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* (black rot) 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 replicates.
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 aqueous Moringa extracts (leaf, bark, and seed) at 3 concentration levels of 60, 100 and 140% were sprayed as foliar applications weekly from 5 weeks after crop emergence in cabbages for the duration of the study. The antibacterial activity for each of the different Moringa plant extracts was evaluated by recording number of totally defoliated plants once every week.
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 pv campestris* black rot disease at varying levels in the cabbage plants ($P < 0.05$). The highest inhibition of black rot disease progression was recorded during 8th week after crop emergence 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). Moringa seed extract had the highest antibacterial activity against the black rot disease in cabbages in this study.
Conclusion: Bacterial black rot disease caused by *Xanthomonas campestris pv campestris* in cabbage can be effectively managed by using either seed, bark, or leaf aqua-based Moringa extract sprays. The 100 and 140% concentration levels were most effective, compared to the 60% concentration level. 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, Antibacterial, Aqua-based extraction,

1. INTRODUCTION

Xanthomonas campestris bacterium can be transmitted either by being seed borne, or at times the infection can be principally spread through wounds caused by insects, natural openings/hydathodes (significant port of entry), 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 because the bacterium attaches itself to the seed before plant establishment. The different pathovars of this bacteria thrive mostly in humid soils and temperatures ranging from 20 to 30^{o}C and a good example is the *Xanthomonas campestris pv. phaseoli* which thrives and causes economic losses at 27^{o}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^{o}C [3]. Vessel damage will ultimately 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^{o}C [4]. These characteristics aid in making *Xanthomonas campestris pv campestris,* 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 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 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].

Currently, no satisfactory chemical control has been established for all the four *Xanthomonas campestris* pathovars [13]. 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]. 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.

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 the efficacy of Moringa aqua-based leaf, seed, and bark extracts against *Xanthomonas campestris pv campestris* in open field grown cabbages. The other aim was to determine which part of the Moringa plant extract was the most effective in suppressing black rot disease in cabbages grown under natural conditions. The study was carried out at Victory Farm, Beatrice which lies in Mashonaland East Province, latitude 18 ^{o} 15'3.72" S, longitude 30^{o} 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 nor inorganic fertilizers 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 3 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 to obtain very fine powders. The aqueous extracts were prepared by suspending 60g, 100g, and 140g powders of each of the extract types separately in 100mls 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 double-layered muslin cloth. This process was repeated to obtain the 3 concentration levels for the study. Based on lack of literature as to the most ideal concentration levels for studies involving botanicals under natural environmental conditions, the highest concentration level of suspending 140g of Moringa extract into 100mls of distilled water was included in this study. The aqueous extracts were the 60%, 100%, and 140% concentration sprays which were then foliar applied to the cabbage plants to run off point.

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 before sowing. 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 obtained from 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 cabbage 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.

2.3 Cabbage Bacterial Inoculation and Data Collection

To inoculate with *Xanthomonas campestris pv campestris* bacteria, the fully expanded top two leaves at the center 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 7 weeks after planting. The data collection exercise was initiated then, and was done on a weekly basis thereafter for the duration of the

study. Totally defoliated plants were counted to evaluate the suppressive efficacy of the different Moringa plant 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 14thEdition. The means were separated using LSD (Least Significant Difference) at 5% level where there were significant differences.

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, flavonoid and tanin which is consistent with the findings by [23].

3. Results and Discussion

3.1 Results

The three Moringa extracts (bark, leaf, and seed) showed great significance ($P < 0.05$) in their antibacterial activity by controlling black rot disease (*Xanthomonas campestris pv campestris)* in this field study. The highest significance was recorded during the 10th 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 Moringa extract types were significantly different from each other in their antibacterial effects on the black rot disease ($P < 0.05$) as indicated by the least leaf defoliation occurring in cabbages under the Moringa seed treatment. Cabbages which were under the Moringa bark extract treatments exhibited an intermediate defoliation rate into the 10th week after emergence, after having succumbed the greatest to the *Xanthomonas campestris pv campestris* bacterium initially. The highest rate of leaf defoliation was exhibited in cabbages under the Moringa leaf extract treatment. Interestingly there were no observed interactions occurring between extract concentration level and ability to suppress *Xanthomonas campestris pv campestris* black rot disease among all the three Moringa extract types. However cabbages under the 60% concentration level for all the three Moringa plant extract type were surpassed in their antibacterial action by the 100% and 140% concentration levels. These two (100 and 140% levels) were not significantly different in their antibacterial action against the test pathogen (Plates 1 – 4) as indicated by the observations obtained in this study.

Table 2: Means showing Effect of Moringa Extracts on *Xanthomonas campestris pv campestris* disease severity in Cabbages

Treatment	Severity Tracking 7WAE	Severity Tracking 8WAE	Severity Tracking 9WAE	Severity Tracking 10WAE
Bark Extract	4.042	2.762	2.92	3.312[b#]
Leaf Extract	2.014	2.969	3.148	3.486[c]
Seed Extract	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: WAE = Weeks After crop Emergence

The results indicated an interesting trend in which the Moringa bark extract at 5 Weeks After crop Emergence (WAE), had the largest *Xanthomonas campestris pv campestris* mean defoliation level compared to both the Moringa leaf and the seed extracts (Figure 1). However by the 8th WAE, its efficacy at suppressing *Xanthomonas campestris pv campestris* disease severity had increased such that it was performing comparably well in relation to Moringa seed extract. At the end of the 10th WAE, Moringa bark extract had managed to surpass the antibacterial 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 *Xanthomonas campestris pv campestris (P < 0.05)*. 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).

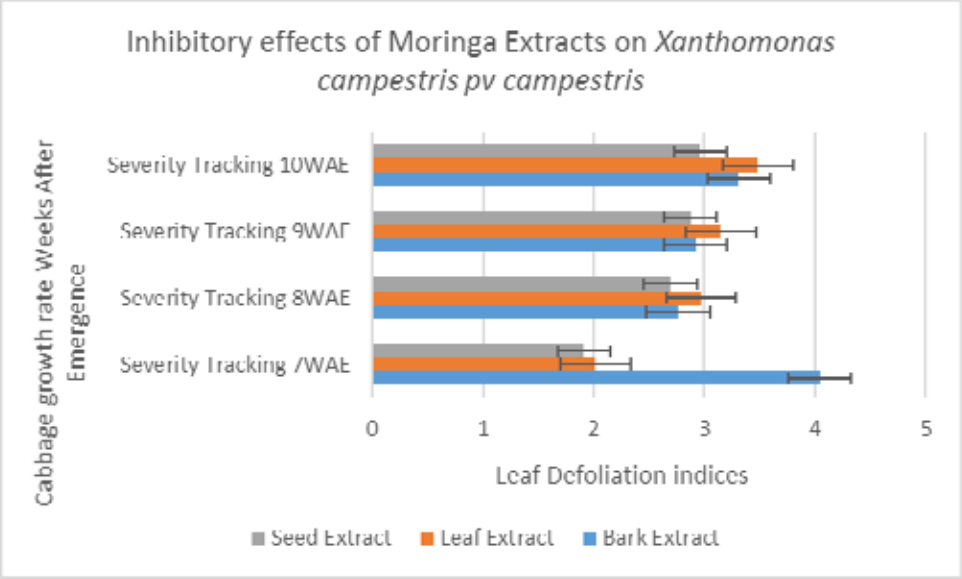
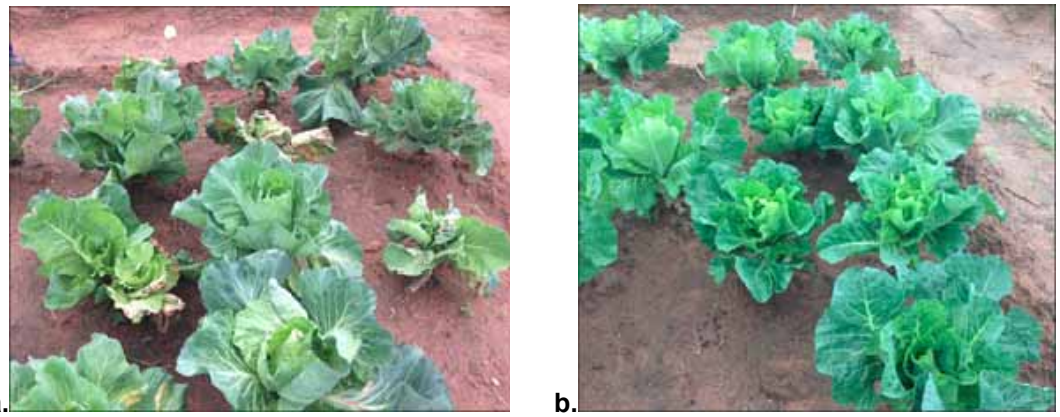


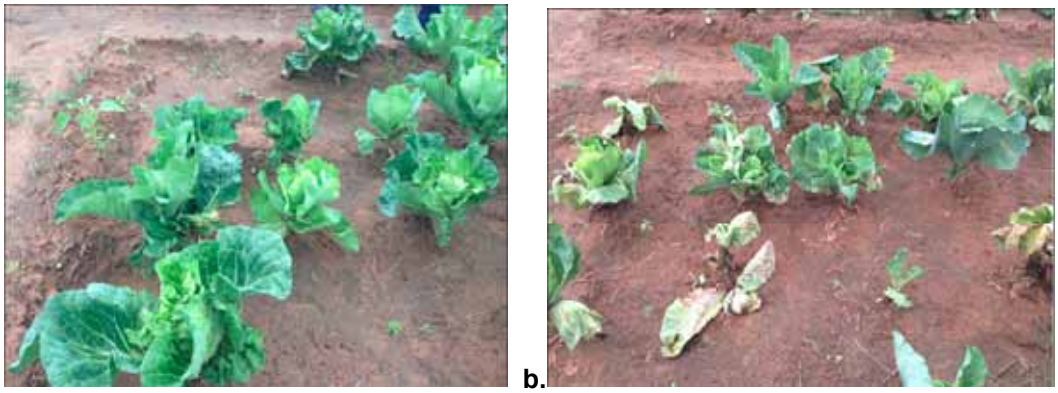
Figure 1: Effect of Moringa seed, leaf and bark extracts on mean cabbage leaf defoliation at 7 - 10 weeks after emergence (Bars at LSD = 0.05). *P* = 0.009. KEY: WAE – Weeks After crop Emergence.

The Moringa extract type effects were significantly different from each other (P < 0.05), 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.

Plates 1a and b: Antibacterial effect of Moringa seed extract in suppressing black rot disease (*Xanthomonas campestris pv campestris*) in field grown Cabbages at 100% and 140% levels respectively.



Plates 2a and b: Antibacterial effect of Moringa bark extract in suppressing black rot disease (*Xanthomonas campestris pv campestris*) in field grown Cabbages at 100% and 140% levels respectively.



Plates 3a and 3: Antibacterial effect of Moringa leaf extract in suppressing black rot disease (*Xanthomonas campestris pv campestris*) in field grown Cabbages at 100% and 140% levels respectively

Plates 4 (a) Moringa seed extract, (b) Moringa bark extract, (c) Moringa leaf extract: exhibiting physiological disorders arising from *Xanthomonas campestris pv campestris* from top left to top right insets; (d) Moringa seed extract, (e) Moringa bark extract and (f) Moringa leaf extract from bottom left to right insets: Showing the secondary infections and rotting effects of *Xanthomonas campestris pv campestris* in cabbage plants. The antibacterial activity was not effective for all the Moringa extract types at 60% concentration levels for all the inset pictures provided.

3.2 Discussion

The presence of bioactive compounds such as phenylpropanoids, alkaloids, chromene neolignans, flavonoid, and tanin 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 only supportive of the findings in this study, but also validate that Moringa is a plant which has antibacterial properties as indicated by its ability to suppress black rot progression in this 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 bio-pesticide 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 *Xanthomonas campestris pv campestris* since this was an open field experiment, where the cabbage plants were exposed to natural conditions. 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] states 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 in 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 [29]. 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. Studies have 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 [31]. 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 *Xanthomonas campestris pv campestris* in this study. Furthermore studies revealed that Moringa seed contains approximately 49.8 – 57.25% oil with undetectable levels of linolenic acid which makes Moringa oil one of the most stable plant oil [27]. 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 aided in keeping the antibacterial action of the seed extract stable and efficient against the *Xanthomonas campestris pv campestris*.

The observations obtained in this study are supported by several studies which have indicated the bacteriolytic effect of Moringa on upon viable myxococci enterobacteria in an invitro study [32], with pterygospermin bioactive compound being identified as being an antibacterial principle acting against a range of bacterial pathogens (Oxford and Singh 1946)[33]. Furthermore, Moringa leaf and seed extracts have been used to inhibit bacterial growth effectively based on their anti-Quorum-Sensing (anti QS) ability, which causes them to exhibit bacteriocidal properties [34]. All these further validate the antibacterial properties observed in this current study. Further studies have indicated the aqueous Moringa leaf extracts as being effective in inhibiting growth of *Escherichia coli* and *Salmonella typhi,* showing diameter zones of inhibition of 20±0.03mm and 18±0.01mm respectively, however, the *Psuedomonas. aeruginosa* pathogen was resistant to the antibacterial action of the Moringa leaf extract [35]. These results are consistent with the findings in this current study whereby Moringa leaf extract has managed to exhibit very low antibacterial action against the test pathogen. It has been further proven that, the part of the plant tissue of Moringa extract being used, and seasonal changes in its growing environment, influence levels of the phytochemical and bioactive compounds present at the time of utilization. This particular study showed that winter samples of Moringa leaf, stem and bark extracts had higher ash, calcium and phenolic compounds and stronger antioxidant activity compared to the winter extracts. However, the Moringa stalk had lower ash, whilst the leaf had lower phenolic compounds. These same studies further revealed that the trend of the antioxidant activity was a function of the part of the

Moringa plant, and it was exhibited thus: leaf > stem > bark, with the Moringa bark extract showing low hydrogen peroxide scavenging activity and low Superoxide Dismutase (SOD) activity in comparison to the other Moringa plant tissues under study [36]. These indicate the potential differences which exist in the percentages of bioactive compounds present in the Moringa plant extracts as influenced by seasonality and plant part positioning on the Moringa plant. This would explain the differences observed in the rate of antibacterial action efficacy shown by the bark, seed, and leaf extracts against the test pathogen in this study. It might also explain why the Moringa leaf extract in this study showed the highest level of disease severity among the cabbages being sprayed with leaf extract. The low amounts of phenolic compounds might have reduced the antibacterial efficacy of the leaf extracts in this current study. It has been argued that Moringa seed extracts contain antibacterial properties which compete favorably with modern day antibiotics in human health pathogens. Studies have indicated that Moringa seed extract contains a niaziridin-rich extract fraction, which enhances the bioactivity of several antibiotics (rifampicin, tetra cycline and ampicillin) that were effective against bacteria [37]. This fact might aid in explaining why the observations in this current study showed that Moringa seed extract exhibited the highest antibacterial action against *Xanthomonas campestris pv campestris* by achieving the least mean number of defoliated cabbage leaves in this current study.

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 the natural phytochemical composition of the Moringa extracts, 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 show thatt *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 in cabbages produced under natural, field conditions. There is need to validate how these same Moringa extracts would perform in other host plants grown under natural conditions. 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 many crops of economic importance and locally in Zimbabwe, organic farmers are the most affected by it as they grapple with the realities of failing to salvage any marketable produce. Hence the need to identify alternative biologically based, non-chemical disease control strategies in Zimbabwe. This work is a step towards that need.

CONSENT

Not applicable for this study**.**

ETHICAL APPROVAL

Not applicable for this study

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