

Antifungal effects of four tropical plant aqueous and ethanol extracts on post harvest rot of tomato (*Lycopersicon esculentum*) in Ado-Ekiti, Nigeria.

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Abstract: Antimycotic effects of aqueous and ethanolic extracts of *Tridax procumbens*, *Venonia amygdalina*, *chromolaena odorata* and *Azadirachta indica* were determined *in vitro* on causative agents of post harvest rot of yam tubers: *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Geotrichum candidum*. All the plants extracts at varying concentrations were effective in reducing the mycelia growth of the rot fungi. One ml of 25 and 15% ethanol as well as 85% and 65% aqueous extracts of different test plants each were used. The radial growths of the fungi were determined after 72 hrs. Each treatment had four replications. *Azadirachta indica* had the highest inhibitory effect accumulatively on the rot fungi using 65% and 80% aqueous extracts of value 51.93 and 61.73% mycelia growth reduction respectively though the effects were not significantly different from that of *Tridax procumbens* and *Venonia amygdalina* using 65% aqueous of mycelia growth reduction value of 47.43 and 47.70% respectively, but significantly different from other 85% aqueous extracts. Fifteen percent ethanolic extract of *Azadirachta indica* had the highest inhibitory effect of value 68.20% as well as 25% *Chromolaena odorata* of value 62.10%. The reality of using these antifungal plants to control tomato rot serves as good option to chemical control. *Azadirachta indica* is most recommendable as bio protective agent on tomato fruits.

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Introduction

Tomato belongs to the kingdom plantae angiosperm, family *Solanaceae*, order-solanales – *Solanum lycopersicum*. Tomato (*S. lycopersicum*) is herbaceous, usually sprawling in the night shade family that is typically cultivated for its edible fruit savory in flavour accordingly termed a vegetable. The fruit of most varieties ripen to distinctive red colour. Tomato plant reach up to 1.3metres (3-10ft) in height, and has weak and wooden stems that often vine over other plants. The leaves are 10-25cm (4-10in) long, odd, pinnate, with 5-9 leaflets on petioles (Acquah, 2002), each leaflet up to 8cm (3 in) long with a serrated margins; both the stem and leaves are densely glandular hair. The flowers are 1-2cm (0.4- 0.8 in) across, yellowish with fine pointed lobes on the corolla: they are borne in cymes of 3-12 together. It

The world tomato producing countries are as follows according to FAO (2008): China, 3381170t, USA, 12575900t, Turkey, 10985t, India, 10 260600t, Italy, 5976912t, Total, 129649883tonnes. Red raw tomato of 100g of fruit has the nutrient content as reported thus: energy 75kg (18k), carbohydrate 4g,

sugar 2.6g, dietary fiber 1 g, fat 0.2g, protein 1g, vitamin C (13mg, 22%) and water content 95g.

In respect to the world production of tomato, diseases range from fungal, bacterial, viral and nematode that poses great challenges to tomato production. Fungal diseases of tomato are as follow: *Alternaria stem canker* caused by *Alternaria alternata* f. sp *lycopersici*, anthracnose caused by *Colletotrichum corcodes*, *C. dematium*, *C. gloesporoides* and *Glomerella cingulata*, black mold rot caused by *A. alternaria*, *Stemphylium botryosum*, *Pleospora tarda*, *P. herbarium*; black rot caused by *Thielavopsis basicola*, *Chalara elegans*; black shoulder caused by *Alternaria alternata*, *Phytophthora capsici*, *P. dreschleri*, *P. nicotiana*.

Cercospora leaf mold caused by *Pseudocercospora fuligena*, *Pythium* damping off and fruit rot by *Pythium aphanidermatum*, *P. debaryanum* and *P. ultimum*. Age long chemicals have being used to control plant pathogens, however, in the recent time, pathogens have developed resistance, Therefore, there is a need for alternative approach to plant diseases control such as the use of plant extracts. Plant extracts are eco friendly, accessible to rural dwellers, cost effective and non or

less phytotoxic. Plant extracts have been successfully used to control a number of plant diseases (Okigbo and Emoghene, 2004; Okigbo and Nmeko, 2005; Amadioha and Obi, 1999; Okigbo, 2009). This research is therefore focused on the efficacy of four selected tropical test plant extracts, through aqueous and ethanol extraction on post harvest rot of tomato fruits.

Materials and method

Collection of infected and healthy tomato fruits

Infected tomato fruits with symptoms of softness were randomly procured locally from Oja-Oba market in Ado-Ekiti. Five samples were collected from each selling point, these were taken and placed in sterile polyethylene bags and conveyed into the laboratory for fungal isolation and subsequent identification. The identified isolates were used to infect healthy tomato fruit to establish their pathogenicity.

Isolation of the fungal organisms

Diseased portion of the yam tubers were cut under aseptic conditions into small bits into a sterile dish with the aid of scissors which was flamed over a Bunsen burner flame and dipped inside methylated spirit (Fawole and Oso 1988). The cut diseased and sterilized bits with 70% ethanol were then placed on Petri dishes containing solidified (potato dextrose agar) PDA. The solidified plates were incubated at room temperature ($28 \pm 2^{\circ}\text{C}$) in the dark for 72 hours. The fungal colonies grown from the incubated plates were sub-cultured into fresh medium until pure culture was obtained.

Microscopic examination was used after examining the colony characteristics. A sterile needle was used in taking a little portion of the hyphae containing spores on the sterile glass slide stained with lactophenol cotton blue and examined under the microscope for fungal structures. The morphology and culture characteristics observed were compared with structures in (Snowdon, 1990).

Pathogenicity test.

Healthy tomato fruits were surface sterilized with 0.1M mercuric chloride (HgCl_2) for 1 minute and washed in five changes of distilled water. A 5ml cork borer was punched to a depth of 4mm into the healthy tomato fruit and the bored tissues were removed. A 5 mm diameter disc from the pure culture was placed back. The wound was sealed with prepared candle wax according to the method of Fawole and Oso (1988). The control was set up in the same manner except that sterile agar disc was used instead of inoculums. The

inoculated tomato fruit were placed in 4 replications at room temperature (28 ± 2) under sterile condition. The pathogens were re-inoculated and identified using the same procedures described earlier.

Preparation of plant extracts.

The following local plants: *A. indica*, *T. procumbens*, *V. amygdalina*, *C. odorata* were air-dried and grinded separately, thirty gram of each sample was added to 15ml of distilled water in separate flasks. This was vigorously stirred and left to stand for 24 hours. The sample was filtered with a Whatman paper (No 1) and the filtrate used as extract.

Effect of plant extracts on fungal growth

Flat bottom flasks were used for the assay. Different percentage of extract solutions each was poured into separate flask containing sterilized potato dextrose broth, with a sterile cork borer, the different fungi were inoculated into separate flask and incubated at room temperature ($28 \pm 2^{\circ}\text{C}$) for 7 days. After the incubation period, mycelia from different broths were taken into pre-weighed filter paper, oven dried at 85% and reweighed, until a constant weight was obtained. The changes in weight were noted. For the control, no plant extract was added to the potato dextrose broth.

Mycelia extension of fungi.

The method of Amadioha and Obi (1999) was used to determine the effect of extracts on mycelia extension of the fungi obtained by placing one disc (3mm diameter) of 5 days old culture of the pathogens in each of five Petri-dishes (1cm diameter) with 170ml PDA medium and 3ml leaf extract. The control experiments were setup with 3ml of sterile distilled water. Five replications of leaf extracts agar per isolate were incubated at room temperatures ($28 \pm 2^{\circ}\text{C}$) for 7 days. Daily measurements of the mycelia extension of the cultures were determined by measuring culture along two diameters mycelia growth inhibition was taken as growth of the fungus on the leaf extract agar expressed as percentage of growth on the PDA. Fungitoxicity was determined in form of percentage growth of colony inhibition and calculated according to this formula:

$$\text{Growth inhibition (\%)} = \frac{(\text{DC} - \text{DT}) \times 100}{\text{DC}}$$

DC = 1

Where DC = Average diameter of colony with control

DT = Average diameter of colony with treatment.

Results

Information on the test plants used.

Common names	scientific names	Family	Parts used
Epo trida	<i>Tridax procumbens</i>	<i>Asteraceae</i>	leaf
Ewe Ewuro	<i>Venonia amygdalina</i>	<i>Asteraceae</i>	leaf
Ewe Akintola	<i>Chromolaena odorata</i>	<i>Asteraceae</i>	leaf
Ewe Dongoyaro	<i>Azadirachta indica</i>	<i>Malicicaae</i>	leaf

Table1: Inhibition % of mycelia growth of fungi in PDA poisoned with 65% and 85% cold aqueous leaf extract concentrations.

Test plants / treatment	% inhibition of radial growth of rot fungi.	
	65%	85%
<i>Chromolaena odorata</i>	42.58b	45.50b
<i>Tridax procumbens</i>	47.43bc	52.65c
<i>Venonia amygdalina</i>	47.70bc	49.18bc
<i>Azardiachta indica</i>	51.93c	61.73d
Control	30.75	30.75

Means in the same column with the same letters are not significantly different at 0.05 levels according to Duncan multiple test range.

Table 2 Inhibition % of mycelia growth of fungi in PDA poisoned with 15% and 25% ethanol plant extracts concentrations.

Test plants /treatments	% inhibition of radial growth of rot fungi (Means.)	
	15%	25%
<i>Chromolaena odorata</i>	51.15b	62.10b
<i>Tridax procumbens</i>	55.63b	57.20b
<i>Venonia amygdalina</i>	54.58b	57.45b
<i>Azardiachta indica</i>	68.20c	31.25a
Control	30.50	31.25

Means in the same column with the same letters are not significantly different at 0.05 levels according to Duncan multiple range test.

The rot fungi isolated from the rotted tomatoes were: *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Geotrichum candidum*. The pathogenicity test carried out on using these organisms on the healthy tomatoes showed that these fungi caused post harvest spoilage of tomato.

The test plants used in this investigation were: *Chromolaena odorata* (leaf), *Tridax procumbens* (leaf), *Venonia amygdalina* (leaf) and *Azadirachta indica* (leaf) (Table 1 and 2). *Azadirachta indica* had the highest inhibitory effect on the radial growth of the rot fungi in the plate using 60% and 80% aqueous leaf extract with the values 51.93% and 61.73% respectively.

The most efficacious property of *A. indica* was also recorded using the 15% and 25% ethanol extract of *A. indica* with the values 68.20% and 84.18% respectively. The effectiveness of *A. indica* was significantly different from other plants in both aqueous and ethanol extract.

The control experiment revealed the uninhibited mycelia growth of the isolated spoilage fungi of tomatoes in storage. All the test plants were inhibitive to the rot fungi of tomato at varying percentage.

Discussion

The spoilage rot identified with tomato rot in storage transit were *A. niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Geotrichum candidum*. Antifungal effectiveness of some tropical plants extracts in controlling several plant pathogens has been reported by several workers (Okigbo and Emoghene, 2004; Tewari and Nayak, 1991, Amadioha, 2000; Okigbo and Nmeka, 2005; Amadioha and Obi, 1999; Okigbo and Ikediugwu, 2000).

This study showed that, *C. odorata*, *T. procumbens*, *A. indica* and *V. amygdalina* have proved effective in the control of storage deterioration of tomato fruits. The antimycotic effects of these test plants have being reported. Natural plant products and their analogues are important sources of new agricultural chemicals used as antimicrobial activities of plant extract (Cardelina, 1982).

Nahed (2007) reported the inhibition of *Fusarium oxysporum*, cause of rot in cucumber using cold aqueous extract of *A. indica*. Paul and Sharma (2002) induced resistance in barley against leaf strip disease with *A. indica* extract. Fokunang *et al.*, (2000) used the extract of *V. amygdalina* and *A. indica* against

cassava anthracnose disease caused by *C. gloesporides*. Wet rot disease of *Amaranthus* sp caused by *Choanephora cucubitarum* controlled with extract of *A. indica* by (Olufolaji, 1999). Yam rot caused by *Rhizopus stolonifer* was controlled using *V. amygdalina* by (Hycenth, 2008). Okigbo and Ajalie (2008) inhibited the growth of some human pathogens with the extract of *C. odorata*. Also, antifungal effect of *T. procumbens* has been reported. The extract of the test plant both aqueous and ethanol can serve as alternative to chemical control because of lack of residual effect and can be applied cheaply

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