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# Isolation and Control of Fungal Rot Pathogen of Tomato Fruit Using Aqueous Leaf Extracts of *Azadirachta indica* in Mubi, Adamawa State

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## Abstract

A study was carried out to evaluate the efficacy of *A. indica* crude/aqueous leaf extracts against post-harvest fungal rot pathogens of tomato fruit (*in vivo*). Samples of tomato fruits (108) were collected from three different markets in the study area (*Kasuwa kuturu, Tsohon kasuwa and Sabon kasuwa*) at random. The fungal pathogen isolated and identified were; *R. stolonifer* and *A. niger* and were confirmed through pathogenicity test to be pathogenic to tomato fruits. *Tsohon kasuwa* had the highest incidence (%) of rot with 22.2%, *Sabon kasuwa* had 16.7% and *Kasuwa kuturu* had 11.1%. *in vivo* control using *A. indica* yielded a positive result in both pathogens. There was significant difference between all the treatments with the extracts as compared with the control at probability level of P<0.005. Treatments with 60% crude extracts of *A. indica* gave the best results with 1.39 mm in *A. niger* and 1.43 mm in *R. stolonifer* as rot diameter. It was recommended that farmers of tomatoes should shorten the distance between harvesting and collection time to reduce chances of fruit exposure to the pathogens.

**Keywords:** Pathogens; Extracts; *A. niger*; *R stolonifer*; *A. indica*; Pathogenicity and *in vivo*

## Introduction

Tomato (*Solanum lycopersicum L.*) belongs to the family Solanaceae and it is an annual sub-tropical fruit vegetable crop. The crop originated from South America and was introduced to Europe in the 16th Century and later to East Africa by colonial settlers in early 1900 [1]. The crop is grown for both fresh domestic and export market but there is increasing demand for processed tomato products [2]. The tomato fruits are largely water (about 94%) but have moderate quantities of soluble sugars or several organic acid (especially citric and malic acid) mineral salt and relatively large quantities of the Vitamin C compared with oranges, tomatoes contain nearly 20 times as much Vitamin A, the same amount of Vitamin B, slightly more Vitamin B2. Effective and efficient management of phyto-diseases is generally achieved by the use of Vitamin C and chemicals [3]. Tomato does well in warm climate with an altitude range of 0-2100 m above sea level. It requires rainfall ranging between 760 mm to 1300 mm and deep fertile loam soil that is well drained, with high content of organic matter and a pH ranging between 5-7 [4]. Fruits are used in salads or cooked as a vegetable, processed into tomato paste, sauce and puree. The nutritional value of tomato makes it a widely accepted vegetable by consumers. Fruits are rich in calcium, phosphorus, magnesium, copper, niacin, iron, folate, Vitamin A, B6, Vitamin E, Vitamin B2, Vitamin C, iron and carbohydrates [1]. Furthermore, the fruit has medicinal value as a gentle stimulant for kidneys, and washing off toxins that contaminate the body systems. It improves the status of dietary anti-oxidants (lycopene, ascorbic acid and phenols) in diet [5]. Tomato juice is known to be effective for intestinal and liver disorders [1].

Post-harvest diseases destroy 10-30% of the total yield of crops and in some perishable crops like tomato especially in developing countries; they destroy more than 30% of the crop yield [6,7]. The quality of tomato is affected by post-harvest handling, packaging, transportation and storage which may result in decay and production by microorganisms which become activated because of the changing physiological state of the fruit [8]. Fruits, due to their low pH, high moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots, may also make them unfit for consumption by producing mycotoxins [9]. Arah et al. reported that handling practices like sorting and grading, packaging, storing and transportation played an important role in maintaining quality and extending shelf life in tomatoes [10]. Research has also revealed that post-harvest loss of fruits due to microbial infections in Nigeria ranges between 50% and 90% [11,12]. In view of the foregoing, there is need to isolate and identify microbes associated with tomato fruits spoilage with the view to proffering suitable solutions of

controlling them before reaching the final consumers, to safeguard human health. The aim of this study therefore focused on isolation and identification of pathogenic microorganisms associated with tomato fruit spoilage in Adamawa state Mubi, Nigeria and their control.

## Materials and Methods

### Description of the study area

Mubi is geographically located between Latitudes 10˚30' and 10˚05'N and Longitudes 13˚10' and 13˚30'E North of the Greenwich Meridian [13]. Mubi exhibits both dry and wet tropical climate type. Also, it occupies an area of 192,307 km and has a population of 260,009 people [13]. The dry season begins in November and ends in March, while the rainy season runs from April to October each year. Rainfall annually is about 900 mm with highest frequencies in July and August. Temperature ranges from warm to hot throughout the year but experience cool period between November and February with gradual increase in January to March. The relative humidity of the area is low but begins to rise from April to August maximally (Figure 1) [14].

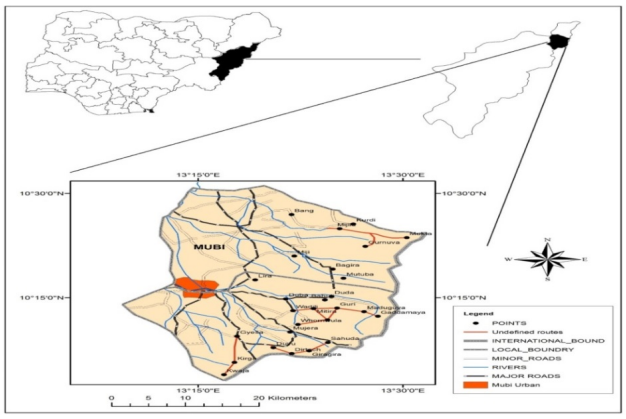


**Figure 1:** Map of the study area (Mubi).

### Sources of sample and sample size

Tomato fruit with the symptoms of rot and decay of the tissue coated and penetrated deep into the fruits were collected from three different markets in the Local Government Area. A total of 108 samples were collected from these three different markets (*Kasuwan kuturu, Tsohon kasuwa* and *Sabon kasuwa*), 12 samples were collected at random from three different traders in different location in each market.

### Determination of diseases incidence in the market

Samples of tomatoes were collected at random from three markets selected, in which samples were collected from different traders at different locations in the markets. The incidence of tomatoes rot in each market was determined by counting the infected tomatoes from the samples collected from each market.

### Isolation and identification of pathogen

The diseased tomato samples were first washed under a running tap water, then dipped into 1% Sodium Hypochlorite to surface sterilize for three seconds and rinsed in three changes of sterile distilled water. They were then blotted dry by using sterile blotting paper. For fungal isolation, direct plating method was used. A sterile scalpel was used to cut 3 mm × 3 mm sections of tissue from the tomato moving from the healthy portions to the decayed portion where the pathogens are likely to be more active. The pieces were dried using sterile filter paper and the dried infected tissues were directly plated on Sterile PDA. They were incubated in the laboratory at room temperature (25˚C) for 5 days. After incubation fungal colonies of different shapes and colours were observed on the plates which were then re-isolated and sub-cultured on separate sterile media.

Fungal identification was done using morphological characteristics and comparing with established keys of [15]. Each isolate was subjected to colony and microscopic examinations during which their morphological features were observed and recorded. Identification of the fungi was based on growth patterns, colour of mycelia and microscopic examinations of vegetative parts.

### Pathogenicity test

Pathogenicity test was carried out using the techniques described by Okigbo et al. [16] and Zakawa et al. [17]. Healthy tomato samples was obtained from the Markets in Mubi, and brought to Botany Laboratory at Adamawa State University Mubi in sterile polythene bags. The tomatoes were then washed under running tap to eliminate dirt from their surfaces. They were surface sterilized in 1% NaCl for three seconds. Thereafter, they were rinsed in three changes of sterile distilled water and wiped dry using a sterile blotting paper. A sterile five (5) mm cork borer was used to punch the tomatoes and the discs removed. The same size of the cork borer was used to cut sections of each of the cultures to be inoculated (fungal pathogens) and the disc was used to inoculate the healthy wounded tomatoes [18]. The wound on the inoculated tomatoes were sealed using sterile transparent adhesive tape. The control was in the same manner but sterile PDA was used without fungal cultures. Three tomatoes were placed in each sterile polythene bag as a treatment, replicated four times and stored at room temperature (25˚C) in the laboratory. Disease development was checked after two days.

### Preparation of leaf crude extract of *Azadirachta indica*

Crude plant extract was obtained from neem leaves. The extraction process followed the procedure described by 19. Handa et al. [19]. Neem leaves were collected from Adamawa State University Ecological Study Area Mubi station in the school premises and brought to Botany Laboratory for drying. The leaves were washed under tap water, rinsed in three changes of sterile distilled water and dried using sterile blotting paper. They were then allowed to dry under the shade. It was grounded into power, 10 g of the dried leaf powder was placed in 90 ml of

sterile distilled water and left to stand at room temperature for 24 hours. The concentrations were gotten by diluting the stock solution (100%), 60 ml of the stock solution was diluted in 40 ml of sterile distilled water to obtain 60% concentration, 40 ml of stock solution diluted with 60 ml of sterile distilled water to obtain 40% concentration and so on.

### Determination of the effects of crude leaf extract on growth of fungal Mycelia (*In vivo*)

Fresh, semi-ripe fruits were washed and surface-sterilized by dripping in 0.1% mercuric chloride solution for 30 seconds then were washed three (3) times with sterilized distilled water and allowed to dry. Tomatoes (three replicates for each fungus, each replicate containing 3 fruits) were wounded with sterile cork borer, a 5.0 mm diameter holes was made on each tomato fruit with cork borer. A disc of each fungus culture (4.0 mm diameter) was soaked for 30 seconds in l ml of leaf extract in sterile petri-dish; and was immediately introduced into the hole, using a sterile mounting needle and forceps; the tissue that was removed from holes was replaced after 2.0 mm had been cut off to compensate for the thickness of the inoculate. The points of inoculation were sealed with Vaseline and inoculated tomato fruits were incubated on clean laboratory table for 7 days at room temperature (25+-30C). Data from lesion size of pathogens were measured using ruler (mm).

### Experimental design and data analysis

The experimental design used was the Complete Randomized Design (CRD) to examine the antifungal activity of the neem leaves on tomato rot and data collected were tested statistically using the one way analysis of variance (ANOVA) [15] and significant means were separated using Duncan Multiple Range Test (DMRT).

## Results, Discussion and Conclusion

### Identification and incidence of pathogens

The pathogens isolated were identified by morphological characteristics and comparing with established keys by 20. Alexopoulas et al. [20] as *Rhizopus stolonifer* and *Aspergillus niger* Plate 2 (a and b). These pathogens were confirmed to be responsible for tomato soft rot through pathogenicity test in which they produced the same kind of rot observed in the original samples from the market. The % incidence of rot showed that soft rot of tomato fruit occurred in all three markets, however, *Tsohon kasuwan* had the highest incidence with 22.2%, followed by *Sabon kasuwa* with 16.7% and lastly *Kasuwan kuturu* with 11.1% (Table 1). The result agrees with those of Chuku et al. [21,22] who reported that *R. stolonifer* and *A. niger* were responsible for soft rot of tomato fruit.

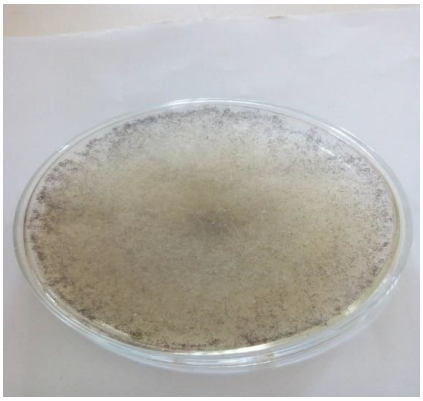

**Plate 2(a):** Pure culture of *R. stolonifer* .

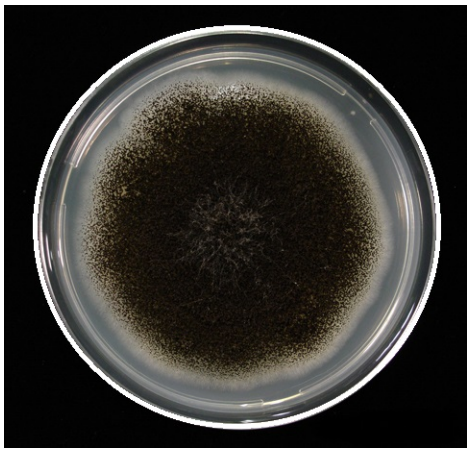

**Plate 2(b):** Pure culture of *A. niger.*

**Table 1:** Incidence of Tomatoes Rot in the Markets

| Markets | Incidence (%) |
|---|---|
| Kasuwan Kuturu | 11.10% |
| Tsohon Kasuwa | 22.20% |
| Sabon Kasuwa | 16.70% |

### Pathogenicity test

The study revealed that the micro-organisms isolated from the infected tomato fruits were pathogenic but with varying pathogenicity as shown in Table 2. When inoculated into healthy tomato fruit, *Rhizopus stolonifer* caused the most rapid (100%) infection where the inoculated fruits were completely rotten by the end of the fourth day of inoculation. The fruits were completely disintegrated with extensive mycelial growth forming a dark colour covering the fruit. The fruits looked water soaked in appearance and wrinkled with depression. Fruits inoculated with *Aspergillus niger* had water soaked lesions with some black mycelia. From the study it was noted that *Rhizopus stolonifer* caused the most rapid rot (70% infection) within the first two days. This observation agrees with the report of Kalyoncu et al.

[23], who recorded that *R. stolonifer* caused the most rapid rot on stored tomatoes in Nigeria. According to Chuku [22], *Rhizopus* recorded the highest rot (80%) on Avocado and pears in Nigeria. It is possible that spreading of the harvested fruits on the ground during the harvesting makes the harvested fruits carry heavy spore load from farms. Mixing of small fruits with large fruits causes more bruises on the small fruits. Soft and overripe fruits were also mixed together with firm fruits making the soft ones to be compressed resulting to losses. Sometimes tomatoes were harvested early in the morning with the morning dew. This increases moisture content that makes them more prone to fungal spoilage [24].

**Table 2:** Pathogenicity of the Pathogens.

| Days | *R. stolonifer* | *A. niger* |
|---|---|---|
| 1 | $0.0^{a}$ | $1.55^{a}$ |
| 2 | $0.78^{b}$ | $1.70^{b}$ |
| 3 | $1.18^{ab}$ | $2.03^{ab}$ |
| 4 | $1.5^{c}$ | $2.15^{c}$ |
| 5 | $1.5^{c}$ | $2.15^{c}$ |
| 6 | $1.5^{c}$ | $2.15^{c}$ |
| 7 | $1.5^{c}$ | $2.15^{c}$ |
| Mean followed by the same superscript letter in the column are not significantly different from each other at P<0.05 (DMRT) | | |

**Table 3:** Effect of Leaf Extracts of *Azadirachta indica* on *in vivo* control of *R. stolonifer* and *A. niger*.

| Treatment | *R. stolonifer* | *A. niger* |
|---|---|---|
| 20% | $1.50^{a}$ | $1.76^{a}$ |
| 40% | $1.44^{b}$ | $1.62^{b}$ |
| 60% | $1.43^{b}$ | $1.39^{c}$ |
| Control | $1.98^{c}$ | $2.00^{d}$ |
| Mean followed by the same superscript letter in the column are not significantly different from each other at P<0.05 (DMRT) | | |

## Effects of aqueous neem-leaf extract on growth of fungal mycelia (*in vivo*)

*In vivo* control on both the pathogens (*A.niger* and *R.stolonifer*) using aqueous leaf extracts of *A. indica* shows that the rate or degree of inhibition increases as the concentration of the extracts increases. The highest level of inhibition was recorded at 60% control in both organisms shown in Table 3. There was statistical significant difference between the control and all other treatments (concentrations) in both pathogens at probability level of P<0.005. In *R. stolonifer*, the treatment with 60% concentration gave the best result with 1.43 mm tomato soft rot followed by 40% concentration with 1.44 mm, 20% concentration had 1.50 mm while the control (0%) had 1.98 mm. the same pattern was recorded in the control of *A. niger* with 60% concentration having 1.39 mm, 40% (1.62 mm), 20% (1.76 mm) and control had 2.00 mm respectively (Table 3). The result agrees with Meena and Mariappan [25] who states that neem leaf extracts inhibited the growth and spore germination of seed microflora including *A. niger* and *R. stolonifer*. The results from this study suggest the presence of anti-fungal compounds in the neem crude extracts which were able to control the growth of fungal pathogens tested. Experiment shows that extracts from the *Azadirachta indica* leaves inhibited conidial germination of radial mycelia growth of number of pathogenic fungi such as *Aspergillus* spp. *Fusarium* spp. and *Cladosporium* spp. [26]. Ijato [27] reported that *Azadirachta indica* leaves inhibited mycelial growth of *Aspergillus* spp and *Botrytis* spp isolated from mango fruit rot in Jimeta-Yola. The Meliaceae specially *Azadirachta indica* (Indian neem tree), contains at least 35 biologically active principals components of which nimbin and azadirachtin are the most active insecticidal ingredients and are present predominantly in the seeds, leaves and other parts of the neem tree [27].

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