EFFECT OF CALCIUM FERTILIZATION ON SOFT ROT (*Pectobacterium carotovora*) DISEASE DEVELOPMENT IN IRISH POTATO (*Solanum tuberosum)*

BY

Tafadzwa Tuhwe

A research project submitted in partial fulfillment of the requirements of Bachelor of Science Honours degree in Agronomy.

Department of Agronomy

Faculty of Natural Resources Management and Agriculture

Midlands State University

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DECLARATION

I hereby declare that this submission is my own work towards the BSc (Agronomy) degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the awarded of any other degree of the University, except where due acknowledgement has been made in the text.

TAFADZWA TUHWE Student No. R115528H

Signature ……..…Date……..…….

CERTIFICATION OF THESIS WORK

I, the undersigned, certify that TAFADZWA TUHWE, a candidate for the Bachelor of Science Agronomy Honours Degree has presented this thesis with the title:

EFFECT OF CALCIUM FERTILIZATION ON SOFT ROT (*Pectobacterium carotovora*) DISEASE DEVELOPMENT IN DIFFERENT IRISH POTATO (*Solanum tuberosum)* VARIETIES

Certified by:

Mr Chagonda ...…… …… (SUPERVISOR) Signature Date……..

Ms Mudyiwa ……..(SUPERVISOR) Signature Date ……..

ABSTRACT

Potato production is being affected by many bacterial and fungal diseases hence most farmers incur heavy pre and post-harvest losses. Among the bacterial diseases soft rot is one of the most severe disease. Soft rot is a potato disease caused by bacteria called *Pectobacterium carotovora.* lt is a seed borne disease and it is difficult to control. The research focuses on increasing resistance of potato to soft rot disease. The experiment was conducted at Africa University campus during the 2013/2014 season. A 3*4 plus control factorial experiment in a randomized complete block design with three replications was carried out in the field. Potato varieties and different calcium sources were the two blocking factors used. The treatments were calcium nitrate (19%ca), calcium chloride (36%ca), and calcium sulphate (22% ca). The control had no calcium. Sprouted tubers of Amethyst, Mondial and BP1 were inoculated with bacterial soft rot solution and then the inoculated tubers were planted with different calcium fertilizers. All the calcium fertilizers were applied basally. Soft rot incidences and severity was significantly low ($P<0.05$) in treatments treated with calcium with calcium chloride having the least incidence and severity. Calcium also increased tuber number with calcium chloride having the highest yield since disease incidence and severity was low. Amethyst and BP1 proved to have lower resistance to incidence and severity of soft rot disease compared to Mondial which proved to be resistance. This study concluded that calcium chloride reduce soft rot disease incidence and severity in potato. Farmers facing the problem of soft rot can use calcium chloride as a basal fertilizer as it has proved to be effective in controlling soft rot bacteria in potatoes.

DEDICATION

This project was dedicated to my family, friends who are in the agriculture industry and my prayer partners.

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CHAPTER 1

1.1 Introduction

Potato is one of the most popular food crops grown in Zimbabwe as a substitute staple, third after maize and rice (Chigumirawa , 2002). It is widely grown because of its varied uses which include chips, crisps, vegetable relish/salad, canning and livestock feed (Manzira.,2010).

In Zimbabwe, potatoes are mainly cultivated in specific areas such as Nyanga, Goromonzi, Mutasa, and Domboshava because they are cool environments which are the best for potato production. Although potatoes have the potential to produce high yields per area, they are prone to a wide range of pathogens that drastically reduce yield and quality. (Makarau, A.1999.)

In Zimbabwe potato growers face the challenge of significant post-harvest losses of tubers ranging from 20 to 80% (Chigumirawa, 2002; Manzira, 2010; Ngadze *et al.,* 2010) leading to significant financial losses. Pathogens of major importance are *Pectobacterium and Dickeya species* which cause the soft rot/blackleg disease complex. Soft rot bacteria produce large quantities of pectolytic enzymes such as pectinases, pectatelyases, cellulases and proteases which cause tissue maceration (Pèrombelon., 2002).

De Boer (2004) hypothesised that vegetative propagules such as tubers are often infected by pathogens associated with the parent; hence seed potatoes need to be produced as a highly regulated crop to keep them free of potential pathogens and pests. Since the government economic blueprint ZIMASSET targets on Food Security and Nutrition and Value Addition and

Beneficiation as one of its four major clusters, there is great need to reduce pre and postharvest losses caused by soft rot disease so as to increase food security in the country.

Of all the potato diseases, potato soft rot caused by *Pectobacterium carotovora* is among the most severe bacterial diseases of potato which affect seed and the plant in the field and this has reduced production globally (Ngadze *et al*., 2012). The pathogen *Pectobacterium carotovora* subspecies *atroseptica* is mainly responsible for soft rot in potatoes. De Boer (2004) and Czajkowisk *et al*., (2011) agree that the pathogens, which cause soft rot, are mainly seed/tuberborne and are readily spread via latently infected tubers. Despite the introduction of pathogen-free mini tubers through tissue culture and micro propagation, the problems with soft rot pathogens remains unresolved and even appear to be increasing in the world over.

On that note, there are many control methods used in practice based on the avoidance of contamination of plants, in particular the use of seed testing programs and the application of hygienic procedures during crop production (Czajkowisk *et al*., 2011) In the field, avoiding excess irrigation and nitrogen, providing proper and drainage prevents the spread of the disease. Cultural methods such as adjusting planting time to avoid hot weather during plant emergence and harvesting the crop before soil temperature rises above 28^0C is recommended. (Potato Health Management. 1993, Randal C., Rowe (Ed) APS.

Plant nutrition is a vital component of natural disease resistance (Agrios., 2005). This is because nutrition affects the plant-pathogen interaction as well as other plant-associated microorganisms. Deficiency of plant essential /macro-elements such as calcium will often result in increased susceptibility to diseases. Calcium is not evenly distributed inside plant parts and in the case of potatoes; potato tubers are often associated with low calcium levels (Czajkowisk *et al.*, 2011).

Increasing tissue calcium content lowers poly-galacturonases and pectolytic enzyme activity (Samson *et al*.,2005). The enzymes produced macerate tuber tissue and induce electrolyte leakage and cell death (Huber and Jones, 2012). The ability of Pectobacterium species to macerate the plant tissue depends on the amount of plant cell degrading enzymes secreted (Mahmoud *et al*., 2007). Adequate calcium is a critical aspect of the mineral nutrition of potatoes since it reduces the virulity of these enzymes. Calcium is involved in both the structure and function of all plant cell walls and membranes. Inadequate supplies of calcium cause growth abnormalities like internal brown spot, hollow heart and easy infection by pathogens (Ngadze *et al.*, 2012). Adequate calcium nutrition can also improve skin color in red potatoes. Abundant tissue calcium also increases the tubers' resistance to soft rot during storage and may improve the performance of seed potatoes (Waterer, 2005).

Calcium has role in cell signaling by acting as secondary messenger and maintains the integrity of plasma membrane (Ngadze *et al*., 2012). It plays a regulatory role in the balance of cation anion (Snijder and Van Tuyl, 2003). Calcium sensing proteins are involved in many cellular processes like cytoplasmic streaming, organelles and vesicles transport, microtubules dynamics, cell division, chromosome segregation, cell elongation, tip growth and morphogenesis (Reddy, 2001).

Calcium influences cellular pH and also act as a regulatory ion in the source sink translocation of carbohydrates through its effects in cells and cell walls. Also is needed for cell wall strengthening and provides protection against biotic and a biotic stresses (Hirsch, 2004; Aranda Peres *et al*., 2009). This research therefore seeks to explore the effects of plant nutrition, particularly different calcium sources on the development of soft rot on potato

1.2 Problem statement

Of all the potato diseases, potato soft rot caused by *Pectobacterium carotovora* is among the most severe bacterial disease of potato which affect tuber and plant in the field resulting in reduced production and yield in Zimbabwe.

1.3 Justification

Soft rot disease is caused by *Pectobacterium carotovora pv, Pectobacterium chrysanthemi* and

Pseudomonas flurescens and these are found wherever potatoes are grown (Agrios.,2005). One way of controlling soft rot is by the manipulation of plant mineral nutrition particularly the macro-element like calcium.

According to Abo- Elyousr *et al*., 2008 minerals like calcium reduce the severity of several diseases caused by root and stem pathogens. A large percentage of the calcium in plant cells is located in the cell wall where it is a key component of the middle lamellae, giving strength to the cell wall. It also plays an important role in maintaining membrane permeability, further increasing the strength and integrity of cells (Fleisher *et al.,* 2011). Soft rot *Pectobacterium* infiltrates host cells through the action of pectolytic enzymes that hydrolyzes pectin between individual cells resulting in the separation of cells and loss of cell structure. A high tissue calcium concentration in the periderm therefore increases the resistance to penetration by the pectolytic enzymes and a delay in the rate of tissue maceration (Czajkowski *et al.,* 2010). The stronger cell walls in the cells just below the periderm and further into the medulla prevent further decay and spread of the bacteria.

Potato production in Zimbabwe is not yet common in most regions. The increased demand for potatoes means that the crop needs to be produced as a highly regulated crop so as to meet the increasing demand; hence this research aims at studying the positive effects of different calcium sources in inducing potato resistance to soft rot disease.

1.4 Main Objective

To determine the effect of different calcium sources; calcium sulphate (22% Ca) ,calcium chloride (36% Ca) and calcium nitrate (19% Ca) in inducing plant resistance on the development of soft rot disease on potato varieties (BP1, Amethyst and Mondial)

1.4.1 Specific objectives

- To determine the effect of calcium sulphate, calcium nitrate and calcium chloride on bacterial soft rot incidence on the different potato varieties.
- To determine the effect of calcium sulphate, calcium nitrate and calcium chloride on bacterial soft rot severity on different potato varieties.
- To determine the effect of calcium sulphate, calcium nitrate and calcium chloride on yield of different potato varieties.

1.5 Hypotheses

There is no significant effect of calcium chloride, calcium sulphate and calcium nitrate on the control of disease incidence of soft rot in different potato varieties.

There is no significant effect of calcium chloride, calcium sulphate and calcium nitrate on the control of disease severity of soft rot in different potato varieties.

There is no significant effect of calcium chloride, calcium sulphate and calcium nitrate on the yield of different potato varieties.

CHAPTER 2

LITERATURE REVIEW

2.1 Economic importance of potatoes

Potatoes are rich in starch and is ranked fourth after maize, wheat and rice. The crop belongs to the Solanaceae - or "nightshade"- family of flowering plants, and shares the genus *Solanum* with at least 1,000 other species, including tomato, eggplant and tobacco (Ozgen *et al*., 2002) The potato (Solanum tuberosum) is a tuber bearing herbaceous plant. The potato is the most important non-cereal crop in the world. It is the largest vegetatively propagated crop in the world, producing vegetative harvest organs for consumption (Abo-Elyousr *et al.*, 2010). Though most potatoes varieties which are being produced nowadays have the potential to yield more than 50t/ha, their potential is largely affected by disease caused by bacteria, fungi and to a lesser extend viruses.

2.2 Causative agent of soft rot

A number of bacterial pathogens are capable of causing rots of potato tubers, most importantly during storage of the crop. The soft rot bacteria are assigned to several species in the genera *Pectobacterium* and *Dickeya* (both were formerly in the genus *Erwinia*), and include *P. atrosepticum, P. carotovorum* subsp. *carotovorum* and subsp. *brasiliensis, P. wasabiae,* and *D. dianthicola* (Lojkowska and Kelman,1994; Ngadze et al.,2012). Though the common causative agent of soft rot is *Pectobacterium carotovorap.v* and also *Pseudomonas Fluorescens* also cause soft rots of fleshy fruits and fleshy vegetables (Agrios, 2005). Bacteria soft rot occurs most

commonly on fleshy storage tissues of vegetables and annual ornamentals such as potatoes, carrots, onions and fleshy fruits such as cucumber and tomato.

2.3 Description of the pathogen

The soft rot bacteria are rod shaped, gram negative, non-sporing, facultative anaerobes they are mobile and have peritrichous flagella (Czajkowski et al.,2011). *Pectobacterium carotovora p.v. carotovora*, *E chrysanthemum* and *Pseudomonas flurescens*. Bacteria *P carotovora p.v carotovora* and *P fluorescens* cause the most common and the most destructive soft rots. *Erwinia carotovora p.v atrosptica*, cause blackleg disease in potatoes while *Erwinia chrysanthemum* affects many hosts and causes many of the soft rot of tropical plants while they are still growing in the field (Agrios2005) . Soft rot bacteria can grow and become active over a range of temperatures from 5 to 30^0c but they can be killed with extended exposure to temperatures above 50^0C (Perombelon, 2002). Soft rot bacteria survive in infected fleshy organs in storage and in the field, in debris on roots or other parts of host plants in ponds and streams used for water irrigation, occasionally in the soil and in the pupae of several insects (Samson *et al*., 2005).

2.4 Development of sot rot disease

The disease may first appear in the field on plants grown from previously infected seed pieces (Hartman and Nesmith, 2008). Some tubers, rhizomes and bulbs become infected through wounds or lenticels after they are set or formed in the soil. The inoculation of bacteria into fleshy organs and their further dissemination in storage and in the field are facilitated greatly by insects. (Flego *et al.,* 2011).

Even when the plants or storage organs are resistant to soft rot and can stop its advance by the formation of wounds cork layers, the maggots destroy the wounds cork as fast as it is formed and the soft rot continues to spread. (McGuire and Kelman.,2009).

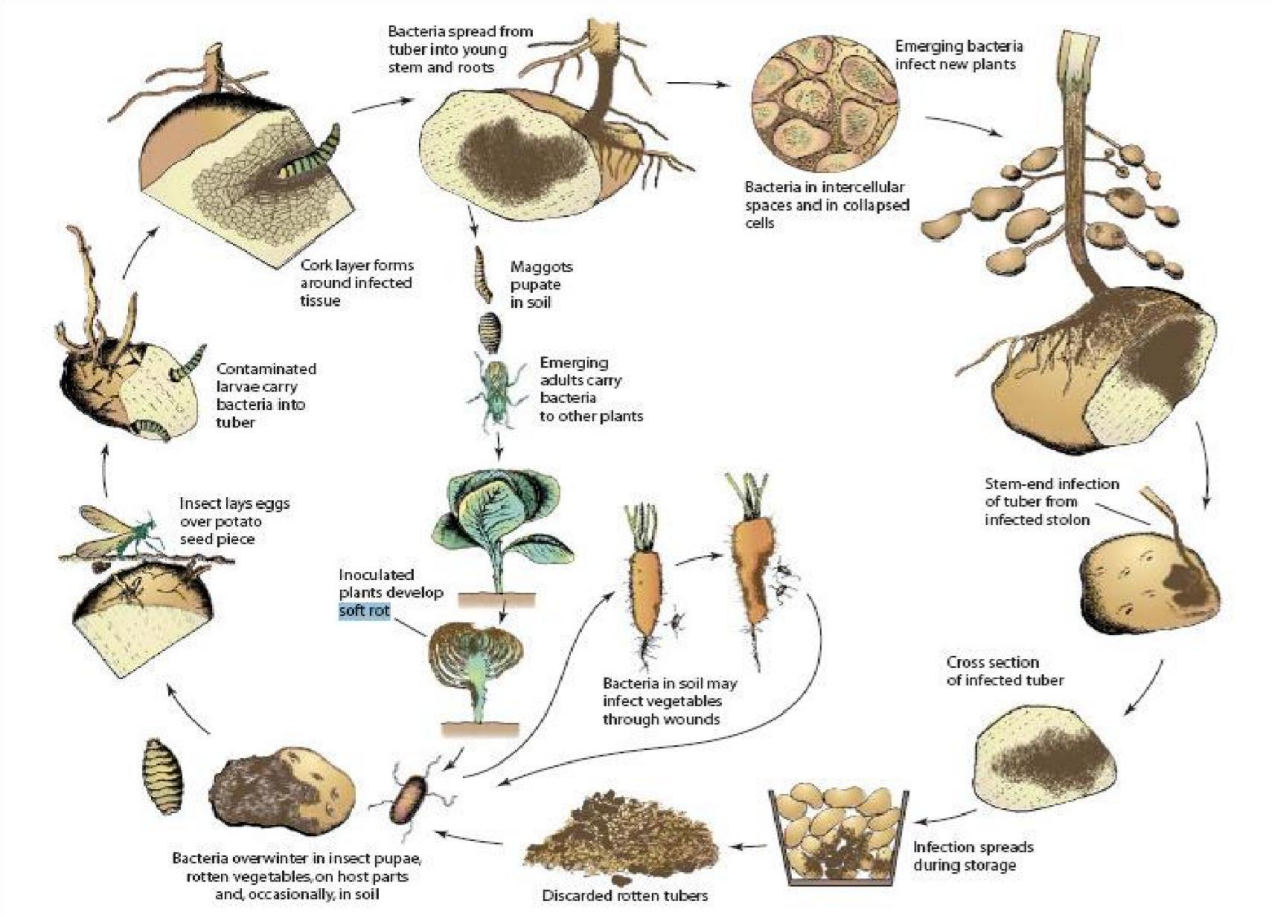
Soft rot bacteria are opportunistic pathogens which produce cell wall degrading enzyme in large amounts, thereby out-competing other pathogens. They are pectinolytic and produce a wide range of enzymes which include proteases, cellulose, pectinases and xylanases (Perombelon, 2002). The pectolytic enzymes breaks down the pectic substances of the middle lamella and bring about maceration of the tissues. Cause of the high osmotic pressure of the macerated tissue, water from the cells diffuses into the intercellular spaces as a result, the cells plasmolyse, collapse and die (Wegner, 2002). The bacteria continue to move and to multiply in the intercellular space, while their enzymes advance ahead of them and prepare the tissues for invasion (Igbal *et al*., 2011). The bacteria do not attack the epidermis of most tissues however, cracks are usually present and the slimy mass extrudes through them into the soil or in storage where it comes into contact with other fleshy organs which are subsequently infected. (Agrios 2005).

2.5 Soft rot disease symptoms

Soft rot symptoms begin as small water soaked lesions, which enlarges rapidly in diameter and depth. The affected area becomes soft and mushy while its surface becomes discolored and somewhat depressed (Toth et al., 2011) .Tissues within the affected region become cream colored and shiny, disintegrating into a mushy moss of disorganized plant cells and bacteria (Czajkowsi et al., 2011). The outer surface layer may remain intact while the entire contents have changed to a turbid liquid, alternatively cracks develop and the slimy moss exudes to the surface

and in air, turns torn gray or dark brown (Agrios, 2005). Whole tuber is converted into a soft, watery, decayed mass within three to five days. When root crops are infected in the field, the lower parts of the stem may also become infected and watery and may turn black and shrived, causing the plants to become stunted, wilt and die (Hellias *et al*., 2009).

2.6 Life cycle of soft rot disease



Agrios, 2005

Fig 2.1 Life cycle of soft rot disease

2.7 Calcium effects on soft rot

Calcium is a key component of cell walls, helping to build a strong structure and ensuring cell stability through its interaction with polygalactic acid (Palta , 2010).Strengthening of the tuber cell wall with calcium can help reduce the severity of *Pectobacterium carotovora* soft rot in storage. Calcium also reduces the level of tuber skin diseases including Black surf and powdery scub leading to better skin finish. McGuire and Kelman (2001) concluded that, increasing the concentration of calcium within potato tubers through calcium fertilization of the growing plants, resulted in a significant increase in tuber resistance to *Pectobacterium carotovora* soft rot.

Calcium in the middle lamella is responsible for promoting gelling in a pectin solution (Conway, 2001) and provide stable reversible inter-molecular linkages between pectin molecules by making the cell wall rigid (Gunter and Palta, 2006). McGuire and Kelman 2010 showed that, at the highest levels of calcium fertilization, tuber surface area decay was reduced by almost half and Abo- Elyousr *et al*., 2010 reported that varieties with the highest amount of rotting tissue. Presumably, calcium bridging of the plasma membrane components reduced electrolyte leakage and maceration by pectolytic enzymes (McGuire and Kelman, 2010) since extracellular calcium is thought to help in maintaining the selective permeability of plasma membrane because of the bridging of calcium ions on phosphate and carboxylate groups of phospholipid head groups at the membrane surface (Gunter and Palta,2008; Geary *et al*.,2010)

Furthermore, adequate calcium has been found to improve cell integrity, thicker skin netting and ensure proper signaling of pathways such as calmodulin (a calcium binding protein that mediates many metabolic activities),thus reducing the incidence and severity of diseases(Rahman and Punja, 2007). Previous study by McGuire and Kelman (2010), supported that calcium localized

in the cell walls may indeed inhibit tissue maceration, as it produces strong structural rigidity by forming cross-links within the pectin polysaccharides matrix and it does so by combining with pectin in the cell walls forming calcium – pectate which resists maceration.

Incidence of tuber soft rot during storage can be reduced by increasing the concentration of calcium in potato tubers thereby increasing the shelf life (Palta, 2010).There is a correlation between tuber cell wall, calcium content and the level of resistance to soft rot (Abo-Elyousr*etal*., 2010). Calcium contributes in maintenance of cell membrane stability and cell wall structure.

2.8 Role of calcium in plants

The use of calcium in crops grown for seed boosts the following crops performance. Calcium improves the growth rate and boosts yield per plant (Perombelon, 2002) Calcium is a key component of cell walls, helping to build a strong structure and ensuring cell stability. Calcium enriched cell walls are more resistant to bacterial of fungal attack (Ozgen *et al*., 2002). It is critical during cell division and expansion therefore essential prior during the rapid growth phase of tubers.

Calcium also helps the plant adopt to stress by influencing the signal chain reaction when stress occurs. It also has a key role in regulating the active transport of potassium for stomata opening (Jones., 2012). It is particularly effective at helping reduce summer heat stress, minimizing wilting and leaf damage. Where this happens, yield improvements of 30% have been recorded (Bussan et al., 2007).

2.9 Control of Soft Rot Disease

There is no chemical control of soft rot disease. Many different methods that farmers use now are cultural control methods and to a lesser extend biological control method.

2.9.1 Biological control method

Experimental biological control of bacterial soft rot of potatoes has been obtained by treating potato seed pieces before planting with antagonistic bacteria or with plant growth promoting rhizobacteria (Czjakowski *et al*., 2011).

2.9.2 Cultural control

Control of bacterial soft rots of vegetables is based almost exclusively on sanitary cultural practices. Wounding should be avoided as much as possible. Potatoes should be dry and humidity and temperature of the warehouse should be kept low (Palta, 2010).

In the field, plants should be planted in well drained areas and at sufficient distances to allow adequate ventilation. Susceptible plants should be rotated with cereals or other non-susceptible crops (Dordas., 2008). Few varieties have any resistance to soft rot and no variety is immune.

Although symptoms of bacterial soft rot do not begin in the field, control of disease does begin in the field (Agrios, 2005). Delaying harvest until the skin is set reduces tuber injuries. This will reduce the entry points for the pathogens (Musarirambi *et al*., 2012). At harvest, watch tuber handling practices and ensure good sanitary procedures to reduce spread of bacteria. Harvesting during wet, muddy conditions generally leads to an increase in bacterial soft rot in storage

(Bussan *et al*., 2007). Proper suberize potatoes to ensure wound healing and reduce the infection sites for the pathogen (Irfan., 2005).

This study focuses on how to control soft rot disease through use of different calcium fertilizer. This type of control method falls under cultural control method whereby resistance of soft rot disease is induced naturally through plant nutrition. Just adding different calcium fertilizers to the potato tuber will strengthen the cell wall thereby increasing resistance to soft rot attack naturally (Ngadze *et al*.,2012)

CHAPTER 3

MATERIALS AND METHODS

3.1 Site Description

The study was carried out at Africa University Farm (AU) in Mutare, Zimbabwe. The area is located 32^0 36'27.9" E and 18^0 53'37,3"S with an altitude of 1104 m above sea level. Average rainfall is above 1000mm. The mean annual temperature ranges of 15-18 ºC, mean minimum temperatures of 10-12 ºC and mean maximum temperature range of 19-23ºC (Makarau, 1999). Orthoferralitic are dominant in the area (Nyamapfeni 1991).

3.2 Planting Materials

Potato cultivars Amethyst, BP1 and Mondial are certified AA potato seeds and were obtained from the Matapiri Seed Sales. Mondial and Amethyst are both late maturing varieties which take to 19-20 weeks to maturity. Mondial is a high yielding variety with a potential of about 40-60 t/ha while Ameythyst can only yield 17-19t/ha. BP1 is an early maturing variety, takes 14- 15 weeks while the yielding potential is 13.5-20 t/ha.

3.3 Experimental design

A 3 x 4 Factorial treatment structure in a Randomised Complete Block Design (RCBD) was used. The experiment consisted of 3 varieties and 4 calcium sources, replicated 3 times. The blocking factor was calcium fertilizers and variety.

Factor A calcium source

1) calcium chloride 2) calcium nitrate 3) calcium sulphate

Factor B Varieties

1) Amethyst 2) Mondial 3) BP1.

The plot size was 15m * 15m

3.4 Treatment Table

Table 3.1 Treatments table

	Calcium sources			
Variety	**Calcium nitrate**	**Calcium sulphate**	**Calcium chloride**	**No calcium**
Amythest	**T1**	**T2**	**T3**	**T4**
Mondial	**T5**	**T6**	**T7**	**T8**
BP1	**T9**	**T10**	**T11**	**T12**

3.5 Application rate for the different calcium fertilizers used.

Table 3.2 Application rates of different calcium fertilizer source.

Treatment	Source of Calcium	Application rate/ kg source/ha	Ca kg/ha
T1	Calcium Nitrate (19% Ca)	250	35.9
T2	Calcium Chloride (36% Ca)	132.19	47.5
T3	Calcium Sulphate (22% Ca)	215.9	40.1
T4	none	0	0

3.6 Isolation of Bacteria

Isolation of bacteria started when tubers and plant samples showing disease symptoms were cleaned, surface-sterilized with 0.5 % sodium hypochlorite solution (for 30 seconds), washed with sterile distilled water, and ground in sterile 0.85 % saline solution using sterile mortar under aseptic conditions. The resulting bacterial suspension was left undisturbed for a few minutes. A loop full of this suspension was then streaked on to plates containing nutrient agar (NA), and incubated at 28^{o}C for 24 hours. Individual colonies (transparent, circular, raised, shiny and creamy white) growing on NA were selected, re-suspended in 0.85 % saline, streaked on NA plates, and then incubated at 28^{o}C for another 24 hours. This was repeated several times in order

to obtain pure cultures. In order to avoid contamination, green pepper fruits were used as an enrichment host for the soft rot Erwinia (*Pectobacterium carotovora*) which were subsequently isolated on NA.

The green pepper fruits were surface-disinfested with 70% alcohol for 30 seconds, followed by treatment with 1% sodium hypochlorite (NaOCl) for 30 seconds and then washed with sterile distilled water. Then, sterile toothpicks were stabbed into soft-rotten tubers. The same toothpicks were then inserted into green pepper (*Capsicum annuum L.)* fruits . The inoculated fruits were maintained in a humid chamber at 28^{o}C for 24-48 hours. Decayed tissue was peeled off with a sterile scalpel and crushed in 0.85% saline. A loop full of bacterial suspension was used to streak the surface of NA plates. Single colonies were harvested and purified. Pure colonies were preserved in 70 % glycerol solution and stored at -20^{0}C. Cultures were also preserved in 0.85 % sterile saline solution and stored at -40^{o}C. When required, each bacterial strain was cultured on LB (Trypton 10gm, yeast extract 5gm, NaCl 10 gm, agar 15 gm, distilled water 1 litre) and incubated at 28°C for 2 days.

3.7 Agronomic Practises

Planting was done on a 30 cm in row and 60 cm interow. Compound S was basally applied at 1500 kgs/ha. The different calcium fertilisers were applied at planting using the different rates in Table 2. AN was applied at a rate of 250 kg/ha after 3 weeks. Irrigation commenced soon after planting and plants were irrigated regularly for the first 4 weeks.

3.8 Data collection

3.8.1 Disease Incidence

To calculate disease incidence (%),

$$\frac{number\ of\ diseased\ plant}{total\ number\ of\ plants} \times 100$$

Plants were randomly picked and 3 plants were selected for sampling from each treatment. All plants it stem rots and tubers showing signs and symptoms of soft rot disease were regarded as diseased plants.

3.8.2 Stem Rot severity

Disease severity was assessed on a scale of 0-3 as reported by Wright et al. (2005) where:

0	no disease symptoms on plant
1	less than 50% of the plant has disease symptoms
2	more than 50% of the plant has disease symptoms
3	plant totally dead

Plants were randomly picked from the plot for sampling and were assigned to the scale accordingly

3.8.3 Marketable yield

Tubers from each treatment were harvested at maturity and they were gathered counted and weighed. All the tubers that had no symptoms of disease were collected as marketable yield. The tonnage per plot or treatment was established.

3.9 Data analysis

Analysis of Variance was done using Gensat 14th Edition statistical package. Discrete data was transformed using the square root prior to analysis. Also separation of means was done using Least Significant Difference (LSD) at 5% level of significance.

CHAPTER 4

RESULTS

4.1 Effects of calcium chloride, calcium sulphate and calcium nitrate on incidence of soft rot disease

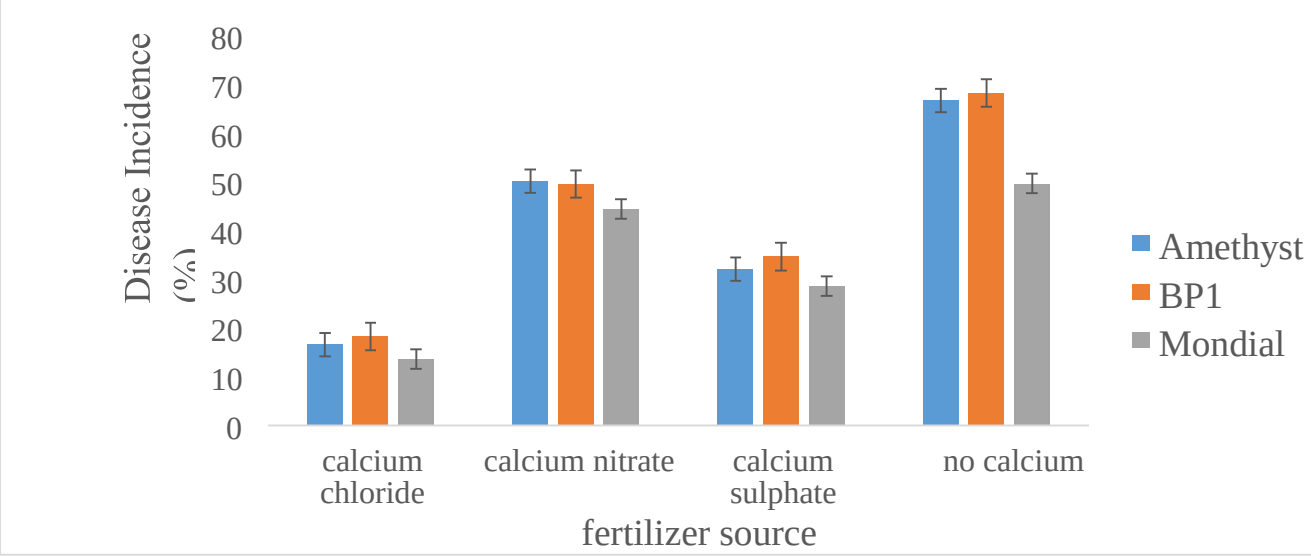


Fig 4.1 Effects of different calcium fertilizers on incidence of soft rot disease

There was an interaction ($P<0.001$) between the three calcium fertilizers and variety on soft rot disease incidence. All the treatments with calcium had lower disease incidence than treatments without calcium with calcium chloride having the least percentage of soft rot disease incidence followed by calcium sulphate and calcium nitrate respectively. The control treatment had the highest percentage of soft rot incidence. From the findings obtained Mondial proved to be more resistant since it had a significantly lower percentage of soft rot disease incidence compared to other Amythest and Mondial respectively.

4.2 Effects of calcium chloride, calcium sulphate and calcium nitrate on severity of soft rot disease

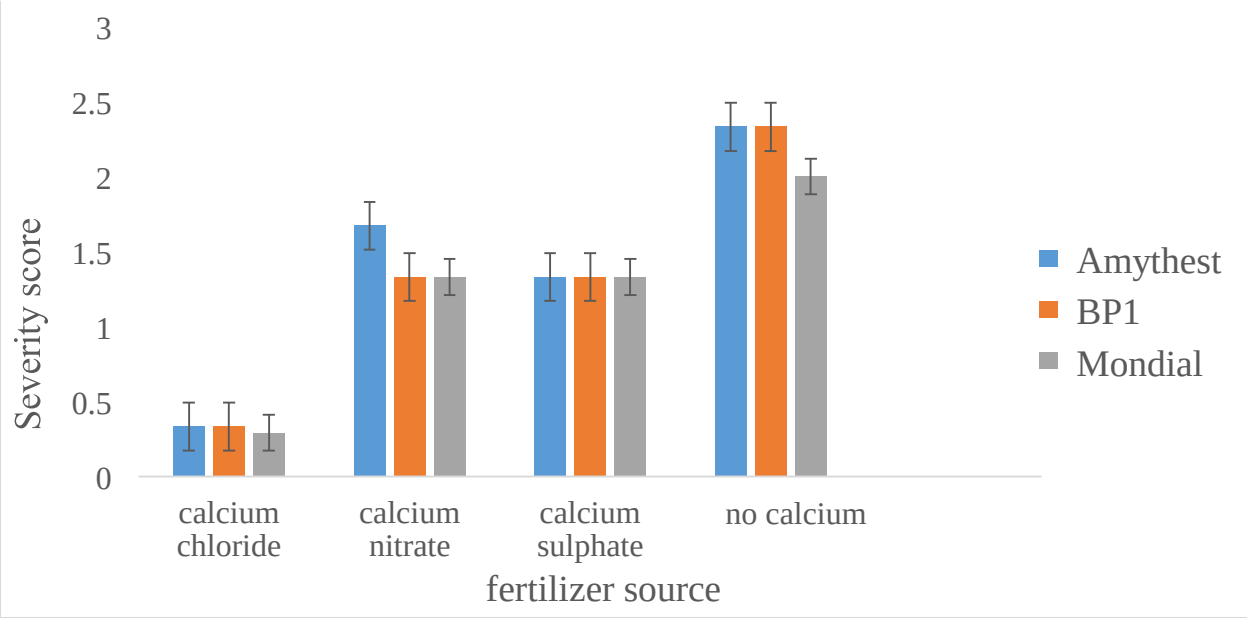


Fig 4.2 Effect of different calcium fertilizers on severity of soft rot

There was an interaction ($P<0.001$) between the three calcium fertilizers and variety on severity of soft rot disease as shown in Fig 4.2. From the results obtained, there was a significant difference between the treatments, with calcium chloride recording the lowest number of plants severely affected by soft rot compared to those with low calcium percentage which are calcium sulphate and calcium nitrate. The treatments with no calcium (control) recorded the higher number of stems that were severely affected by stem rot. Mondial had the lowest number of plants that very severely affected by soft rot disease in all treatments compared to Amethyst and BP1. Though there was no significant difference among the three varieties where calcium sulphate was applied.

4.3 Effects of calcium chloride, calcium sulphate and calcium nitrate on marketable yield

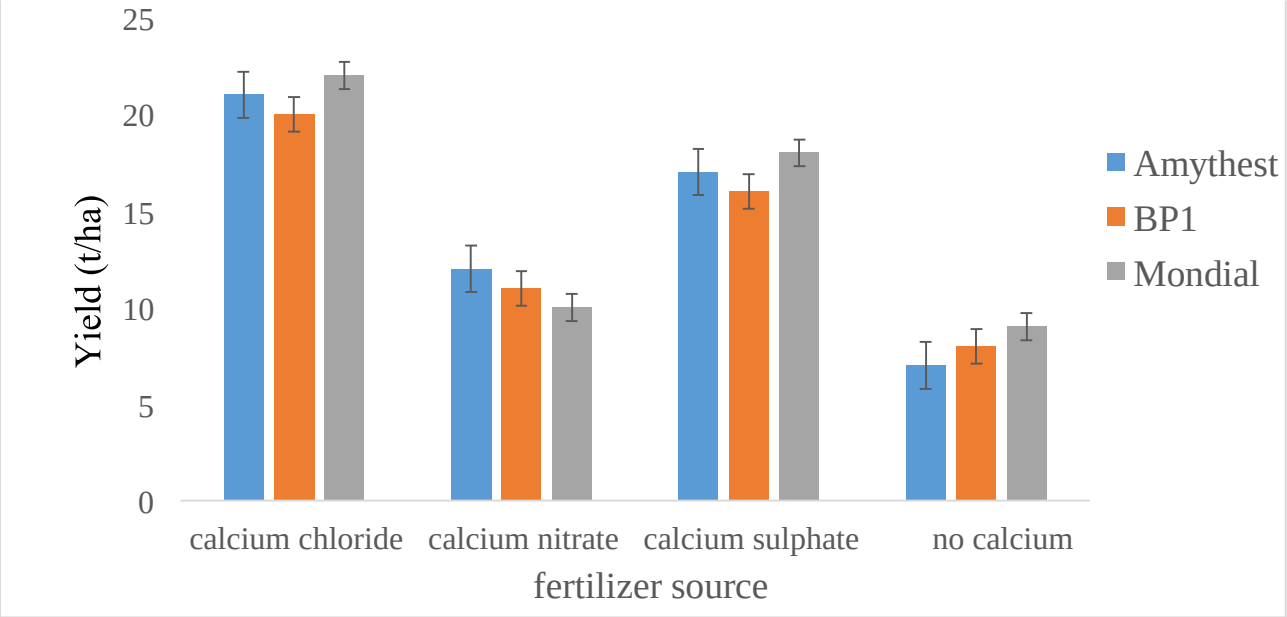


Fig 4.3 Effects of different calcium sources on marketable yield

There was an interaction ($P<0.005$) between the three calcium fertilizers and variety on marketable yield. Treatments with calcium had higher yield compared to treatments without calcium. There was significant difference between treatments, with calcium chloride recording the highest yield, followed by calcium sulphate and calcium nitrate. The treatment with no calcium (control) alone recorded the lowest yield. Mondial had the highest yield than the other varieties, BP1 and Amythest.

CHAPTER 5

DISCUSSION

5.1 Effects of calcium chloride, calcium sulphate and calcium nitrate on incidence of soft rot disease.

Results have shown that tubers with high calcium had a lower percentage of disease incidence than treatments without calcium. Since tubers with high calcium have an improved structural integrity of both the plasma-lamella and cell wall materials as compared to tubers with low calcium content (Dordas, 2008). Calcium in tubers is thought to inhibit the multiplication and spread of the bacterial pathogen throughout the tissue (Ngadze *et al.*, 2012). This theory supports the final results obtained from the experiment where all treatments treated with calcium had a relatively lower percentage of soft rot incidence than treatment without. Calcium chloride had the lowest percentage of soft rot incidence while calcium sulphate and nitrate followed respectively. This was attributed by the high calcium content in calcium chloride (36%).

Also according to Palta (2010) the activity of polygalacturonase (PG) a cell-wall-degrading enzyme produced by bacterial and fungal pathogens can be inhibited by calcium and hence cannot breakdown the pectin in the plant tissue cell walls. Mondial had the least percentage of disease incidence than Amythest and BP1. This may be due to their difference in genetic make-up. Also Amythest and BP1 were released in 1977 and 1972 respectively and there are the most widely grown cultivars in Zimbabwe so with time these two varieties are losing their genetic viability to resist pathogen infection.

5.2 Effects of calcium chloride, calcium sulphate and calcium nitrate on severity of soft rot disease.

The results showed that treatments with calcium had the least severity score of soft rot disease than treatments without calcium. Calcium chloride had the least number of affected plants followed by calcium sulphate and calcium nitrate respectively. Results also suggested that calcium is an essential mineral in many physiological processes such as plant defense. Presumably, calcium bridging of the plasma membrane components reduced electrolyte leakage and maceration by pectolytic enzyme (McGuire and Kelman, 2005) since extracellular calcium is thought to help in maintaining the selective permeability of plasma membranes because of the bridging of calcium ions on phosphate and carboxylate groups of phospholipid head groups at the membrane surface (Gunter and Palta, 2008; Geary et al., 2010). This is thought to inhibit the multiplication and spread of the bacterial pathogen throughout the tissue thus making the plant healthier.

Other studies showed that calcium in the middle lamella is responsible for promoting gelling in a pectin solution (Abo- Elyousr et al. 2010) and provide stable reversible intermolecular linkage between pectin molecules by making the cell wall rigid. This may also be the reason why treatments with calcium chloride proved to be resistant to soft rot disease severity than the other treatments with calcium sulphate and calcium nitrate. The higher the calcium percentage in the tuber, the lower the severity of the disease.

Mondial performed better than Amethyst and BP1. This may be attributed to the fact that Mondial is a more recent variety than Amethyst and BP1 which were released in the early 1970's, so their genetic viability to resist disease is becoming weaker with time.

5.3 Effects of calcium chloride, calcium sulphate and calcium nitrate on marketable yield.

From the results obtained calcium chloride had a higher yield than other calcium sulphate and calcium nitrate with control having the least number of tonnes. This may be due to the fact that calcium chloride had a higher calcium percentage (36%) compared to calcium sulphate (22%) and nitrate (19%) respectively, hence performing better in resisting soft rot disease which in turn increased yield It is known that calcium nitrate helps increase yield in potatoes, but it was not possible in this research because most of the plants were severely damaged since disease incidence and severity was high in treatments where calcium nitrate was applied.. Also the application rate of calcium chloride was the highest (132.19 kg/ha) followed by calcium nitrate (250kg/ha) and calcium sulphate (216kg/ha), this has attributed to the effectiveness of calcium chloride. Since calcium plays an important role in the resistance of plants against bacterial pathogens (Perombelon, 2002) it means a high calcium content in crops is often positively related to increased resistance against bacterial diseases, including potato blackleg and soft rot disease.

Also calcium sensing proteins are involved in many cellular processes like cytoplasmic streaming, organelles and vesicles transport, microtubules dynamics, cell division, chromosome segregation, cell elongation, tip growth and morphogenesis (Reddy, 2001) thus treatments with calcium chloride performing way better than others.

Mondial had the highest yield since it had a high yielding potential of 40-60 t/ha. Amethyst and

BP1 have a yielding potential of up to 20 t/ha under good management, that’s why they yielded lower than Mondial.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Calcium fertilizer proved to be very effective in reducing severity and incidence of soft rot disease hence increasing yield. Among the calcium fertilizers calcium chloride was the most effective compared to calcium sulphate and nitrate.

Mondial yielded more than Amethyst and BP1 because of its high yielding potential. Also Mondial proved to be more resistant to soft rot disease severity and incidence than Amethyst and BP1.

6.2 Recommendation

Since the use of calcium fertilizers is relatively easier and cheaper compared to biological control method, farmers can consider applying calcium chloride at planting to reduce incidence and severity of soft rot disease. Farmers are advised to grow newly introduced varieties that are on the market since they have proved to be better in resisting soft rot disease than the old varieties.

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APPENDICES

Appendix 1

Variate: **ANOVA FOR SOFT SEVERITY**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	2.2039	1.1019	1.54	
block.*Units* stratum					
fertilizer_type	3	10433.3978	3477.7993	4864.23	<.001
variety	2	515.6239	257.8119	360.59	<.001
fertilizer_type.variety	6	268.0406	44.6734	62.48	<.001
Residual	22	15.7294	0.7150		
Total	35	11234.9956			

Appendix 2

Variate: **ANOVA FOR SOFT ROT INCIDENCE**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	7.9350	3.9675	6.21	
block.*Units* stratum					
fertilizer_type	3	5122.1964	1707.3988	2673.19	<.001
variety	2	59.5117	29.7558	46.59	<.001
fertilizer_type.variety	6	27.1728	4.5288	7.09	<.001
Residual	22	14.0517	0.6387		
Total	35	5230.8675			

Appendix 3

Variate: **ANOVA FOR YIELD**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	0.00722	0.00361	0.28	
block.*Units* stratum					
fertilizer_type	3	60.67111	20.22370	1555.07	<.001
variety	2	4.21556	2.10778	162.07	<.001
fertilizer_type.variety	6	1.06889	0.17815	13.70	<.001
Residual	22	0.28611	0.01301		
Total	35	66.24889			