ISOLATION OF FUNGI ASSOCIATED WITH THE SPOILAGE OF POST-HARVEST TOMATO AND THE USE OF LEAF EXTRACT OF NEEM TREE IN THEIR MANAGEMENT

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ABSTRACT

Tomato is an important vegetable crop in Nigeria. It is widely grown for home consumption and for sale. The demand for fresh tomato is high both for domestic use and markets. However, tomato post-harvest losses are a threat to the harvested tomatoes. There is no well documented current knowledge on the nature and status of post-harvest losses on tomato in Nigeria particularly with regard to pests and diseases. In areas where post-harvest losses have been documented the figures vary considerably such that their usefulness is short lived. Periodic surveys are therefore necessary to help understand the severity of losses in a specific place at a specific time. The aim of this study was to isolate and identified the fungi associated with tomato fruit spoilage in Geidam. The study also aimed at evaluating the efficacy of plant crude extracts against post-harvest tomato damaging pathogens. Disease causing micro-organisms that were suspected to cause the post-harvest damage were isolated, identified and re-inoculated to wounded surface sterilized fresh harvested ripe tomato to establish pathogenicity. Crude plant extracts from neem leaves, were tested for the control of the most potent fungal pathogens. An in vivo experiment was carried out where healthy ripe tomato fruits were dipped into the selected crude plant extracts and disease development on them monitored and compared with the untreated tomato samples. Data was analysed using one way ANOVA. The in vivo study demonstrated that the extracts could be applied to control the rots on the tomato fruits. Results of this study showed that plant extracts had antimicrobial compounds such as diallyl disulphide, azadrachtin that acted against the test pathogens and can be an important step in developing plant based bio-pesticides for the management of fruit rots because the plants are readily available, and affordable. The study recommends that farmers shorten the distance between harvesting and collection time to reduce chances of fruit exposure to the pathogens.

Keywords: Tomato, fungi, spoilage, Microscopic,

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). 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). Moreover Tomato crop 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- (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). However Inadequate storage, distance and time consuming market distribution, poor access to the market, post-harvest spoilage microorganisms 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. Moreover 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.

MATERIALS AND METHOD

Study Area

The study area is Geidam town, which is geographically located between Latitudes 12°53' 39'N and Longitudes 11°55'36'E North of the Greenwich Meridian. Geidam exhibits both dry and wet tropical climate type. Also, it occupies an area of 4, 3571 km and has a population of 153,295 people at 2006 census. The dry season begins in November and ends in March, while the rainy season runs from June 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 main occupation of people of Geidam town includes agricultural practices such as crop production, fishing, irrigation agriculture, trading, inland water transportation. hing, crop production, animal husbandry, aviculture and forestry. Other socio-economic characteristics includes pottery, irrigation agriculture, inland water transportation, trading.

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Sterilization of Glass Wares

All the glass wares such as Conical flask, measuring cylinder, beaker etc. that were used in this study, would be washed with detergent, rinse in clean water, and make it dry using drying cabinet and were sterilized in the hot air oven at 160^{o}C for 2 hours as described by (Cheesbough 2000).

Sample Collection Site

For the purpose of this research, spoiled Tomato samples were collected from three (3) different markets within Geidam town namely: Geidam main market, Geidam motor park and Geidam women market and were conveyed to the Department of science laboratory and technology research centre , Mai Idriss Alooma polytechnic Geidam laboratory.

Method of Sampling

A total of fifteen (15), spoiled Tomato samples was collected from three (3) different markets within Geidam town namely: Geidam main market, Geidam motor park and Geidam women market in to a sterilize polythene bag and were transported to the research laboratory of school of science, department of science laboratory technology, of Mai Idriss Alooma polytechnic Geidam for preparation and analysis as described by (Apha 1992).

Media Preparation

The media used for this research were prepared according to the instruction provided by Ibrahim and Rahma, (2009), About 27 grams of potato dextrose agar (PDA) was weighed and poured into a clean 500ml conical flask, 475ml of distilled water would be added and stirred vigorously to dissolve, Chloramphenicol was added to inhibit any bacterial growth to shun contamination, The conical flask were sealed with fuel paper and masking tape, The content were autoclaved at 121^0C for 15minutes and then allowed to cool at room temperature, Appropriate amount is be poured in to sterilized petri dishes

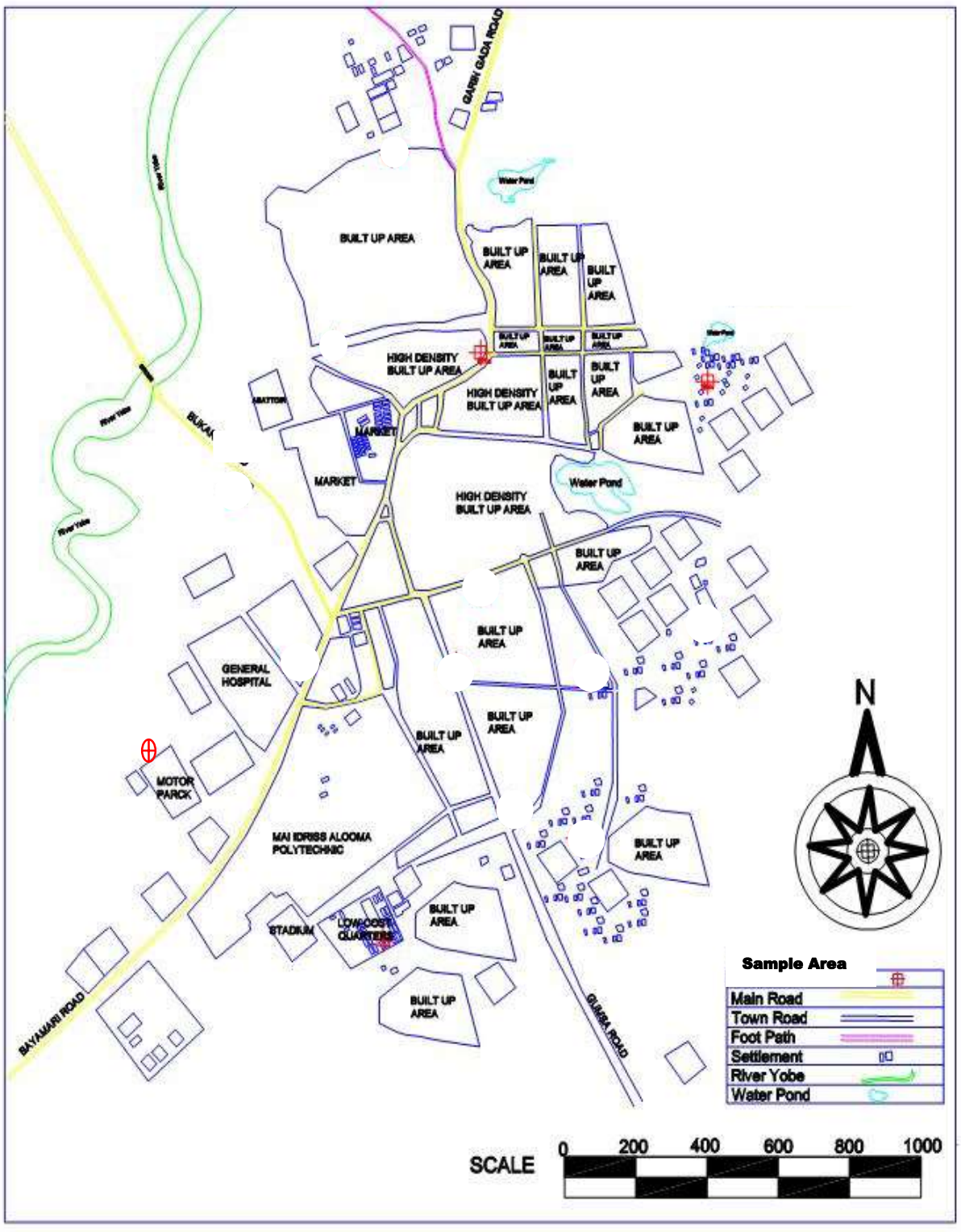

Figure1.A Map of Geidam local government area (showing the study site)

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Fungal Isolation

Isolation of the fungi was carried out as described by (Amusa et al., 2007). Firstly, the fruits were washed with running water and the surface contaminant was removed. Serial dilution and washing off methods were carried out individually for fungi isolation, by incubation of the intact fruits after injuring their surface.

Serial dilution method: - Stock solution were prepared by dissolving 10gm of spoiled part of fruit pulp and fruit peel in 90ml of distilled water, Shake vigorously with the help of stirrer to mixed properly. Five (5) test tube containing 9ml of distilled water placed on test tube stand. One (1) ml from stock solution was transferred aseptically into the first test tube and vortex mix. One (1ml) from first test tube was

transferred to the second test tube and mix. This procedure would be repeated till the last test tube. One 1ml solution from last tube would be discarded, 0.1ml solution from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilution would be spread on solidified PDA medium (Spreading plate method), Chloramphenicol were added as antibiotic to inhibit the growth of bacteria, the inoculated plates were incubated at room temperature for 5 days to observe fungal growth, Subculture were made from the fungal colonies in order to obtain pure colonies.

Identification of Isolated Fungi

Identification was based on macroscopic and microscopic examination as employed by Kora et al., (2007) as follows:

Macroscopic Examination

This was based on the nature of the appearance of the colony and morphological characteristics of the isolated fungi.

Microscopic Examination

A few drop of lactophenol cotton blue solution was placed on the surface of clean grease free glass slide, Small portion of the isolated fungi was picked and placed in to lac tophenol cotton blue with the help of sterile wire loop and emulsified, a cover slip would be placed at the centre of the suspension, air bubbles would be avoided. The prepared slides was examined using x10 0bjective lens and x40 for confirmation, Identification of the fungal species using –standard mycological atlas would be employed as described by Cheesbrough (2000).

Pathogenicity Test

Intact and matured tomato fruits were purchased from 3 different markets 5 from each, the samples were surfaced sterile with 1% sodium hypochlorite and rinsed with distilled water. The samples were replicated 3 times with the control and one side of each of the replicates would be carefully punctured with a sterile scalpel beyond epidermal layer. The identified isolates were introduced in to the punctured portions with a sterile needle and sealed with Vaseline to avoid being contaminated by microorganisms. All samples were incubated at room temperature for daily observation for 7 days. (Elmougy et al, 2004).

Preparation of leaf extract of Neem Tree (Azadirachta indica) Crude plant extract was obtained from neem leaves. The extraction process followed the procedure described by Handa et al. (2008). Neem leaves were collected from Mai Idriss Alooma polytechnic Ecological Study Area Geidam Station in the school premises and brought to Laboratory for drying. The leaves were washed under tap water, rinsed in three changes of sterile distilled water and dried in an oven at 50 degree Celsius and prepared by blending 50g of the dried leaf with 100ml of methanol for 10m. The crude extract was filtered through muslin followed by whatman No. 1 filter paper prior to autoclaving (121 degree Celsius for 15mins) before storage at -20 degree Celsius.

Experimental design and data analysis

The experimental design used was Complete Randomized Design (CRD) to examine the antifungal activity of the neem leaves on tomato rot and data collected was tested statistically using the one-way analysis of variance (ANOVA).

Result

Total Fungal count (TFC) of Tomato sample from Geidam main Market range from 8.0 $x10^2$ to 4.8 $x10^6$ CFU/g, Geidam Motor Pack range from 6.3 $x10^2$ to 3.4 $x10^6$ CFU/g ,and Geidam women Market range from 5.7 $x10^2$ to 2.7$x10^6$ CFU/g, respectively.

Table 1: Total fungal Count (CFU/g) of "Tomato Sample" obtained from the Three Cardinal Points of Geidam town.

S/N		SAMPLES TFC (Cfu/g)		
		GMM	GMP	GWM
1.	A1	$8.0x10^2$	B1 $6.3x10^2$	C1 $5.7 x10^2$
2.	A2	$6.4x10^3$.	B2 $4.7 x10^3$	C2 $4.9 x10^3$
3.	A3	$5.3x10^4$.	B3 $4.0 x10^4$	C3 $3.7 x 10^4$
4.	A4	$5.0x10^5$.	B4 $3.7 x10^5$	C4 $3.0 x10^5$
5.	A5	$4.8x10^6$.	B5 $3.4x10^6$	C5 $2.7 x10^6$

Result

Isolation and identification of fungal contaminants associated with the spoilage of tomato was conducted, where five (5) fungal pathogens where identified based on macroscopic (colony appearance) and microscopic identification with the help of Standard Mycological Atlas.

Colony appearance, microscopic characteristic and frequency of occurrence of the Fungi identified at Geidam Main Market.

Table 2 and 3 shows the colony appearance of the identified fungal pathogens associated with the spoilage of tomato in Geidam main market and their frequency of occurrence respectively, where (Tafinta 2013)

Table 2 Colonial appearances and identified fungal species in Geidam main market.

Colony appearance	Identified fungal species
Fairly distinct with woolly appearance, blackish Flat and coarse.	Aspergillus niger

Colonies with distinct velvety yellow green and sometimes brown colour. The brown colour is prominent in older culture.	A. flavus
Green fluffy mycelia with some white sporongiospore, flat and coarse	Penicillium sp
Colonies are fluffy rise, and whitish colony which grow very fast.	Rhizopus. Stolonifer[
Colonies are cotton like, usually white turning pink Violet or brown at the centre.	Fusarium sp.

Table 3; Frequency of occurrence of identified fungal contaminants associated with spoilage of Tomato at Geidam main Market.

Identified Fungal Isolate	Number of isolates	Frequency of occurrence
A. niger	7	28%
A. flavus	4	16%
R. stolonifer	3	12%
Penicillium spp	5	20%
Fusarium sp	6	24%
Total	25	100%

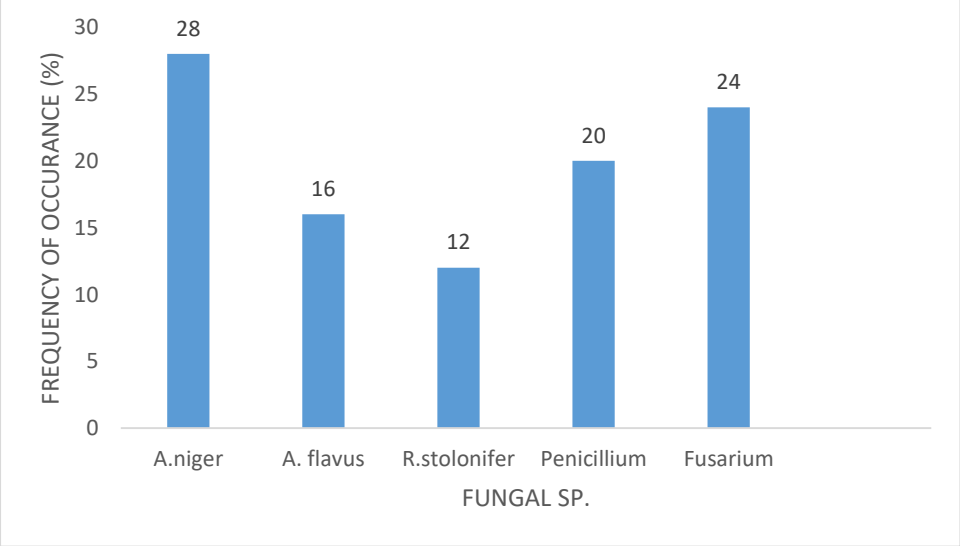

Figure 2 showing the colony of appearance of the identified fungal pathogens with their frequency of occurrence in Geidam main market.

Colony appearance, microscopic characteristic and frequency of occurrence of the Fungi identified at Geidam Motor Park.

Table 4 and 5 showed the colony appearance of the identified fungal pathogens with their frequency of occurrence in Geidam Motor Park as well. (Tafinta 2013)

Table 4 **Colony appearance of identified fungal species isolated on Tomato in Geidam Motor Park.**

Colony appearance	Identified fungal species
Fairly distinct with woolly appearance, blackish Flat and coarse	Aspergillus niger
Colonies with distinct velvety yellow green and sometimes brown colour. The brown colour is prominent in older culture.	A. flavus
Colonies are fluffy rise, and whitish colony which grow very fast.	Rhizopus. Stolonifer

Table 5: Frequency of occurrence of identified fungal contaminants associated with spoilage of Tomato at Geidam Motor Park.

Identified Fungal Isolate	Number of isolates	Frequency of occurrence
A. niger	7	30.43%
A. flavus	6	26.1%
R. stolonifer	10	43.47%
Total	23	100%

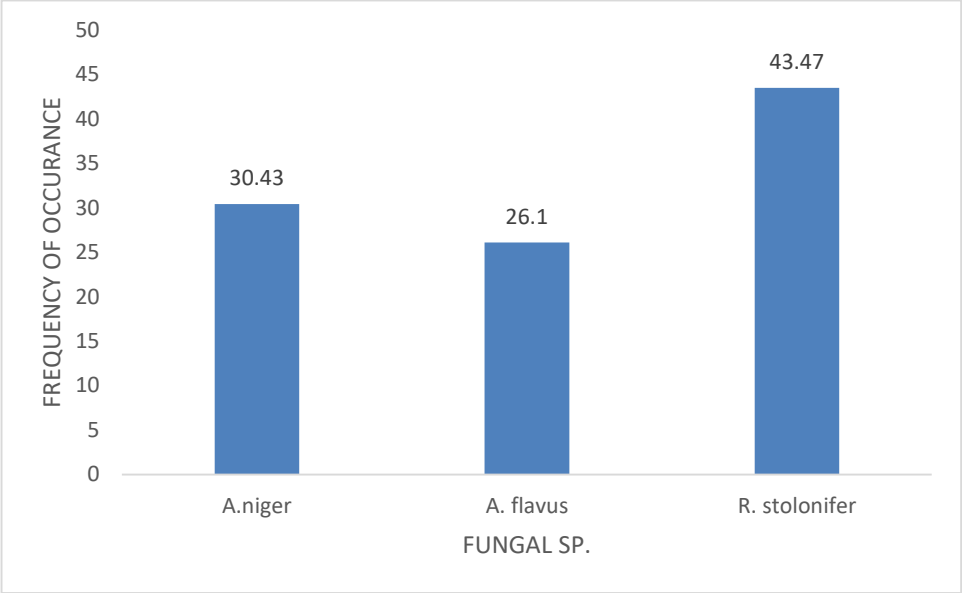

Figure 3 showing the colony of appearance of the identified fungal pathogens with their frequency of occurrence in Geidam motor pack.

Colony Appearance, Microscopic Characteristic and Frequency of Occurrence of the Fungi Identified at Geidam Women Market. (Tafinta 2013)

Table 6 and 7 indicating the colony appearance of the identified fungal pathogens responsible for the deterioration of tomato fruit sold at Geidam women market with their frequency of occurrence respectively.

Table 6: Colony Appearance and Identified Fungal Species at Geidam Women Market.

Colony appearance	Identified fungal species
Colonies with distinct velvety yellow green and sometimes brown colour. The brown colour is prominent in older culture.	A. flavus
Green fluffy mycelia with some white sporongiospore, flat and coarse.	Penicillium sp
Colonies are fluffy rise, and whitish colony which grow very fast.	Rhizopus. Stolonifer

Table 7: Frequency of occurrence of identified fungal contaminants associated with spoilage of Tomato at Geidam women market.

Identified Fungal Isolate	Number of isolates	Frequency of ccurrence
A. flavus	7	31.8%
Penicillium sp	9	40.9%
R. stolonifer	6	27.3%
Total	22	100%

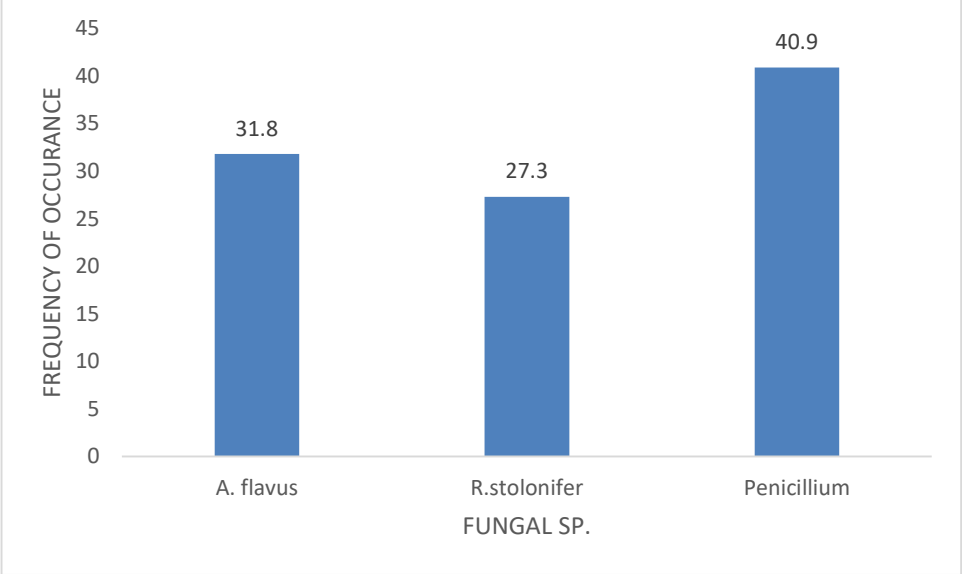

Figure 4 showing the colony of appearance of the identified fungal pathogens with their frequency of occurrence in Geidam women market.

Colony appearance and Determination of Percentage Frequency of Occurrence

The colony and frequency of the occurrences of different types of isolated fungal pathogen associated with spoilage of tomato were obtained. The percentage of the occurrence was calculated using the formula as used by (Morsy 2009).

$$\% \text{ Frequency} = \frac{\text{Number of the isolated isolate}}{\text{Total number of the isolated fungi}} \times 100$$

Pathogenicity test

The study revealed that the micro-organisms isolated from the infected tomato fruits were pathogenic but with varied pathogenicity. When inoculated into healthy tomato fruit, R. stolonifer spp. caused the most rapid (100 %) infection where the inoculated fruits were completely rotten by the end of the second day after inoculation. The fruits were completely disintegrated with extensive mycelial growth forming a dark color covering the fruit skin. The fruits looked water soaked in appearance and wrinkled with depression. Fruits inoculated with Fusarium spp. had water soaked lesions with some white to pink mycelia. while Samples inoculated with A. niger spp. had small water soaked lesion with slightly brownish appearance on the inoculated areas while tomato fruits inoculated with penecillium spp. had small hard dark lesion around the inoculated area. Moreover, fruits inoculated with A. flavus spp. also had water soaked lesions around the inoculated areas while fruits inoculated with Botrytis spp. had water soaked lesions with a dark appearance on the inoculated areas. The pathogens were isolated and identified as described earlier to confirm pathogenicity.

Discussion

The findings of this research revealed that various fungal pathogens were responsible with the deterioration of harvested tomato fruit sold in Geidam. Five (5) fungal pathogens were identified which include the following; Aspergillus niger, A. flavus, Rhizopus stolonifer, Penicillium sp. And Fusarium oxysporum. This finding is in conformity with that of Akinmusere (2011), Ogawa et al., (1995), Wogu and Ofuase (2014) Ghosh (2009), Dimphna and Nneka (2016) among many other researchers whose revealed in their research that the identified fungal pathogens mentioned above were responsible with the spoilage of harvested tomato at different part of the world .According Kader (2002),pathogens infect fruits during prolonged periods of rainfall and high humidity, especially when fruits are poorly packed. A.niger, A. flavus, Penicillium, R.stolonifer and Fusarium oxysporum were identified associated with the spoilage of tomato in Geidam main Market which may be due to the provision of the suitable growing condition or due to unhygienic environmental condition in the studied sites. Among the five pathogens identified, A. niger was found to have highest frequency of occurrence (28%). This is to say that A. niger was the major fungal pathogen associated with the spoilage of tomato fruit sold in Geidam main Market,

which might be due to the presence of all the necessary growing factors required for this particular pathogen followed by Fusarium sp. With (24%), Penicillium sp (20%), A. flavus(16%)and finally R. stolonifer with the least percentage of occurrence (12%).A.niger, A.flavus and R.stolonifer were found to be responsible with the spoilage of tomato sold in Geidam motor park. R. stolonifer was found to be of highest percentage of occurrence (43.47) and considered to be the most prevalent pathogen causing spoilage of tomato fruit in Geidam motor park, followed by A.niger with (30.43%) and A. flavus with the least frequency of occurrence (26.1%). A. flavus, Penicilllium sp. and R.stolonifer Were identified as the fungal pathogens responsible with the spoilage of tomato sold in Geidam women Market. Penicillium sp. was found to be the most occurring fungal pathogen associated with the spoilage of tomato in Geidam women market with (40.9%) percentage of occurrence as a result of poor handling and storage of the produce followed by A. flavus with (31.8) and R.stolonifer with the least percentage of occurrence (27%). The survey carried out revealed that factors such as poor grading, packing containers, means of transport, duration between harvest and transport to the market, pests and diseases have significant impact on post-harvest losses. Tomato fruits were usually spread on the ground waiting for grading after harvest. Mixing of healthy and infected tomato fruits during harvesting possibly increased chances the spread of disease causing micro-organisms to healthy fruits. Harvested fruits were usually thrown on the ground or dropped into the harvesting containers and the impact could cause bruises on the fruits that may act as routes for secondary infections. Heaping of fruits in the farm results to squeezed fruits causing injuries that allow entry of micro- organisms that cause decay.

Conclusion

From the findings of this research, it can be concluded that various fungal pathogens were found associated with the spoilage of tomato fruit sold in Geidam with varying frequency of occurrences. The fungal pathogens identified were A. niger, A. flavus, R. stolonifer, Penicillium sp. and Fusarium oxysporum. Among the identified fungi, A. niger, R.stolonifer and Penicillium were the major destructive pathogens. These fungal pathogens causes serious loss to this economically valuable plant product and affect food security as well. The deterioration of tomato can be due to poor transportation, storage, packaging, or other environmental factors such as temperature.

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References

1. Abdel-Mallek, A. Y., Hemida, S.K. and Bagy, M.M. K. (1995). Studies on fungi associated with tomato fruits and effectiveness of some commercial fungicides against three pathogens. Mycopathologia 130: 109-116.

2. Aran, N. Alperden,I. and Topal,O. (1987). Mould contamination problem in tomato paste and risk analysis system in the critical control place. Journal of food Industry 2(3): 43-47.

3. Asan, A. and Ekmeki, S. (2002). Contribution to the colonial and morphological characteristics of some Aspergillus species isolated from soil. Journal of Faculty of Science 25:121-139

4. Beuchart, L.R. (1995). Pathogenic Microorganisms Associated with fresh produce. Journal of Food Production 50(2): 204-216.

5. Bashir, O. B., Habib, U., Odunayo, O.J., Opeyemi, A.S.,Alafe, A.H. and Owode, T.M.(2006). Microorganisms causing post-harvest tomato fruit decay in Nigeria. Journal of Entomology and Zoology Studies 4(1): 374-377.

6. Bihn, E.A. and Gravani, R.B. (2006). Role of good Agricultural practices in fruit and Vegetable safety. In: Microbiology of fresh Produce (Edited by K.R. Mathews). ASM press, Washington D.C. 328pp.

7. Eni, A.O., Ibokunoluwa, O., Oranusi, U. (2010). Microbial quality of fruits and vegetables. African Journal of Food Science 4(5): 291-296.

8. Gosh, A. (2009). Identification of Microorganism responsible for spoilage of tomato (Lycopersicum esculentum) fruit. Journal of Phytology 1(6):414-416.

9. Ibrahim, A.D.,K., Musa, K., Sani, A., Aliero,A.A., Yusuf, B.S. (2011). Microorganisms associated with the production of volatile compounds in spoilt tomatoes. Research in Biotechnology.2: 82-89

10. Kimura, S. and Sinha, N. (2008). Tomato (Solanum lycopersicum): A model fruit Bearing crop. CSH Protoc. Doi: 10.1101/pdb.emo 105

11. Mbajiuka, S.C. and Enya, E. (2004). Isolation of Microrganisms associated with deterioration of Tomato (Lycopersicum esculentum) and Pawpaw (Carica papaya) fruits. International journal of Current Microbiology and Applied Sciences 3:501-512

12. Onuorah, S. and Orji, M.U. (2015). Fungi Associated with the Spoilage of Post-harvest Tomato Fruits Sold in Major Markets in Awka, Nigeria. Universal Journal of Microbiology Research 3(2): 11-16

13. Pitchaon M., Maitree S. and Rungnaphar P.(2007). Assessment of phenolic content and free radical-scavenging capacity of some indigenous plants. Food Chemistry 100:1409-1418.

14. Rashad, R.A., Ahmed, R.A. and Saleh, A.M. (2011). Isolation and identification of some fruit spoilage fungi:screening of plant cell wall degrading enzymes. African Journal of Microbiology 5(4):443-448.

15. Rodriquez P.,Soares C.,Kozakiewicz, Z.,Paterson R.R.M., Lima, N. and Venancio A. (2017). Identification and Characterization of Aspergillus flavus and toxins. Communicating Current Research and Educational topics and trends in Applied Microbiology 527534.

16. Sravanthi, J., Gangadhar, S. R. (2015). Phytochemical and antioxidant composition in Lycopersicum esculentum. Journal of Medicinal Plants Studies 3(4): 107-110

17. Ugwu, C. O., Chukwuezi, F.O. and Ozougwu V. C.O.(2014). Microbial Agents of tomato spoilage in Onitsha metropolis. Advances in Biological Research 8(2):87-93.

18. Wogu, M.D, Ofuase, O.(2014). Microorganisms responsible for the spoilage of tomato fruits, Lycopersicum esculentum, sold in markets in Benin City, southern Nigeria. Scholars Academic Journal of Bioscience 2(7): 459-466

19. Wokoma E.C.W. (2008). Preliminary report on disease of tomatoes in Choba, Rivers State. J. Appl. Sci.Environ. 12(3):178-121.

20. Yaltao, T. (1985). Physiology of mature tomatoes and harvest. Proceedings of the last Congress Raising and Evaluating of Tomatoes Technology,(CRETT'85). British Journal of Botany 4(3):23-33

21. Zain, M.E. (2011). Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15: 129-144.

22. Ibrahim, S. and Rahma, M. A. (2009). Isolation and identification of fungi associated with date fruits (phoenix dactylifera, linn.) sold at Bayero University, Kano, Nigeria. Bayero Journal of Pure and Applied Sciences, 2(2): 127 - 130