EVALUATION OF BACTERIAL BLACK ROT DISEASE (*XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*) TOLERANCE IN CABBAGE VARIETIES (*BRASSICA OLERACEA* VAR. *CAPITATA*)

A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE HONOURS IN AGRICULTURE AND NATURAL RESOURCES

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DECLARATION

I…… do hereby declare that this work done is my original work undertaken at Africa University, Mutare, Zimbabwe in partial fulfillment of the requirements for the Bachelor of Science in Agriculture and Natural resources (honours degree) and has not been submitted nor is being currently submitted to any university for the award of any other degree.

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Student's signature

Date …/……/……..

Approved for submission

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Signature

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ABSTRACT

Bacterial black rot disease of Brassicas caused by bacterium *Xanthomonas campestris* pv. *campestris* has increasingly become the major constraint to sustained production of cabbage worldwide. Black rot problem is aggravated by continuous cropping due to cultivation of susceptible varieties and lack of suitable disease management strategies. Other challenges faced by smallholder farmers in the management of black rot include problems in seed quality. Field studies were conducted at Africa University farm situated in Mutare to evaluate black rot tolerance in cabbage varieties: Potomac, Star 3301, and Star 3311and Star 3316 from mid February to early May 2012. Cabbage seedlings were raised in the greenhouse and transplanted into the field plots on the 18th of February 2012. The experiment set up was laid out in a randomized block design with three replications and four varieties in each block (RCBD). Compound D was applied as a basal fertilizer at the rate of 30 grams per plant station and Carbaryl was applied on day of transplanting to control cutworms (*Agrotis* spp.). Data on disease incidence and severity was collected as from the 8th week after transplanting for four consecutive weeks. A Bacterial Black Rot Disease Score on Brassicas was used for data recording and calculating disease incidence and disease severity. Raw data on disease severity was used to prepare graphs and to calculate area under disease progress curve (AUDPC). Results showed that the variety Potomac was highly susceptible with AUDPC of 54 cm^2 for the 3 replications as compared to the other 3 varieties. Potomac had higher disease incidence for week 1 it was 8.3%, week 2 25%, week 3 37.5% and rose to 58.5% in the fourth week. Data on disease severity was subjected to ANOVA in Minitab version 15 after transforming the data using the √(x+1) transformation. All varieties showed no significant difference in disease severity levels at P¿0.05 but in terms of disease incidence there were notable differences. This work established that the variety Star 3311 has high tolerance to bacterial black rot disease while variety Potomac was more susceptible. One would recommend farmers to choose among the Star varieties especially Star 3311 which is highly tolerant to black rot disease.

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DEDICATION

I dedicate this project to my Mother (Sheila Muendo), my fiancée Mazvita Chikwengo and my sister Rudo who greatly account for my strength and education, for their love, devotion and support. To my sister Rudo Gwanzura who was by my side throughout difficult periods of my studies.

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CHAPTER ONE

1.0 INTRODUCTION

Cabbage production is practiced by both smallholder and large scale commercial farmers and bacterial black rot disease is the most important disease constraint and one of the most destructive diseases of cabbage globally. Black rot is a major disease constraint of cabbage production for smallholder farmers in Africa where substantial crop losses are experienced especially during the warm and wet seasons (Onsando, 1992). Black rot is caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Xcc) and is a significant disease of cabbage and other crucifer crops worldwide. The disease was first described in New York State on turnips in 1893, and has been a common problem for growers since then. The pathogen thrives in warm (26.7 to 30.6 ^{o} C), moist wet weather, spreading from plant to plant by splashing water, windblown water droplets, and by workers or animals moving from infected fields to healthy fields. The bacterium can spread rapidly during transplant production in greenhouses or seed beds, and spreads long before any symptoms are observed. The pathogen can also survive in cruciferous weeds, such as yellow rocket, Shepherd's purse, and wild mustard, as well as in soil and crop debris in the field (Dzhalilov, 1995,)

1.1 Black rot symptoms

The bacterium is introduced to fields on infected seeds or transplants. Seeds are considered to be the most important source of inoculum of Xcc on cabbages (Russell, 1898). Once present, it may survive on decomposing crop residue for up to two years, or indefinitely on weeds in the crucifer family.

Black rot can be recognized by large, V-shaped, yellow-to-brown areas in the leaves, starting at the leaf edge. Plants can be infected during any growth stage and the symptoms resemble nutritional deficiencies (McGrath, 1994). The heads of the infected plants remains small and its

quality is reduced making it unfit for marketing. Bactericides have been used to control the disease but they tend to reduce the quality of the outer leaves and hence less marketability. An integrated approach is needed to manage black rot successfully and this includes use of tolerant varieties, sanitation in fields, use of disease-free certified seeds and weed-insect control to reduce field infection. Use of black rot tolerant varieties is the best method to control the disease. Cabbage varieties with partial resistance (tolerance) to black rot should be used as a control measure and these include 'Atlantis', 'Blue Dynasty', 'Bronco', 'Cecile', and 'Ramada' Japanese cabbage cultivar, Early Fuji, "Riana F1 Hybrid"(McGrath, 1994)

The research sought to evaluate the level of tolerance to the disease in four cabbage varieties namely Star 3301, Star 3311, Star 3316 and Potomac. The trial was conducted in a field experiment where natural bacterial infection occurred.

1.2 Statement of the problem

Among the major drawbacks of sound cabbage production bacterial black rot disease is the most important constraint and one of the most destructive diseases in cabbage production globally. Huge losses are experienced by smallholder farmers in Africa where the disease occurs (Onsando, 1992) due to unreliable "clean seed' and high susceptibility of popular local cultivars to the disease. This disease remains the greatest impediment to cabbage cultivation and production to farmers worldwide (Williams, 1980). Bacterial Black rot is endemic and the cause of much damage in cabbage production (Onsando, 1992). Varieties that are completely resistant haven't been found but there are some varieties that can be tolerant to the disease.

1.3 Justification of study

Numerous measures have been suggested as a solution to reduce infection in the fields and these include use of disease-free seed, use of seeds that are firstly hot water treated, fumigation of the seedbeds, sites that are well drained and sunny to reduce length of time the seedlings are wet from dew or rain (Russell, 1898). These measures are effective to a limited extent and huge losses continue to be experienced worldwide in cabbage production, hence the measure of using

tolerant varieties could be the best solution. The research seeks to assess and evaluate the level of bacterial black rot tolerance in four different cultivars of cabbage. Some varieties like Potomac and Star 3301 and Star 3311 are said to be tolerant to the disease. A bacterial black rot resistant variety would be a cheaper and long term solution towards bacterial black rot disease control to small holder farmers and commercial farmers worldwide. Evaluating bacterial black rot tolerance in the four cabbage varieties will help assure a long term alternative and more natural solution towards curbing the problems posed by bacterial black rot on cabbages. It will in turn help reduce chemical use on the cabbages which reduce its marketability as the outer leaves are affected. Hence the project will assist in bringing out the most tolerant variety to the disease among the four selected varieties. The use of bactericides is expensive to the smallholder farmers and they are not environment friendly and they reduce quality of cabbage outer leaves posing danger to human health during consumption (Massomo, 2002). Host plant resistance remains the most feasible strategy for managing black rot. Improved levels of resistance could give added yield benefit by reducing crop losses in the rainy seasons.

1.4 Objective of the study

- To identify a variety that is most tolerant to the bacterial black rot disease among the four varieties which are Star 3311, Star 3317, Potomac and Star 3301

Specific objective:

- To assess the level of tolerance in the four varieties at all stages of growth

1.5 Hypothesis

The four cabbage varieties show significant levels of tolerance to Bacterial black rot disease

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of cabbage production

Cabbage in Tanzania is produced by smallholder farmers in contrast to countries such as the Republic of South Africa and Zimbabwe where cabbage and other Brassicas are mostly produced by the large-scale commercial farming sector (Sibanda et al., 2000). The common cabbage is a very familiar garden plant and parts of the plant are widely used as a vegetable all over the world. Cabbage is a popular cultivar of the species *Brassica oleracea Linne* (Capitata Group) of the Family Brassicaceae (or Cruciferous) and is a leafy green vegetable. It is a herbaceous, biennial, dicotyledonous flowering plant distinguished by a short stem upon which is crowded a mass of leaves, usually green but in some varieties red or purplish, which while immature form a characteristic compact, globular cluster (cabbage head). Greeks and Romans praised this vegetable for its medicinal properties, declaring that "It is the cabbage that surpasses all other vegetables." Cabbage was developed by ongoing selective breeding for suppression of the internodes' length. The English name derives from the Normanno-Picard *caboche* (head), leaves. Cabbage is used in a variety of dishes for its naturally spicy flavor. The so-called "cabbage head" is widely consumed raw, cooked, or preserved in a great variety of dishes. It is the principal ingredient in coleslaw and Sauerkraut. Cabbage is an excellent source of vitamin C. It also contains significant amounts of glutamine, an amino acid that has anti-inflammatory properties. Cabbage can also be included in dieting programs, as it is a low calorie food (McKay and Philips 1990)

2.2 Constraints to cabbage production

Weeds and diseases are a constraint to cabbage (*Brassica oleracea* L.) in most parts of Africa where cabbage is grown. Moreover, the highly competitive weed species, *Ambrosia artemisiifolia* L., increases the risk of white mold caused by *Sclerotinia sclerotiorum*. A major

constraint of cabbage production in Kenya and Tanzania are insect pests, especially the Diamondback Moth (DBM), which is resistant to almost all commonly used pesticides. Among the many destructive diseases affecting the cabbage and often other members of the cabbage family are:

- blackleg or black stem, caused by certain fungi (such as *Phoma lingam*); lesions in the stem near the soil surface become sunken and dark, and may girdle the stem
- black ring or black ring spot, caused by a virus; necrotic, dark and often sunken rings on the leaf surface
- Cabbage yellows or cabbage wilt, caused by a fungus (*Fusarium oxysporum* or *Fusarium conglutinans*); yellowing and dwarfing,
- black rot, caused by a bacterium (*Xanthomonas campestris*), of economic importance to smallholder farmers

2.3 Bacterial black rot disease of brassicas

Bacterial black rot is caused by the bacterium *Xanthomonas campestris* which belongs to the genus that causes diseases on at least 124 monocotyledonous and 268 dicotyledonous plant species (Dzhalilov, 1995). This disease was first recorded by botanist and entomologist Harrison Garman in Lexington, Kentucky, USA in 1889. Cabbage in the vicinity of Lexington in 1889 was badly affected with bacterial black rot. The bacterial pathogen was described from rutabaga in Iowa in 1895 by Pammel (Pammel, 1895). At about this time it became destructive in numerous cabbage-growing areas in the United States and was studied intensively in Wisconsin by Russel (Russel, 1898) and in this and other States by Smith (Smith, 1897). The pathogen was one of the first of the bacteria shown to be seed-borne; this was brought out by Harding *et al* in 1904 in studies, of the cabbage seed plants on Long Island. Owing undoubtedly to seed transmission of the pathogen, the disease has been reported to occur, often destructively, in many parts of the world. It occurs on many cultivated and wild crucifers. In developing countries such as those in South and Eastern Africa, black rot remains the greatest impediment to cabbage cultivation due to unreliable “clean seed’, multiple cropping annually and high susceptibility of popular local varieties to the disease. Although the disease is usually of minor importance, when conditions are suitable it may become serious and many growers sustain severe economic loss. In

warm and wet conditions black rot losses may exceed 50% due to the rapid spread of the disease. The disease is usually most prevalent in low lying areas and where plants remain wet for long periods. Conditions favoring plant-to-plant spread of the bacterium has led to a total loss of crucifer crops. Bacterial black rot disease is a major constraint to cabbage production in Tanzania (Day *et al*., 1992; Mgonja and Swai, 2000; Massomo, 2002). Farmers in northern Tanzania experienced yield losses of up to 100%, during *El-Niño* rains in 1998 (Massomo, 2002). On-farm surveys were carried out in northern Tanzania to gain an insight on cabbage production practices in relation to the prevalence of black rot. The studies revealed a rapid increase in cabbage spread occurrence of black rot. The black rot problem seemed to be aggravated by continuous cropping due to shortage of land, cultivation of susceptible varieties and lack of suitable disease management strategies. Other challenges faced by farmers in the management of black rot included problems in seed quality, choice of cabbage varieties. However, control of the disease in the prevailing farming systems under humid tropical conditions of Tanzania has usually been difficult. For instance, all popular cultivars of cabbage grown in Tanzania are highly susceptible to black rot (Massomo *et al*., 2004). Black rot is also the main disease constraint for brassicas grown by smallholder farmers in Kenya, Malawi, Mozambique, South Africa, Uganda, Zambia and Zimbabwe (Onsando, 1992; Mguni, 1996; Anonymous, 2000). Black rot is widespread in Zimbabwe where it is considered the most important disease of Brassicas (Mguni, 1996). The pathogen was found in crucifer crops from the five agro-ecological regions of the country. Disease incidence was higher during 1994 (10-80%) than during 1995(10-50%), (Mguni, 1996).

2.4 Disease Cycle and spread

The causal bacterium overwinters on and in seed and crop debris left in the field. The organism survives especially well in cabbage and Brussels sprout refuse, in plants stored for seed production, and in numerous weeds including black mustard, field mustard, charlock, shepherd's purse, Virginia pepper weed, and cress (Dzhalilov, 1995). The bacteria are spread by splashing or flowing water, blowing of detached leaves or dust particles, shipping and handling of infected plants, and insects. The bacteria are seed borne and thus are disseminated worldwide. As few as three infected seeds in 10,000 (0.03%) can cause black rot epidemics in a field (McGrath, 1994.)

In the spring, when seedlings emerge, bacteria pass from the cotyledons into young leaves directly or through the stomata. The bacteria move intercellularly until they reach the xylem tissue and from there spread throughout the plant. The pathogen is spread from plant to plant by splashing rain, or in films of water moved by people, equipment, insects, and other animals. The bacteria enter the plant through hydathodes along the leaf margin. The result is initially a small, wilted, V-shaped infected area that extends inward from the leaf edge toward the midrib. The pathogen can also enter the plant through insect-feeding injuries, hail, or other mechanical wounds or other mechanical water pores, and in very susceptible crops, such as cauliflower, directly through the stomates (McGrath, 1994.)

The optimum temperature for growth of the organism is from (25° to 30°C), the minimum is (5°C), and the maximum is (35°C). Free moisture in the form of dew, fog, or rain is required for infection and disease development. Under the optimum conditions, symptoms may appear on plants 7 to 14 days after infection. At lower temperatures, symptoms develop more slowly. It does not usually spread in dry weather and is inactive at temperatures below 10 degrees Celsius The bacteria can survive in the soil for a year and may spread in surface water or through irrigation. Seed infection usually varies from 0.25 to 80% (Shrestha 1977), Schaad and Dianese, 1981).

2.5 Symptoms and biology

Symptoms of black rot generally begin with yellowing at the leaf margin, which expands into the characteristic "V" shaped chlorotic to lesions at the margin of leaves and blackened vascular tissues. As the disease progresses, parenchyma cells surrounding vessels in the main stem also turn black and the plant becomes wilted, stunted and finally rots (Williams, 1980; Alvarez, 2000). Diseased leaves and heads have a poor market value, and are unsuitable for storage as they quickly rot. Blackening of the vascular tissue is typical in severe infections. If infection occurs in young seedlings, the disease is usually much more severe as main stems become infected. These plants remain stunted and the veins in the stems are black. Heads from these plants deteriorate rapidly after harvest. Diseased areas enlarge and progress toward the base of the leaf, turn yellow to brown, and dry out. The veins of infected leaves, stems, and roots turn black as the pathogen multiplies. The infected lower leaves of cabbage are usually stunted, turn

yellow to brown, wilt, and drop prematurely. Occasionally, diseased plants have a long bare stalk topped with a small tuft of leaves. In extreme cases, heading may be prevented (Miller, Sahin, and Rowe. 1996.)

2.6 Control of bacterial black rot disease

Sherf and MacNab (1986) reported that the disease could be controlled by growing transplants from seed treated with hot water and through a combination of sodium hypochlorite and "Thiram". Belder and Bartha (1983) found that a copper-based spray applied every 7-10 days effectively controlled black rot once symptoms appeared. Bacteria can persist in plant debris for more than one year so soils used should not have had crucifers grown in them for two years. Crops should be grown in soils with good drainage and good sanitation practices such as removing infected plants, ploughing debris into soil after harvest and the use of clean equipment should be employed (Sherf and MacNab, 1986; Snowdon, 1991). Chinese cabbage varieties such as WR Green 60, Spectrum and Green Rocket have been found to be tolerant of bacterial rots (McKay and Phillips, 1990).

Recently, good control of black rot was achieved when antagonistic *Bacillus* endophytes were seed dressed, applied as root dip at transplanting or as foliar sprays. They evaluated the bio-control efficacy of several *Bacillus* antagonistic strains from Tanzania against Xcc in cabbage and the influence of methods of application on the bio-control efficacy of *Bacillus* strains under field conditions. The number of diseased leaves as well as black rot severity in the foliage stems and heads of a highly susceptible cultivar Copenhagen Market were significantly reduced, especially when antagonists were applied through the roots. Mguni (1996) evaluated the effect of antagonists on the control of the black rot pathogen in trials conducted in the greenhouse and in the field in Zimbabwe. Good bio-control activity was obtained when antagonistic Bacillus spp. was applied as seed dressings or root treatments to transplants. A reduction in disease severity of 65-80% was obtained in seed treatments evaluated in the greenhouse and 69-90% in root-treated transplants in the field.

The control of black rot of cabbage has become very difficult for smallholder growers in Tanzania. Thirty-one local and introduced cabbage varieties were screened for resistance to

black rot under greenhouse and field conditions in Tanzania. Greenhouse evaluation at the seedling stage permitted the elimination of highly susceptible varieties, but did not distinguish varieties with moderate resistance. Differences in resistance among varieties were observed in the field in terms of both foliar and internal stem/head reactions. Generally, varieties with good foliar resistance also showed less black rot in stems and heads. Open-pollinated cultivars were highly susceptible to black rot. Partially resistant F1 varieties included; T-689, N66, Bravo, JK-1, Maja, Ducati, Fortress, Riana, N-9690, Adelita, Blue Thunder, Rotan and Amigo. However, some of these cultivars had undesirable characteristics (head splitting, poor taste and loose or flat heads) for adoption in Tanzania, (Massomo et. al, 2004).

2.7 Disease Management

Use certified disease-free seed and transplants. If the seed lot is known or believed to be contaminated, hot-water treatment is recommended. Crucifer seeds should be treated at 122º F. Soak seeds of cabbage for 25 minutes. Employ good sanitary practices to minimize the risk of pathogen introduction and carry-over. In transplant production, use new or bleach-sanitized trays. Avoid clipping transplants in order to prevent plant-to-plant spread of the black rot pathogen, and don't purchase transplants that have been clipped. Do not handle plants when they are wet – this will reduce the risk of pathogen spread. Destroy crop residue by disking or deep-turning at season's end, particularly if black rot has occurred in a field. Manage insects and weeds (especially weeds in the crucifer family). The following cruciferous weeds are susceptible to black rot: birds rape mustard (*Brassica rapa*), Indian mustard (*B. juncea*), black mustard (*B. nigra*), short pod mustard (*Hirshfeldia incana*), Virginia pepper weed and other pepper grasses (*Lepidium* spp.), shepherds purse (*Capsella bursa-pastoris*), radish (*Raphanus sativus*), wild radish (*R.raphanistrum*). Rotate crucifers with crops that are not susceptible to black rot. Do not plant cruciferous crops in the same field in consecutive years. Plant varieties of cabbage with partial resistance (tolerance) to black rot that have been reported to be tolerant include 'Atlantis', 'Blue Dynasty', 'Bronco', 'Cecile', and 'Ramada'. The broccoli varieties 'Arcadia', 'Eureka', and 'Green belt' have shown tolerance to black rot in tests conducted at Cornell University. Some cabbage varieties that are tolerant to the disease include the following Starke Ayres Star varieties:

- STAR 3301

  All season hybrid with good field holding ability with intermediate resistance to Black Rot (*Xanthomonas campestris* pv. *campestris*). Maturity from transplant, 80 - 85 (summer), 110 - 120 (winter)

- STAR 3311

  Heat tolerance and high resistance to Black Rot (*Xanthomonas campestris* pv. *Campestris*). Maturity from transplant 75 - 85 days (summer)

- STAR 3316 (NEW)

  All season, large framed hybrid with excellent uniformity and intermediate resistance to Black Rot (*Xanthomonas campestris* pv. *campestris*). Maturity from transplant 90 – 115 days (summer) and 120 - 130 (winter)

- Chemical control of bacterial black rot disease

The effectiveness of chemical control for black rot has been inconsistent in university trials over the years. The spread of black rot in fields may be slowed in some instances through applications of fixed coppers. These materials are inexpensive and are available to both home gardeners and commercial producers. Actigard, a chemical that induces resistance to certain diseases in some plants, is labeled for suppression of black rot on commercially grown crucifers; however, results have been disappointing with this material in general. Refer to the University of Kentucky production guides listed in the reference list for products and rates, but keep cultural practices and sanitation at the forefront of any disease management program for crucifers.

Suggested Control measures for bacterial black rot disease

A. For transplant growers:

- Purchase only certified and pathogen-free seed.
- Treat all crucifer seed with hot water. Seed lots should be entirely free of black rot bacteria before planting; this is critical. Because of the difficulties in treating seed, most

growers prefer to buy seed already treated. Proper hot water treatment also helps to eliminate seed-borne infections of other diseases such as blackleg, Alternaria leaf spot, anthracnose, Fusarium yellows, and downy mildew.

- Seedbeds or greenhouses should be at least 1/4 mile from crucifer production fields.
- The soil to be used for seedbeds should have had no crucifer production for at least three consecutive years. If rotation of the plant bed is impossible, disinfest the soil using heat or fumigation.
- Seedbeds and greenhouses must be kept free of crucifer weeds and should receive regular applications of pesticides to insure freedom from diseases and insect damage.
- When watering plant beds, avoid sprinkling the foliage. Sprinkling is one of the most common means of disseminating black rot bacteria. Also, do not overcrowd or plant in poorly drained soil.
- All tools and equipment used in seedbeds or greenhouses should not be used on other crucifer crops or should be decontaminated before bringing them back into the seedbed area.
- Transplants should not be "topped" to fit into shipping containers or sprayed or dipped in water prior to transplanting.
- Only new crates or crates not previously used for crucifers should be used for shipping transplants.
- The transplants should be certified as disease-free by the State Department of Agriculture inspectors before shipping.

B. For field growers:

- Purchase your own seed and verify that the seed has been hot-water treated, certified free of the black rot bacterium, and documents that transplants were not trimmed and that only new packaging material was used. Information such as seed lot number and source, dates of pulling and shipping, pest control schedules, and transit conditions is also useful in judging the health of plant materials and helping to identify the source of disease case problem.
- Be sure that the transplants are certified as disease-free.

- Grow plants in fields that have not been in a crucifer crop for at least three consecutive years. Locate crucifer plantings where both air and soil drainage is good.
- Do not work in the seedbed or fields when plants are wet. Use clean or new harvest containers that are smooth and flexible.
- Control all crucifer weeds which may serve as a source of inoculum.
- Wherever feasible, clean up and burn or cleanly plow down all crop debris immediately after harvest.
- Cabbage, rutabaga, turnip, kale, and black mustard varieties are available that have varying degrees of resistance. New varieties are constantly being developed with improved disease resistance. Some cabbage varieties with resistance to black rot include Guardian, Defender, and Hancock, Gladiator, Bravo, Supermarket, and Blue boy. Consult current seed catalogs and trade publications for additional varieties.
- Maintain balanced soil fertility in both seedbed and field, based on a soil test.
- Control cabbage root maggots, cutworms, cabbage worms, and other insects to prevent injury which can serve as a point of infection for black rot

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Trial site

The study was carried out at the Africa University farm along Nyanga Road in Old Mutare in Manicaland Province. It is located in Natural region II and. The average annual temperature is 19^{o}C. The coldest month is July with minimum temperatures of 8.5^{o}C and maximum 20.5°C. The minimum temperature is 17^{o}C and maximum is 26^{o}C. The latitude for Mutare is 18.97 South and the longitude is 32.670 East. The annual rainfall is 818mm (*Retrieved from: http://en.wikipedia.org/wiki/Mutare)*. Rain falls mostly in the months December to February. The soils at Africa university farm research block are Sandy Clay Loam (SCL) in terms of the texture meaning that it is well drained.

3.2 Field experimental setup

Seedlings were transplanted on the 18th of February 2012. The experiment was laid out in a randomized block design with three replications. Four varieties used were Star 3301, Star 3311, Star 3316 and Potomac as the control. The inrow spacing was 45 cm, interow spacing of 50cm. Spacing between blocks was 1.5m and between plots in a block was 1m. Compound D fertilizer was applied at transplanting the rate of 30g per planting station. Each block had four plots of the different varieties with each plot carrying 30 plants. Plots were immediately irrigated after transplanting. Carbaryl was applied three days after planting to control cutworm (*Agrotis* spp.). First weeding was done at week four mainly to control nutsedge *Cyperus rotundas* and couch grass, *Cynodon dactylon.*

3.3 Trial layout

The trial was laid out as shown in table 3.1 and plate 3.1

Table 3.1 Physical layout of the trial

BLOCK 1	BLOCK 2	BLOCK 3
Star 3316	Potomac	Star 3316
Potomac	Star 3301	Star 3311
Star 3311	Star 3316	Star 3301
Star 3301	Star 3311	Potomac

Plate 3. 1 Field layout of the trial showing the three blocks, with block 2 at the centre.

3.4 Data collection and Analysis

Data on disease incidence and disease severity was collected as from the 8th week after transplanting for 4 consecutive weeks after carrying out the bacterial Ooze test in the laboratory to confirm the disease on the cabbage leaves. Readings were only collected from twelve plants within the net plot that is all the outside lines from all angles were discarded. Disease severity was determined using a scale of 0 to 9, in which 0= no disease and 9= whole plant dying. A scoring sheet was used to record the bacterial black rot disease incidence; severity and disease index (see Appendix 1). Raw data on disease incidence was presented on a table in the appendices and a column graph was prepared. Data on disease severity was used to calculate the AUDPC which were subjected to ANOVA on Minitab version 15 after transforming the data using the $\sqrt{(x+1)}$ transformation. Graphs were prepared using Microsoft Excel.

CHAPTER FOUR

4.0 RESULTS

4.1 Disease confirmation

On the 8th week after noticing some water soaked lesions on one leaf of the variety Star 3301 in the field (Plate 4.1) bacterial Ooze test was carried out in the laboratory. A small part of the affected leaf was cut and a slide was prepared and bacterial ooze was observed under a compound microscope and disease was confirmed as results were observed.

Plate 4. 1 “V” shaped lesion showing bacterial black rot symptoms on Star 3301

4.2 Disease incidence for the four cabbage varieties

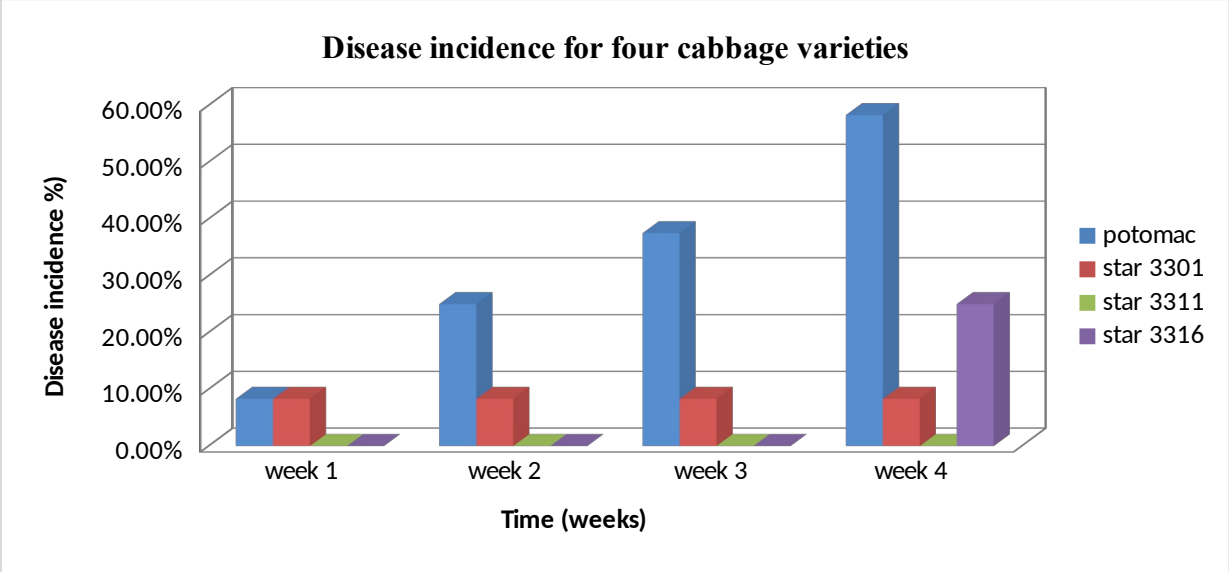

Figure 4. 1 Disease incidence on the four cabbage varieties starting from week 8 after transplanting.

In the first week of recording Potomac had only one leaf out of twelve affected so the disease incidence was lower and the same applies for Star 3301 incidence was 8.3% as for the other varieties there was no disease incidence. Disease incidence rose in the second week for Potomac to 25% and Star 3301 remained at 8.3% with only one plant affected in all the three replications whereas the other two Star varieties maintained incidence at zero. Readings for 3rd week showed a marked increase from 25% to 37.5% in disease incidence in variety Potomac showing its susceptibility levels and Star 3301 had the same incidence percentage, Star 3311 and Star 3316 had zero incidence percentages. In the fourth week which was the final week for collecting data, Potomac disease incidence rose from 37.5% to 58.3% showing that disease incidence is higher towards cabbage head formation. Star 3301 had the same percentage, Star 3311 had zero incidences throughout and first incidence was recorded in Star 3316 and it was 25%. Results showed that Potomac had a higher incidence as compared to the other varieties (Figure 4.1). Star 3311 is the most tolerant to bacterial black rot as it had zero disease incidences during the four weeks of data collection.

4.3 Rainfall pattern during period of study (mm per month)

The pattern of rainfall during the period of evaluation is shown in figure 4.2.

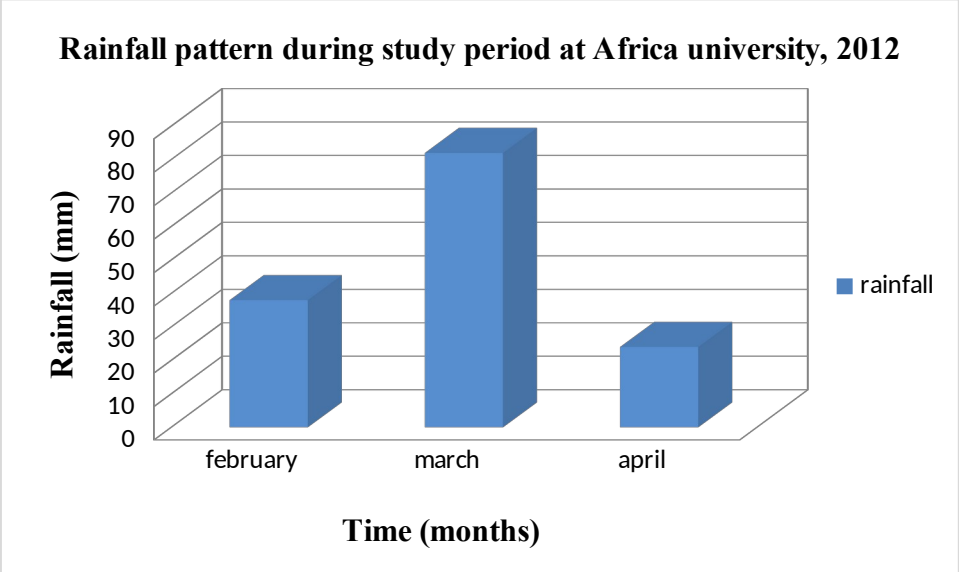

Figure 4. 2 Rainfall pattern during the period of study

The disease does not usually spread in dry weather conditions it favors warm moisture conditions. It thrives well at 80-100% humidity. Total month rainfall for February was 38mm, March was 82mm and April 24mm. The relationship between disease incidence and rainfall pattern in this case is that there was low rainfall hence low disease incidence as conditions were dry and not favorable for the bacterium. For example in April it was 24 mm total month rainfall meaning that moisture levels were very low for the bacterium. The weather conditions were too dry.

4.4 Disease severity among the four varieties

Disease severity was calculated for all the varieties and table of results was prepared. Graphs for each variety were prepared on weekly basis and area under disease progress curve was calculated and data was presented in a table. Area under disease curve was done so as to which variety was hit hard by the disease.

4.5 Data analysis using ANOVA

Using Minitab version 15 to analyze the data, varieties were entered as the factor and output of disease scores (square roots calculated from area under disease curve) were entered as the response on the work sheet. Variety Potomac was assigned as number 1, Star 3301 as 2, Star 3311 as 3 and Star 3316 4. For the responses square root of every replication was entered that is each variety had three replications. Normality test was first conducted to check if the data was normally distributed the response column was the variable and Anderson Darling test was used. Data was found to be abnormally distributed on the line the reason being the huge differences in disease severity scores as some varieties were having zero scores for example Star 3311 had zeros throughout as compared to Potomac with a score of 3. The data was transformed using the √(x+1) transformation to normalize the data before being subjected to Analysis of variance (ANOVA) after testing for normality (Figure 4.3).

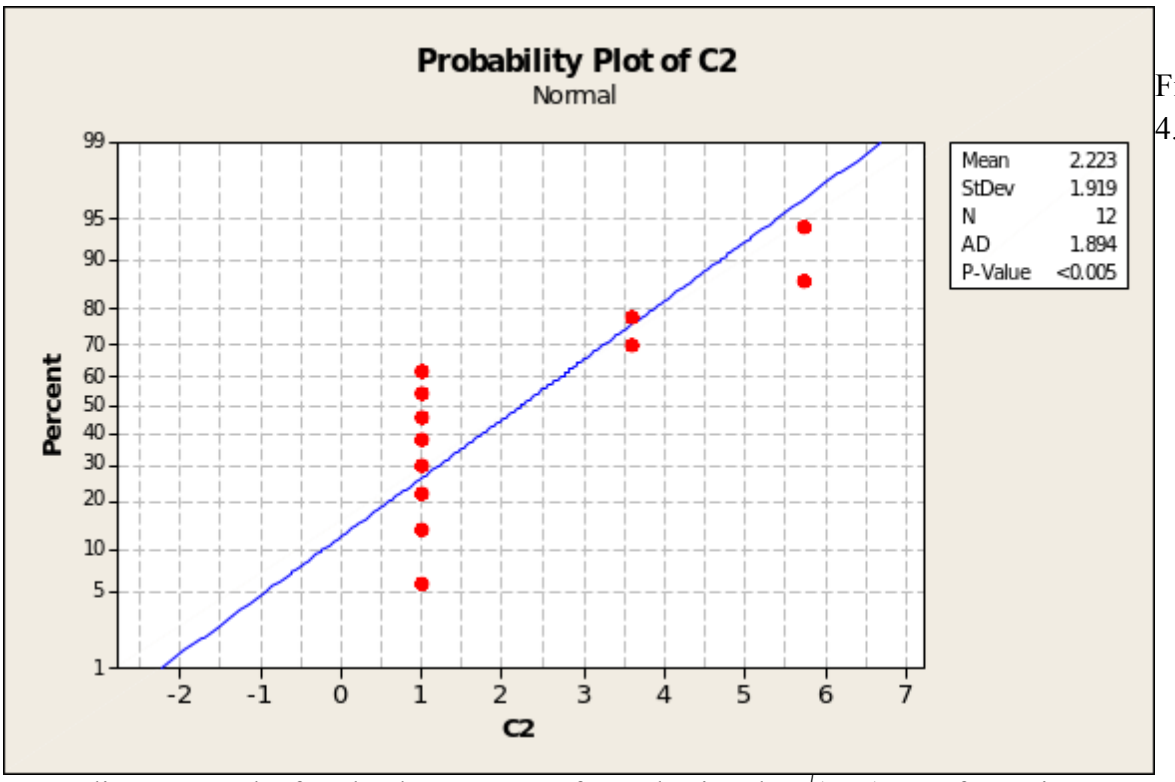

Figure 4. 3 Normality test graph after the data was transformed using the √(x+1) transformation

Actual analysis was done using one way ANOVA. Response (disease scores) was the second column and factor (varieties) was the first column.

4.6 Analysis of ANOVA RESULTS

Table 4. 1 ANOVA test on disease severity for the four varieties

Variety	AUDPC (cm^2)[1]± SE
Potomac	3.447 ±2.374 a
Star 3301	2.580 ±2.737 a
Star 3311	1.000± 0.000 a
Star 3316	1.867 ± 1.501 a

[1]Figures in the same column followed by the same letter are not significantly different at P ¿0.05.

CHAPTER FIVE

5.0 DISCUSSION

Results showed that the chosen cabbage varieties are more tolerant to bacterial black rot disease as they had a low disease severity score (Table 4.1). Potomac, Star 3301 and Star 3316 had an average score of 3 whereas Star 3311 had a score of zero meaning that there was no disease incidence on the variety. Hence variety Star 3311 proved to have a high resistance to bacterial black rot as stated on its characters in the literature review on disease management. Disease symptoms were noticed in the 8th week after transplanting due to use of tolerant varieties the disease is pushed further than occurring in the early season and also intensity is reduced. Lengthening time before disease occurs is increased; disease severity and incidence will be low as shown by the results of the study (Table 4.1 and figure 4.1). Disease incidence was very low among the varieties except for variety Potomac which was susceptible among the four cabbage varieties. Disease incidence for Potomac rose from 8.3% from the first week of recording to 58.3% (Figure 4.1) in the fourth week of recording showing that it was the susceptible variety among the varieties used in the study and disease incidence is more severe when cabbage heads are coming out and heading may be prevented (Miller, Sahin, and Rowe. 1996) resulting in yield losses. Potomac was used as the control but it proved to be the more susceptible among the four varieties since it had the highest disease incidence percentage and larger AUDPC.

The disease causative agent (*Xanthomonas campestris* pv. *campestris* (Xcc) survives well especially in cabbage and Brussels sprout refuse, in numerous weeds including black mustard, field mustard, charlock, shepherd's purse, Virginia pepper weed and cress (McGrath, 1994), these weeds were not dominating the trial site hence low bacterial black rot inoculum in the field and hence low disease incidence among the varieties used for the study, the predominating weed on the site is purple nutsedge (*Cyperus rotundas*) and does not harbor the bacterium. Pathogen thrives well in free moisture in the form of dew, fog or rain which is required for infection and disease development, the pathogen is spread from plant to plant by splashing rain, and does not usually spread in dry weather (Shrestha, 1977, Schaad and Dianese, 1981). The disease is usually most prevalent in low lying areas and where plants remain wet for long periods (Day *et al*., 1992;

Mgonja and Swai, 2000; Massomo, 2002) the trial site is not on a low lying area and the soils at trial site (Africa university farm research block) are Sandy Clay Loam (SCL) in terms of the texture meaning that it is well drained and disease inoculum is very low.

The rainfall pattern during the period of study was low (Figure 4.2) for disease development. In Mutare rain falls mostly in the months December to January and fewer rains in the month February. In February it was 32 mm and in April it was very low (24mm) hence dry conditions are not favorable for the pathogen survival and spread and hence low disease incidence percentages among the cabbage varieties used, incidence is high where plants remain wet for long periods. The period when the evaluation was carried out was not suitable for bacterium proliferation and maybe results were distorted as conditions were not suitable though these varieties have tolerant characteristics to bacterial black rot therefore evaluations should be carried out during rainy seasons. Due to dry conditions temperatures could have been higher than the optimum temperatures and inhibiting bacterium survival leading to very low levels of disease occurrence in the field.

Disease severity was very low as indicated by the figures obtained on AUDPC with Potomac as the highest with 54 cm2 for the three replications and Star 3311 with the lowest figure 0 cm2 hence high tolerant to the disease. The percentages of disease incidences on the disease incidence graph (Figure 4.1) show the level of tolerance of each variety to the disease. STAR 3301 has intermediate tolerance to Black Rot (*Xanthomonas campestris* pv. *campestris*), STAR 3311 high tolerance to Black Rot, STAR 3316 intermediate tolerance to Black Rot and Potomac with the least tolerance as compared to the other three varieties. Therefore the Starke Ayres Star varieties have high tolerance to bacterial black rot disease.

The ANOVA analysis done on Minitab version 15 the results (Table 4.1) showed that the in terms of severity there is no significant difference but in terms of incidence there are some notable differences. Overlapping in the lines shows that there is no significant difference in tolerance levels though variety Potomac has a higher level of disease severity but there is no significant difference from the rest. So all the varieties are on the tolerant side but Star 3311 is highly tolerant. ANOVA test (Table 4. 2) on disease severity for the four varieties shows that the

varieties have the same letter a hence they are not significantly different at P ¿0.05 meaning that they are all falling in the same category (tolerant). The reason for no significant differences in tolerance levels could be attributed to the fact that all the varieties used are on the tolerant side.

In Tanzania and other countries where cabbage is largely produced by smallholder farmers, host plant resistance remains the most feasible strategy for managing black rot in studies done by Massomo and others. Cabbage cultivar Amigo F1 is the only recommended black rot resistant cultivar in the Arumeru region in Tanzania (Massomo et al., 2004). This study also demonstrated that the use of varieties (Potomac, Star 3301, Star 3311 and Star 3316) that have plant host resistance to bacterial black rot reduces disease incidence and severity hence curbing problems of yield losses among the smallholder farmers. Therefore cabbage varieties with partial resistance to bacterial black rot should be used as a control measure to manage black rot successfully.

Seed infection usually varies from 0.25 to 80% (Shrestha 1977), Schaad and Dianese, 1981) so the seeds of varieties used are certified seeds so they are free from seed-borne bacterium therefore there was no disease occurrence in the seedlings and as a result low disease incidence in mature plants as evidenced by the findings, infection on the varieties affected could have been from the soil debris.

CHAPTER SIX

6.0 CONCLUSION AND RECCOMMENDATIONS

It can be concluded that there was no significant difference in tolerant levels to bacterial black rot disease among the four varieties since the results of the ANOVA analysis showed overlapping in lines hence all the varieties are falling in the same category (tolerant). Star 3311 had zero disease incidence and score for disease severity was a zero hence zero AUDPC therefore it can be regarded as the most tolerant variety to the disease among the four varieties used for the study. The results showed that there was no significant difference to disease tolerance among the varieties as opposed to the hypothesis hence hypothesis is not true. All the four varieties had high levels of black rot tolerance at all stages of growth except for Potomac which had high disease incidence towards cabbage head formation hence disease is more severe when the heads are coming out. The rainfall pattern determines the levels of infection as disease pathogen favors wet and rainy conditions in the study disease incidence and severity were very low due to low rains therefore conditions were not favorable as they were dry. Potomac was the control but it was more susceptible to the disease hence the Star varieties are more tolerant. Improved levels of plant host resistance could give added yield benefit by reducing crop losses in the rainy seasons. Substantial benefits can immediately be realized by the introduction, testing and dissemination of black rot resistant varieties to the farmers.

Recommendations:

- Farmers are recommended to choose Star 3311 as it is highly tolerant to bacterial black rot disease.
- Trials for evaluating cabbage varieties for bacterial black rot tolerance should be held during the rainy season especially from December to January when there is a lot of rainfall and moisture (favorable conditions for the pathogen).
- Choice of cabbage varieties: a wider range of varieties should be used when conducting evaluation trials the study did not include a highly susceptible variety for comparison, all of the varieties used are falling in the tolerant category only.

CHAPTER SEVEN

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APPENDICES

Appendix 1 : Bacterial Black Rot Disease Score on Brassicas

Date……

Trial ……..

Plot number…….....

Treatment…….

Plant number	Disease incidence	Disease severity	Disease Index (Incidence X Severity)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
Mean			

Scoring scale:

0= no disease

3= 25 % of cover leaves

5= 50 % of cover leaves

7= 75% of cover leaves

9= whole plant dying

Appendix 2 : Disease incidence table (%)

VARIETY	week 1	week 2	week 3	week 4
Potomac	8.3%	25%	37.5%	58.3%
Star 3301	8.3%	8.3%	8.3%	8.3%
Star 3311	0%	0%	0%	0%
Star 3316	0%	0%	0%	0%

Appendix 3: Disease severity table in scores

	Potomac			Star 3301			Star 3311			Star 3316		
Weeks	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	3	0	0	0	3	0	0	0	0	0	0	0
2	3	0	0	0	3	0	0	0	0	0	0	0
3	3	0	3	0	3	0	0	0	0	0	3	0
4	3	0	3	0	3	0	0	0	0	0	3	0
Mean score	3	0	3	0	3	0	0	0	0	0	3	0

Appendix 4: Area under disease progress curves (cm^2)

Variety	Replication 1	Replication 2	Replication 3
Potomac	32	0	12
Star 3301	0	32	0
Star 311	0	0	0
Star 3316	0	12	0

Appendix 5: Data after normalizing with $\sqrt{(x+1)}$ transformation

Variety	replication 1	replication 2	Replication 3
Potomac	5.74	1	3.6
Star 3301	1	5.74	1
Star 3311	1	1	1
Star 3316	1	3.6	1

Appendix 6 : ANOVA ANALYSIS One-way ANOVA: C2 versus C1

Source	DF	SS	MS	F	P
C1	3	9.74	3.25	0.84	0.507
Error	8	30.75	3.84		
Total	11	40.50			

S = 1.961 R-Sq = 24.06% R-Sq (adj) = 0.00%

Individual 95% CIs for Mean Based on Pooled StDev

Level	N	Mean	StDev	--------+---------+---------+---------+-
1	3	3.447	2.374	(----------*----------)
2	3	2.580	2.737	(----------*----------)
3	3	1.000	0.000	(----------*----------)
4	3	1.867	1.501	(----------*----------)

--------+---------+---------+---------+-

0.0 2.0 4.0 6.0

Pooled StDev = 1.961

Fisher 95% Individual Confidence Intervals

All Pair wise Comparisons among Levels of C1

Simultaneous confidence level = 82.43%

Appendix 7: Yields per plot

Pumphouse **hostels**

South

1.6 m

4 14.39 kg	3 -	2 11.7 kg	1 14.04 kg **1.1 m**
8 13.43 kg	7 12.24 kg	6 13.66 kg	5 12.02 kg
12 11.22 kg	11 12.21 kg	10 14.78 kg	9 12.62 kg

North