in salads or as an extract or sauce in many dishes. Tomato and tomato-based foods provide a wide variety of nutrients and many health- related benefits to the body [3]. Losses of up to 50% have been recorded in tomato production. Tomato rots caused by *Aspergillus flavus* are a common cause of tomato loss due to reduction in the quantity and quality of tomato during sale and storage [4].

Azadiratcha indica belongs to the family Meliaceae. It is a tall tree of about 30m with a short straight trunk and girth of about 2.5m. The leaves are broad and evergreen. A decoction of the leaves is used in treating boils, ulcers and eczema [5]. *Vernonia amygdalina* belongs to the family Compositae and is a small and erect branched shrub of 1 m – 4.5 m height found mainly in the South and Savannah zones of Nigeria and other African countries [6]. It is used medicinally in the treatment of diabetes and hypertension, laxatives and to prevent stomach ache [6,7].

The stem bark of medicinal plants is reported as the principal plant part for use as biopesticide [8]. The stem bark extracts of Neem (*Azadirachta indica* A. Juss.) and bitter leaf (*Vernonia amygdalina* Del.) are natural products which are reported to exhibit fungitoxicity effects against pepper fruit anthracnose caused by *Colletotrichum capsici* [9]. The present study evaluated the effects of the stem bark extract of *A. indica* and *V. amygdalina* on the quality of healthy tomato fruits inoculated with *A. flavus*.

2. MATERIALS AND METHODS

2.1 Study Location

The experiments were conducted at the Pathology Laboratory of the Federal University of Agriculture, Makurdi, Benue State, Nigeria located between Latitude 07° 45'- 7° 50'N and Longitude 08° 45' - 08° 50'E.

2.2 Sample Collection

The samples of the stem bark of *Azadirachta indica* (A. Juss.) and *Vernonia amygdalina* (Del.) were collected by digging and cutting the plant part from a single adult parent of the identified plant using a cutlass and hoe. The samples were collected from Kwande Local Government Area of Benue State, Nigeria. The collected samples were peeled with a scalpel, thoroughly washed in running tap water, rinsed in distilled water, sun- dried for one week and ground to powder.

2.3 Isolation of Fungal Organisms

Infected tomato fruits collected from North bank market in Makurdi Local Government Area of Benue State, Nigeria were used for isolation of fungal organisms associated with tomato fruits. Several small sections (3-5 mm^2) were cut from the edge of the infected lesions to contain both diseased and healthy looking tissues to avoid culturing saprophytes [10]. The tissue pieces were sterilized for 1minute in 1% Sodium hypochlorite solution after which they were rinsed in three changes of SDW (Sterile Distilled Water) and blotted dry on sterile filter papers. The tissue pieces were plated on PDA plates (four pieces per plate). The plates were then incubated on the laboratory bench at ambient conditions of light and temperature (30± 2°C) for 3 days. Pure cultures were obtained by sub culturing unto fresh PDA plates. The pure culture of the fungus was obtained by culturing the hyphal tip of each fungus selected from the periphery of actively growing colony and inoculating them unto fresh PDA plates under aseptic conditions. Pure cultures of *A. flavus* was identified microscopically with the use of reference manual [11] and further confirmed at the Germplasm Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

2.4 Preparation of Aqueous Plant Extracts

The sun- dried plant materials were ground into powder using a hand grinder after which 5% w/v, 10% w/v and 15% w/v concentration were made from the plant extracts by weighing out 25 g, 50 g and 75 g separately in a 1 Litre conical flask, soaked in 500 mL of cold distilled water and left to stand for 48 hrs. The suspension of each plant extract was filtered using cheese cloth.

2.5 Treatment and Experimental Design

The experiments were conducted using two plant extracts Neem (*Azadirachta indica*), bitter leaf stem bark (*Vernonia amygdalina*) at three concentrations (5% w/v, 10 w/v % and 15 w/v %) and a control (where no extract was added). The experiments were a completely randomized design replicated three times.

2.6 Fungitoxic Effect of the Stem Bark Extract of *Azadirachta indica* and *Vernonia amygdalina* on the Mycelia Growth of *Aspergillus flavus*

Four grams of PDA was added to each extract (at three concentrations 5, 10, and 15% w/v) and the sealed conical flasks were autoclaved at 121°C for 15 minutes [10]. Streptomycin sulphate was added when the medium cooled to about 40°C. The media was dispensed to labeled sterile 9 cm Petri dishes. Seven days old pure cultures of *A. flavus* was used in the inoculation. A sterile cork borer disc of 1mm was used to introduce the fungi inoculum into the center of the medium containing the plant extract in Petri dishes and also on the control plates (without plant extract). The treatments were replicated thrice and incubated on the laboratory bench for seven days. Observations were made and mycelia growth in centimeter was recorded.

2.7 Effect of Stem Bark Extract of *Azadirachta indica* and *Vernonia amygdalina* on the Control of *A. flavus* Growth on Tomato Fruits

In the experiment to control the fungi on tomato fruits, the stem bark extracts of *A. indica* and *V. amygdalina* were used at three concentrations (5% w/v, 10% w/v and 15% w/v), a control (where the tomato fruit was untreated but inoculated with *A. flavus*) and a check (untreated, un-inoculated tomato fruit).

2.8 Application of Plant Extracts and Inoculation of Pathogen on Tomato Fruits

Tomato fruits at ripe stage free from blemish and bruises were washed with water, surface sterilized with 10% sodium hypochlorite and rinsed in sterile distilled water. Fruits were dipped separately in 5% w/v, 10% w/v % and 15% w/v % stem bark extract of *A. indica* and *V. amygdalina* for five minutes.

The sterilized tomato fruits were inoculated with 7 day old pure cultures of *A. flavus* using the agar plug method. A sterilized 3 mm cork borer was used to punch into the healthy tomato fruits and the bored tissues were removed. A sterile 1 mm cork borer was used to bore holes on pure culture agar plates of *A. flavus.* Each agar plug was placed in the hole bored on the tomato fruits, the pathogen was inoculated and the hole covered with the tissue (three tomato fruits were inoculated per replicate).

The inoculated and un-inoculated fruits were placed on a moistened sterile filter paper in a plastic container with perforated cover, arranged in a completely randomized design and replicated three times (three fruits per replicate). The containers were incubated on the laboratory bench at ambient conditions of light and temperature (30±2°C) [12,13].

2.9 Data Collection

2.9.1 Fruit weight loss

Fruit weight loss was determined by weighing tomato fruits from each treatment individually before and after the experiment. Weight changes were calculated using the following formula:

% weight loss = (Initial weight –Final weight)/ Initial weight x100

2.9.2 Firmness decrease

Firmness decrease rating was done using a modified rating scale of 1-5 [14]:

Where

1-2.99 = < 10% fruit soft
3- 4.99 = 25% of fruits soft
5 = ≥50% of fruits soft

2.10 Data Analysis

All data collected were statistically analyzed using SAS version 9.2 statistical software [15] and significant treatment means separated using Duncan New Multiple Range Test (DNMRT).

3. RESULTS

The morphological and microscopic description of *A. flavus* is presented in Plate 1. Colonies on PDA at 30°C attained a diameter of 9cm within 7 days with dense felt yellow- green conidiophores. Conidial heads were typically radiate, later splitting into several loose columns. Conidiophores were hyaline coarsely roughened, up to 1.0 mm in length. Phialides were borne directly on the vesicle 6-10x4.0-5.5 µm, conidia were globose to subglobose.

3.1 Fungitoxic Effect of the Stem Bark Extract of *Azadirachta indica* and *Vernonia amygdalina* on the mycelia growth of *Aspergillus flavus*

The mycelia growth of *A. flavus* treated with the stem bark extract of A. *indica* and *V. amygdalina* is presented in Table 1. All the plant extracts tested at all concentrations significantly reduced the growth of A. *flavus* when compared with the control.

Table 1. Mycelia growth of fungi treated with the stem bark extract of neem and bitterleaf

Treatment	Mycelia growth (cm)
***Azadirachta indica* (%)**	
5	7.48^{b} (16.88)
10	6.37^{bc} (29.22)
15	5.87^{bc} (34.77)
***Vernonia amygdalina* (%)**	
5	6.15^{bc} (31.67)
10	5.40^{c} (40.00)
15	4.62^{c} (48.67)
Control (Distilled water)	9.00^{a}

Values in parenthesis represent % reduction of mycelia growth

Higher concentration of the plant extracts resulted in higher percentage inhibition of the rot pathogen. *Vernonia amygdalina* at 10% and 15% w/v significantly (P≤ 0.05) reduced the mycelia growth of *A. flavus* (40.00%) and (48.67%) respectively compared with *A. indica* at 5% (16.88%) and the control.

3.2 Effect of the Stem Bark Extract of *Azadirachta indica* and *Vernonia amygdalina* on the Lesion Diameter of *Aspergillus flavus* on Tomato Fruits

The lesion diameter of *A. flavus* on tomato fruits treated with the stem bark extract of *A. indica* and *V. amygdalina* is presented in Table 2. The lesion diameter of *A. flavus* on tomato fruits treated with the stem bark extracts of *V. amygdalina* and *A. indica* was not significantly (P ≥ 0.05) different among the treatments and the control. However the lesion growth produced by *A. flavus* on tomato fruits treated with 15% *Vernonia amygdalina* was lower (2.27 cm) compared with all other treatments and the control (2.37 cm).

3.3 Effects of Plant Extract Concentration on Tomato Fruit Quality

Data presented in Table 2 shows the effects of plant extracts on the firmness decrease and weight loss percentages of tomato fruits. Tomato fruits treated with 15% *A. indica* significantly (P≤ 0.05) reduced weight loss by 67.11% compared with the control. Tomato fruits treated with 10% *A. indica* reduced weight loss by 59.87%. The application of the stem bark extracts of *V. amygdalina* at 15% had a weight loss reduction of 46.06% while dipping tomato fruits in 5% concentration of *V. amygdalina* resulted in

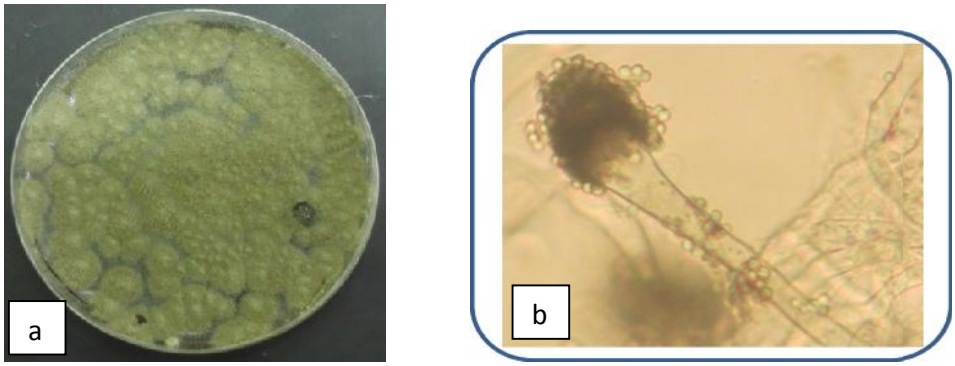

Plate 1. Morphological and microscopic description of *Aspergillus flavus* showing (a) Colonies yellowish- green (b) Globose or subglobose conidia in vesicle

Table 2. Lesion diameter (cm) of *A. flavus* on treated tomato fruits inoculated with *Aspergillus flavus* at 33°C in Makurdi

Treatment	Lesion diameter (cm)
***A. indica* (%)**	
5	3.18[a]
10	3.19[a]
15	2.40[b]
***V. amygdalina* (%)**	
5	3.14[ab]
10	2.65[ab]
15	2.27[b]
Control (T+P+N)	2.37[b]
Check (Tomato only)	0.00[c]

TPN = Tomato+ Pathogen+ No extract

a weight loss reduction of 33.63%. Untreated tomato fruits had a weight loss of 13.27% indicating the effect of environmental conditions such as temperature on weight loss. Lower concentrations of the plant extracts resulted in greater reduction in fruit firmness. *Azadirachta indica* at 5% concentration reduced firmness decrease by 44.33% while 10% concentration of *A. indica* and *V. amygdalina* reduced firmness decrease by 22.33% respectively.

4. DISCUSSION

Aspergillus flavus is a storage fungus of great concern because it is capable of producing secondary metabolites and mycotoxins which are harmful to man [16,17]. *Aspergillus flavus* has been reported in both fresh and minimally processed fruits and vegetables including tomato [16]. The higher level of inhibition at higher concentrations of the plant extracts indicates the availability of more active ingredients than at the lower concentrations. This is in line with the report of [18] which reported that the inhibitory action of extracts on mycelia growth increased with increasing concentration. The reduction of mycelia growth of *A. flavus* in this study agrees with the report of [5] in which neem leaf extract completely inhibited the production of aflatoxin produced by *A. flavus* on cotton seed. Also [19] observed that neem extract were effective against *Curvularia lunata, Fusarium spp*. on wheat seed. [20] and [21] attributed the inhibition of mycelia growth of *A. flavus* by *A. indica* to the presence of Azadirachtin in the plant extract.

The post harvest quality of fruits and vegetables depends on the rate at which stored food reserves are used and the rate of water loss during storage [22]. The application of plant extracts in this study significantly improved tomato fruit quality. The reduced weight loss in treated fruits could be attributed to the activity of the chemical constituents of the plant extracts which may have decreased sensitivity to ethylene and resulted in reduction of water loss. This finding is collaborated by the report of [23] which reported reduced weight loss in avocado fruits treated with ethylene. [9] had earlier reported that fungitoxic activity of *Azadirachta indica* and *Vernonia amygdalina* on *Colletotrichum capsici* was due to the presence of taninnins, glycosides and saponins.

Percentage weight loss varied according to the concentration of plant extract applied. Increase in concentration of the extracts of *A. indica* and *V. amygdalina* resulted in decrease in weight loss. [24] also related weight loss in tomato to cultivar variation such as size of fruit, thickness of cuticle.

Table 3. Effects of *Azadirachta indica* and *Vernonia amygdalina* on the quality of tomato fruits inoculated with *Aspergillus flavus* seven days after inoculation at 33°C in Makurdi

Treatments	Firmness decrease	Weight loss (%)
***A. indica* (%)**		
5	1.67[bc] (44.33R)	38.17[b] (38.17R)
10	2.33[b] (22.33R)	24.77[d] (59.87R)
15	3.00[ab] (0.00 No effect)	20.30[e] (67.11R)
***V. amygdalina* (%)**		
5	5.00[a] (40.00S)	40.97[b] (33.63R)
10	2.33[b] (22.33R)	35.20[bc] (42.98R)
15	5.00[a] (40.00S)	33.30[c] (46.06R)
Control (T+P+N)	3.00[ab]	61.73[a]
Check (Tomato only)	5.00[a]	13.27[f]

Values in parenthesis represent % reduction (R) or stimulation (S) of firmness decrease or percentage water loss by the plant extracts used

They noted that tomato had a relatively thick waxed cuticle that protects the fruit from losing water. The higher weight loss recorded by the lower concentrations of the plant extract at ambient condition could be due to lower active ingredients which encourages faster metabolism resulting in shriveling [25]. [26] also reported high percentage weight loss of 70% of pepper fruits stored at ambient conditions 8 days after storage due to relative humidity and temperature of the storage environment. According to [27] high temperature of about 60 – 80°C destroys vegetative cells of organisms resulting in increased metabolism, softening of tissues and disease infection. The weight loss could also be attributed to the type of surfaces and underlying tissues of the fruit. Also differences in morphology, physiological behavior, form and structure may account for different rates of water loss in horticultural crops. [24] noted that tomato had a relatively thick waxed cuticle that prevents the fruit from losing water and that a maximum weight loss of between 6 and 7% is considered as acceptable weight loss before tomato becomes non- saleable. Furthermore, [24] agreed that water loss increased pathogen invasion, causes post harvest deterioration leading to economic loss of tomato especially when sold by weight. Also, water loss usually results in the impairment of the visual, composition and eating quality of tomato [24]. Weight loss and visual quality attributes such as firmness is highly and significantly correlated. An increase in weight loss resulted in decrease in firmness during storage of fruits and vegetables [24]. [27] also reported that fruit firmness was reduced by hydrolytic enzymes which cause softening of fruits.

5. CONCLUSION AND RECOMMENDATION

The results of this study demonstrate that the aqueous extracts of the stem bark of *A. indica* and *V. amygdalina* exhibited fungitoxicity against *A. flavus*. *Azadirachta indica* at 15% w/v improved tomato fruit quality by reducing weight loss of tomato in storage.

The stem bark extract of *V. amygdalina* at 15% w/v concentration is recommended for the suppression of mycelia growth of *A. flavus*. This is because this treatment resulted in the highest inhibition of the colony growth of *A. flavus*. *Azadirachta indica* at 15% w/v concentration is recommended for improvement of tomato fruit quality in storage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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