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Full Length Research Paper

Investigation of the role of calcium in prolonging the shelf life of fluted pumpkin (*Telefairia occidentalis* Hook F.)

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A 16-week study using 2 certified land races of *Telfairia occidentalis* Hook F plants, to ascertain the effectiveness of calcium in prolonging the shelf life of the leaves and identify the pathogens that cause leaf rot of the plant was carried out. The two varieties were subjected to 5 levels of calcium carbonate treatments (0.25, 0.50, 0.75, and 1 g and control (0 g) in 20 kg polythene bags and grown in the field for 120 days using completely randomized block design. The results obtained were subjected to analysis of variance at probability of 0.05. The rates of chlorophyll disintegration, respiration (CO_2 evolution) and calcium accumulation were measured from Days 1 to 6 after harvest using the 5th upper most fully expanded leaves (120 days old) for each variety per treatment. However, plants that received 1 g $CaCO_3$ died two weeks after germination. Significant differences were observed in the chlorophyll concentrations of the two varieties that received different calcium carbonate treatments and control (untreated leaves). On Days 5 and 6, the levels of chlorophyll were lowest in control and highest in leaves that received 0.5 and 0.75 g $CaCO_3$. The varieties did not vary significantly in chlorophyll disintegration on different days of storage and in different calcium treatments. The two varieties with different treatments showed a gradual decrease in chlorophyll concentrations from Days 1 to 6 and from Treatments 0 to 0.75 g. However, there was no significant variation in the two varieties on the rate of respiration (CO_2 production). Both varieties showed a gradual increase in CO_2 evolution from day 1 to 6 and the leaves treated with 0.5 and 0.75 calcium carbonate fertilizer produced least CO_2, while the highest CO_2 evolution was observed on untreated plant. The results showed that the two varieties did not differ significantly on the rate of calcium accumulation. Leaves treated with 0.5 and 0.75 g $CaCO_3$ accumulated the highest calcium, 0.25 g accumulated low calcium while the control had the least calcium 120 days after planting. The pathogens isolated include *Erwinia carotovora* and *Pseudomonas syringae.* The sensitivity test of calcium treatments on the growth of the pathogens, showed that calcium did not inhibit the growth of the isolated pathogens.

Key words: *Telfairia occidentalis,* vegetable, preservation, calcium, disease, shelf-life, pathogens.

INTRODUCTION

Telfairia occidentalis vegetable is a good source of vitamin, folic acid, mineral salt, protein and fibre. It contains some phytochemicals such as saponin, alkaloid, phenol and tannin (Sofowora, 1996) which have curative properties. *T. occidentalis* vegetable and fruit enhance regular bowel movement, prevent constipation, heart diseases, stroke, high blood pressure and accumulation of cholesterol (Etukudo, 2003). Consumption of the vegetable is limited by its short shelf life and many technical problems in maintaining its quality during storage. The amount of vegetable loss annually worldwide during post harvest period is estimated at 20 to 30% (Coursey, 1983) and this could feed millions of people. In all, approximately 25 species of fungi and bacteria are responsible for the major forms of decay in

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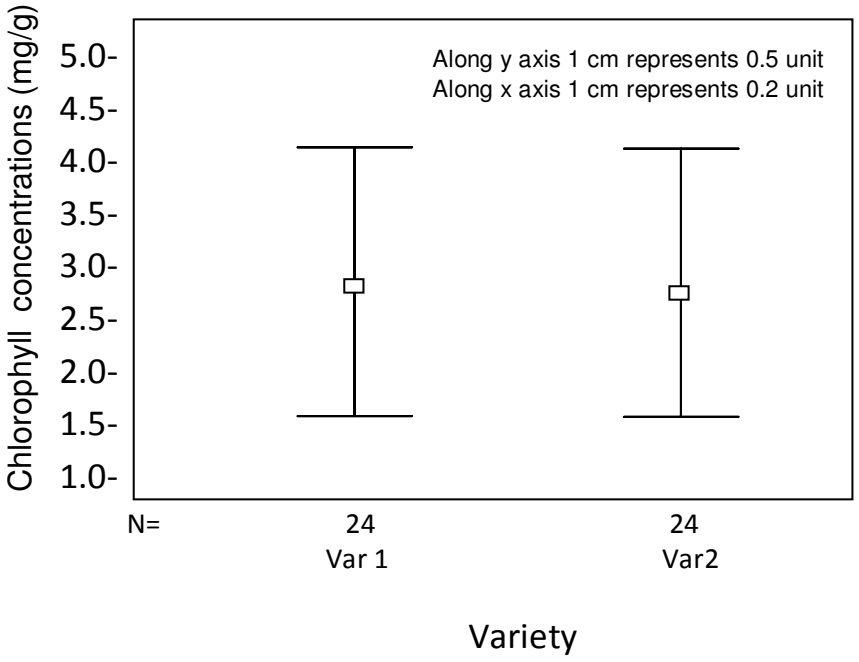

Figure 1. Chlorophyll concentrations of harvested leaves of two varieties of *T. occidentalis.*

plant products after harvest (Eckert and Ratnaya, 1983). Cutting of vegetable destroys the epidermis and internal components, thereby exposing the interior tissues to air, heat and chemical degradation (Anderson et al., 1991).

Several methods have been used for storage of green vegetables. These include refrigeration and sun drying. Preservation of this leafy vegetable in refrigeration is very expensive and unreliable because of incessant power failure in Nigeria. Refrigeration causes chilling injury and loss of flavor (Cabreva and Saltviet, 1990). Sun drying causes nutrient loss, the problem of weather and storm during rainy season limits this method of storage. The involvement of calcium in prolonging shelf life of post harvest vegetables and fruits have been reported by several workers (Miklus and Beelman, 1996; Pooviaiah, 1986; Vitor and Martins, 1995). These studies show that calcium prolongs the shelf life of vegetables and improves post harvest colour and texture of mushroom. Calcium is reported to influence various senescence characteristics such as ethylene production, protein disintegration in harvested leaves (Miklus and Beelman, 1996) . In addition, calcium is shown to confer rigidity to cell wall, reduce permeability of membrane to electrolytes and decrease the disintegration of cell components (Pooviaiah, 1986).

Hence the need for a detailed study to clearly determine whether or not calcium prolongs the shelf life of harvested leaves of *T. occidentalis*. The objective of the study is to: (1) investigate if calcium has any effect on post harvest decay of *T. occidentalis* leaves, (2) identify organisms that cause leaf rot of *T. occidentalis*, (3) test the sensitivity of the isolated pathogens to calcium.

MATERIALS AND METHODS

Soil preparation and calcium application

Certified seeds of two land races of *T. occidentalis* Hook F. Mbaise, with larger leaves and Ohaji, large leaves were used for the study at Abia State University botanical garden. Loam soil sieved with 2 mm mesh was treated with calcium carbonate (AR grade) using 0.25, 0.5, 0.75 and 1.0 g $CaCO_3$ and control (0 g) per polythene bag containing 20 kg soil and watered and left in open field for one week before planting the seeds in the bags. After germination, the soil was maintained at a field capacity until 120 days after sowing. The soil was analysed to determine its calcium requirement using $Ca(OH)_2$, titration method according to Chapman and Pratt (1961). The pH value of the soil – water suspension was determined using all glass electrode pH meter. The graph of pH value against the milliequivalent of calcium added per 100 g of soil was plotted to determine the amount of calcium needed to bring the pH to the desired level.

Sample collection

The fifth uppermost fully expanded leaves of each variety per treatment were randomly collected for analysis.

Rate of chlorophyll disintegration

The rate of chlorophyll disintegration of harvested leaves (120 days old) was measured by adding 10 ml of 85% acetone to 1 g of

ground leaves and centrifuged at 200 rpm. The absorbance of the decanted supernatant was measured at 643 and 663 nm wavelength as described by the Association of Official Analytical Chemists (AOAC, 1990).

Rate of respiration (CO_2 production)

The rate of respiration of harvested leaves (120 days old) was measured by monitoring the rate of CO_2 production on days 0 to 6 by NaOH titration method as described by AOAC (1994). 5 g of the leaves was weighed into a tight sealed plastic container. 10 ml of 0.5 N HCl was pipetted into a separate container and placed on the water soaked sample. The 10 ml NaOH was titrated with 0.5 N HCl at different monitoring intervals using phenolphthalein indicator, and calculated as the CO_2 absorbed by the NaOH which is released from the plant material during decomposition.

Calcium accumulation

Calcium accumulated in the two varieties grown under different calcium concentration and was determined by Multiple Nutrient wet – acid digestion method as described by James (1995). Then the calcium in the digest was estimated by Resonate EDTA complexiometry as described by Analytical Chemistry of foods (1995).

A fresh sample weighing 0.2 g was transferred into an extraction flask, and 5 cm^3 of the extracting solution was added and allowed to stand for 16 h at room temperature. The sample was placed on a digestion hot plate set at 30°C to heat for 3 h then 5 ml of concentrated $HClO_4$ was added to the flask and placed back on the hot plate and heated until digestion was completed, 20 ml of distilled water was added to the sample and warmed and allowed to cool and made up to 50 ml in a volumetric flask.

Pathological studies

Isolation of bacteria: The decaying leaves of *T. occidentalis* were washed with 1 L distilled water and 100 ml saline to dislodge the bacteria species. Pure culture of bacteria species was obtained by serial dilution of bacteria in suspension of sterilized distilled water and 1 ml of the solution was streaked in a plate and individual colonies isolated.

Isolation of fungi: The decaying leaves of *T. occidentalis* were washed with 1 L of distilled water and 0.1 ml of the suspension was placed on sterile SDA containing two drops of lactic acid and incubated at 25°C for 2 days. The spores were subcultured on freshly prepared SDA severally to obtain pure uncontaminated isolates.

Pathogenicity tests: Pure isolates were inoculated into healthy leaves of *T. occidentalis*. The leaves were washed and surface sterilized, rinsed in sterile distilled water and allowed to dry. Wounds were made with needle and two drops of suspension of bacteria and fungi (15×10^{-4}) were placed around the wounds. The points of inoculation were sealed with petroleum jelly. Control were set up using 2 drops of sterile distilled water. After 1 week the leaves were examined.

Sensitivity tests of pathogens isolated from leaves of *T. occidentalis* to calcium carbonate solution: A standard calcium carbonate solution was prepared and diluted in series to obtain 10^{-3}, 10^{-2}, 10^{-1} of 2 M calcium carbonate solution. Circular wells were made on the media with the aid of sterile cork borer. Into each well 0.4 ml of the test calcium carbonate solution was deposited carefully and allowed to permeate the media on which the pathogen had been seeded. The plates were examined for the presence of zones of inhibition around the wells. The extent of inhibition in each case was determined by the diameter of inhibition zones a measured with the aid of a meter rule (Agar well technique)

RESULTS

The plant that received 1 g calcium carbonate died two weeks after germination. The harvested leaves (120 days old) were stored at room temperature, untreated plants and plants that received 0.25 g calcium carbonate, most of the leaves rotted during the period of storage (Plate 1).

Chlorophyll disintegration

Significant differences between treated leaves and untreated leaves were observed (Figure 2, Plate 1). Both varieties did not differ significantly in chlorophyll retention, during the period of storage (Figure 1, Plates 1 and 2). In both varieties, untreated plants had the lowest chlorophyll level while plants treated with 0.5 and 0.75 g calcium carbonate had the highest chlorophyll level during 6 days of storage. The plant that received 0.25 g calcium carbonate did not differ from the control plant in chlorophyll retention during storage. The lowest chlorophyll concentrations were observed on the 5th and 6th days of storage on both varieties.

Rate of respiration (CO_2 production)

Significant differences between treated and untreated leaves were observed in the rate of CO_2 evolution (Figure 4, Plates 1 and 2). Untreated plant had the highest CO_2 production, but plants which received 0.5 and 0.75 g calcium carbonate had the lowest CO_2 production. Both varieties did not vary in the rate of CO_2 production during the period of storage (Figure 3). The highest level of CO_2 was produced on Days 5 and 6 while the least CO_2 production was observed on Days 1 to 4 on both treated and untreated plants (Figures 3 and 4).

Calcium accumulated in the leaves of *T. occidentalis* 120 days after planting

In both varieties, statistical analysis showed significant differences in the concentrations of calcium accumulated in the leaves that received different calcium treatments (Figures 5 and 6). The least concentration was observed in the control while the leaves that received 0.25 g calcium had less calcium concentration, but the leaves that received 0.5 and 0.75 g calcium carbonate, accumulated the highest calcium (Figures 5 and 6). Both varieties that received the same treatments did not differ

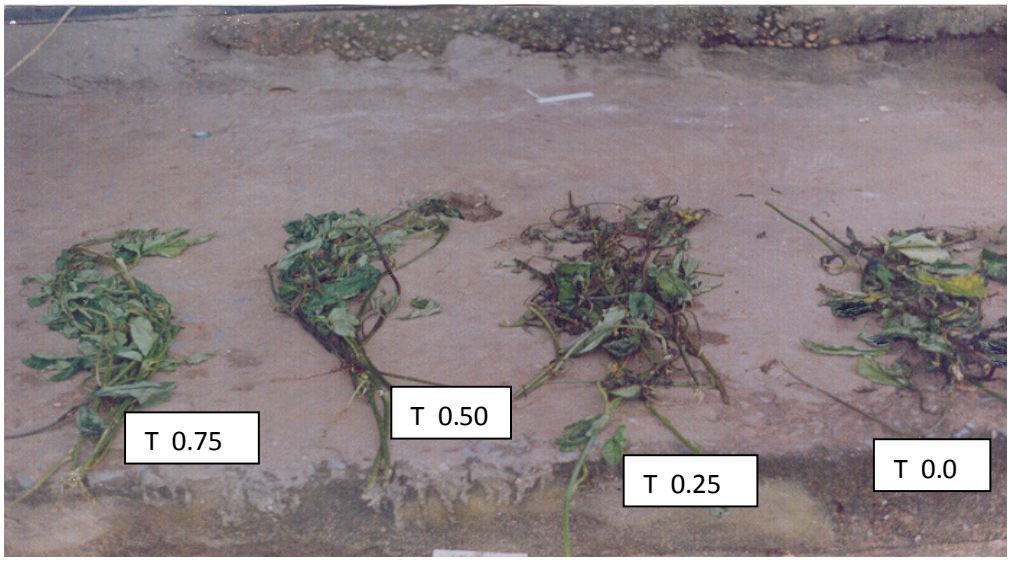

Plate 1. Leaves stored for 6 days.

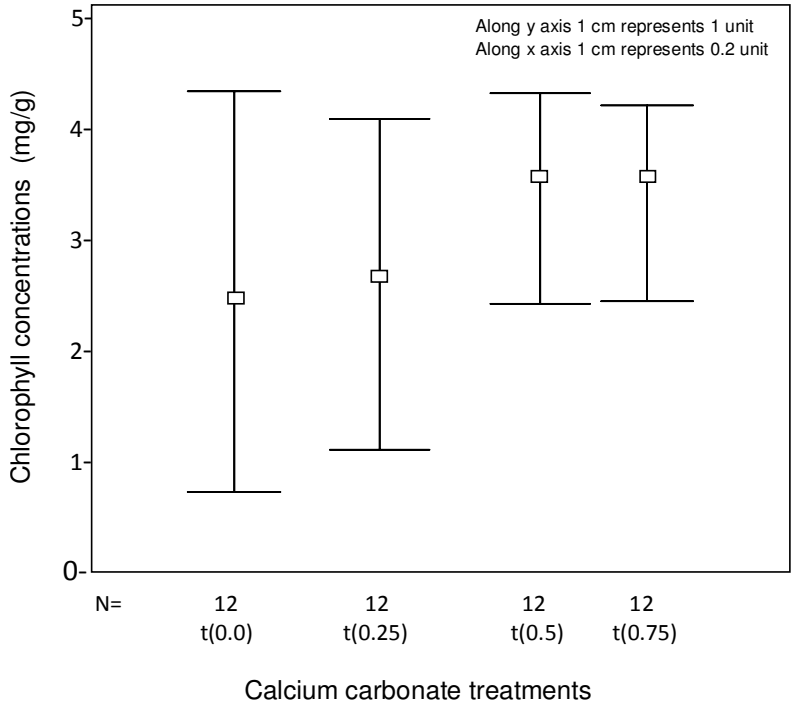

Figure 2. Chlorophyll disintegration of harvested leaves of *T. occidentalis* with different concentrations of calcium carbonate (mg/g).

significantly in the rate of calcium accumulations. The incorporation of calcium carbonate fertilizer in *T. occidentalis* plant increases calcium content of plant tissues and consequently prolonged the shelf life of harvested leaves.

Pathogenicity tests

Erwinia carotovora and *Pseudomonas syringae* were identified as pathogens that cause rots fluted pumpkin leaves. These pathogens, which were originally isolated

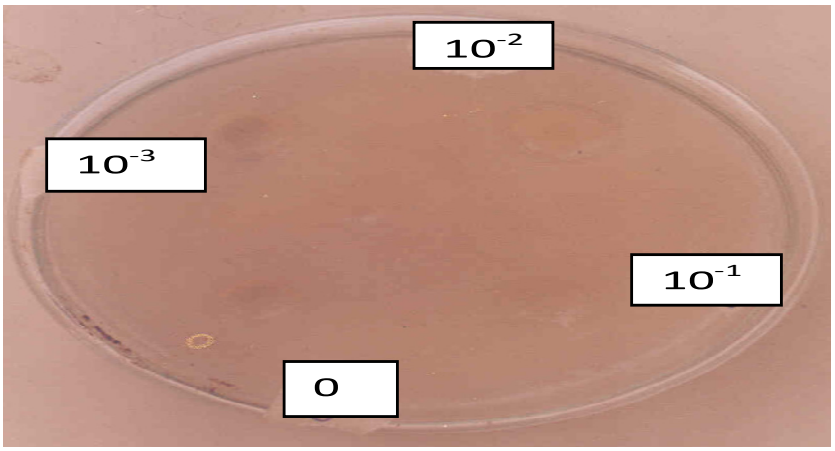

Plate 2. Sensitivity of *Erwinia carotovora* to different concentrations of $CaCO_3$ solution.

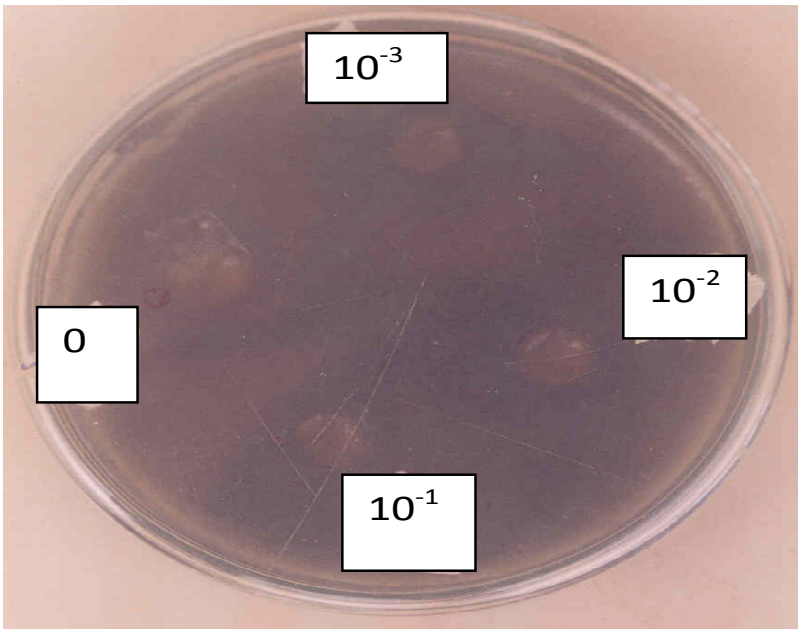

Plate 3. Sensitivity of *Pseudomonas syringae* to different concentrations of $CaCO_3$ solution.

from the rotten leaves caused the same symptoms. The symptoms observed were water soaking, chlorosis, flaccidity and pungent odour. *E. carotovora* caused water soaking and soft rot on the leaves while *P. syringae* caused leaf spots. It was observed that the majority of the rotting leaves were infected from the point of attachment of the leaf to the stem and wounds created on the leaves during harvesting. During the pathogenicity tests *E. carotovora* caused spoilage on the 3rd day while *P. syringae* caused spoilage on the 4th day. *Penicillium spp, mucor spp, Aspergillus niger* did not cause spoilage until the leaves began to decay.

Sensitivity test results

The results showed that $CaCO_3$solution did not inhibit the growth of the isolated pathogens (Plates 2 and 3).

DISCUSSION

Chlorophyll disintegration

Reduction in chlorophyll concentration of harvested leaves observed during storage, particularly after five days is in agreement with Montalbini and Buckanan (1974)

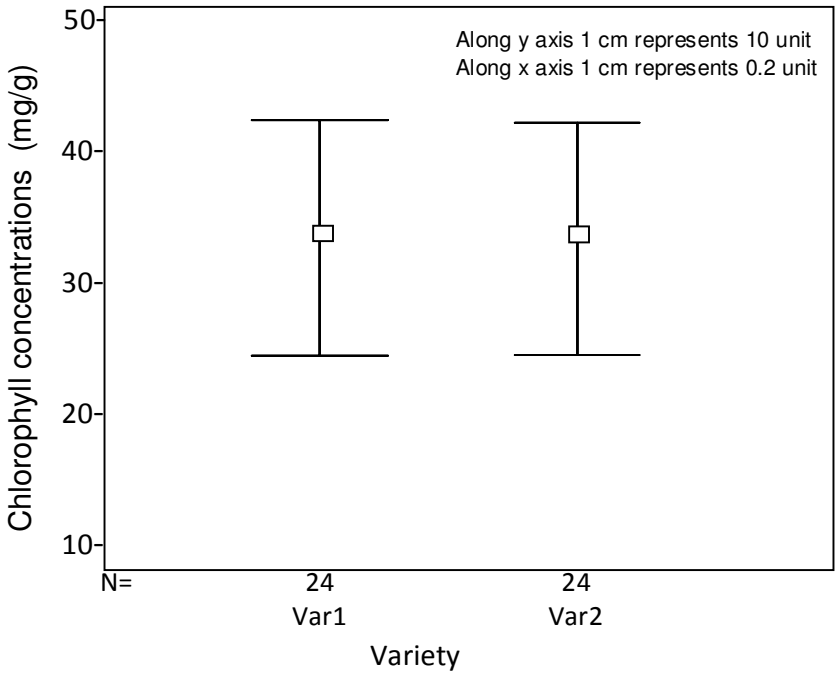

Figure 3. Carbondioxide production of harvested leaves of two varieties of *T. occidentalis.*

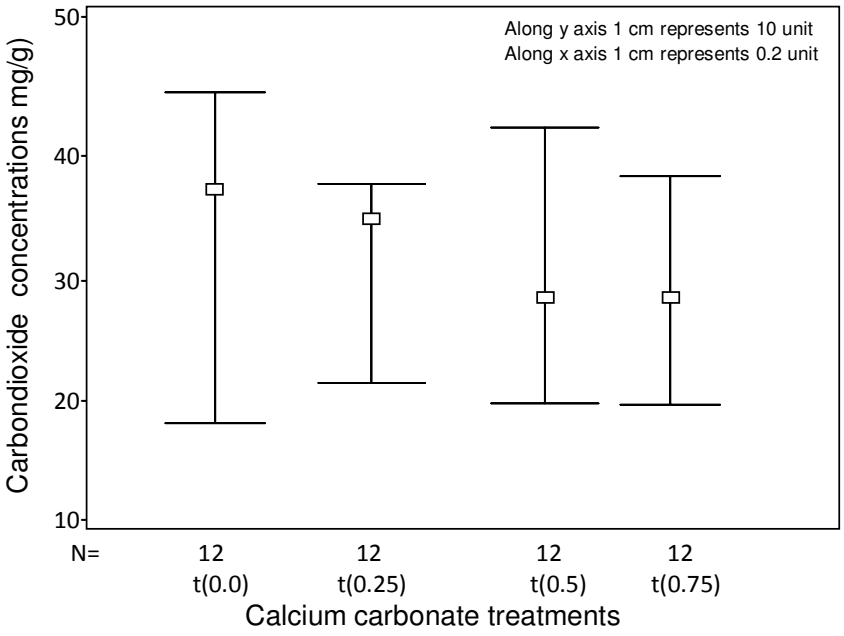

Figure 4. Carbondioxide production by harvested leaves of *T. occidentalis* with different concentrations of calcium carbonate.

who argued that chlorophyll disintegration is caused by deterioration of chloroplast, cell components and by alteration in the pathway of diffusion of CO_2 into and within leaves. This is consistent with the view that low chlorophyll concentration is caused by destruction of photosynthetic machinery, feedback inhibition of photosynthesis following carbohydrate accumulation and pathogen sequestration of inorganic phosphate from the

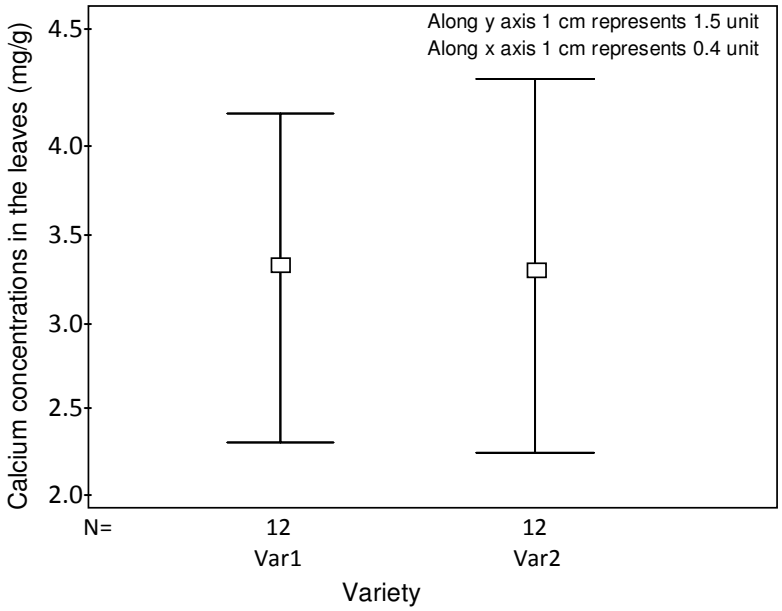

Figure 5. Calcium accumulated in the harvested leaves of two varieties of *T. occidentalis*.

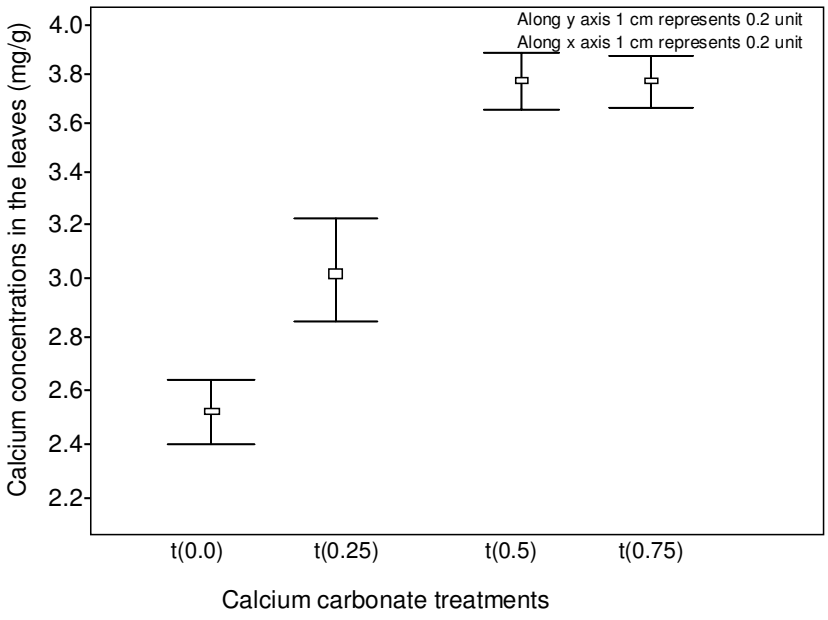

Figure 6. Calcium carbonate treatments.

decayed leaves (Steven and Buchanan, 1983).

Respiration (CO_2 production)

Calcium was also found to affect respiration rate as shown in the decrease in CO_2 evolution in treated leaves compared to control. The observed decrease in CO_2 evolution in calcium treated leaves during 6 days of post harvest storage may be due to the role of calcium in preserving cellular compartments, maintaining membrane integrity and inhibiting the activity of mitochondrial and pectic enzymes (Wills and Rigney, 1979). This is in agreement with the report that avocado pear containing high calcium spoilt later and produced less CO_2 at the climacteric stage than banana with less calcium (Tingwa

and Young, 1974). Increased rate of respiration as shown by increase in CO_2 produced on Days 1 and 2 may have been due to wound respiration associated with enhanced consumption of ATP, NADH and other energy – rich compounds (Daly, 1976). He also argued that the mechanism of the respiration increase in wounded plants may result from uncoupling of oxidative phosphorylation, increase in the rate of synthetic reactions requiring energy and these raise the level of ADP and inorganic phosphorus in tissues which result in an increase in the rate of respiration.

The sharp decrease in CO_2 production on Day 3 may be attributed to swelling (alteration) in chloroplast and mitochondria (sites of ATP synthesis), which affects ATPase activity and change in energy flow and inhibition of photophosphorylation (Uritani and Asahi, 1980). The highest level of CO_2 produced by both varieties on day 5 and 6 after harvest may be due to increase in anaerobic respiration as opposed to mitochondria respiration (Daly, 1976). Similarly (Yoder, 1980) reported that increase in respiration may be due to increased level of enzymes involved in carbohydrate degradation to simple sugars by invading microorganisms. This is consistent with the report that in barley infected with *Erysiphe graminis*, the respiration increase is greater on the centre of the mildew colonies than in the tissues adjacent to a colony and it was suggested that the respiration of the host was stimulated by substances diffusing from the infection site (Daly, 1976). In addition, Yoder (1980) found that extra-cellular hydrolytic enzymes produced by *Pseudomonas solanacearum* not only affect structural integrity of the host cells but increased O_2 consumption of diseased tissues.

Effect of calcium on pathogen growth (sensitivity test)

The result of the experiment showed that calcium did not inhibit the growth of the isolated pathogens. The insensitivity of pathogen to calcium observed in this study was in agreement with the report that calcium seems to have a specific inhibitory effect on polygalacturonase (pg) production without reducing bacterial growth or the production of other extracellular enzymes (Diana et al., 1997). Calcium is reported to have a direct effect on the pathogen life cycle, for example calcium inhibited zoospore release in *Phytopthora parasitica* (Von Broemsen, 1997). In contrast, Mcguire and Kelman (1984) were of the opinion that increased plant calcium content did not lead to a significant decrease in maceration of tobacco leaf tissues by a cellular enzyme preparation. The mechanism of calcium action in reducing pathogen attack involves total blocking of the accumulation of the endopolygalacturonase transcripts (Diana et al., 1997). He also found that 10^{-3} M calcium concentration reduced the severity of pectic lyase produced by *Hyphomyces solani* on potato discs.

Isolated pathogens

The bacteria species isolated in the work were *E. carotovora* and *P. syringae*. Meanwhile, *E. carotovora* was similarly isolated from waterleaf by Custa and Ponighia (2000). Meanwhile, Nemeth and Kovacs (1995) reported that *P. syringase* caused spoilage in soybeans leaves in Hungary. Therefore, the observation of *P. syringae* and *E. carotovora* on *T. occidentals* leaves could be appropriate. *A. niger, Aspergillus flavus, Mucor* spp*, Penicillium* spp observed in this work could be contaminants. However, Hao and Brackell (1993) also reported that *A. niger, Mucor* spp*, Penicillium* spp were found in spoiling pumpkin, pepper and cabbage.

The decay process might have modified the conditions of the leaves to favour the growth of the other microorganisms, which were not initially pathogenic to the crop. The application of calcium carbonate fertilizer during the growth of *T. occidentalis* plant increased the shelf life of the leaves by delaying chlorophyll breakdown. This suggests that the effect of calcium was physiological and perhaps inhibiting stages of disease process for example penetration.

REFERENCES

Association of Official Analytical Chemists AOAC (1994). Washington D. C. pp. 25-27

Cabreva RM, Saltviet ME (1990). Physiological response to chilling temperature of intermittently warmed cucumber fruits. J. Am. Soc. Hort. Sci., 115: 256-261.

Chapman HD, Pratt FF (1961). Methods of analysis for soils, plants and waters. Univ. Agric. Sci., pp. 10-15.

Custa PI, Poniglia I (2000). The occurrence of California. Division bacteria and fungal species in spoilage of pumpkin leaves. J. Plant Pathol., 8: 211-217.

Daly JM (1976). The carbon balance of diseased plants. Changes in respiration, photosynthesis and translocation. In: Heitefuss and Pitt Williams, eds. Physiological plant pathology, Encyclopedia plant physiology New York Spring Verlag, 14: 450-479

Daly M (1987). Mechanism of action of toxin in plant disease. In: Heiterfuss and Pitt Williams, eds. Physiological plant pathology, Encyclopedia plant physiology, New York, 12: 331-394

Diana F, Minna P, Hanna S, Tuula K (1997). Control of virulence gene expression by plant calcium in phytopathogen *Erwinia carotovora.* Mol. Genet., 3: 16-18.

Etukudo I (2003). Ethnobotany, Conventional and traditional uses of plants. Nigeria, Verdict Press, p. 191.

Hao YY, Brachell (1993). Influencing modified atmosphere on growth of vegetable spoilage bacteria in media. J. Food Prot., 52: 225-229

James CS (1995). Analytical chemistry of foods. New York Chaplain and Hall, p. 45.

Mc Guire R, Kelman (1984) A Reduced severity of Erwinia soft rot in potato tubers with increased calcium content. Phytopathology, 74: 250-1256.

Montalbini P, Buchanan BB (1974). Effect of a rust infection on photophosphorylation by isolated chloroplast. Physiol Plant Pathol., 4: 191-196.

Nemeth, Kovacs AN (1998). Occurrence of *Pseudomonas syringae* pathovar on soyabeans in Hungary and their differenciation. Acta Phytopathol. Entomol., 33: 245-254.

Poovaiah BW (1986). Role of Calcium in prolonging storage life of fruits and vegetables. Food Tech., 40: 86-89.

Sofowora A (1996). Medicinal plant and traditional medicine in Africa. Ibadan, Nigeria Spectrum Books, 2: 12

Steven H, Buchanan B (1983). Bioenergetic and metabolic disturbances in diseased plants. In: Callow JA. ed. Biochemical plant pathology. John Wiley and Sons Ltd, pp. 336-338.

Swamura ME, Bruiswa J (1998). Levels of endogenous ethylene, Co_2 and soluble pectins and activities of pectin methylesterase and polygalacturonase in ripening tomato fruits. Plant Cell Physiol., 19: 1061-1069.

Tingwa PO, Young RE (1984). Effect of calcium on ripening of avocado fruits. J. Am. Soc. Hort. Sci., 99: 540-542.

Uritani I, Asahi T (1980). Respiration and related metabolic activity in wounded and infected tissue. In: Stump PK. and Conn EC. Eds. Biochemistry of plants metabolism and respiration. New York, Academic Press, 2: 463-487.

Vitor W, Martin D (1995). Effects of calcium and calmodulin in spore germination and appressorium development in *Collectotrichum trifolii.* Appl. Environ. Microbiol., 10443: 74-79

Von Broemson S, Deacon JW (1997). Calcium interferes with zoospore biology and infectivity of p*hytophthora parasitica* in nutrient irrigation solutions. Phytopathology, 87: 522-523.

Wills RH, Rigney CY (1997). Effect of calcium on activity of mitochondria and pectic enzymes isolated from tomato fruits. J. Food Biochem., 3: 103-110.

Yoder OC (1980). Toxins in Pathogenesis. Ann. Rev. Phytopathol., 18: 103-128.