Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 4(12) pp. 923-928, December, 2015 Special Anniversary Review Issue.
Available online http://garj.org/garjas/home

Full Length Research Papers

The Effect of Three Natural Preservatives on the Growth of Some Predominant Fungi Associated With the Spoilage of Fruits (Mango, Pineapple and Cucumber)

***M.A. Sani[1], N. Usman[1], F. Kabir[1] & A.S. Kutama [2]**

[1] Department of Science Laboratory Technology, Sch. of Tech. Kano State Polytechnic, Kano, Nigeria
[2]Department of Biological Sciences, Federal University, Dutse, Jigawa State Nigeria.

Accepted 20 December, 2015

The study was carried out to determine the effect of three natural food preservatives; vinegar, garlic oil and olive oil on the growth of some predominant fungi associated with the spoilage of three common edible fruits, pineapple, cucumber and mango. Five fungal species were isolated from the spoilt fruits; *Aspergillus sp, Fusarium sp, Rhizopus sp, Saccharomyces sp*, and *Penicillium sp. Rhizopus sp.* had the highest rate of occurrence among the isolated fungi, followed by *Aspergillus sp.* and *Saccharomyces* species while *Fusarium sp* and *Penicillium sp* were the least isolated fungi. Pathogenicity test revealed that all the isolated fungi were pathogenic when re-inoculated to fresh fruits, hence could be responsible for the spoilage of the fruits. Invitro studies to determine the effective concentration of antifungal activity of the three preservatives on the growth of the isolated fungi revealed that vinegar exhibited greater potencies by inhibiting the growth of the entire tested organisms followed by garlic oil that inhibited some of the isolated fungi and the least effective was olive oil.

Keywords: Fungi, fruits, preservatives and pathogenicity test.

INTRODUCTION

Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health. They are widely distributed in nature and can play an important role in health through the prevention of heart disease, cancer and diabetes. Fruit and salad vegetables are different from most foods that we buy because they are not cooked and they are usually eaten in raw. One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by pathogens attacked. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby 2006, Zhu 2006).

Over the years, there has been an increase in theneed to identify and isolate the fungi associated withtheir spoilage.Spoilage refers to any change in the condition of food in which the food becomes less palatable, oreven toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture(Paul and Diana 1981).

The occurrence of fungal spoilage of fruitsis also recognized as a source of potential health hazard to man

*Corresponding Author's: muhammadahmadsani@ymail.com

and animals. This is due to their production of mycotoxins (naturally occurring toxic chemical often of aromatic structure) compoundswhich are capable of including mycotoxicoses in man following ingestion or inhalation. They differ in theirdegree and manner of toxicity (Effiuvwevwere, 2000).

The contamination of fruits and vegetables byfungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation.

Due to the economic impacts of spoiled foods and the consumer's concerns over the safety of foods containing synthetic chemicals, a lot of attention has been paid to naturally derived compounds or natural products (Amusa*, et al.*, 2002). Recently, there has been considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods which are considered as human-safe and environmentally friendly (Chuku, *et al*,. 2008).

Natural volatile compounds, including vinegar and essential oil have been extensively investigated and demonstrated to elicit as antibacterial and antifungal activities (Gupta and Parthak, 1986).

It is in this vein that this research was conducted to isolate and identify fungal genera that are responsible for the spoilage of three common fruits; Mango, Cucumber, and Pineapple and to determine the susceptibility of the fungal isolates to some natural preservatives. This is with the view to finding if they could reduce decay and maintain better quality and shelf life of the fruits.

MATERIALS AND METHODS

Sample Collection

Three different spoiled fruits; Mango, Pineapple, and Cucumber both fresh and spoilt ones werepurchased from three different markets within Kano metropolis in Kano State Nigeria. A total of twenty seven fruits comprising nine fruits from each fruit type were purchased from Na'ibawa, Jakara and Rimi markets and transported to the laboratory in an ethanol sterile polythene bag for analysis. Among each of the nine fruits, three were spoilt and the remaining six were healthy looking i.e. altogether there were nine spoilt fruits.

Isolation and Identification of Fungi

Isolation of the fungi was carried out as described by Amusa*et.al* (2007). Segments (3-5cm) oftissues from the margins of the spoilt fruits were cutwith a sterile scalpel and placed on sabouraud dextrose agar in Petri dishes and incubated at room temperature for 5 days. Subculture were made from the fungal colonies isolated to obtain pure colonies The resulting isolates were identified microscopically by their characteristics under x 40 objective. In accordance with Cheesbrough (2000) and David Ellis *et al*., (2007).

Inoculum Standardization

Pure cultures of the isolated fungi were standardized according to the European Standard (1997).Conidia were detached from the culture surface using a glass spatula and transferred into 10 ml sterile distilled water in a flask containing glass beads (3–4 mm diameter). After shaking for 1 min, the suspension was filtered through a filter (40–100 m porosity).

Pathogenicity of Isolated Fungi

This was carried out as described by Baiyewu *et al*. (2007) and Chukwuka *et al*. (2010). Each of the fungal isolates was tested on healthy fruits (mango, pineapple and cucumber) for its ability to induce spoilage. A sterile 4mm cork borer was used to make holes in each of the fruits. One set of the healthy fruits were inoculated with each of the standardized fungal isolates by injecting 1ml of the suspension into the hole made. The remaining set of the fruits was used as control without being inoculated with the isolates. All the inoculated fruits and the controls were placed in clean polyethylene bag (one fruit per bag) each and incubated at room temperature for 3 days. After 72 h, the inoculated fruits were observed for symptom development. The causal agents were re-isolated from the infected fruit and compared with the original isolates.

In Vitro Antifungal Assay:

Each of the preservatives (garlicoil, olive oil and vinegar) were mixed with sterile sabouraud dextrose agar (SDA)to obtain five different concentrations(10%, 20%, 30%, 40% and 50%). 1ml of the suspension of each isolate were placed in 90mm Petri plate and then the mixture of preservative and SDA mixture was poured into the Petri plate, incubated for 7 days at room temperature and thenobserved for fungal growth (Hadizadeh *et al*., 2009).

RESULTS

Five fungal genera *Aspergillussp, Rhizopussp, Fusariumsp, Saccharomyces sp,* and *Penicilliumsp* were isolated from a total of nine spoilt fruits. *Aspergillus sp.* was isolated from *Cucumis Sativus* and *Mangiferaindica, Fusarium sp*. was isolated from *Cucumissativus, Rhizopus sp*. were isolated from *Mangiferaindica, Cucumissativus* and *Ananascomosus, Saccharomyces sp*. was isolated from *Ananascomosus* and *Cucumissativus* while *Penicillium sp.* was isolated only from *Cucumissativus*. The

Table 1: Frequency of occurrence of fungal pathogens associated with the spoilt fruits

Fruit	*Rhizopussp*	*Saccharomyces Sp*	*Aspergillus sp*	*Fusarium sp*	*Penicillium sp*	Total
Ananascomosus (Pineapple)	3	3	0	0	0	6
Mangiferaindica (Mango)	3	0	3	0	0	6
Cucumissativus (Cucumber)	3	3	3	3	3	15
TT.no of genera	9	6	6	3	3	27
Frequency (%)	33.33%	22.22%	22.22%	11.11%	11.11%	100%

frequency of occurrence shows that *Rhizopussp* had the highest frequency occurring in all the nine fruits tested recording 33.33%.*Saccharomyces sp* and *Aspergillus sp.* recorded 22.22% each while *Fusarium sp*. and *Penicilliumsp* had the least frequency occurring in only one of the fruits ie *Cucumissativus.* Recording 11.11% each as shown in Table 1.

Pathogenicity tests revealed that all the five isolatedfungi were pathogenic. They were able to produce the same spoilage signs in the healthy fruitsinto which they were re-inoculated as shown in Table 2.

Results of the effect of three natural preservatives tested against the isolates showed that vinegar is highly effective against all the five isolates by inhibiting their growth at all the concentrations used, while garlic oil is only moderately effective against Fusarium *sp*. at 40% and 50% concentrations. Similarly olive oil was found to be moderately effective against *Penicillium sp* at 40% and 50% concentrations (Table 3).

DISCUSSION

The result of this study shows that five (5) fungal species; *Aspergillus, Fusarium, Penicillium, Rhizopuss* and *Saccharomyces* were isolated from the spoiled fruits (mango, pineapple and cucumber).The most frequently occurring was *Rhizopussp* which was found in all the spoilt fruits. All the five isolated fungal species were isolated from cucumber.The fungi found to be associated with the spoilage of mango in this study were Aspergillus and Rhizopus which are the same genus Pantistico (1975) reported to be the most common fungi that attack mangoes in addition to *Fusarium. Rhizopus and Saccharomyces* were observed to be associated with the spoilage of pineapple; this is in line with the work of Effiuvwevwere (2000) who reported that *Aspergillus sp.* and *yeast sp.* are responsible for the rotting of pineapple.

All the five organisms isolated were confirmed to be responsible for the spoilage of the fruits but in varying degrees. Findings showed that among the isolated fungi, *Rhizopus* was highly pathogenic leading to rapid disintegration of treated fruits in 4-5 days while *Aspergillus* and *Saccharomyces* were moderately pathogenic and *Fusarium*and *Penicillium*were least pathogenic, and cause the least amount of rot on the fruits. When these isolates were aseptically inoculated into healthy susceptible fruits, the characteristic symptoms originally observed were also noticed. The prevalence of fungi as the spoilage organism of fruit and vegetables is due to a wide range of factors which are encountered at each stage of handling from pre-harvest to consumption and is related to the physiological and physical conditions of the produce as well as the extrinsic parameters to which they are subjected (Effiuvwevwere, 2000). Damage inflicted on produce at the time of harvest is a major cause of infection since most of the spoilage microorganisms invade the produce through such damage tissue; similarly, the extent of deterioration is influenced by the depth of the wound. Furthermore, the incidence of infection is worsened by poor sanitary practices such as cross-contamination, contact infection during the transportation of produce (Effiuvwevwere, 2000). The result of the Pathogenicity tests carried out show that all the organisms were pathogenic and were the actual causal agents of spoilage of the different fruits and can also infect different fruits other than their original host. The tests also established the fact that fungi cause deterioration of the fruits when they gained entrance into them through mechanical injuries such as bruises and wound as reported by Zitter (1985).

Although essential oils from various wild plants have been reported in the past to be effective against a wide

Table 2: Pathogenicity test result

Fungal specie	Fruit inoculated	Spoilage pattern
Rhizopus	*Ananascomosus*	Sunken depression
	Cucumissativus	Brownish necrotic patches with cottony growth.
	Mangiferaindica	Water soaked wrinkled with depression.
Saccharomyces	*Ananascomosus*	White deposit, fruit becoming spongy with gas production
	Cucumissativus	Fruit becoming with gas production and sunken spots.
Aspergillus	*Cucumissativus*	Brownish, necrotic patches.
	Mangiferaindica	Brownish necrotic patches with cottony growth.
Fusarium	*Cucumissativus*	Whitish-pinkish mycelia growth.
Penicillium	*Cucumissativus*	Brownish necrotic patches with cottony growth.

Table 3: Inhibitory effect of garlic oil against the fungal isolates

	Concentrations of garlic oil				
Organism	**10%**	**20%**	**30%**	**40%**	**50%**
Aspergillus	-	-	-	-	-
Fusarium	+	+	+++	+++	+++
Saccharomyces	+	+	+	++	++
Rhizopus	-	-	-	-	-
Penicillium	+	+	+	+	+

range of microorganism invitro. (Chang *et al.,* 2008), the ability of the three preservatives used in this study to inhibit the growth of the isolated fungi in vitro could be observed in Tables 3,4 and 5. Vinegar is the most effective preservative among the three used in this study by inhibiting the growth of all the fungal isolates at all the five tested concentrations (ie. 10%, 20%, 30%, 40%, and 50%) as shown in table 5. This indicates that vinegar contains active antifungal agent capable of inhibiting the growth of the isolated fungi. Garlic oil had no effect on *Aspergillus sp.* at all the concentrations However the effect of garlic on *Fusarium sp.* is highly effective at 30%, 40% and 50%

Table 4: Inhibitory effect of olive oil against the fungal isolates

Concentrations of olive oil					
Organism	**10%**	**20%**	**30%**	**40%**	**50%**
Aspergillus	-	-	-	-	-
Fusarium	-	-	+	++	++
Saccharomyces	+	+	+	++	++
Rhizopus	-	-	-	-	-
Penicillium	-	-	+	++	++

Table 5: Inhibitory effect of vinegar against the fungal isolates

	Concentrations of vinegar				
Organism	**10%**	**20%**	**30%**	**40%**	**50%**
Aspergillus	+++	+++	+++	+++	+++
Fusarium	+++	+++	+++	+++	+++
Saccharomyces	+++	+++	+++	+++	+++
Rhizopus	+++	+++	+++	+++	+++
Penicillium	+++	+++	+++	+++	+++

+++ = highly effective ++ = moderately effective+ = less effective - = not effective

concentrations which is in contrast with the work of Sa'ad and Soad (2005), who reported that garlic oil, rosemary, rose, dill and peppermint were not effective on *Fusarium sp*. Furthermore all the concentrations of garlic used in this study were not effective on *Rhizopussp* and *Aspergillus sp.* but less effective on *Penicillium sp.* as shown in Table 3. On the other hand olive oil was not effective on all the concentrations used on *Aspergillus sp.* and *Rhizopus sp.* but moderately effective against *Fusarium, Penicillium* and *Saccharomyces* species (Table 4).

CONCLUSION

In conclusion five different fungal species; *Aspergillus, Fusarium, Penicillium, Rhizopus* and *Saccharomyces* were isolated from the spoiled fruits which were alsoconfirmed to be responsible for the spoilage of the fruits. The most predominant fungi associated with the spoiled fruits is *Rhizopus.* Vinegar was the most effective preservative among the three preservatives (Olive oil, Garlic oil and Vinegar) tested against the fungi isolated in this study, hence could be used in the preservation of these fruits. This is followed by Garlic oil and the least effective was Olive oil.

REFERENCES

Amusa NA, Kehinde IA, Ashaye OA (2002). Bio-deterioration of Breadfruit (Artocrarpuscommunis) in storage and its effects on the Nutrient Composition. African Journal of Biotechnology, 1(2): 57-60.

Baiyewu RA, Amusa NA, Ayoola OA, Babalola OO (2007). Survey of the post harvest diseases and aflatoxin contamination of marketed Pawpaw fruit (*Carica papaya* L) in South Western Nigeria. *African Journal of Agricultural Research*; 2(4): 178-181.

Cheesbrough M (2000). District Laboratory Practicein Tropical Countries Part 2, Cambridge University Press, Cambridge. P. 47-54.

Chuku EC, Ogbonna DN, Onuegbu BA, Adeleke MTV (2008). Comparative Studies on the Fungi and Bio-Chemical Characteristics of Snake Gaurd (Trichosanthescurcumerinalinn) and Tomato (Lycopersiconesculentus mill) in Rivers state, Nigeria. Journal of AppliedSciences, 8(1): 168-172

Chukwuka KS, Okonko IO, Adekunle AA (2010). Microbial Ecology Of Organisms Causing Pawpaw (*Carica Papaya L*.) Fruit Decay In Oyo State, Nigeria. *American-Eurasian Journal of Toxicological Sciences*; 2 (1): 43-50

David E, Stephen D, Helen A, Rosemary H, Robyn B, (2007). Description of Medical Fungi 2nd edition, Published by the Authors Mycology Unit Women's and Children's Hospital North Adelaide 5006 AUSTRALIA.

Droby S (2006). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *ActaHorticul*.709: 45–51.

Effiuvwevwere BJO (2000) Microbial Spoilage Agents of Tropical and Assorted fruits and Vegetables (An Illustrated References Book).Paragraphics publishing company, Port Harcourt; pp: 1-39.

European Standard (ES) (1997). Chemical disinfectants and antiseptics. Basic fungicidal activity. Test method and requirements. EN 1275.Bruxelles, Belgium: European Committee for Standardization.

Gupta AK, Parthak VN (1986). Survey of fruit Market for Papaya fruit Rot by Fungi pathogens; Indian J. Mycol., 16: 152-154.

Hadizadeh I, Peivastegan B, Hamzehzarghani H (2009). Antifungal activity of essential oils from some medicinal plants of Iran against Alternaria alternate, *American journal for applied sciences*.

Paul S, Sainsbury D (1981). Dictionary of Microbiology. John Wiley and Son's Publication, New York.

Sa'ad R, El-Lemity, Soad MA (2005). Antifungal activity of some essential oils and their major chemical constituents against some phytopathogenic fungi, pesticide chemistry dept. , faculty of Agriculture. (chatby), Alex University. *J. pest cont and environ. Sci.* 13 (1): 61-72.

Zhu SJ (2006). Non-chemical approaches to decay control in postharvest fruit. In: Noureddine B, Norio S. (Eds.), Advances in Postharvest Technologies for Horticultural Crops. Research Signpost, Trivandrum, India; pp. 297–313.

Zitter TA (1985). *Bacterial Diseases of Tomato*. Fact Sheet. Department of Plant Pathology, Cornell University. 1985; pp 735-750.