European Journal of Biotechnology and Bioscience

www.biosciencejournals.com

E J B B

European Journal of Biotechnology and Bioscience

ISSN: 2321-9122
www.biosciencejournals.com
EJBB 2014; 2 (2): 24-27
Received: 21-07-2014
Accepted: 05-08-2014

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Identification and control mycopathogens associated with storage of five horticultural produce in evaporative coolant-vegetable basket

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Abstract
Five freshly harvested horticultural produce: two fruit vegetable-tomato and okra and three leafy vegetables- Celosia, *Amaranthus* and *Telfairia* were stored in the vegetable basket –an evaporative coolant system. Disease incidence .frequency and severity were scored after the storage of each produce. Spoilage micro-organisms were isolated and identified. Fungicides: Benlate, Thiram and one botanic leaf powder-*Peperomia pellucida* plant were used for control of pathogens. In tomato: *Fusarium moniliforme* and *Rhizopus* spp were isolated. *Fusarium moniliforme* was isolated from okra fruits; *Aspergillus* niger, *Fusarium moniliforme* and *Cladosporium fulvum* were isolated from Celosia leaves while *Fusarium moniliforme* and bacterium spp were isolated from *Amaranthus* leaves. In *Telfairia* spp: *Helminthosporium* spp was isolated. The fungicides showed no significant effect in controlling the disease spread in the test materials.

Keywords: Isolation, Identification, Control, Mycopathogens, Horticultural produce, and Vegetable basket

1. Introduction
Post harvest loss is defined as any change in the availability, edibility, wholesomeness or quality of food that present it from being eaten by man (Mbuk *et al*. 2011). A post-harvest loss is the bane of agricultural production in Nigeria. Availability data reveal that large quantities of fruits and vegetables are produced during the growing season, but due to lack of effective storage facilities most of the produce are wasted and millions of Naira are spent in importing their concentrates (Aworh, 2011). This then makes post-harvest management of fruits and vegetables of great importance. This will help to stabilize prices by carrying produce period of high production to period of low production (Agoda *et al*. 2011).
Mycopathogens is one of the major cause of post-harvest deterioration in the humid regions of West Africa (Obetta *et al*. 2011). Many bacteria and fungi can cause postharvest decay of fruits and vegetables. It has been well established that the major post-harvest losses of fruits and vegetables are caused by species of such pathogens as *Alteriaria*. *Botrytis Diplodia Monilinia, Penicillium, Phenopsis, Rhizopus Sclerotinia, Fusarium, Geotrichum, Heminthosporium, Curvularia* and of bacteria *Erwinia* and *Pseudomonas (*Correl *et al 1986)*.
Fruits and vegetables are rotted by organisms that either infect the produce while still immature or attached to the plant or during the harvesting and subsequent handing and marketing. Rotting is greatly aided by mechanical injuries on the surface of the produce such as fingernail scratches, abrasions, insect punches, and abscission layers and cut stems (Morris *et al*, 1987). Although some pathogens can penetrate the skin of healthy produce e.g. *Collectotricum* spp. Therefore, avoiding mechanical injuries by using appropriate harvesting methods is very critical in reducing postharvest losses. Losses of fruits and vegetables stand at an alarming rate of 50% and above in Nigeria (Agoda *et al*. 2011, Nwufo *et al*. 1990). It is not sufficient to plan for food sufficiency programmes, if appropriate postharvest interventions are not identified and specified for minimizing post-harvest losses in the field and in marketing (Idah *et al*. 2007). Harvesting technique and adequate handling is a very good preventive measure against postharvest losses (Shulka *et al.* 2010, Imonikebe, 2013). To achieve this adequate storage, appropriate technologies should be applied (Amrat *et al*.2013).

Appropriate technologies involve those that are adequate, available, affordable and easily adaptable to the farmers. Such adequate, cheap and adaptable technology is the vegetable basket (NSPRI 1991, Aworh 2011).

Evaporative cooling effect as a result of the evaporation of liquid such as water is based on the principle of adiabatic cooling of unsaturated air when in contact with water for sufficiently long period of time. Then evaporation takes place when molecules of water acquire enough kinetic energy to overcome attractive force of the neighbouring molecule and escape into space (Liberty *et al.* 2013b).

The principle of evaporative cooling has been applied for the storage of fresh agricultural produce by lowering the temperature of the storage chamber (Amrat *et al.2013*). It also maintains the produce at high relative humidity. The high relative humidity prevents excessive loss of moisture from produce and therefore helps to prolong the shelf lives of the produce (Liberty *et el.* 2013a). However, the incidence of mycopath otogenic infection cannot be ruled out, since the high relative humidity provides a favorable environment for the growth of the fungi pathogens that can attack the produce right in the chamber (Obetta *et al*. 2011)

Hence, the objectives of this study are to identify the mycopathogens associated with the storage of produce in evaporative coolant system and to apply some control measures.

2. Materials and Methods

The vegetable basket was originally designed, built and promoted by Nigerian Store Product Research Institute (NSPRI 1990) which works on the evaporative cooling principle was used to store five horticultural produce. This consisted of three leaf vegetable and two fruit vegetables. These were Telfairia, Amaranthus, Celosia, Tomato and Okra. The five vegetables were grown in the research farm of the Department of crop science, University of Nigeria Nsukka. Equal quantities of the produce were used in each vegetable basket. The produces were stored one after the other. The cooling effect was obtained by sprinkling the vegetable baskets with water from an open stream-water.

2.1 Isolation and Identification

Spoilt and infected fruits and vegetables were sampled from the storage devices and surface sterilized with 1% alcohol and rinsed two times with sterile water. A sterile knife was used to remove tissues from the interface region between the healthy tissue and diseased part of the produce into a water agar (WA) medium. After four days, a sub-culture was transferred from the water agar to potato dextrose agar (P.D.A). Two drops of lactic acid was added to one litre of the PDA to prevent bacterial contamination. The sub-culturing was repeated until pure cultures were obtained. Then the pure organisms were stained with lactophenol, viewed under compound microscope and subsequently identified.

2.2 Disease Frequency

The total number of spoilt fruits after storage was counted. The spoilt fruits due to pathogenic factors were sorted out according to the similarities of the symptoms observed on them. Also the percentage incidence of each disease type was calculated as follows:

$$\frac{\text{Number of spoilt fruits due x}}{\text{Total number of spoilt fruit}} \times 100$$

Where x = type of disease (symptom)

2.3 Disease Incidence

This was also calculated thus:

$$\frac{\text{Number of spoilt fruits due to x}}{\text{Total number of fruits sampled}}$$

For leafy vegetables, hundred leaves of each vegetable type were sampled and inspected for symptoms of spoilage due to pathogenic infection. The incidence and frequency were also calculated as in fruit vegetables

2.4 Pathogenicity Test

A 3 mm cork borer was used to obtain discs of the mycelium of each identified fungus from the periphery of a 4-day old P.D.A. culture. These discs were placed in the wound made on a healthy tissue of the test produce with a cork borer. The rim of the wound was sealed with jelly to prevent contamination. Ten healthy, firm fruits were used for each produce. The produces were then left for five days on the laboratory bench. They were examined for any symptom development. For the control, sterile PDA discs were used on the wounded surfaces of the produce. The test was done for all the identified fungi. For the leaf vegetable surface wounds were used for the inoculation.

2.5 Application of Fungicides

Two synthetic and one botanic fungicides were used at a concentration of 0, 10, 20 and 30 mg ml^{-1} of sterile water. The dipping method was used. Ten fruits of tomato and Okra which were inoculated with each of the identified fungi were used including ten leaf blades for the leaf vegetable. In case of controls, sterile water was applied. The synthetic fungicides used were Thiram and benomyl while the botanic extract was *Peperomia pellucida* crude powder.

3. Result

The isolation and identification carried out showed that the majority of the spoilage micro-organisms were fungi pathogens.

The result showed that from Celosia was isolated the highest number of fungi pathogens-Fusarium spp, Cladosporium spp and Aspergillus spp followed by *Telfairia* and Tomato which had two fungi organisms respectively, while one fungi pathogen and a bacterium spp were isolated fom Amaranthus, but Okra had only one fungi pathogen *(Fusarium moniliforme*) (Table 1).

Table 1: isolated pathogens from five diseased produce and their symptoms

Produce	Observed Symptoms of Diseases	Pathogens Isolated
Tomato	Watery rot	*Fusarium semitectum, Rhizopus spp*
Okra	Slimy mucilage and soft rot	*Fusarium moniliforme*
Celosia	Dry not	*Fusarium moniliforme, Cladosporium fulvum* and *Aspergillus niger*
Amarathus	Dry leaf rot	Fusarium moniliforme & Bacterium spp
Telfairia	Translucent leaf spot	*Curvularia Lunata, heminthosporium* spp

3.1 Pathogenicity Test

Most of the pathogens isolated showed high degree of virulence when they were inoculated into healthy produce, and they showed similar symptoms as was observed in the diseased produce after the storage. The symptoms observed were watery exudates and maceration in tomato fruits and slimy rot in okra fruits. Necrosis and dry rot were observed on the leafy vegetables. There was a rapid increase in the spread of *Rhizopus* spp inoculated into tomato fruits. It was continuous until the whole produce was covered with the mycelia of the pathogens. But in leafy vegetables the disease spread was not rapid and continuous as observed in fruit vegetables.

3.2 Fungicidal Efficacy

All the fungicides used performed better than the no treatment in controlling the disease spread in all the test produce but there was no significant difference among the three fungicides at p = 0.5 (Table 2).

Table 2: Effects of fungicide on disease spread of artificially inoculated tomato fruits.

Mgml-1

Fungicides	0	10	20	30	Mean
Benlate	3.35	3.33	3.30	3.28	3.30
Thiram	3.46	3.43	3.41	3.40	3.42
Peperomia spp Power	3.48	3.65	3.63	3.62	3.46
Mean	3.47	3.45	3.43	3.41	

F-LSD (0.05) NS

- The means shown are the means of three of replicates.

3.3 Disease Incidence and Frequency

In tomato fruits *Rhizopus* spp hah higher disease incidence and frequency (0.52and52%) respectively than Fusarium spp which had 0.24and 24% respectively ln okra the disease incidence and frequency for Fusarium spp the only isolated pathogen were 0.60and 60 % respectively. Form the hundred leaf blades sampled form each bunch, *Hemithosp oruim* spp had 0.57 disease incidence and 57% disease frequency in *Telfaria* spp while Curvularia spp had 0.38 disease incidence and 38 disease frequency. ln *Amaranthus* spp the only fungal pathogen, *Fusarium moniliforme* had a disease Incidence and frequency of 0.49 and 49% and respectively. Celosia spp with the highest rot pathogens isolated where Fusarium spp had a disease incidence and frequency of 0.46 and 46% respectively followed by a Aspergillus spp which had 0.32 and 32% and Cladosporium spp which had 0.12 and 12% respectively.

4. Discussion

4.1 Disease Incidence and Frequency

From the list of pathogens isolatd Fusarium spp occurred most as the spoilage micro-organism. This is similar to earlier work by (Leonian, 2010) who identified Fusarium moniliforme from corn fruits. The result showed that all the fungal organisms isolated showed high pathogenicity. This result was similar to the report of Correl *et al.* (1986), who isolated and identified fungi organisms as major spoilage pathogens in horticultural produce.

The disease incidence showed that *Rhizopus* spp caused the highest degree of spoilage in tomato fruits the reason is quiet obvious. This pathogen thrive very well in very humid environment and the high moisture content of the tomato fruits provided a suitable condition for its growth (Obetta *et al*. 2011). ln leafy vegetables such as the *Amaranthus* spp, Fusarium spp occurred most. The reason could be because this fungi is quite ubiquitous. lts high prevalence could have resulted from the fact that the seeds from which the vegetables were grown were not treated before sowing into the nursery The fungi which is highly prevalent in the southern Agricultural zone of Nigeria (Ora *et al.* 2011) must have resulted from seeds which were transferred to the leaves during the growing in the field.

4.2 Fungicidal Efficacy

The three fungicides did not have a significant effect in controlling the disease spread after inoculating the healthy produce with the pathogens. This could have been as a result of low concentration of the fungicides used.

Also according to (Adekunle and Uma, 2005), *Rhizopus* oryzae have developed resistance to binomial, lmazalil and Femopanil. These fungicides had only little effect on *Geotrichum candidum*. Fungicidal resistance have also been reported by Wild (1983) in Australia. He observed that *Penicillium digitatum* have developed resistance to Guazatine and Benomyl at certain dosages. Therefore the result obtained could have been as a result of fungicidal resistance. According to Wills [1998], it is more advisable to treat the produce after harvest with sodium hypochlorite mixed with the washing water or with other protective chemicals to prevent the product from being infected by spoilage micro-organisms before storage This implies that the fungicides could have been effective if they were applied before the produce were infected with the fungi pathogens.

5. References

1. Agoda S, Atanda S, Usanga OE, Ikotun I, Isong IU. Postharvest food losses rduction in maize production in Nigeria. Afr J Agric Res 2011; 6(21):4833-4839.

2. Amrat LB, Samuel DVK, Vimala B. Evaporative cooling system for storage of fruits and vegetables. J Food Sci Technol 2013; 50(3):429-442.
3. Atanda SA, Pessu PO, Agoda S, Isong IU, Ikotun I. The concepts and problems of postharvest food loss in perishable crops. Afr J Food Sci 2011; 5(11):603-613.
4. Aworh CO. Reducing postharvest losses of horticultural commodities in Nigeria through improved packaging. IUFOST Ontario Canada, 2011.
5. Correl JC, Uhalla JEP, Schneider. Identification of Fusarium oxysporium, F.sp apii on the basis of colony siz ,virulence and vegetative compatibility American Phytopathological Society, 1986, 7, 396-399.
6. Idah PA, Ajisegiri ESA, Yisa MG. Fruits and vegetables handling and handling and transportation in Nigeria. Agric J Technol 2007; 10(3):175-183.
7. Imonikebe BUN. Measures for minimizing postharvest food losses: Steps towards ensuring food security in Delta State, Nigeria. Int J Food Sci 2013; 2(2):23-27.
8. Kay JS, Pallas JE. Postharvest physiology of perishable plant produce. University of Georgia, USA, 1991, 226.
9. Leonian LH. The Pathogenicity and the variability of Fusarium moliniformi from corn. Digitized Internet Archive, American Libraries, 2010, 16.
10. Liberty JT, Okonkwo WI, Echiegu SA. Evaporative cooling: A postharvest Technology for fruits and vegetables preservation. Int J Sci Eng Res 2013a; 4(8):2257-2266.
11. Liberty JT, Ugwuisuwu BO, Pukuma SA, Odo CE. Principles and application of Evaporative cooling System for fruits and vegetables preservation. Int J Curr Eng Technol 2013b; 3:3.
12. Mbuk EM, Bassey NE, Udo ES, Udo EJ. Factors influencing postharvest loss of tomato in Uyo Urban market in Uyo Nigeria. J Agric Food Environ 2011; 7(2):40-46.
13. NSPRI. Technology for reduction of postharvest losses in fruits and vegetables, Ilorin No.4, 1990, 1-38.
14. Nwufo MI, Obiefuna JC, Emebiri LC. Storage technique and seed viability in fluted pumpkin (Telfaria occidentals). A paper presented at the symposium of conservation of plant genetic resource held at 11TA, Ibadan, 1990, 16-18.
15. Obetta SE, Nwakonobi TU, Adikwu OA. Microbial effects on selected stored fruits and vegetables under Ambient Makurdi, Benue State, Nigeria. Res J Appl Sci Eng Technol 2011; 3(5):393-398.
16. Ora N, Faruq AN, Islam MT, Akhtar N, Rahman MM. Detection and Identification of seed borne pathogens from some cultivated hybrid rice varieties in Bangladesh, Middle-East Journal of Scientific Research 2011; 10(4):482-488.
17. Shukla S, Boman BJ, Ebel RC, Robert P, Dand HEO. Reducing unavoidable Nutrient losss from Florida's Horticulture crops. Hort Technology 2010; 20(1):52-56.
18. Wild BL. Double resistance by Citrus green mould *Penicillium digitatum* to the fungicides, quazatine and benomyl. Association of Applied Biologist, Department of Horticultural Postharvest Laboratory, New South Wales Gosford, Australia, 1983, 1326-1328.
19. Wills RBM, Graham R, Loyce D. Postharvest: An introduction to the physiology and handling of fruits and vegetables and ornamentals CAB International, Newyork, USA, 1998, 1225.