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Effect of Neem Leaves and Garlic Bulbs Extracts on Fungi Causing Post-Harvest Spoilage of Tomatoes in Sokoto, North-Western Nigeria

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Abstract
This study attempts to overcome the challenges of postharvest losses of tomato fruits by examining some botanical extracts as alternative means to the use of synthetic chemicals. Step by step procedures were followed during conducting this work. Two locations were chosen (Sokoto and Shuni) and the samples of spoiled tomato fruits were collected randomly, materials that were used for the analysis were sterilized. Fungal pathogens were isolated at Mycology Laboratory of Usmanu Danfodiyo University Sokoto. The pathogens were also identified after isolation. The identified pathogens were, Aspergillus niger, Aspergillus fumigatus, Saccharomyces cerevisiae, Preparation of crude extracts of neem leaves and garlic bulb and determination of the efficacy of these extracts on identified pathogens were conducted. Each identified pathogens were treated with three different concentrations of both neem and garlic extracts, at 100mg/ml, 200mg/ml, and 300mg/ml and ketoconazole was used as control. The zones of inhibition were observed after 24hours in each and recorded in each sample.

Keywords: *Neem leaves and Garlic Bulbs Extracts, Fungi, Post-Harvest Spoilage, Tomatoes*

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I. 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 (Wamache, 2005). Nigeria is the 14th largest producer of tomatoes in the world. On the continent, the country is ranked second after Egypt with about 1.8 million metric tons (MT), which she produces annually. With over 48 million tomato farmers across the country, Nigeria accounts for 65 per cent of tomatoes produced in West Africa (Eno-Abasi *et al.*, 2018). Ironically the country is the largest importer of tomato paste in the world, importing an average of 150,000mt of concentrate per annum, which value at $170m. (Eno-Abasi *et al*., 2014) Tomato plays a vital role in meeting the nutritional food requirements, generation of income, foreign exchange earnings and creation of employment (Sigei *et al.*, 2014). The crop is grown for fresh domestic but there is increasing demand for processed tomato products (Mungai and Heden, 2000). 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 – 2100m 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 (Rice *et al.*, 1994). 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 (Wamache, 2005). 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 (George *et al*., 2004). Tomato juice is known to be effective for intestinal and liver disorders (Wamache, 2005).

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 (Kader, 1992). 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 (Kader, 1992). 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 (Kader, 1992).

According to FAO (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 (Kader,1992; Okezie, 1998). 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 varies 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.

Most of the tomato fruits are also damaged after harvesting because of inadequate handling and preservation methods (Wills *et al.,* 1981). 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 (Stinson *et al.,* 1981; 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.

Problem Statement

Tomatoes are an attractive cash crop for small scale farmers and provide potential source of employment to many rural and urban Nigerians. The tomato fruits have been marketed freshly picked from the field and is the bestselling fresh market vegetable crop (AVRDC, 2006). Despite the human need of tomato, damage as a result of post-harvest spoilage micro-organisms has been of serious concern. Microbial decay is one of the main factors that determine losses and compromises the quality of the produce. The extent of the losses especially through microbial decay has not been quantified in most areas and where this has been quantified the results are short lived. Therefore, the study aims at evaluating ways of managing the post-harvest losses of tomatoes using crude plant extracts.

Justification

Several kinds of synthetic fungicides have been successfully used to control the post-harvest decay of fruits and vegetables (Adaskaveg *et al.,* 2000; Kanetis *et al.,* 2007). However, there are three major concerns: Firstly, the increasing consumer concern over pesticide residues on foods which are toxic and carcinogenic. Secondly, predominance of fungicide resistant strains of fungi due to excessive use of fungicides and thirdly, environmental pollution.

Therefore, there is need for new effective means of post-harvest disease control that possess 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 (Cardelina, 1995; Okigbo, 2009). It has been reported that plants are sources of natural pesticides that lead in new pesticide development. Arokiyaraj *et al.* (2008), Shanmugavalli *et al.* (2009), Swarnalatha and Reddy (2009). Anti-fungal and anti-bacterial compounds of neem plant leaf and garlic bulb crude extracts on rot pathogens of post-harvest tomato fruits are the main target of this study.

Objectives of the Study

The objective of the study was to evaluate the effect of the selected crude plant extracts on major micro-organisms causing post-harvest spoilage of tomatoes in Sokoto, and specifically the study was aimed at:

- Isolation and identification of pathogenic fungi causing post-harvest spoilage of tomato
- Determining the effect of crude plant extract on growth of fungal mycelia.

II. Materials and Methods

Experimental Location

The research was conducted at Usmanu Danfodiyo University Sokoto, at Mycology Laboratory for fungicidal effects of neem leaves and garlic bulbs extracts respectively. Sokoto is located in the Sudan savannah zone in the extreme northwestern part of Nigeria. It lies between latitudes 12^0N, 13^0N and 58^0N and longitudes 4^0E, 8^0E and 5^0E (Mamman *et al.,* 2000). Sokoto has low humidity and high solar radiation, it records annual rainfall between 300mm-800mm and mean temperature of 34.5^{o}C. The dry season temperatures do exceed 45^0C during the day time which is the highest recorded in Nigeria, (Adegboyega, *et al.,* 2016).

Sample Collection

In this analysis, four (4) spoiled tomato fruit samples were collected from different places. Two samples from Sokoto vegetable market (Kasuwan Daji) in Sokoto metropolis, and the remaining two (2) others from

Dange Shuni Local Government. Infected tomato fruit samples were identified by physical examination and then collected randomly in a sterilized polythene bags. The samples were then brought to Usmanu Danfodiyo University at Microbiology Laboratory for bacterial analysis and Mycology Laboratory for fungal analysis.

Sterilization of Materials

Different Laboratory materials that were used for this analysis were first washed with detergent, rinsed with clean water and air dried. The Petri dishes were sterilized in a hot air oven at a temperature of 160^0C for 1hour. For test tubes, 9ml of distilled water were measured using sterilized syringe and poured into the test-tubes. The test tubes were then inserted into an autoclave heater and heated at a temperature of 121^0C for 15minutes.

Media Preparation

Sabouraud Dextrose Agar (SDA) was the standard medium that was used to isolate fungal pathogens from the tomato samples. Preparations of the Medium was conducted according to the manufacturer's instruction.

6.5g of SDA (Sabouraud Dextrose Agar) was measured using sensitive weighting scale and poured into a conical flask. Then 0.1g of streptomycin was measured using the same scale and poured into the same conical flask. 100ml of distilled water was also measured using measuring cylinder and poured into the conical flask containing SDA and streptomycin. The conical flask was covered with cotton wool and foil paper and placed on a hot plate for not more than 15minutes with slight agitation to completely dissolve the media residues. The conical flask containing the media was then inserted into an autoclave heater and heated at a temperature of 121^oc for 15mins. The media was cold for 15minutes, and then taken to the incubation room together with the sterilized Petri dishes for pouring. Pouring means to pour 25mls of the media in each Petri dish and incubated at room temperature for 24hours.

Isolation of Pathogenic Fungi from Rotten Tomato Fruits

For fungal isolation, the infected tomato samples were taken to the incubation room and the surface of each sample was cleaned using ethanol and cotton wool to surface sterilized the sample. The inoculation needle was also being sterilized using heat generated from the bouncing burner and it was used to collect some infected portions from the tomato samples and inoculated into Petri dishes. Each Petri dish was labeled as S_1, S_2 representing Shuni and SK_1, SK_2 representing Sokoto with date of inoculation. After four days, different colors were observed in each Petri plate and subculture separately.

Identification

Fungal identification was done using morphological characteristics and comparing with established keys (Bernnet and Hunter, 1999). Each isolate was subjected to colony and microscopic examinations were carried out during which their morphological features was observed and recorded. Identification of the fungi is based on growth patterns, color of mycelia and microscopic examinations of vegetative and reproductive structures.

Motility Test

This was carried out as described by (Singleton, 1997). Motility can sometimes be inferred from the way organism growing on solid media. A speck of each isolate was stab on triple sugar phosphate agar which was incubated at 37^0C for 24hours. Motility was observed by spreading of the organism outward from the stab area.

After identifying different organisms, fungi on samples collected, the Petri dishes were labeled, wrapped with masking tape and kept in a refrigerator before preparing the media for anti-fungal tests.

Preparation of Plant Crude Extracts

Crude plant extracts were obtained from neem leaves and garlic cloves. The extraction process was followed the procedure described by Handa *et al*. (2008). Neem leaves were collected from Usmanu Danfodiyo University Plantation and were brought to Agricultural Physical 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 placed in the oven and dried at a temperature of 40^0C for three days. Garlic bulbs were obtained from University mini market and brought to the same laboratory. Garlic cloves were peeled washed in sterile distilled water and dried using sterile blotting papers. The cloves were cut into smaller pieces and placed in the oven to dry at a temperature of 40^0C for seven days. The neem leaves were grounded to powder using sterile mortar and pestle so as to rapture leaf tissues and cell structures to release the active cell contents. The extracts were placed in sterile specimen bottles. The garlic was also grounded into powder by use of a sterile motor and pestle and

place in the sterile specimen bottles. This was done to maximize the surface area which in turn enables the mass transfer of active ingredients from the plant material to the solvent.

Fifty (100gms) of each of the powder was put into separate sterile conical flasks and 300ml of distilled water was added to each of the plant powder ensuring that the powder is completely immersed into the solvent, then it was shaken vigorously and allowed to stand on the bench at room temperature but with continued shaking at different intervals for two days. A sterile funnel was placed into a 500mls conical flask and then a Whitman's (No.2) filter paper was folded and placed into the funnel. The extract was poured gradually into the filter paper and allowed to trickle into the conical flask. The filtrate was then poured into stainless plate and covered with a foil paper. The stainless plates were labeled and taken to biochemistry laboratory, Usmanu Danfodiyo University and dried in an oven at 40°C for four days until a powder like substance remains at the bottom of the stainless plates.

Effects of Crude Plant Extracts on Growth of Fungal Mycelia.

Screening of Extracts for Anti-Fungal Activities

26g of SDA and 0.4g of streptomycin were measured and poured into 500ml conical flask, 400ml of distilled water was measured and poured into the same conical flask, the conical flask was covered with cotton wool and foil paper and was shaken slightly till the media dissolved completely. The media was then separated into three different conical flasks of 250ml size. 130ml of the media was poured in each conical flask and sterilized at temperature of 121^0c for 15mins.

After preparing the media, three different fungi species that were identified were brought out of refrigerator, unwrapped and in each of the plates, 10mls of distilled water was measured and poured into each, the Petri dishes were shaken until organisms were mix with the distilled water. 2mls of each organism was dispensed into each conical flask. The conical flasks were labeled according to the organism dispensed in them. 27 Petri dishes were sterilized and in every nine Petri dishes media containing single organism was poured in them, the media was solidified and after 24hours and the cork borer of 7 diameters was used to make a hole in each Petri dish.

Different concentrations of the crude extracts were prepared by weighing them separately; 1g, 2g and 3g replicated 3 times of both garlic and neem powder respectively. Each powder was dissolved into 5ml sterile of distilled water to form solutions of different concentrations. Each test tube containing dissolve extract was labeled according to name of the extract and quantity dispensed.

2mls of the extract was collected from each of the test tubes and dispensed into the holes in the Petri dishes. The Petri dishes were labeled according to the organism inoculated, name of the extract and quantity dispensed. Zones of inhibition were observed in each Petri dish and measured using ruler after 24hours.

Data Analysis

The biocidal activities of the plant extracts on susceptibility of the tomato samples to the pathogens was analyzed using completely randomized design (CRD) and least significant difference (LSD) was used for mean separation.

III. Results and Discussions

Isolation and Identification of Fungi Associated with Post-Harvest Losses of Tomatoes

The pathogenic fungi that were isolated and identified were *Aspergillus spp* and *Saccharomyces cerevisiae*. *Aspergillus spp* were the most prevalent with two species (*A.niger* and *A. fumigatus*) constituting 75%, while *Saccharomyces cerevisiae* constitute the remaining 25% of the total population.

Table 1. Frequency of occurrence of fungi associated with postharvest spoilage of tomatoes in Sokoto, 2019.

Organism	Frequency	Percentage%
Aspergillus niger	3	37.5%
Aspergillus fumigatus	3	37.5%
Saccharomyces cerevisiae	2	25%
Total	8	100%

The fungal organisms that were isolated and identified in this research were also reported by other researches in similar studies in the past. It was mentioned in the international journal of science: Basic and Applied Research (IJSBAR, 2018) that five 5 fungi species were identified after isolation from two different tomato fruit samples, which include *Aspergillus niger, Candida albicans, Fusarium oxysporum, Aspergillus fumigatus* and *Rhizopus stolonifers.* (Sani *et al.,* 2018). In the Universal Journal of Microbiology Research (UJMR, 2015). A research on fungi associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka, Nigeria made mention of *Saccharomyces cerevisiae* among the fungi pathogens identified after isolation. The pathogens were; *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum,*

Saccharomyces cerevisiae, Alternaria alternata, Penicililum digitatum and *Geotrichum candidum.* (Onuorah *et al.,* 2015).

Aspergillus niger.

Aspergillus niger largely exist as saprophytes, which means that they obtain their nutrition from a variety of dead and decaying material such as leaves, fruits and other vegetation. As such, they also contribute to the decay of various food products, given that their sources of vegetations are readily available, virtually everywhere. *A. niger* are widely distributed and common in many geographical areas. However, they are known to be particularly prevalent in areas with higher temperatures. Research had shown that the black spores of *A. niger* help protect them from the sun's radiation, which in turn allows this species to thrive in warm areas. (Wang, 2014).

Aspergillus fumigatus

A. fumigatus is one of the most prevalent *Aspergillus* found in most environments. One of the most unique characteristics that separate *A. fumigatus* from the rest of the *Aspergillus* is that it can survive very high temperature (it is thermos tolerant) which is the reasons as to why it is more prevalent. *A. fumigatus* also exist as saprophyte that plays an important role in the cycle of carbon and nitrogen in nature. (*http;//creativecommons.org/licenses/by-sa/2.5*). This citation had shown that, A. *fumigatus* can also be found on tomatoes since they are saprophytic and can be found in most environments.

Saccharomyces cerevisiae

S. cerevisiae is a species of yeast. It has been instrumental in winemaking, baking, and brewing since ancient times. It was believed to have been originally isolated from the skin of grapes (one can see the yeast as a component of the thin white film on the skins of dark-colored fruits such as plums; it exists among the waxes of the cuticle). It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model bacterium. (Fieldman *et al.*, 2010).

Occurrence of Pathogenic Fungi on Different Tomato Fruit Samples.

Two different tomato fruit samples used in this project have shown two different pathogenic organisms, *Aspergillus niger* appears only on samples collected from Shuni local government and *Aspergillus fumigatus* appears on samples collected from Sokoto vegetable market, while *Saccharomyces cerevisiae* appears on both samples.

Table 2: Occurrence of fungal species on different tomato samples

S/N	Organisms	Samples	
		Shuni	Sokoto
1	*Aspergillus niger*	+	_
2	*Aspergillus fumigatus*	_	+
3	*Saccharomyces cerevisiae*	+	+

+ Organism present
- Organism absent

Effect of Crude Plant Extract on Identified Fungal Species

The Table below shows the efficacy of both neem and garlic on isolated fungal pathogens from the tomato samples**.**

Table 3 Efficacy of Neem and Garlic extract on isolated fungal pathogens

S/N	Name of plant used	Part of plant used	Extractant	Conc. in mg/ml	Diameter of inhibition in mm/fungal spp.		
					A. niger	*A. fumigatus*	*S. cerevisiae*
1	Neem	Leaves	H_2O	100	00	11.6	00
				200	00	16	00
				300	00	20.6	00
2	Garlic	Bulbs	H_2O	100	00	00	00
				200	00	00	00
				300	00	00	00
3	Control	Ketoconazole		5	12	11	15

Size of cork borer = 7mm

The study revealed that among all the three fungal pathogens that were identified, it was only neem extract that shown inhibition on one pathogen which was *Aspergillus fumigatus.* The zone of inhibition

increases with increase in concentration of the crude extract, at 100mg/ml the zones of inhibition on three different plates inoculated with *A. fumigatus* were 12m, 12m and 11m, at 200mg/ml the zones of inhibitions were 15m, 17m and 16m, at 300mg/ml the zones of inhibition were 20m, 22m and 20m. This research had shown that neem extract can substitute synthetic chemical in the control of *A. fumigatus.*

Garlic extract does not show inhibition on any of the organisms identified. However, several research conducted by many scientists has shown a positive result in inhibiting the growth of pathogenic fungi by garlic extract. As mentioned in the Journal of Basic Medical Sciences, a sulphur containing compound in garlic known as di-allyl thiosulfinate (allicin), is the active component in inhibition of the growth of fungi and bacteria. (Farzad *et al.*, 2014). Fresh aqueous extract of garlic shows antifungal activity specifically against some *Aspergillus* Spp., including *A. fumigatus, A. terreus, A. nidulans and A. niger* (Farzad *et al.*, 2014). Showing less effect of neem and the negative effect of garlic maybe as a result of overheating of extracts during drying which might lead the extract contents to denature. A research in the journal of nutrition on the influence of heating on anticancer properties of garlic shown that when garlic is over heated it may lead its active constituents to destroy, it was mentioned that boiling garlic at 100^0C for 20 minutes produces a significant decrease in the inhibitory effect of fungi (Yin and Cheng, 1998).

Table 4: Effect of Neem and Garlic extracts on isolated fungi species from tomato fruit samples.

Treatments	*Aspergillus niger*	*Aspergillus fumigatus*	*Saccharomyces cerevisiae*
Neem	0.00^b	16.10^a	0.00^b
Garlic	0.00^b	0.00^c	0.00^b
Control	10.50^a	9.50^b	12.00^a
SE±	0.05	1.75	0.25
Significance	*	*	*

SE= Standard error of difference, *= significant at 5% level of significance.

Result of analysis of variance shows that there was significant ($p<0.05$) difference of neem and garlic extracts on isolated fungi species. Control produce statically better than both garlic and neem extract with a mean 10.50 and 0.00 respectively with respect to *Aspergillus niger*. With respect to *Aspergillus fumigatus* neem extract recorded the highest mean 16.10 while control produced statically higher than garlic which had a mean of 0.00. However, *Saccharomyces cerevisiae* both the garlic and neem extract recorded the least means 0.00 while control recorded the highest mean 12.00 (Table 4).

IV. Summary, Conclusion and Recommendations

Summary

This research was conducted to analyze the efficacy of neem leaves and garlic bulbs extracts on identified fungi isolated from tomato fruit samples collected from Sokoto vegetable market and Shuni local government Sokoto State. Saboraud dextrose agar was used for the fungi isolation at Mycology at Usmanu Danfodiyo University Sokoto.

Three fungal species were identified after the isolation and both were treated with the extracts that were prepared at different concentrations of 100mg/ml, 200mg/ml and 300mg/ml. The identified fungal species were *A.niger, A. fumigatus* and *S. cerevisiea,* among these organisms it was only *A. fumigatus* growth that was inhibited when treated with neem extract.

Conclusion

At the end of this study, it was observed that there are fungal micro-organisms that cause post-harvest losses of tomatoes in both Shuni and Sokoto. The identified pathogens were *A. niger, A. fumigatus* and *S. cerevisiae.*

Neem leaves and garlic bulb extracts were found to have potential anti-microbial compound that inhibit the growth of pathogens isolated on tomato fruits at various concentrations. The evaluated concentrations were effective against the tested pathogens, although all concentrations from both extracts have not shown any significant ($P>0.05$) effect on two out of the three pathogens, (*A. niger* and *S. cerevisiae*). Result of this study can be an important step in developing plant bio pesticides for management of fruit rots.

Recommendations

This study therefore recommends that;

1. Farmers should disinfect the tomato fruits after harvesting to reduce chance of infection by pathogens. This could be done by use of sodium hypochlorite.
2. Further research needs to be carried using higher concentrations of extracts than the quantity used in this research, which might be enough to substitute the used of synthetic chemicals.

3. Further research need to be conducted to found other alternatives for control of *A. niger* and *S. cerevisea* apart from synthetic chemicals in order to reduce ingesting chemical residues by people and to prevent polluting the environments.
4. Government and other research organizations need to sponsor researches on this aspect since these medicinal plants are abundant and affordable, this can help greatly in reducing several diseases such as cancer that might occur as a result of ingesting chemical residues.

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