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Rhizoctonia solani Control in Field Grown Cabbage (*Brassica oleracea*) Using *Moringa oleifera* Extracts, Beatrice, Zimbabwe

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Authors' contributions

This work was carried out in collaboration between all authors. Author MG designed the study, carried out the field work, collected the field data, analyzed the data and wrote the first draft of the manuscript. Author PM managed the literature searches and the whole experimental process, proof-read the final draft manuscript and author AG identified the species of plant pathogens, developed the laboratory processes and edited the final draft manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the antifungal activity of *Moringa oleifera* leaf, seed, and bark extracts in suppressing *Rhizoctonia solani* disease in field grown cabbage (*Brassica oleracea*).
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 in the November 2015 to April 2016 season at Victory Farm in Beatrice, Zimbabwe to evaluate the efficacy of *Moringa oleifera* leaf, bark and seed aqueous extracts in controlling bottom rot caused by *Rhizoctonia solani* in cabbages.
Methodology: Bottom rot and root rot diseases are mainly caused by soil-borne pathogens such as *Rhizoctonia solani*. The fungal pathogen was isolated from diseased samples, identified and

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cultured. Cabbage plants were inoculated with the pathogens 5 weeks after crop emergence. Three Moringa extract concentrations of 60%, 100%, and 140% were sprayed as foliar applications weekly from week 7 after crop emergence until the week 11 after crop emergence. The antifungal activity for each of the different Moringa extract efficacy was evaluated by recording number of totally defoliated plants once every week for the duration of the study.

Results: Moringa extracts were significant in reducing the growth of fungi in cabbages (*P* = 0.05). The leaf and seed extracts which were not significantly different form each other in their antifungal activity. They both revealed a high level of control of *Rhizoctonia solani* with indice means of 1.552 and 1.697 respectively *(P* = 0.05*)*. The bark extract with a mean of 2.075 differed significantly from the leaf and seed extract in its antifungal properties (*P* = 0.05) and had the highest disease mean.

Conclusion: *Rhizoctonia solani* fungi growth on cabbage can be effectively reduced by using either seed or leaf extract sprays. Moringa seed and leaf extracts contain antifungal properties which suppressed *R. solani* progression in field grown cabbage.

Keywords: Moringa plant extracts; antifungal; leaf defoliation rate.

1. INTRODUCTION

Rhizoctonia solani is a very common soil inhabitant with a wide host range among which are lettuce *(Lactuca sativa)*, rape (*Brassica napus)*, cabbage (*Brassica olearacea*), potato (*Solanum tuberosum*), onion (*Allium cepa)*, dry bean *(Phaseolus vulgaris)*, wheat (*Triticum spp*), corn (*Zea mays*), and several weeds [1]. It is a fungal pathogen of economic importance worldwide [2].The pathogen survives between host crops such as cabbages, as sclerotia or mycelium in soil and crop debris and can spread into fields by wind- or water-disseminated spores (basidiospores); it is both seed borne and soil borne and present in most soils [3]. The fungus *Rhizoctonia solani*, not only causes *Rhizoctonia* root or bottom rots, and is also a key part of a group of fungi responsible for damping off disease in most crop seedlings, but it also causes stem, bottom and root rot diseases [4]. *R. solani* is grouped among notorious soil-borne fungal pathogens which are difficult to control because there are no registered fungicides for its effective control [5]. Control of *R. solani* among horticultural crops is made even more difficult because these crops are normally grown on a large scale using monoculture production systems, making conditions even more conducive for this soil inhabiting pathogen [6]. Current strategies being employed to manage this pathogen is by intensive alternation of various chemicals which is proving ineffective [7]. The intensified fungicide usage has resulted in fungi resistance to chemicals and detrimental health issues to animal, fauna and humans [8]. Not only has chemical usage led to environmental concerns and degradation, it has also resulted in a shift in disease dynamics, with minor problem diseases emerging to become major epidemics as a result of chemicals now being developed for particular targeted pathogens [8]. *R. solani* causes significant losses in cabbages, which results in lowered returns per unit area, despite intensified fungicide usage among vegetable market gardeners in Zimbabwe.

Cabbages are an important leafy vegetable in Zimbabwe as they are used for many social gatherings and functions such as weddings, funerals and also in many restaurants and food outlets. Cabbages are also an important crop for vegetable growers in small scale, peri-urban and back yard market gardeners in Zimbabwe as they are an alternative source of nutrition for sustained livelihoods [9]. Leafy vegetables in Zimbabwe among the peri-urban, small scale and market gardening growers, are economically important as a source of income for marginalised and resource poor families [10]. Cabbages are listed second after Rape (*Brassica napus*) among the most commonly grown exotic leafy vegetables in Zimbabwe [11], because they are an alternative and cheaper source of the side dish (relish), which accompanies the Zimbabwean staple food, maize corn (*Zea mays)* thick porridge. However, the major constraint being faced by this group of leafy vegetable growers is that of disease epidemics mainly due to them being resource poor farmers who can only manage to acquire low amounts of agricultural inputs towards their farming activities [12]. These farmers also possess very little knowledge on implementing integrated pest management (IPM) strategies and as a result, they intensify usage of one chemical resulting in resistance build up and further crop yield losses [13]. There is great need therefore to train vegetable farmers in IPM strategies which should

include use and adoption of natural bio-agents in disease management, which would be a cheaper alternative for them. Small scale commercial farmers in Zimbabwe currently, make up the majority of leafy vegetable producers and this group of farmers face major financial constraints [14], which prevent them from effectively implementing proper chemical disease control schedules.

The detrimental impacts to the environment as a result of extensive chemical usage, coupled with the financial constraints faced by the majority of leafy vegetable growers, indicates the need to identify alternative, environmentally sustaining strategies to manage *R. solani* infestations in cropping areas. These methods should not necessarily disturb the ecosystem and the balance of nature but should be sustainable crop protection systems such as use of biological methods. *Moringa oleifera*, a multipurpose tree with antimicrobial properties [15] can be one such disease management strategy. There is strong need to identify and develop natural biocontrol agents in disease management as these are environmentally friendly [16] and will aid in reducing the carbon foot print in the face of climate change. Agriculture is changing fast and with it, the agro-landscape in which diseases occur in response to population growth. In reaction to the high demand for agricultural production due to population expansion, farmers are resorting to new, more novel methods of dealing with disease dynamics [17]. These methods include breeding of more resilient, adaptable crop varieties which are also bred for particular disease resistance traits, however, despite all these efforts, disease epidemics are still occurring worldwide for instance the rhizomania disease of sugar beet in the UK, citrus canker in Florida and the cassava mosaic in West Africa [17]. It therefore is evident that the pathogen-specific chemicals which are currently in use, are not being effective because of their specificity, the need for natural, bio-pesticide strategies is fast becoming a reality and the best possible alternative for broader disease control strategies. It is argued that the need to identify best chemical, genetic and biologically sustainable strategies for disease management is remarkably high [17]. Biological control strategies using natural bioactive compounds present in many medicinal plants need to be identified and developed for large scale application [18]. Natural biocontrol agents are more environmentally friendly, have different modes of action thus do not result in pathogen resistance developing, and pose less risk to humans and other life forms [19].

In particular, studies involving Moringa in pathogen suppression, most of which are invitro, have revealed the ability of Moringa to suppress growth of fungal pathogens such as *Fusarium solani, Fusarium oxysporum* and bacterial pathogens such as *Shigella shingi, Pseudomonas aeruginosa* and *Shigella sonnei* [20]. There is need to validate these antifungal properties in field trials involving horticultural crops and pathogens of economic importance, of which *R. solani* is one such pathogen. Moringa is the plant of choice based on its documented antimicrobial properties [20] and the fact that it has naturalized locally in Zimbabwe [21], thus making it easily accessible to resource poor farmers as well.

This study aimed to evaluate the efficacy of Moringa leaf, seed, and bark on suppressing bottom rot disease caused by *R. solani* in field grown cabbages.

2. MATERIALS AND METHODS

2.1 Experimental Site and Methodology

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 1900 msl and receives an average rainfall of 450 -600 mm/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 *R. solani*. This farmer is an organic farmer who uses organic fertilizers and natural botanicals to manage pests and diseases in his field crops.

2.2 Fungal 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 [22]. 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 60 g, 100 g and 140 g powders of each of the extracts separately in 100 mls 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. 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 4 m x 2 m. The beds were pre-watered to field capacity. The cabbage seeds were sown at a rate of 3 kg /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 Fungal Inoculation and Data Collection

To inoculate with *R. solani*, the top most two fully expanded leaves of each cabbage seedling were bruised using a sterilized 5mm gauge hypodermic needle, 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 fungus was sprayed onto each individual plant using 1 liter hand sprayers to run off point. The inoculation was done at 5 weeks after crop emergence. Disease severity or severity tracking (ST) for *R. solani* were collected based on the scoring in Table 1.

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. 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.

Table 1. Scoring used for *R. solani* leaf defoliation assessment in cabbage (*Brassica capitata var. sugar loaf*)

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 [23]

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of Moringa extracts on *R. solani* disease progression

Moringa extracts were significant (p = 0.05) in reducing the growth of fungi in cabbages (p = 0.05). The leaf and seed extracts which were not significantly different from each other in their antifungal activity, revealed high level of control of *Rhizoctonia solani* with means of 1.552 and 1.697 respectively. The bark extract with a mean of 2.075 differed significantly from the leaf and seed extract in its antifungal properties, and exhibited the lowest ability to suppress *R. Solani* in field grown cabbage (p = 0.05) (Table 2).

3.1.2 Antifungal activity of Moringa plant extracts on *R. solani*

However, the results also indicated that from week 10 after crop emergence, the antifungal action of all the extracts (leaf, bark, and seed) was not being exhibited upon the test pathogen, and the means were increasing. Moringa leaf extract exhibited the least antifungal action on *R. solani* from 10 weeks after crop emergence into 11 weeks after crop emergence (Fig. 1).

Table 2. Moringa leaf, seed and bark extract effect on cabbage leaf defoliation on *Rhizoctonia solani*

Treatment	8WAE	9WAE	10WAE	11WAE
Bark extract	1.9	2.075[b]	2.762	2.893
Leaf extract	1.41	1.552[a]	2.969	3.157
Seed extract	1.52	1.697[a]	2.694	2.923
P Value	0.205	0.047	0.105	0.518
Least significant difference 5%	1.021	0.614	0.5278	0.7281
Covariance %	11	4.8	2.5	7.1

 # Means with similar letters are not significantly different at P = 0.05.
Key: WAE = Leaf defoliation indices 8 – 11 Weeks After crop Emergence

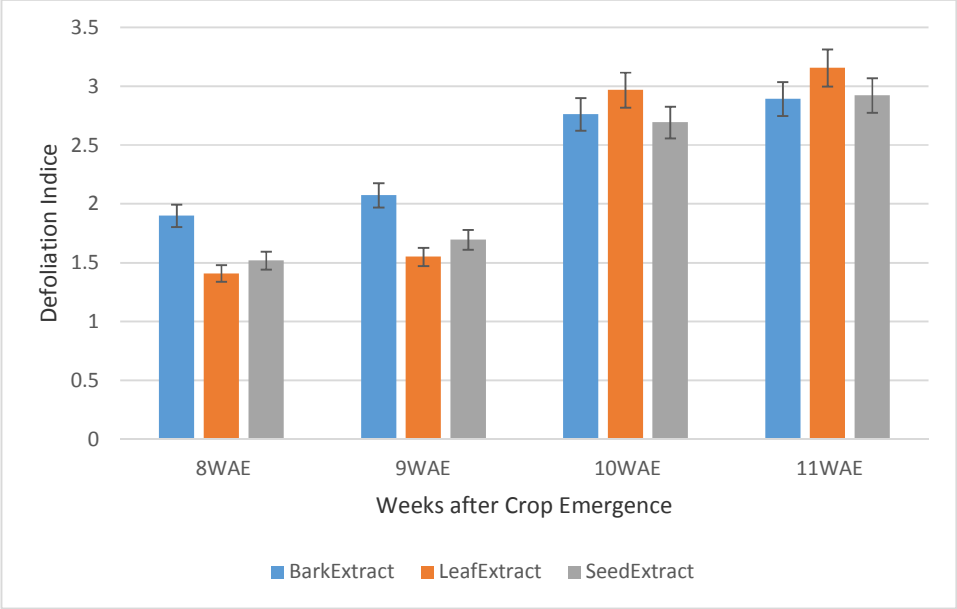

Fig. 1. Effect of Moringa seed, leaf and bark extracts on mean cabbage leaf defoliation at 8 - 11 weeks after emergence (bars at LSD *P* = 0.05)
Key: 8 – 11 Weeks after Emergence

3.2 Discussion

3.2.1 Effect of Moringa extracts on *R. solani* disease

Moringa seed and leaf extracts were able to suppress *R. solani* disease progression during week 8 and 9 after crop emergence due to the presence of flavonoids and phenolic compounds which infer antimicrobial activity to the extracts [24]. Moringa seed contains a chitin binding protein (Mo_CBP_3) which inhibited the growth of *R. solani, F. oxysporum* but not *Pythium oligandrum* by causing structural plasma membrane disarrangement in pathogen cells [25]. The same effect might have occurred in this current study. Further studies indicated that the Mo_CBP_3 might have caused permeabilization of *F. solani* cells and interference with the plasma membrane [26]. However, the actual antifungal mechanism is not fully understood and also there is need to study the relationship between Mo_CBP_3 binding proteins, with other carbohydrate binding domain families, as this relationship is unknown [26]. The mechanism of how the antifungal activity is exhibited needs further research because further studies state that the Mo_CBP_3 acts by inhibiting germination and mycelial growth of phytopathogenic fungi [27]. Other studies have indicated the ability of Moringa extracts to achieve interference with metabolic pathways of the pathogen, thus achieving control [28]. There arises therefore, the need for further study to identify the mechanism of the antifungal action and how it relates to other protein binding compounds within the host plant.

The ability of the Moringa seed extract to suppress *R. solani* at a rate higher than the other Moringa plant part extracts, might have been as a result of the seed possessing a thermostable protein which is resistant to pH changes [23].Thus given the fluctuations in weather patterns which are experienced between November (rainy season – hot, wet and humid for prolonged periods) and April (towards cooler periods, less rainfall) [29] in Zimbabwe, it is possible that the effectiveness of the leaf and seed extracts were reduced in their virulence due to fluctuating weather conditions from week 10 after crop emergence onwards. Heat has been shown to reduce the efficacy of the antifungal property of Moringa leaf extract as evidenced by failure of Moringa leaf extract to show any inhibitory effect on *Shigella shinga*, *Pseudomonas aeruginoso* and *Shigella sonnei* after having used hot water in their extraction process [24]. This observation indicates the need for further study to determine the best extraction method for the Moringa plant extracts which do not reduce the antifungal activity of the extracts and the most ideal application stage of plant growth of these extracts. The aspect which needs to be determined is the level of pulverization which is most ideal to enhance the extractive process of the bioactive properties of the Moringa extracts. Studies have indicated level of Moringa powder pulverization as having an influence on the strength of extraction solution attained [30]. The rate of Moringa extract pulverization was not taken into consideration during the present study, hence the differences in the particle sizes of the powders used in three extract processes, might have influenced the rate of extraction of the bioactive compounds for each of the different Moringa plant parts used in the study.

3.2.2 Antifungal activity of Moringa plant extracts on *R. solani*

The different levels of antifungal activity exhibited by Moringa extracts validate the fact that each Moringa plant part has got varying percentages of different bioactive compounds which influence their antimicrobial properties. Moringa seed is the only part of the plant which contained the highest concentrations of 4-(-α-l-rhamnopyranosyloxy)-benzylglucosinolate, the roots contained high concentrations of both 4-(-α-l-rhamnopyranosyloxy)-benzylglucosinolate 4-(-α-l-rhamnopyranosyloxy)-benzylglucosinolate and benzyl glucosinolate, the leaves contained 4-(-α-l-rhamnopyranosyloxy)-benzylglucosinolate and three monoacetyl isomers of the benzyl glucosinolate whilst the bark contained only trace amounts of 4-(-α-l-rhamnopyranosyloxy)-benzylglucosinolate [28]. Moringa leaves further revealed presence of quercetin-3-O-glucoside [31]. The phytochemical composition of each of the compounds might affect the antifungal efficacy of each extract, hence the differences in levels of antifungal action exhibited against *R. solani* in this current study. The inability of bark extracts to have high levels of antifungal effect on *R. solani*, are consistent with other studies in which the Moringa bark extracts did not exhibit any inhibitory effect on *Aspergillus* and *Penicillium* fungal *spp* in an invitro assessment [32]. Moringa plant therefore, contains different concentrations and types of bioactive compounds depending on the plant part under study.

Further studies have indicated that different phytochemical properties are present in each different plant part, occurring in varying proportions which might affect their antifungal efficacy as observed in this current study [33]. The observations of this current study are supportive of other findings which indicated the antifungal and antibacterial properties of Moringa in an invitro study [34]. The efficacy of Moringa plant extracts antimicrobial activities were further validated in other *in vitro* studies were *Pseudomonas aeruginosa,Colletotrichum spp*, *Staphylococcus aureus,* and *Bacillus subtilis, vibrio* were suppressed by the antibacterial action of Moringa. These same studies exhibited Moringa extracts as having antifungal activity against pathogenic fungi *Alternaria spp, Colletotrichum spp, Curvularia spp* and *Fusarium spp* [35]. These antimicrobial properties of Moringa plant extracts are further validated by [36], who investigated and summarized on the pharmacological properties of Moringa constituents against similar test organisms and recorded their ability to inhibit microbial growth in invitro studies done. The antimicrobial properties of Moringa plant extracts are being further utilized to reduce bacterial contamination of water. The efficacy of Moringa antibacterial action reduced growth of *Escherichia coli* (a gram negative) and *Bacillus subtilis* (a gram-positive) bacterial pathogens in contaminated water to levels of up to 93%, making the water potable [37]. However, the increase in studies relating to Moringa antimicrobial properties is mainly in artificially controlled and created environments, there is need to carry out more agricultural field trials in natural, cropping

environments to validate these results. The other area of study would be on identifying extraction methods which would not render these antimicrobial actions of Moringa plant extracts ineffective. According to studies done, Moringa leaf cold water extracts were able inhibit *Shigello shinga, Pseudomonas aeroginosa* and *Shigello sonnei pathogens* growth, whilst the hot water extracts did not exhibit any inhibitory action against the test pathogens [38]. This should be an area of concern in terms of identifying sustainable, low-cost and easy extraction methods which would enhance, and not damage these natural antimicrobial properties of *Moringa oleifera* plant extracts.

4. CONCLUSION

Moringa leaf and seed extracts can be effectively used in disease management strategies to control diseases such as damping off, root and stem rots caused by *Rhizoctonia solani* in cabbages (*Brassica oleracea*). However, further studies are needed to verify extraction and application methods and stage of plant growth which would improve efficacy of these antifungal properties. Further studies are also needed to determine level of plant extract pulverization for most effective extraction of bioactive phytochemicals from Moringa extracts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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