***Original Research Article***

# Black rot (*Xanthomonas campestris pv. campestris*) control in field grown Cabbage (*Brassica oleracea* var. Sugar loaf) with *Moringa oleifera* extracts


## ABSTRACT

**Aims:** To evaluate if the antibacterial compounds present in Moringa were significant enough to effect suppressive effect on *Xanthomonas campestris pv campestris* in field grown cabbages (*Brassica oleracea*), in an open field experiment.
**Study design:** The experimental design was a 3 x 3 factorial laid out in a split plot in two blocks with three replications.
**Place and Duration of Study:** Field experiments were carried out for 6 months during the October 2015 to April 2016 season at Victory Farm in Beatrice, Zimbabwe
**Methodology:** Three Moringa extract concentrations of 60, 100 and 140% were sprayed as foliar applications weekly and the antibacterial activity for each of the different Moringa extract efficacy was evaluated by recording number of totally defoliated plants once every week. The experiment was laid out as a split plot design in two blocks with three replications.
**Results:** The results indicated high significance in antibacterial activity of all the three Moringa extracts as they were able to achieve control of *Xanthomonas campestris* at varying levels in the cabbage plants ($P$ = 0.05). However, Moringa seed extract had the highest antibacterial activity against the black rot disease in cabbages in this study.
**Conclusion:** In conclusion bacterial growth on cabbage can be effectively reduced by using either extracts sprays. Further studies need to be carried out to assess if the utilization of the Moringa seed extract as a seed dressing would not increase its antibacterial effects against the test pathogen since it is an important seed borne disease of brassicas and crucifers.


*Keywords: Moringa plant extracts, Antifungal, Xanthomonas campestris, field-cabbage*

## 1. INTRODUCTION

*Xanthomonas campestris* bacterium can either be seed borne, or at times the infection can be principally spread through wounds caused by insects, hydathodes (significant port of entry), or through fluid filled stomata openings [1] or even via irrigation water [2]. However, seed is the main effective and most important vehicle of transmission for this pathogen. The different pathovars of this bacteria thrive mostly in humid soils and temperatures ranging from 20 to 30°C and a good example is the *Xanthomonas campestris pv. phaseoli* which thrives and causes economic losses at 27°C in common beans (*Phaseolus vulgaris*) [3]. The bacteria, however, exhibits a much more reduced spread in dry weather and is usually less active at temperatures above 50°C [3]. The *Xanthomonas campestris* species has approximately 125 pathovars, has a polar tail-like structure (flagellum) for motility and can also manufacture extracellular polysaccharides (xanthan) which exhibits plugs that can cause damage to the plant's vascular bundles especially the xylem vessels [4]. Vessel damage will in the end inhibit water transportation which leads to wilting due to water stress. The bacteria's generation time is temperature dependent and one bacterium needs approximately 2 hours to produce 2 bacteria by fission, and its shortest generation time occurs at approximately 27°C [3]. These characteristics aid in making *Xcc*, a serious pathogen in Zimbabwe since it is located in the tropics which experience warm to hot climates most of the year. *Xanthomonas campestris* pathovars exhibit a wide host range, with each pathovar being specific and exceptionally distinctive to particular host plants. *Xanthomonas* pathovars are listed as number five among the Top 10 list of plant bacterial pathogens of economic importance worldwide [5]. This indicates their ability to cause devastating diseases of colossal economic importance. The pathovars *campestris, vesicatoria*, *malvacearum* and *phaseoli* are among the detrimental pathogens which belong to this group of species.

Black rot is caused by the bacterium *Xanthomonas campestris* pv *campestris,* is a very deleterious disease by its ability to persist in organic matter and the huge losses incurred as a result of its devastating symptoms (damage) [6], which results in crop produce which is unfit for human consumption or even marketing. The disease has been known to cause up to 40% yield losses and in Zimbabwe, it poses a huge threat to both smallholder and commercial farmers in Agro-ecological regions II, III and IV [7] which are the hubs of vegetable production. The most common methods of control being currently implemented include cultural and preventative control methods which include use of certified seed, control of weeds and volunteer plants, and removal and destruction of diseased plants. Biological control using *Bacillus subtilis* has been effectively used in Zimbabwe on *Xanthomonas campestris pv. campestris* on different Brassicae species in dry and short rainy seasons [8]. Black rot is a disease affects all crucifers worldwide, that is caused by the bacterium *Xanthomonas campestris pv. campestris*. Black rot is a seed borne, vascular disease which favors warm and humid conditions [6]. Black rot is a major disease constraint for vegetable production by smallholder farmers in many parts of Africa including South Africa, Kenya and Zimbabwe with devastating impacts on productivity. 100% losses were encountered by smallholder farmers during the Elnino rains in 1998 in Tanzania [9]. In Zimbabwe, black rot disease causes problems in all the five agro-ecological regions and disease incidence can be as low as 10% to as high as 80% [8]. The black rot disease mainly affects the above ground parts of the plant and proceeds fast in plots with multi-focal inoculation than in those with uni-focal inoculation. Plants are susceptible to black rot at any stage of their growth, with the initial, usual and characteristic symptom being the V-shaped, small, wilted lesions which appear on the leaf margins. The lesion patch usually has small black veins and in severe cases, vein discoloration sometimes even reaches the stems. After reaching the stem the bacteria moves systemically to other healthy uninfected plant parts [10]. The infected areas will eventually enlarge, progress towards the leaf base, turn yellow to tan and then dry out. Diseased leaves may become stunted on one side and then drop prematurely [11]. One of the downs of this disease is that it inevitably paves way to soft rots which produce a very unwelcoming smell and cause quick rotting even before harvesting the produce and in extreme cases the crop might even fail to be produced [12]. Xanthomonas can survive in organic matter in the soil, infecting disease free seedlings thus it quickly brings about epidemics of the black rot disease in many cropping areas [13] which make it even more difficult to manage and reduce its spread.

Currently, no satisfactory chemical control has been established yet for all the four *Xanthomonas campestris* pathovars. This is mainly because most chemical applications are done when crop has already been infected and symptoms have been observed rendering this method marginally ineffective in disease suppression, and in some cases, the chemicals may even cause pathogen resistance [14]. No satisfactory chemical control has been established yet and the only feasible means of managing the diseases caused by the *Xanthomonas* species are the cultural and preventative control methods such as use of certified seed, control of weeds and volunteer plants, and removal and destruction of diseased plants [14]. Biological control initiatives of bacteria such as *Bacillus subtilis* has been used in Zimbabwe and it proved to be effective in controlling black rot on different Brassicae species in dry and short rainy seasons in Zimbabwe. Further studies have used *Pseudomonas* and *Bacillus* strains to control bacterial blight causal agents, and this proved to be slightly effective [15]. Black night shade has also been reported to be very effective in controlling black rot [16]. However, though these control methods aid in reducing black rot virulence, they are not effective in achieving its control. This is mainly because once the produce has been infected by the different *Xanthomonas* species, the disease caused renders a larger percentage of the economic parts incapable of fetching high market prices and unfit for storage or shipment [17]. There is need therefore, to identify other biological control agents to aid in combating black rot, without necessarily resorting to extensive and over usage of chemicals, and Moringa has got great potential given it antimicrobial properties, if properly harnessed to be utilized effectively as an alternative means to manage black rot disease.

In Zimbabwe, the Moringa tree has naturalized in the northern parts of the country, which are drier (Binga, Zambezi valley, Victory Falls) and more conducive to its establishment [18], thus based on its varied utilizations, the local communities can further benefit from it a bio-pesticide and not only as a food source, livestock feed or hedge plant [19]. Moringa is a multipurpose tree with antimicrobial properties whose potential needs to be harnessed and implemented in sustainable pest control strategies. Studies have indicated that plant extracts can be used to obtain the above objectives and *Moringa oleifera* is one of the many plants that have been utilized for its anti-microbial properties [20].

## 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

### 2.1 Experimental Site and Methodology

The main objective of this study was to evaluate if the antibacterial compounds present in Moringa were significant enough to effect suppressive effect on *Xanthomonas campestris pv. campestris* in field grown cabbages, in an open field experiment. The other aim was to determine which part of the Moringa extracts was the most effective in suppressing black rot disease in field grown cabbages. The study was carried out at Victory Farm, Beatrice which lies in Mashonaland East Province, latitude 18 ° 15'3.72" S, longitude 30° 51'9.96E, approximately 1900msl and receives an average rainfall of 450 -600mm/annum. The soils in this area are predominantly sandy loam soils. This farm is an under organic farming and no chemicals are used in their production practices.

#### 2.1.1 Experimental Design and Factors

The experiment was laid out in a Split – Plot, 3 x 3 factorial, in a Randomized Complete Block Design with 4 replicates. Moringa extract type was the main plot factor at 3 levels that is, leaf, bark and seed, whilst the subplot factor were the Moringa extract concentration levels at 3 levels, 60%, 100% and 140%. The control factor was the use of Neem extract in controlling *Xanthomonas campestris pv campestris* black rot disease.

### 2.2 *Xanthomonas campestris* Pathogen and Moringa Extract Preparation

The pathogen was prepared by collecting diseased leaves from an infected cabbage plant. These were isolated, purified, cultured and multiplied after Kochs postulate procedure [21]. These were then stored until they were needed for inoculation of the study plants.

To prepare the plant extracts, firstly the Moringa seed, leaf and bark powders were obtained from Mutoko District, Zimbabwe were they had been ground using a pestle and mortar, then sieved into to obtain very fine powders. The aqueous extracts were prepared by suspending 60g, 100g and 140g powders of each of the extracts separately in 100 ml of sterile water. The samples were shaken and stirred continuously for 30 minutes and allowed to sediment at room temperature for 24 hours, after which they were strained with a muslin cloth. The aqueous extracts were the 60%, 100% and 140% concentration levels.

The land was ploughed using a tractor and the beds were made using hand hoes. Two blocks A and B each with 3 beds were prepared. The beds measured 4m x 2m. The beds were pre-watered to field capacity. Liquid organic fertilizer was applied as fertigation through sprinkler irrigation 3 times per week to field capacity. The farm has a biodigestor hence the liquid manure is the waste which comes cattle, sheep, goat and pig droppings. The irrigation system is also run using biogas generated from the biodigestor. The farm is a model farm which trains farmers in sustainable permaculture and farming practices through efficient use of renewable energy. The rape seeds were sown at a rate of 3kg /Ha (actual sown were 5 seeds per planting station). The seedlings were then thinned to leave one plant per station at four weeks after planting.

The land was ploughed using a tractor and the beds were made using hand hoes. Two blocks A and B each with 3 beds were prepared. The beds measured 4m x 2m. The beds were pre-watered to field capacity. The cabbage seeds were sown at a rate of 3kg /Ha (actual sown were 5 seeds per planting station). Fertigation was achieved by sprinkler irrigation using liquid organic fertilizer obtained from the holding tanks from biodigestor waste material 3 times weekly until field capacity was reached. The seedlings were then thinned to leave one plant per station at four weeks after planting.

### 2.3 Cabbage Bacterial Inoculation and Data Collection

To inoculate with *Xcc* bacteria, the fully expanded top two leaves of each cabbage seedling were pricked and a third of a section of the leaf was cut using sterilized, stainless steel scissors, to improve the penetration of bacteria. The freshly prepared inoculum suspension from the cultured bacteria was sprayed onto each individual plant using 1 liter hand sprayers to run off point. The inoculation was done at 5 weeks after planting.

The three Moringa extracts at concentrations of 60, 100 and 140% were foliar applied achieving full cover spray at each application, on cabbage plants once weekly basis from 5 weeks after planting and data collection was initiated then, and was done on a weekly basis. Totally defoliated plants were counted to evaluate the suppressive efficacy of the different Moringa extracts using the scoring method modified from [22] (Table 1).

**Table 1: Disease Severity scoring table for *Xcc***

| Scale | Disease Severity |
|---|---|
| 1 | No symptoms |
| 2 | Very few symptoms, 1-3 small lesions on 1/2 leaves |
| 3 | 3-5 leaves with more than 3 yellow lesions |
| 4 | Enlarged lesions on 3 or more leaves |
| 5 | Coalescing lesions forming wilted tissue. |
| 6 | Necrosis, with the veins turning black or brown |
| 7 | Plants completely defoliated and dying. |

**Modified from Anjorin, Jolaoso, and Golu. (2013).**

The data was analyzed using Excel and GenStat 14th Edition. The means were separated using LSD (Least Significant Difference) at 5% level where there was significant differences.

## 3. Results and Discussion

### 3.1 Results

The three Moringa extracts (bark, leaf and seed) showed a great significance ($P$ = 0.05) in their antibacterial activity by controlling black rot disease, which is caused by *Xcc* bacterium in a field study. The highest significance was recorded during 8th week after crop emergence (Table 2) with the seed extract recording the least mean leaf defoliation of 2.965 followed by the bark extract (3.312) and lastly leaf extract (3.486). The extracts levels were not significantly different from each other in their antibacterial effects on the black rot disease ($P$ = 0.05). Interestingly there were no interactions which were observed between extract concentration level and ability to suppress *Xcc* black rot disease in this study.

**Table 2: Effect of Moringa Extracts on *Xcc* disease severity in Cabbages**

| Treatment | ST5WAE | ST6WAE | ST7WAE | ST8WAE |
|---|---|---|---|---|
| BarkExtract | 4.042 | 2.762 | 2.92 | 3.312[b] |
| LeafExtract | 2.014 | 2.969 | 3.148 | 3.486[c] |

Define these terms.

| | | | | |
|---|---|---|---|---|
| SeedExtract | 1.912 | 2.694 | 2.875 | 2.965[a] |
| P value | 0.739 | 0.069 | 0.087 | **0.009** |
| LSD5% | 0.8791 | 0.5278 | 0.3617 | 0.09 |
| CV % | 8.2 | 2.5 | 2.1 | 1.1 |

**Means with different letters are significantly different at P = 0.05**

**Key:** STWAE = Severity Index …. Weeks After crop Emergence

The results indicated an interesting trend in which the Moringa bark extract at 5WAE had the largest *Xcc* mean defoliation level compared to both the Moringa leaf and the seed extracts (Figure 1). However by the 6th week after emergence, its efficacy at suppressing *Xcc* disease severity had increased such that it was performing comparably well in relation to Moringa seed extract. At the end of the 8th week, Moringa bark extract had managed to surpass the efficacy of Moringa leaf extract in suppressing black rot disease in the cabbage plants. The results indicated significant differences in the antibacterial activities of each of the three Moringa extracts from each other in their antibacterial properties against *Xcc*. Moringa seed extract had the lowest defoliation mean, followed by Moringa bark extract and the highest mean defoliation was recorded for Moringa leaf extract (Figure 1).

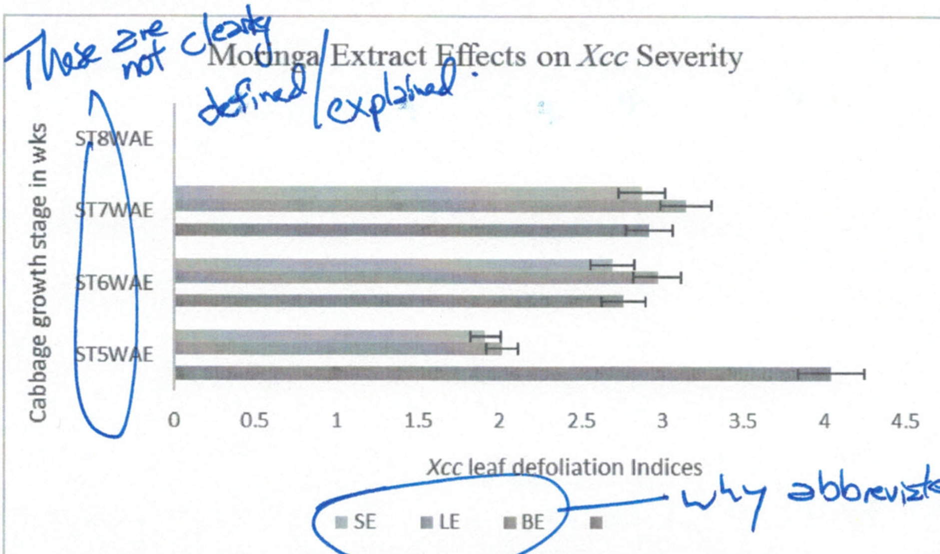



**Figure 1: Effect of Moringa seed, leaf and bark extracts on mean cabbage leaf defoliation at 5 - 7 weeks after emergence (Bars at LSD 0.05). *P* = 0.009.**

**SE** – Seed Extract **LE** – Leaf Extract **BE** (Blue) – Bark Extract

The Moringa extract type effects were very significant, affecting the level of defoliation and devastation of the crop not only statistically (Table 2 and Figure 1), but these significant differences were also exhibited physically in the field. Plates 1 – 4 are showing the physical manifestation of the effect of Moringa extract type on black rot disease severity and impact on cabbage growth, defoliation and incidence of secondary physiological conditions such as rotting and plant death.

a.  b. 

**Plates 1a and b: Antibacterial effect of Moringa seed extract in suppressing black rot disease (*Xanthomonas campestris pv campestris*) in field grown Cabbages**

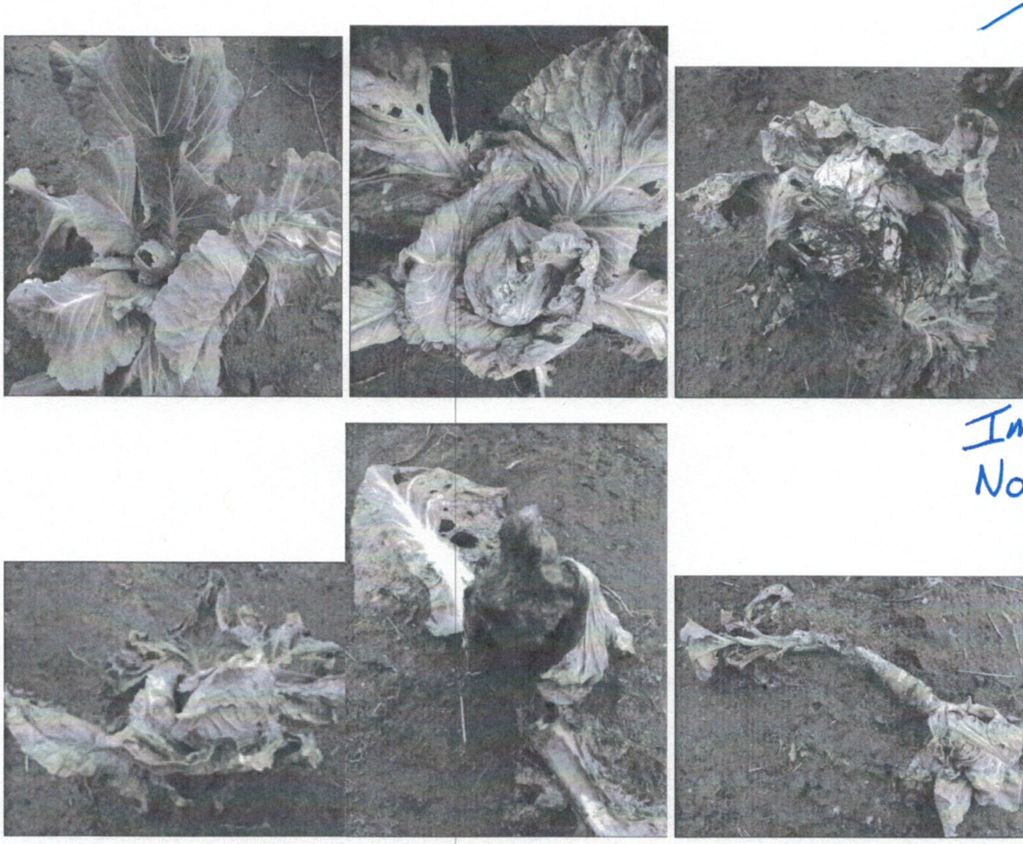
a.  b. 

**Plates 2a and b: Antibacterial effect of Moringa bark extract in suppressing black rot disease (*Xanthomonas campestris pv campestris*) in field grown Cabbages**

a.  b. 

**Plates 3a and 3: Antibacterial effect of Moringa leaf extract in suppressing black rot disease (*Xanthomonas campestris pv campestris*) in field grown Cabbages**

Which ones (ie. A or B) had the extract?



**Plates 4 a, b, c, d, e and f from the top left to the bottom right: Showing the damaging effects of *Xanthomonas campestris pv campestris* in Cabbage plants were the antibacterial activity was not effective for Moringa leaf extract at 60% concentration level.**

### 3.2 Discussion

A GC/MS phytochemical analysis which was carried out on the Moringa extract sources revealed the presence of bioactive compounds such as phenylpropanoids, alkaloids, chromene neolignans, flavonoids and tanins which is consistent with the findings by [23]. These compounds are responsible for the antimicrobial activity found in Moringa. The flavonoid eugonol in particular, is antibacterial in its activity against pathogens [23]. These findings are not supportive of the findings in this study, but also validate that Moringa is a plant which has antibacterial properties as indicated in current study. This tree has been used for decades as part of an important medicinal plant traditionally, mostly being used to remedy all sorts of human conditions and illnesses [24]; and as such, its utilization as a biopesticide would be easily adopted. This is because over 80% of rural populations rely on traditional medicines or remedies which are within their affordability range and readily available [25]. Based on the findings of this study, it would be important to engage local farmers in a wider study involving the larger majority of the farming communities located in various agricultural zones to validate its efficacy under a wide range of climates and natural environments. The current study was carried out from October 2015 until March 2016, this period overlapped with two contrasting seasons in Zimbabwe. During the October to November period, Zimbabwe experiences its hottest months as a country, which is then followed by its rainy season from the months of December until March when the rains tail off and eventually come to an end [26]. It is possible that this state of affairs influenced the efficacy of the Moringa extracts at varying on their antibacterial activity against *Xcc* since this was an open field experiment, where the cabbage plants were exposed to the mercy and effects of nature. Moringa extract efficacy might have been affected by contrasting extremities in weather which occurred during this study period, the intense heat followed by the prolonged wet and rainy periods. These conditions might have influenced the ability of one extract to express its antibacterial properties over the other type. [27] state that the phytochemical and bioactive compounds present in Moringa are affected by varying degrees of temperature. According to these same studies, the amounts of phenols within the Moringa seed peaked with increase in temperature, hence the improved efficacy of seed extract as an antibacterial agent in comparison to leaf and bark extracts might be attributed to this phenomenon. Studies by [28] however are in contradiction with this assertion as they found no significant negative influence of temperature on the antibacterial activity of Moringa leaf extract against *Staphylococcus aureus* and *Salmonella typhi* pathogens.

Moringa seed extracts in this study exhibited high antibacterial properties by suppressing deleterious progression of the black rot disease in the cabbage plants. This is supportive of studies carried out by [29] in invitro screening of Moringa extracts for their antibacterial potential against enteric pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, E. coli and Vibrio cholera,* and the results revealed the strong antibacterial potency of Moringa against several gram negative and gram positive bacteria. This antibacterial action of Moringa can be safely attributed to the presence of phenols (hydroxybenzoic acids, 2-hydroxy benzoate) and flavonoids (kaemperferol, quercetin, isorhamnetin, L-rhamnose) which infer antimicrobial properties to the plant [30]. The bioactive compound tannin, which is also present in Moringa also imparts antimicrobial activity to the plant. Phytochemical compound *p*-hydroxybenzoic acid for instance reduced the growth of *Clostridium botulinum* types A and B, whilst the naturally occurring ethyl *p* - methoxycinnamate within plants, reduced mold growth in another study. [16]. Further studies revealed the ability of *p* - coumaric acid and ferulic acid to increase the lag phase and inhibit the lag phase of *Saccharomyces cerevisiae* pathogen respectively. The enhanced efficacy of Moringa seed extract as an antibacterial agent compared to leaf and bark extracts might be linked to the higher concentrations of these bioactive phytochemicals in the seed extract compared to the bark and leaf extracts. [31] indicated that Moringa leaf has the lowest concentration of tannins, which might explain the low antibacterial action the leaf extract exhibited compared to the performance of the bark and seed extract. The presence of tannins at cellular level, not only result in a firmer, drier and thermally more stable structure [16], but it also contains high levels of antimicrobial activity due to the presence of trihydroxy benzoic acid. Thus the low concentration of this bioactive compound in the leaves might have reduced its antibacterial action against *Xcc* in this study. Furthermore, [27] stated that Moringa seed contains approximately 49.8 – 57.25% oil with undetectable levels of linolenic acid which makes Moringa oil the most stable plant oil. This stability might have enabled the phytochemical compounds present in Moringa seed extract not to break down due to the contrasting heat and wet conditions, which might have decreased its antibacterial properties.

There however, exist quite a number of factors which infer the need for further studies to provide more valid information on some aspects such as at which plant growth stage would the Moringa extracts be more efficient as a biological control agent against pathogens? What would be the most ideal application method for these extracts in field grown crops? At which level of Moringa extract pulverization would the extraction process be most efficient? And lastly which extraction method would result in Moringa extracts whose phytochemical composition of the naturally occurring bioactive compounds remaining in their natural form as much as possible? Many questions concerning improving efficacy and sustainable, climate smart utilization of *Moringa oleifera* remain unanswered.

## 4. CONCLUSION

The observations from this current study, ~~though proving conclusively~~ that *Xanthomonas campestris pv campestris* bacterial disease (black rot) growth on cabbages can be suppressed more effectively by using Moringa seed extracts compared to Moringa bark and leaf extract, it also brings to light areas which need further studies. This current study has validated that Moringa does possess antibacterial properties which are effective against black rot produced in open field grown brassicas such as cabbage. There is need to validate how these same Moringa extracts would perform in other host plants grown in the open field. Although a lot of research has been done to validate the antimicrobial properties of Moringa, not enough open field studies have been done. Hence there is still that need. Black rot is a devastating disease of