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Research Article

Investigation on causes and control of post-harvest spoilage of tomatoes (*Solanum lycopersicum*) using some selected botanical extracts

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Abstract

This study was carried out at Microbiology laboratory of Bauchi state university Gadau to isolate and identify pathogenic micro-organisms associated with deterioration of tomatoes fruits. Fruits samples of infected and non-effected tomatoes were collected from an open market Azare in Bauchi, Nigeria. The infected tomatoes were directly plated on potato dextrose agar (PDA) for fungal growth, incubated at room temperature, and observed from 24 hours to 7days. Also each tomato flesh was streaked on nutrient agar (NA) for the Bacterial growth and incubated at 37°C for 48 hours. Identification using slide culture technique was followed after which different colonies were obtained. The study also aimed at evaluating the efficacy of some selected plant crude extracts against the post-harvest tomato damaging pathogens. The organisms identified that cause's post-harvest tomatoes spoilage were Bacillus subtilis, *Staphylococcus aureus* and Geotrichum candidum, causes the most rapid destruction followed by *Staphylococcus aureus* and Bacillus subtilis causes the least rot demonstrating that it is not one of the most damaging pathogen. Ginger, Garlic and Neem crude plant extracts were found to have potential anti-microbial compounds that inhibit tomato fruit rots at various concentrations. The evaluated concentrations were effective against the test pathogens but efficacy varied with the concentration. The most effective crude extract was Garlic being effective in each concentration compared to others, followed by Ginger and then Neem. The extracts also were effective in controlling tomatoes rot.

Keywords: *Solanum lycopersicum,* PDA, *Geotrichum candidum*, Microbiology, Extracts

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the family *Solanaceae* which 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 (Wamache, 2005). In Nigeria, tomato plays a vital role in meeting domestic and nutritional food requirements, generation of income, foreign exchange earnings and creation of employment (Sigei et al., 2014). The crop is grown for both fresh domestic and export market but there is increasing demand for processed tomato products (Mungai et al., 2000). Tomato production in Nigeria is about 1.5 million tons of tomatoes a year (Google source). Nigeria is the 14th largest producer of tomatoes in the world. It is also the largest producer of tomatoes in sub-Saharan Africa. Tomatoes are mostly produced in Northern part of Nigeria followed by Southwest part of Nigeria (Google source). Farmers from these zones take their tomatoes to markets where Nigerians can easily buy them. Though it's only tomatoes from Northern part are always available throughout the year in the markets. Tomatoes from South-west are only available for a while in the market. The crop is grown either on open field or under greenhouse technology. Open field production account for 95% while greenhouse technology accounts for 5% of the total tomato production (Seminis, 2007). Tomato crop does well in warm climate with an altitude range of 0-2100 m above sea level. Fruits are used in salads or cooked as a vegetable, processed into tomato

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paste, sauce and puree. Tomato production is constrained by factors such as poor pre-harvest practices, adoption of poor production techniques, rough handling and moisture condensation causing pathogen infestation. Packaging in bulk without sorting and grading of produce, damage during transport and storage due to mechanical injuries are other factors contributing to post-harvest losses. Inadequate storage, distance and time consuming market distribution, poor access to the market, post-harvest spoilage micro-organisms and cultivars disposition to diseases causes high post-harvest losses of tomatoes.

According to Food and Agricultural Organisation (2002), records of post-harvest losses do not exist and if available they do not cover enough period of time and the figures are only estimates made by observers. It has been estimated that 20-50% of tomato fruits harvested for human consumption are lost through microbial spoilage while other losses result from damage by dynamic stresses during transit, and through rough handling during loading and unloading. Thirupathi et al. (2006) estimated the magnitude of post-harvest losses in fresh fruits to be 25-80%. Post-harvest decay remains a major challenge in tomato production. The magnitude of post-harvest losses vary from one country to another, one season to another and even one day to another (Mujib et al., 2007). There are numerous micro-organisms that cause post-harvest decay of tomatoes. Among these, fungi and bacteria are the most destructive. 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 Moss, (2002). Mycotoxins are potential health hazards to man and animals and in most cases they are unnoticed. Control of fruit rot also remains a major challenge in tomato production estimates of post-harvest losses in Azare and Gadau, Bauchi state Nigeria County, identify pathogens involved in post-harvest decay of tomato fruits and evaluate ways of managing them using crude plant extracts. Several kinds of synthetic fungicides have been successfully used to control the post-harvest decay of fruits and vegetables (Adaskaveg et al., 2002; Kanetis et al., 2007). However, there are three major concerns:

(a) The increasing consumer concern over pesticide residues on foods which are toxic and carcinogenic, (b) predominance of fungicide resistant strains of fungi due to excessive use of fungicides, (c) environmental pollution. Therefore there is need for new effective means of post-harvest disease control that poses less risk to human health and the environment. Natural plant products and their analogues have been found as important sources of agricultural bio-pesticides which serve as anti-microbial properties of the plant extracts (Okigbo & Emoghene, 2009). Arokiyaraj et al. (2008), Shanmugavalli et al. (2009), Swarnalatha & Reddy (2009), reported that plants are sources of natural pesticides that lead in new pesticide development. Anti-fungal and anti-bacterial compounds of neem plant leaf, ginger rhizome, and garlic bulb crude extracts on rot pathogens of post-harvest tomato fruits were also targeted in this study.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Azare, Bauchi state Nigeria. The rainfall in Bauchi state ranges between 1300 mm per annum in the south and only 700 mm per annum in the extreme north. This pattern is because in the West Africa sub-region, rains generally come from the south as they are carried by the southwester lies. There is, therefore a progressive dryness towards the north, culminating in the desert condition in the far north. So also is the case in Bauchi state. The annual temperature ranges between 9.7-21.6°C. Bauchi state is one of the states in the northern part of Nigeria that span two distinctive vegetation zones, namely, the Sudan savannah and the Sahel savannah. The Sudan savannah type of vegetation covers the southern part of the state. Here, the vegetation gets richer and richer towards the south, especially along water sources or rivers, but generally the vegetation is less uniform and grasses are shorter than what grows even farther south, that is, in the forest zone of the middle belt. There is not much rainfall in Azare town all year long. The average annual temperature is 26.4°C, about 768 mm of precipitation annually (**Figure 1**).

Survey of post-harvest losses in Azare and Gadau

The survey was carried out in Azare area of Bauchi state in July, 2017 for the collection of tomatoes fruits samples and to determine the causes of the post-harvest losses in the area.

Collection of infected fruit samples and isolation of pathogens

Collection of infected fruit samples:

Market center in Azare area was targeted for the survey. Infected tomato fruit samples were identified by physical examination and then collected randomly from the local markets. Twelve (12) fruits with various rot symptoms were collected, placed in polythene bags and brought them to the Microbiology laboratory, Bauchi state University Gadau for further analysis.

Isolation of pathogenic fungi and bacteria from rotting fruits:

Potato dextrose agar (PDA) and Nutrient agar (NA) were the standard media used to isolate the fungal and bacterial

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Figure 1: Study location of Azare town Katagum. (Source: Google maps).

pathogens, respectively from the fruits. The infected tomatoes samples were first washed under a running tap, and then dipped into 1% Sodium Hypochlorite to surface sterilize for three minutes and rinse in three changes of sterile distilled water and then blotted dry by using sterile blotting paper.

For bacteria isolation, a sterile wire loop was used to get some cells of the fruit tissue and streaked on the NA in Petri dishes and incubated at 35°c respectively. Colony formation was observed after the second day.

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 to dry the juice. The dried infected tissues were directly plated on sterile PDA and then incubated in the laboratory at room temperature (25°C) for 7 days. After incubation fungal and bacterial colonies of different shapes and colors were observed on the plates and were re-isolated and sub-cultured on separate sterile media. The different isolate were characterized using the slide technique, microscopic examination was carried out after gram staining the bacteria isolates, while Lacto phenol blue staining was carried out for fungi isolates.

Identification of pathogens

Fungal identification:

Fungal identification was done using morphological characteristics and comparing with established keys (Barnnet & Hunter, 1999). 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, color of mycelia and microscopic examinations of vegetative and reproductive structures.

Bacterial identification:

The bacteria were first identified using colony color and morphology on nutrient agar (NA) according to Schaad (1980), followed by Gram staining (De Boer & Kelman, 1975).

Plant materials

Preparation of plant crude extracts:

Crude plant extracts were obtained from neem leaves, garlic cloves and ginger rhizomes. The extraction process procedure followed was described by Handa et al. (2008).

Neem leaves was collected from Bauchi state University Gadau Botanical Garden to Bauchi state University Gadau Microbiology Laboratory for drying and further analysis. The leaves were washed under running tap water, rinsed in three changes of sterile distilled water and dried using sterile blotting paper. They were placed in the oven and dried at a temperature of 40°C for three days.

Ginger rhizomes and garlic bulbs were bought from Azare market and brought to the laboratory. Garlic cloves were peeled washed in sterile distilled water and dried using sterile blotting papers. They were cut into smaller pieces and placed in the oven to dry at a temperature of 40°C for three days. Ginger rhizomes were also washed under tap water and rinsed in three changes of sterile distilled water. They were blotted dry using sterile blotting papers, peeled, cut into smaller pieces and placed in the oven at the temperature of 40°C for three days.

The neem leaves, garlic and ginger were grounded to powder by the use of a sterile mortar and pestle so as to rapture tissues and cell structures to release the active cell contents. The powdered extracts were placed in sterile specimen bottles. This was done because to maximize the surface area which in turn enables the mass transfer of active ingredients from the plant material to the solvent. Twenty grams (20 gms) of each of the powder was put into separate sterile conical flasks and 60 ml of methanol was added to each of the plant powder ensuring that the powder was completely immersed into the solvent, then shaken vigorously and allowed to stand on the bench at room temperature but shaken at different intervals for two days. A sterile funnel was placed into a 500 mls conical flask and then a Whitman's (No.2) filter paper was folded and placed into the funnel. The extracts were poured gradually into the filter paper and allowed to trickle into conical flask. The filtrate in the conical flask were poured into beaker and placed in a water bath for 60 minutes at 50°C to concentrate the extracts by evaporating the beaker. The concentrated crude extracts were dried in an oven at 40°C for two days until a powder like substance remained at the bottom of the universal bottles. The labeled universal bottles containing the powder were stored in the refrigerator at 4°C.

Effects of crude plant extracts on growth of fungal mycelia and bacterial colonies:

The effectiveness of the crude extracts in controlling rots was evaluated with the four most damaging pathogens as determined during the pathogenicity tests. The experimental design was a completely randomized design, replicated four times. The method of Amadioha & Obi (1999) was used to determine the effects of the crude extracts on the fungi.

Different concentrations of the crude extracts were prepared by weighing separately 1 mg, 2 mg and 3 mg of ginger, garlic and neem powder respectively. Each powder was dissolved in 1ml sterile distilled water to form solutions of different concentrations. A sterile media of PDA and NA were prepared and each of the different extract concentrations are dispensed into four Petri-dishes replicated four times. Three (3) ml of water was mixed with the media for the negative controls. The media was allowed to cool and solidify. The bacteria were streak on each of the amended media. The colony forming units (CFU) were observed after 48 hr (Bernett HL & Hunter BB, 1999).

For the fungal treatment 5 mm fungal culture discs from one week old cultures was cultured at the center of each Petri dish per replicate and incubated at room temperature. Radial growth from each of the treatment was observed after the second day and repeated at an interval of 24 hr. up to the seventh day.

Effects of plant extracts on post-harvest tomato disease development:

Healthy tomato samples were obtained from market at Azare and brought to Microbiology Laboratory, Bauchi State University. The samples of fruits were washed under running tap water but were not surface sterilized so as not to interfere with surface pathogens. The fruits were then dipped into the treatments prepared by dissolving 3 mg of each of the crude extract into 1 ml of sterile distilled water for five minutes and then air-dried. For each extract three treated tomatoes were placed into a bowl and replicated four times.

The experimental design was a completely randomized design replicated four times. The control treatment was immersed in tap water in a basin, dried and placed into the plastic bowls without being dipped into the crude extracts. The fruits were left uncovered in the laboratory at room temperature and disease development observed. The number of rotting fruits was counted after twelve days.

RESULTS

Isolation and identification of pathogens associated with post-harvest losses

The pathogens that were isolated and identified were *Bacillus subtilis, Staphylococcus aureus* and *Geotrichum candidum*. Among the pathogens, *Geotrichum candidum* (fungi) was the most occurring organism with 56.3%, *Staphylococcus aureus* with 25% and *Bacillus subtilis* with 18.7% (bacteria) shown in **Figure 2**.

Geotrichum candidum:

The fungus colony grew on PDA being low, flat, white and leathery with no reverse pigmentation. Hyphae were hyaline septate, branched and broke up into chains of hyaline, smooth, one-celled, subglobose to cylindrical, slimy arthroconidia (ameroconidia) by the holoarthric fragmentation of undifferentiated hyphae. The arthroconidia, were quite variable in size, aerial, erect or recumbent, cylindrical, hyaline, unicellular and barrel shaped.

Staphylococcus aureus:

The bacteria colony grow on NA media is circular, pinhead colony which is convex with entire margins. The colony appears the colour of the agar.

Bacillus subtilis:

The bacteria colony was dry, flat and irregular with locate margins. The colony colour appears creamy shown in **Tables 1 and 2**.

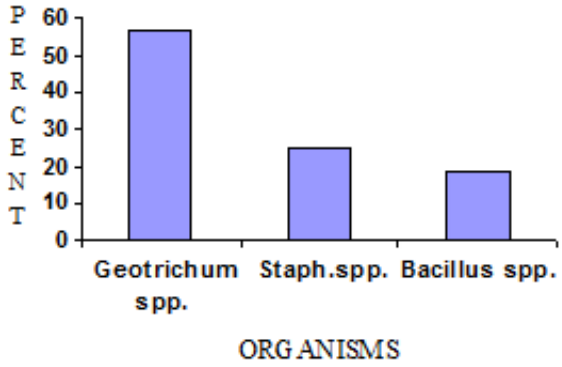

Figure 2: Percentage of organisms causing tomatoes spoilage.

Table 1. Bacteria (Gram Staining).

S/N	Sample Name	Media Used	Gram Reaction	Colonial Appearance	Shape	Formation	Organism Suspect	Number of colony
1.	Tomato UTC	N/A	+	Purple	Spherical	Cluster	*Staphylococcus aureus*	5
2.	Tomato ROMA	N/A	+	Purple	Rod	Cluster	*Bacillus subtilis*	3

Table 2. Fungi (Lacto-Phenol Blue Stain).

S/N	SampleName	Macroscopic Appearance	Microscopic Appearance	Organism Suspect	Number of colony
1.	Tomato UTC	Whitish colour	Butryous circular	*Geotrichum candidum*	4
2.	Tomato ROMA	Whitish colour	Butryous circular	*Geotrichum candidum*	4

Pathogenicity test

Pathogenicity test was carried out using the techniques described by Okigbo & Emoghene (2009). Healthy tomato samples were obtained from the Azare market, and brought to Bauchi state University Gadau, Bauchi State. The tomatoes were then washed under running tap to eliminate dirt from their surfaces. They were surface sterilized in 1% NaoCl for three minutes. 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 of the previously isolated fungal pathogens and the discs were used to inoculate the healthy wounded tomatoes (El-mougy et al., 2004). The wound on the inoculated tomatoes was sealed using sterile transparent adhesive tape. The negative control was also set 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. The pathogens were re-isolated and identified as described earlier.

For bacterial isolates a sterile loop that had been dipped into the culture isolate was used to introduce the bacteria into wounded healthy tomatoes. The negative control was also set in the same manner but sterile NA was used without bacterial cultures. Three tomatoes were placed in each sterile polythene bag, replicated four times and stored at room temperature in the laboratory. Disease development was checked after 48 hours. The pathogens were isolated and identified as described earlier.

Determination of tomato fruit damage by the isolated pathogens

This study revealed that the most damaging pathogen was *Geotrichum* spp. causing rot in both UTC and Roma it disintegrated the entire fruit after the fourth days of inoculation. *Staphylococcus* spp. was the second most damaging pathogen but has more effect on UTC tomatoes. The rot damage differed significantly in the two cultivars. *Bacillus* spp. was the third damaging pathogen and has more effect on Roma tomatoes.

Effect of crude plant extracts on post-harvest pathogens

Geotrichum spp.

The results indicated that the three plant extracts can inhibit radial growth on *Geotrichum* spp. as compared to the control but the rate of inhibition varied with concentrations. 1 ml concentration of the three plant extracts differed significantly in their effects on *Geotrichum* spp. as indicated. Garlic was most effective followed by ginger. At the concentration of 2 ml the effects on *Geotrichum* spp. also differed significantly. At concentration of 3 ml all the extracts were found to be effective. The effectiveness of

neem differed significantly at different concentrations. The most effective concentration was 3 ml. The effect of ginger also differed significantly at different concentrations and 3 ml concentration was the most effective. Garlic was effective in all the concentrations and therefore was the best for controlling *Geotrichum spp.*

Effect of crude plant extracts on *Staphylococcus spp.*

The study revealed that in different concentrations of the extracts, the number of colony forming units of *Staphylococcus* spp. on the amended NA varied significantly as compared to the control. At 1 ml of all the extracts, garlic was the most effective with the least colony forming units. Neem extract was the second most effective while ginger was the least effective. At 2 ml garlic extract was the most effective where there were no colony on amended NA. Ginger was the second most effective while neem was the least effective. However, at 3 ml the three extracts were effective. The effectiveness of neem extract at different concentrations differed significantly with the 3 ml being the most effective as indicated by no growth on the amended Nutrient agar. At 2 ml the colony forming units were less than 1 ml. The effect of ginger crude extract on the growth of *Staphylococcus* spp. differed significantly at different concentration and also comparing to the control. 3 ml was the most effective while the 1 ml was the least effective. The effectiveness of garlic also differed significantly when compared to the control. Three mg/ml and 2 ml concentrations had the same effect such that no growth occurred on the amended NA. A few colony forming units were on the NA with 1 ml. On evaluation of the three extracts, garlic was the most effective but ginger and neem crude extracts did not differ significantly (Wamache A, 2005).

Effect of different extracts on *Bacillus subtilis*

The study revealed that the effectiveness of the extracts at 1 ml differed significantly with the garlic extract being the most effective. At 2 ml the effectiveness also differed significantly with garlic extract being the most effective. At 3 ml there was no growth in all the treatments.

Effect of crude extracts in controlling tomato rots

All the extracts from the three plants were effective in controlling the rot pathogens on tomato fruits as compared to the control.

CONCLUSION

From this study, it was concluded that causes of post-harvest losses on tomatoes fruits occur as a result of micro-organisms (fungi and bacteria). In this study, Geotrichum spp., *Staphylococcus* spp., *Bacillus* spp. was identified. Among the pathogens identified *Geotrichum* spp. was the most destructive pathogen, followed by *Staph.*spp. While *Bacillus* spp. caused the least rot demonstrating that it is not one of the most damaging pathogens.

Ginger, garlic and neem crude plant extracts were found to have potential anti-microbial compounds that inhibit tomato fruit rots at various concentrations. The evaluated concentrations were effective against the test pathogens but efficacy varied with the concentration. This can provide an alternative means for the control of tomato fruit rot by farmers. Results of this study can be an important step in developing plant based bio-pesticides for the management of fruit rots because the plants are readilavailable and affordable.

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