Abstract: Thermal control effect on the incidence of some post-harvest rot pathogens of Solanum tuberosum (potato) was investigated in this study. Three cultivars of potato tuber whose local names are, Patiska, Mai Bawondoya and Nicola were used for the study. Five pathogenic fungi viz: Botryodiplodia theobromae, Fusarium redolens, Fusarium oxysporum, Penicillium sp. and Rhizopus oryzae associated with post harvest storage rot of root-tubers, were isolated from diseased potatoes. Among the three species of potatoes used in the study, Patiska was found most resistant followed by Mai Bawondoya, while Nicola was the least resistant. Increase in substrate (i.e. soluble starch or CMC) concentration enhanced a proportional increase in mycelial growth and in the amount of extracellular enzymes produced. Some of these test pathogens were found to produce cell wall degrading enzymes (i.e. amylase and cellulase). Preferential utilization of carbohydrate sources was established in this study based on the growth of test pathogens. Growth on potato broth medium was highest followed by growth on Cocoyam and Sweet potato broth media and least on Cassava broth medium. Growth of the test pathogens on carbohydrate sources was found at variant.

The use of hot water treatment at different temperatures was found to significantly reduce post-harvest fungal populations on the surface of root-tubers. The efficacy of blanching in hot water at 60°C was significantly higher than that of blanching in hot water at other temperatures. The control method adopted in this study showed that the problems of potatoes’ rot disease in storage (especially by the peasant farmers) can be eradicated by thermal treatments without reducing the quality of the Irish tuber.

Key words: potato tuber, diseases, thermal treatment.
Introduction

After poverty itself, the third world’s most pressing problem is food scarcity. The International Food Policy Research Institute (1977) predicted a bleak future for the world’s food situation. The food deficit in the developing market economics is projected to rise from 12 million metric tons in 1975 to 70-85 million by 1990, Asia accounts for 40% of the total projected, North Africa/Middle-East about 25%, Sub-Saharan Africa over 20%, and Latin America over 10%”. Equally alarming is the rate of some 15 million of the world’s Children who die each year of malnutrition and related diseases (FAO, 1997). Most of this mortality is concentrated in the developing countries, which are said to home about 75% of the world’s population. Even where the “green revolution” has made its mark, many children are malnourished (Villareal, 1982). This is because their diets are largely based on cereal grains, cassava and bananas; all starchy and low in protein. It has been established that a child’s small stomach cannot hold enough of such food to provide the protein the body needs let alone the necessary vitamins and minerals (Villareal, 1982; FAO, 1997). In view of this, it is imperative that a solution is found which will check the besetting problems of low production, post-harvest storage deterioration/spoilage and dependence on crops of relatively low nutritional value.

In the quest for food and the struggle for human survival, Irish Potato has historically played important role and is suitable in addressing the problems previously highlighted. This is due to their yield per unit area (hectare) per unit time and their nutritional value (i.e. ratio of protein to carbohydrate) (Tewe, et al., 2003)). African farmers are faced with several constraints in the production of food and cash crops. Some of these constraints are poor soils, poor farm practices, use of local varieties, land tenure and damages by diseases and pests (FAO, 1997). Irish potato tubers like many other crops are attacked by various pathogens which cause diseases in them. Such pathogens are fungi, bacteria and viruses. The greatest of these constraints is that of post-harvest spoilage of farm produce, out of the several tons harvested, just a fraction is utilized while most of the amount is lost to post harvest diseases especially rot diseases. (Alexandratos, 1995). These pathogens cause great losses and reductions in the value of these crops but with a good system of control this can be eradicated. Pathogenic fungi associated with post-harvest Irish potato tuber rot are Alternaria solani (early blight), Rhizogospora subtranea (Powdery Scab), Fusarium roseum and Fusarium solani (fusarium rot disease), Helminthosporium solani (skin blemishes disease), Colletotrichum atramentarium (black rot disease), Aspergillus niger, Pythium ultimum (Pythium tuber rot disease), Phytophthora erythroseptica (Pink rot disease), and Phytophthora infestans (late blight disease) (Clark and Moyer, 1988). The mechanisms by which diseases are produced
Thermal control of rot pathogens on potato vary considerably with the causal agents and sometimes with the plant. Parasitic diseases commonly arise as a result of an interchange of metabolites between a parasite and the invaded host plant (Agrios, 1998). This is because enzymes are the first substances secreted by the pathogen on establishing contact with the surface area of their hosts. Many plant pathogenic and non-pathogenic organisms are capable of producing an array of cell-wall-degrading enzymes.

Biological control methods encompass the utilization of organisms other than man (Campbell, 1989). This involves the selection and breeding of plants for resistance to particular pathogens or the use of other microorganisms that are either antagonistic to the pathogen or parasitize the pathogen itself (Agrios, 1998; Whipps and McQuiken, 1993; Kukik, 1995). Although, the use and breeding of resistant varieties (Biological control method) is the oldest, cheapest and overall best means of controlling plant diseases, some are not economically feasible. The use of physical agents, such as temperature (high or low) and various types of radiation in controlling plant diseases (Agrios, 1998) is another means of control that has not been adequately explored and information on its use suggest great promise for future development. Hence this study shows the effect of thermal method of control application on the incidence of some post harvest rot pathogens of Irish potato tubers.

Materials and Methods

Survey and Collection of Sample

Six local markets Bodija, Oje, Sabo, Ojoo, Dugbe (in Ibadan, Southern Nigeria) and Bukuru (in Jos, Northern Nigeria) were visited to collect both healthy and diseased samples. All infected tubers were inspected for rotted areas and were stored in clean polyethylene bags. All samples were brought to the Laboratory for further analysis.

Preparation and Sterilization of Culture Media

Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) (Difco or Oxoid) were used for the isolation and cultivation of different fungi generally associated with Post-Harvest spoilage/deterioration of Irish potatoes.

Isolation of Associated Fungi

Diseased samples were surface sterilized with 1% sodium hypochlorite solution for 1 minute, rinsed in five successive changes of sterile distilled water and then blotted dry. These tubers were cut aseptically from healthy region to necrotic region. Discs of about 4mm diameter were made from the advancing
edge of the rot as well as from the middle of lesions and plated on media (PDA and MEA) into which 0.1% streptomycin was added to suppress bacterial growth. These were incubated at room temperature i.e. 28±2°C for the growth of organisms. The organisms were to grow for 96 hours after which they were sub-cultured to obtain pure cultures. The pure fungi cultures were placed on slants in Mac Cartney bottles and stored in a refrigerator for further use.

Identification of Associated Fungi

Using a sterile inoculating needle, small portions of each mycelia colony were aseptically taken and placed on clean microscope slides and teased in a drop of lacto phenol cotton blue. Fungi species that developed were identified with the aid of a compound microscope, by reference to compendium of soil fungi, Domsch et al., (1980), Barnett and Hunter (1972), and by comparing with identified species of the same genera in the microbial collection of the Department of Botany and Microbiology, University of Ibadan, Nigeria.

Establishment of Pathogenicity of Isolated Fungi

All fungi isolates were tested for their ability to induce rot in healthy tubers. The healthy tuber surfaces were swabbed with cotton wool soaked in 1% sodium hypochlorite and washed thrice with sterilized tap water. Holes were dug using sterilized 5mm cork borer and the plug was pulled out. 3mm mycelial discs of the test pathogen were placed at the bottom of the hole. Portions of the plugs were cut off to compensate for mycelial disc thickness before replacing in the hole and the wound sealed with vaseline to prevent extraneous infection. The controls consisted of sterile PDA discs of 3mm wells made on healthy tubers. The tubers were incubated in desiccators for 2,4,6,8 and 10days respectively at 28°C ±2°C. All treated tubers were prepared in triplicate. After a period of 10days, the tubers were cut across by means of sterilized scapel along the plane of inoculation and the percentage rot was assessed using the formula below according to Ajiboye (2004):

\[
\frac{\text{Rotted surface Area} \times 100}{\text{Total surface Area}}
\]

Effect of CMC concentration on mycelia growth of the test pathogens:

Carboxyl Methyl Cellulose (CMC) medium was prepared at various concentrations of 0, 0.25, 0.5, 0.75 and 1.0%. Each medium was dispensed measuring 30mls of each into 150-ml flasks. After sterilization, the flasks were inoculated and incubated at 30°C for 6 days. The cellulase activity of the filtrates was then determined at 540nm using an SP 600 Spectrophotometer Transmittance. The pH of the filtrates was also measured.
Effect of soluble starch concentration on mycelia growth of the test pathogens:

A soluble starch medium for enzyme synthesis was prepared with various concentrations of starch, 0, 0.25, 0.5, 0.75 and 1.0%. Each medium (pH 6.9) was dispensed 30mls each, into 150ml conical flasks. Five flasks of each concentration were sterilized, inoculated and incubated at 30°C for 6 days. The mycelium produced in each flask was then filtered in pre-weighed filter paper, dried in an oven at 85°C for 24hrs, cooled in a desiccator and weighed. The amylase activity of each filtrate was determined at 30°C. The pH of the culture filtrates was also measured. Each filtrate was tested for the presence of reducing sugar by boiling 2 ml of the filtrate together with 2ml of Benedict’s solution for 5 min.

Effect of different carbohydrate sources on mycelia growth of fungal isolates

The effects of different carbohydrate media were tested on the mycelia growth of the fungi and enzyme activities. The carbohydrate sources used were:
- Cocoyam;
- Sweet Potatoes;
- Irish potatoes;
- Cassava.

Extracts of the different carbohydrate sources were prepared by boiling 250g each of the carbohydrate sources for 1 hour in 1 litre of distilled water. The supernatant was collected into 1 litre-measuring cylinder and made up to 1000ml with distilled water. Each extract was supplemented with 20g glucose. The mixture was then dispensed into each of the 30 and 150ml conical flasks; the process was repeated for the other broths. All flasks and contents were autoclaved at 121°C for 15 minutes; after the content had cool, 5mm mycelial disc of one of the test pathogen was used to inoculate 3 flasks of Cocoyam Dextrose Broth (CDB). The same process was repeated for the rest of the test pathogens for CDB. The above procedures were carried out for the rest of the broth media. All flasks were incubated at 30°C for 7 days. At the end of incubation period, the mycelia mats were harvested unto pre-weighed filter papers respectively and oven dried at 80°C for 24 hours. They were then cooled in desiccators and weighed. The dry weight of mycelia mats was obtained by subtracting the weight of filter paper from total weight. The filtrates were collected for enzyme assays as earlier described.

Determination of the thermal Level

Hot Water Treatment:

The root tubers were blanched in 25 Litres containers containing 20 Litres of hot water at 28, 60 and 100°C for 25 minutes. The water was changed several times to prevent inoculum build up. The root-tubers were subsequently air dried.
and stored in well aerated storage sheds with temperature ranging between 12°C to 18°C. The root-tubers were well spread out. Tubers to be dehydrated were placed in boxes and sun dried for 5 days.

Results and Discussion

Incidence of Pathogenic Infection on Irish potatoes tubers

Ten fungal isolates were cultured from the rotted Irish potatoes. A total number of five (5) properly identified fungi species were randomly selected for use. Those selected are Botryodiplodia theobromae; Fusarium redolens; Rhizopus oryzae; Fusarium oxysporum; and Penicillium sp.

Key
Ro = Rhizopus oryzae,
Fr = Fusarium redolens,
Bth = Botryodiplodia theobromae,
Fo = Fusarium oxysporum
Psp. = Penicillium sp.

Level of Susceptibility of potato tubers to the Different Pathogens isolated

All fungi isolated from rotted Irish potatoes were pathogenic at varying degrees to healthy Irish potatoes when re-inoculated into them (Plates vi-xx). Fusarium redolens and Botryodiplodia theobromae were the most aggressive and virulent followed by Rhizopus oryzae and Fusarium oxysporum while Penicillium specie was the least pathogenic (Fig.1).

![Degree of infection (%)](image)

**Fig. 1. - Level of Susceptibility of Irish potatoes to Different Pathogen**
Thermal control of rot pathogens on potato

Mycelia Dry Weight

The mycelia dry weight of all the test pathogens increased gradually with incubation period when cultured on CMC except *Fusarium oxysporum* which had reduced dry weight values on the 8th day. Also, *Penicillium* sp. had the highest mycelia dry weight followed by the *Rhizopus oryzae, Botryodiplodia theobromae, Fusarium redolens and Fusarium oxysporum* when cultured on soluble starch medium (fig.2) while *Fusarium redolens* had the highest mycelia growth followed by *Rhizopus oryzae, Fusarium oxysporum, Botryodiplodia theobromae* and *Penicillium* sp. in cellulose basal medium (fig.3).

![Fig. 2. - Mycelia dry weight of test pathogens in soluble starch culture filtrate](image)

See Fig. 1 for the key

![Fig. 3. - Mycelia dry weight of test pathogens in CMC culture filtrate](image)

See Fig. 1 for the key
Effects of Increased Substrate (Starch or CMC) Concentrations on the Mycelia Growth and Enzyme synthesis

Both mycelia growth and enzyme synthesis increased as substrate concentrations increased (fig.4-7). This shows that Mycelia growth and enzyme synthesis are influenced by the concentration of the substrates. *Penicillium sp.* had the highest mycelia growth and enzyme synthesis followed by *Botryodiplodia theobromae*, while it was found least in *Fusarium oxysporum* when increased starch concentration was considered. However, in increased concentration of CMC, *Fusarium redolens* had the highest followed by *Rhizopus oryzae* and found least in *Botryodiplodia theobromae*.

Fig. 4. - Effects of increased Starch concentration on Mycelia growth

![Graph showing effects of increased Starch concentration on Mycelia growth](image1)

Fig. 5. - Effects of increased CMC concentration on Mycelia growth

![Graph showing effects of increased CMC concentration on Mycelia growth](image2)
Fig. 6. - Effects of increased soluble starch on enzyme synthesis

Fig. 7. - Effects of increased CMC concentration on enzymes synthesis
Effect of Different Carbohydrate Sources on Mycelia Growth of Fungi Isolates

Growth of the test pathogens was observed on all carbohydrate sources used in this study. The highest growth of the test pathogens was found on potato followed by Cocoyam, Sweet potato while it was least on Cassava (fig.8). Overall, Botryodiplodia theobromae recorded the highest growth followed by Fusarium redolens while Rhizopus oryzae recorded the least growth except on Irish potato source where Fusarium redolens was found highest (fig.8).

Effects of Thermal Control/Treatment on Root-tubers

At 28 °C, 8%, 28%, 60% and 96% of the tubers had rotted in 2, 4, 6 and 8 weeks respectively while at 60 °C, 4% of potatoes rotted in 14 weeks (fig. 9). At 100°C, all the root-tubers rotted under a week (fig. 9). However, when the tubers thus treated, were sun dried, 12%, 32%, and 44% of potato rotted in 10, 12, and 14 weeks respectively (fig. 9).

Losses from Post-harvest disease of stored potatoes are of great economic importance to nations and they are warring against food security in the developing world (Swaminathan and Sawyer, 1982). These diseases also occur during different seasons of the year, thus, potatoes are available for consumption only during part of the year due to inadequate storage. Reduction of post-harvest food losses, which are particularly high in perishable crops like potatoes in developing countries, is important not only from an obligation to avoid waste but because the cost of preventing food losses in general is considered to be lesser than producing a similar additional amount of food of the
same quality (Burton and Booth, 1982). Considering the number and virulence of the organisms isolated from the root tubers of the Irish potatoes, the importance of plant and pathogen interaction in food production and in post-harvest management cannot be over emphasized (Ajiboye, 2004). Of all the organisms isolated from diseased potato root tubers in this study, five have been found to cause spoilage of root tubers in storage facilities. The ability of these pathogens to colonise a wide range of plant materials, shows their ubiquity and non-host specific nature. In this study, *Fusarium oxysporum* had the highest frequency and it occurred on all potatoes types. This can be attributed to its non-host specific nature. This is in line with the findings of Anderson, (1985); Therberge, (1989); Tell, (1991); and Adewolu (1999). It has been observed in this study again that pathogenic organisms establish their contacts with their hosts by utilising enzymatic substances. This is due to the fact that all the test pathogens isolated in this work had increased mycelia weight and growth when cultured on different carbohydrate and carbon sources. This has also been discovered by Palmer (2001) and Okolo et al. (1995). These organisms were also found to respond to increased concentrations of culture filtrates used in this study, which they used to synthesise amylase and cellulose which are cell wall degrading enzymes. Rabinorich et al. (2002) and Salami (1999) also discovered this in their studies. Also discovered in this study is the fact that infection occurs as a result of wound created on the tuber, this is because wounded tuber rotted easily more than the unwounded tuber. Thus, tubers should be handled carefully during harvest to prevent wounding (Rabinorich et al., 2002).

Hot water treatment has been proved to be very effective for preventing the inocula of all the organisms isolated from Irish potato in this study without injuring them. This has been noted by Sherwood (1970) and Pullman, et al. (1981). Differences in sensitivity to temperature has also been established in this work, as the temperature at which the hot water prevented potato was found to be at 60°C for 25 minutes, whereas it varies in others. This has been ascribed to natural variance between populations from different climates (Dashwood et al., 1991; Harikrishnan and Yang, 2004). Dipping of ware potato tubers in hot water at 60°C for 25 minutes in this humid tropical environment has been found to be an effective means of reducing the spoilage of potato tubers during storage without adversely affecting the quality of the tubers. This is in line with what Burnett et al. (1988) and Ranganna, et al. (1998) discovered. However, potato cultivars differ in sensitivity to hot water treatment, the temperature detected in this work seems to be tolerated by these seed tubers especially in this tropical environment, though for a limited period of time, without affecting their sprouting and subsequent growth and yield. This method has also been discussed by FAO, (1990) and Lealam and Gashe, (1994) as one of the methods approved to extend the shelf life of fresh potato tubers.
Conclusions

The control of Irish potato post harvest storage rot by thermal treatment have proven to be very effective in checking post harvest storage rot in order to prolong their shelf life in this study. This control method is culturally, economically and socially suitable for the farmers. However, physical damage to the root-tubers should be avoided at all cost since they serve as major entry points for pathogens. Contact between the harvested root-tubers and the soil should also be avoided, in order to prevent re-infection, since the soil is the natural reservoir for pathogenic organisms. When in storage, root-tubers should either be well spread out on wood or on clean concrete floor. The use of heated soil which is free of pathogens could be considered as an alternative to water where the latter is scarce.

References


TERMĲKA KONTROLA NEKIH UZROČNIKA TRULEŽI KRTOLA KROMPIRA U SKLADIŠTU

Abiodun Olusola Salami1 and Olumide Omololu Popoola2

Rezime


Pri ispitivanju uticaja izvora ugljenika na porast kolonija, utvrđeno je da je porast kolonija bio najveći na čorbi od krompira, a potom na čorbi od Cocoyama i slatkog krompira, a najslabiji na čorbi od kasave. Potapanjem krtola

1 Abiodun Olusola Salami, Plant Science Department, Obafemi Awolowo University, Ile-Ife
2 Abiodun Olusola Salami Botany And Microbiology Department, University Of Ibadan, Ibadan
Thermal control of rot pathogens on potato

Kromira u vodu zagrejanu na različitim temperaturama značajno je redukovana populaciju patogena na njihovoj površini. Efikasnost tretiranja krtola topom vodom zagrejanom na 60°C je bila značajno viša nego pri teretiranju sa topom vodom zagrejanom na drugim temperaturama. Način tretiranja krtola krompira, ispitivan u ovom radu je pokazao da patogeni uzročnici truleži uskladištenog krompira (posebno kod seljaka) mogu biti uništeni bez smanjenja kvaliteta krtola krompira.