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Moringa oleifera Extracts Effect on *Fusarium solani* and *Rhizoctonia solani* Growth

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Authors' contributions

This work was carried out in collaboration between all authors. Author MG designed and carried out the field study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PM and AG managed the analyses of the study and supervised the phytochemical analysis of the Moringa plant extract materials used in this study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: An *in vitro* study was conducted to test the effect of concentration levels of *Moringa oleifera* leaf and seed extracts in controlling the growth of *Rhizoctonia solani* and *Fusarium solani* pathogens.
Study Design: The experimental design was a 2*7 factorial laid out in a Completely Randomized Design. Potato Dextrose Agar was amended with Moringa leaf extract and seed extract, and mycelial growth of *R. solani* and *F. solani* were measured.
Place and Duration of Study: University of Zimbabwe pathology laboratory during 2014/ 2015 season.
Methodology: The concentrations levels of 10%, 15%, 20%, 25% and 30% from each extract were used. Distilled water (0%) was used as negative control, whilst 10% copper oxychloride was the positive control. Potato Dextrose Agar was amended with Moringa leaf extract and seed extract, and mycelial growth of *R. solani* and *F. solani* were measured.

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Results: All extracts showed a significant effect on reducing fungal growth (*P*=0.05). The higher the extract concentration level, the less the mycelial growth and no mycelial growth occurred on the positive control (10% copper oxychloride). Maximum percentages of inhibition of 45 and 50% was recorded against *R*. *solani* using Moringa seed extract at 25 and 30% concentrations, respectively. Both Moringa extracts gave 50% inhibition growth of *F*. *solani* at the 30% concentration level.
Conclusion: Moringa leaf and seed extracts contain antifungal properties which inhibited growth of *R*. *solani* and *F. solani*. Moringa extract concentration levels influenced the antifungal efficacy of the extracts, with higher concentration levels exhibiting an increased antifungal ability against the test pathogens. The phytochemical analysis of Moringa leaves and seed solvent extracts showed presence of alkaloids, flavonoids, glycosides, tanin and phenolic compounds, terpenoids, etc.

Keywords: Moringa extract concentration; antifungal; Rhizoctonia solani; Fusarium solani.

1. INTRODUCTION

Fusarium solani and *Rhizoctonia solani* are important pathogens that infect many different genera of plants; They mainly cause serious root rot and stem rot on vegetables and ornamentals [1]. Root-rot caused by *Fusarium solani* is considered among the most deleterious diseases, resulting in major losses in brassicas and cruciferous vegetables in many parts of the world [2]. *Fusarium* species play an important role as plant pathogens, causing a wide range of disease such as vascular wilts and stem rots in a diversity of hosts [3]. The fungus *Rhizoctonia solani* causes damping-off and wire stem of cabbage, cauliflower, other crucifer seedlings in the seedbed; and bottom rot and head rot of older plants in the field [4]. Further studies have indicated *F. solani* and *R. solani* as being the most important soil borne fungal pathogens which cause the majority of the devastating diseases which exhibit symptoms of bottom rot and root rot diseases to a wide range of vegetables and other cruciferous crop plants including lettuce [5]. *Fusarium solani* and *Rhizoctonia solani* are listed among the major problematic soil-borne plant pathogens causing drastic reductions in yield and quality of crops in agricultural production world-wide and are also responsible for causing damping off, root-rots and wilts of vegetables [6]. The deleterious effects of these harmful parasites on crop stand, quantity and the quality of crops produced has resulted in an intensive usage of fungicides as a way of managing them. However, these management strategies are not only ineffective in achieving satisfactory control of the root diseases, but the intensity of their usage has raised concerns over their negative impact on the environment, fauna and flora, and human health [6]. This has also impacted negatively on overall productivity of cropping enterprises as a result of the increased input costs acquiring fungicides. There arises an urgent need therefore, to identify alternative methods and strategies to manage these soil-borne pathogens which are environmentally friendly and cost-effective in the long run. In order to identify such alternative strategies, medicinal plants such as *Moringa oleifera* need to be researched upon and validations made regards their antimicrobial properties [7]. This aim of this study was to evaluate the efficacy of Moringa leaf and seed extracts against *Fusarium solani* and *Rhizoctonia solani* as an *in vitro* experiment. There was also the need to determine at which concentration level the extract was most effective in inhibiting pathogenicity of the test pathogens. Validation of the antimicrobial action of the Moringa leaf and seed extracts would then enable further research into improving on extraction, identification and proper isolation of the bioactive compounds to enhance their effectiveness in field applications in agriculture.

Moringa is a multi-purpose tree which is being utilized for various applications in industry, traditionally and recently, a lot of research has gone into its medicinal use against human pathogens [8]. However, not much research has been carried out regards the efficacy of Moringa as a Natural Bio-Agent against devastating crop pathogens of economic importance [9]. Research on Moringa medicinal properties and its effectiveness against human diseases, its benefits to human and animal health and on human pathogens has been accumulating over the years as researchers are more fascinated with its anti-carcinogenic, antiulcer properties, and improved human life [10-12]. Further studies have revealed Moringa as an ideal supplementation in livestock feed formulations as a result of its high nutritional constituent [13] and other yield benefits such as increased milk production dairy animals [14]. The focus therefore has been, and continues to be, on

improving human livelihoods and health using natural plant sources and this has overshadowed its importance and potential application as a bio-pesticide. Besides being packed with antioxidants, minerals, vitamins and having proven medicinal value for both human and animal benefit [15], Moringa can be effectively utilized as a natural bio-pesticide and should therefore, be included in integrated pest management strategies. Moringa can also be used as the crop disease management strategy in organic farming systems due to the various bioactive ingredients it contains, that act in different ways against pathogenic infection within the plant [16] which might even prove more effective. Intensive and continued fungicide usage is associated with development of resistance by fungi to systemic fungicides and the specificity of fungicide formulations which affect only one pathway in the biosynthesis of fungi pathogens [17], processes which reduce the efficacy of fungicides. Hence, use of biological agents such as Moringa in control of soil-borne fungi pathogens notorious for root-rot diseases, might prove to be more effective compared to fungicides [6]. Not only are these bio-agents environmentally friendly compared to chemical methods, they have been used in various *in vitro* studies to effectively inhibit pathogen growth [18].

Moringa has been used in an *in vitro* study, for the control of *Rhizopus* pathogen which is a problem organism causing food spoilage and huge losses [19], with many other *in vitro* studies exhibiting Moringa efficacy against disease pathogens, such as; *Salmonella typhi, Citrobactor spp, Shigella dysenteriae, Escherichia coli, Salmonella paratyphi* and *Pseudomonas aeruginosa* [20]. Due to increased negative impacts associated with pesticides usage, much attention is being focused on alternative methods of pathogen control. There is a need to examine possible non-synthetic chemical approaches for disease management [21] in agricultural applications. Research has demonstrated that biological disease control is a potentially feasible alternative to the use of pesticides [22]. *Moringa oleifera* leaf extracts in combination with other bio-control agents have been successfully used as a seed treatment against *Scerotinia rolfsii* which causes damping off and stem rot in cowpea [23]. Based on these preliminary studies, it is imperative that Moringa bioactive agents efficacy be evaluated *in vitro* against a wide range of crop pathogens which are deleterious in their impact on quantity and quality of produce and also which have proven to easily develop fungicide resistance (e.g. *Fusarium solani* and *Rhizoctonia solani,* the study organisms in this study). It is only after *in vitro* studies have validated the effectiveness of Moringa bioactive agents against crop pathogens, can it then be effectively utilized by developing and adapting efficient extraction methods, correct formulations and determine correct application methods, rates and intervals. There are still so many areas of research regards *Moringa oleifera* based on its constituents, botany and phytochemical traits which need to be carried out to improve utilization of the bioactive phytochemicals present in Moringa as a bio-pesticide and a natural bio-agent in agricultural applications. Furthermore, the Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals that can inhibit the development of these pathogenic fungi [24] once adequate studies have been exhausted in field research.

2. MATERIALS AND METHODS

2.1 Experimental Design

The experimental design which was used to carry out this study was a 2*7 factorial laid out in Completely Randomized Design replicated three times. Factor A was *Moringa oleifera* leaf and seed extracts at two levels; factor B was the concentration levels of the extracts (10, 15, 20, 25, and 30%). Negative control 0% concentration level and positive control 10% copper oxychloride (this is the main fungicide used locally for fungal disease control by most farmers in Zimbabwe). The Moringa leaf and seed plant extracts were analyzed using the GM/GC technique using three different solvents to identify the bioactive compounds and phytochemical constituents.

2.2 Preparation of Medium and Sub-culturing

Medium was prepared by mixing 54 grams Potato Dextrose Agar (PDA) with 1000ml distilled water, in sterilised conical flasks. The medium was later autoclaved at 121℃ and 15 psi for 20 minutes and left to cool before pouring into 9 cm petri dishes. Pouring was done under aseptic conditions using the lamina flow cabinet and 10 ml of medium were poured in each petri-dish and stored for future use.

2.2.1 Isolation of *Fusarium solani*

The pathogen was isolated from cabbage which had yellows symptoms. The infected leaves were surface sterilized in 10 percent hypochlorite for 5 minutes and rinsed three times in sterile water and blot dried with sterile filter paper. The pieces were plated onto Potato Dextrose Agar and incubated at 25°C for 7 days. The *F. solani* was then purified by sub-culturing the whitish cottony mycelium with some tinge of pink [25] and canoe shaped conidia which are slightly curved and septate or aseptate.

2.2.2 Inoculum preparation

One-week old culture of *F. solani* was used. The culture was carefully teased with a sterile inoculating loop to dislodge the spores. A concentration of approximately 10^8 cfu per ml sterile distilled water was used. An Improved Neuber Haemocytometer was used to count the spores. The fungal strains were then introduced into the medium by cutting a 5 mm square disc from pure culture of the strain and placing them upside down in PDA and incubated at 25℃.

2.3 Preparation of *Moringa oleifera* Seed and Leaf Extracts

The leaves and seeds for preparing the Moringa extracts were obtained from Herbal Health Centre, an organic grower of Moringa and herbal plants in Harare. Both leaves and seeds were first thoroughly washed with distilled water to remove any dust debris and later air dried at room temperature under dark conditions for seven days. After drying, both the leaves and seeds were ground to a fine powder using mortar and pestle as described by [26].

Aqueous extracts were prepared by separately mixing grounded seed and leaf with distilled water. Leaf and seed powder weighing 10, 15, 20, 25 and 30 grams were placed in sterilised beakers and 100 ml of distilled water was added to make 10, 15, 20, 25 and 30% extracts as described by [27]. The mixtures were then stirred using a sterile glass rod and allowed to stand at room temperature for 24 hours. The extracts were separately filtered through a sterile Whatman number 1 filter paper placed in a funnel as described by [28]. The extracts were further filtered through the microfilter for further purification to avoid contamination.

The five concentrations for each plant part and one control (distilled water) were mixed with prepared PDA in separate sterile petri dishes and later inoculated with fungal pathogens. 1 ml extract of each concentration (10, 15, 20, 25 and 30%) of seed and leaf *M. oleifera* were added in each petri-dish containing potato dextrose agar. Fungal strains were inoculated by cutting a square piece from a pure culture and placing it centrally upside down into the PDA (Fig. 1). The plates were later incubated at 25℃ after which they were examined daily for the presence or absence of fungal growth.

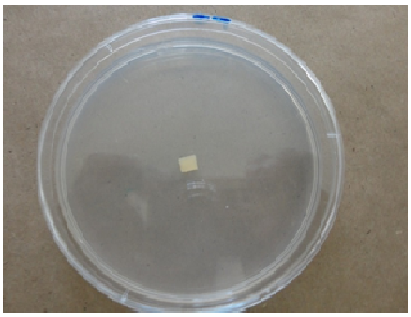

Fig. 1. Fungal plating

The Moringa leaf and seed extracts were analyzed using the GC/MS method to identify the bioactive and phytochemical compounds of the Zimbabwean Moringa accessions as described by [29]. The analysis was carried out at the Central Analytical Facilities, Stellenbosch University in the Mass spectrometry Unit, at Matieland, South Africa. Two different solvents (dichloromethane (DCM), methanol (Me OH)) were used for the plant extraction process namely, solid phase micro extraction (SPME).

2.4 Mycelia Growth of the Fungal Pathogen

Mycelia growth of the fungal pathogens was measured in millimetres once a day for 7 days. Observations on colony diameter for the fungal pathogen were recorded and percent growth inhibition was worked out by using the following formula suggested by [28]

$$\text{Percentage inhibition} = \frac{R_{1-R_2}}{R_1} \times \frac{100}{1} \qquad (1)$$

Where,

R_1 = Furthest radial distance of fungus in control plates (PDA only).

R_2= Furthest radial distance of fungus in treatment plates.

2.5 Data Analysis

The effectiveness of *Moringa oleifera* leaf and seed extracts was analysed using Genstat (version 14) statistical package and significant results were subjected to a LSD test at 5% significance level.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of concentration levels of Moringa extracts on radial growth of *R. solani* and *F. solani*

The effect of concentration levels was significant (*P*<0.001) on pathogen growth mean of *R. solani* and *F*. s*olani* compared to the control (Table 1). The effect of extract was more pronounced at higher concentrations: the higher the extract concentration the less the mycelia growth and least colony mycelium growth was formed on PDA amended with copper oxychloride (10 ml). There was no significant difference for Moringa extracts of 25 and 30% on radial growth of *F*. *solani*.

Table 1. Effect of concentration levels on radial growth of *R. solani* and *F. solani* growth

Concentration levels (%)	Radial growth (cm)	
	R. Solani	***F. Solani***
Distilled water (0)	7.82^{f}	10.62^{f}
Copper oxychloride (10)	0.06^{a}	0.50^{a}
Moringa extracts (10)	6.36^{e}	7.91^{e}
Moringa extracts (15)	5.32^{d}	7.52^{d}
Moringa extracts (20)	4.70^{c}	6.77^{c}
Moringa extracts (25)	4.35^{bc}	6.35^{b}
Moringa extracts(30)	4.05^{b}	6.06^{b}
P-value	P<0.001	P<0.001
L.S.D	0.5	0.42
C.V%	11.2	7.3

NB: means with the same letter are not statistically different from each other at P = 0.05

3.1.2 Interaction of Moringa extracts and concentration levels on radial growth of *F. solani*

Results in Fig. 2 indicated that there was significant interaction effect of Moringa extracts and concentration levels (*P*<0.01), with decrease in *F. solani* growth with increase in extract concentration. Moringa seed extracts showed better inhibition of radial growth of *F. solani* at concentration levels of 20 and 25%. There was no significant effect on pathogen growth for both extracts at concentration levels of 10 and 15%.

3.1.3 Effect of concentration levels of Moringa extracts on percent inhibition of *R. solani*

The recorded percent growth inhibition for *R. solani* is presented in Table 2. There was significant (*P*<0.001) response on the effect of concentration levels of Moringa extracts on pathogen growth. Moringa extracts at 30% concentrations showed stronger antifungal activity against *R. solani* than all other concentrations. Moringa extracts of 10% concentration levels had the least percent growth inhibition as compared to the positive control. The highest percent inhibition growth was recorded on the positive control with 10% copper oxychloride.

Table 2. Effect of concentration levels on percent inhibition of *R. Solani*

Concentration (%)	Percent inhibition ***R. solani***
Distilled water (0)	0^{a}
Copper oxychloride (10)	93.97^{g}
Moringa extracts (10)	19.33^{b}
Moringa extracts (15)	33.26^{c}
Moringa extracts (20)	40.44^{d}
Moringa extracts (25)	45.96^{c}
Moringa extracts(30)	50.33^{f}
P-value	P<0.001
L.S.D	2.8
C.V%	14.4

NB: means with the same letter are not statistically different from each other at P = 0.05

3.1.4 Effect of Moringa extracts and concentration levels on percent inhibition *of F. solani*

The observations indicated that leaf and seed extracts significantly had an effect on the growth of *Fusarium solani* (*P*<0.001) at different concentration levels (Fig. 3). However, seed extract exhibited better growth inhibition compared to leaf extract at all concentration levels. Moringa extracts inhibitory effects on *F. solani* growth were more pronounced at their higher concentration levels, the higher the concentration the lower the percentage inhibition growth recorded.

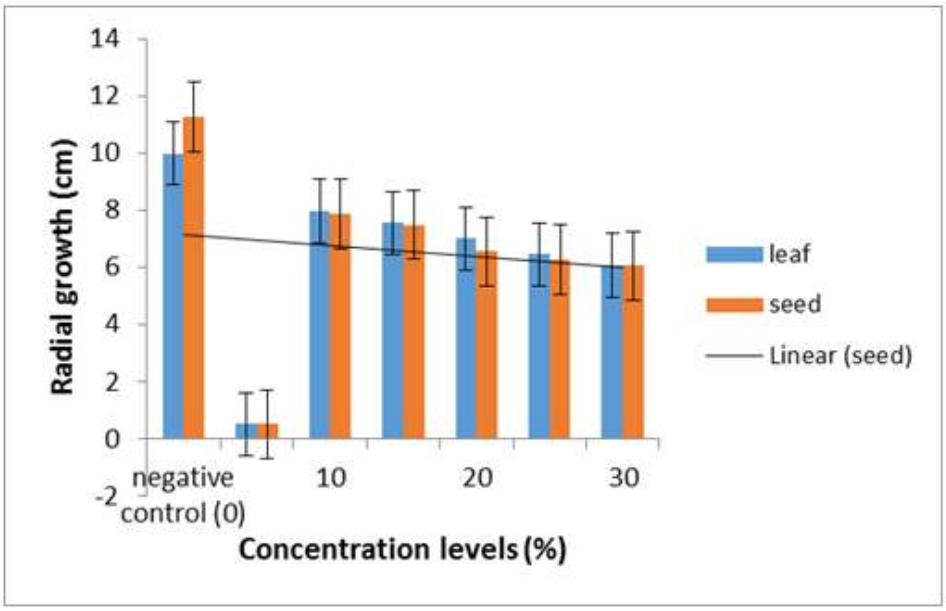

Fig. 2. Interaction effects between Moringa extracts and radial growth of *F. solani*, June 2015
Bars represent least significant difference at P = 0.05

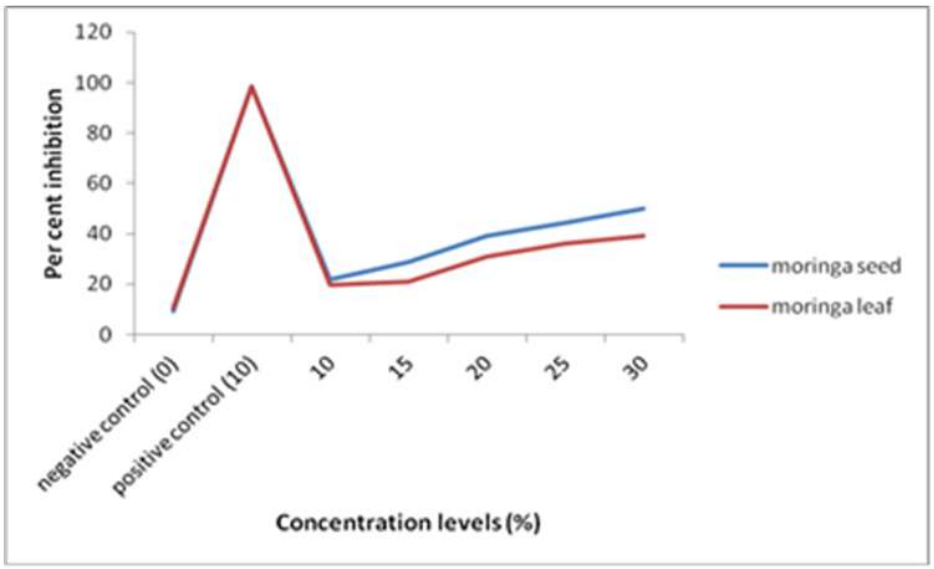

Fig. 3. Effect of Moringa extracts and concentration levels on percentage inhibition of *Fusarium solani*, June 2015
Bars represent least significant difference at P = 0.05

3.2 Discussion

Seed and leaf extracts both exhibited antifungal activity against both fungal pathogens, *Rhizoctonia solani* and *Fusarium solani* at all extract concentration levels. However, leaf extract was not as effective in its inhibitory properties compared to the seed extract, leading to the assumption that the seed contained more antifungal bioactive compounds which made it more effective. This could be because seed extract is said to contain more of *Pterygospermin* which is a fungicidal compound [7]. Results further indicated a reduction in pathogen growth

occurring gradually, with increase in the concentration levels of extracts in the growth medium. The increase of the concentration of the extract implied an increase in the active ingredients of the bioactive compounds which acted upon the fungus thereby affecting its physiological processes and consequently lowering the growth of the fungus. Studies using Moringa extracts, indicated antioxidant and anti-inflammatory activity as being influenced by the dosage rate used; with the higher dosage (300 mg/kg) exhibiting enhanced activity over the lower dosage (100 mg/kg) in the test subjects [30], These findings are also consistent with work done by [31] which revealed higher protection against castor-oil induced diarrhoea at dosages of 800 mg/kg compared with 400 mg/kg using *Detarium microcarpum* plant extracts as antidiarrheal agents. The highest records of reduction on radial growth of pathogen were recorded for Moringa seed and leaf extract at a concentration of 30%.

Moringa oleifera leaves contain some crystalline alkaloids, fatty acid, proteins, glycosides and niazirins which are believed to possess antimicrobial properties [32]. The phytochemical analysis which was carried out for the Moringa plant extracts used in this study revealed the presence of a wide range of bioactive compounds; among which were flavonoids/bioflavonoids such as 2-phenylchromen-4-one (2-phenyl-l,4-benzonpyrone), 3- phenylchromen-4-one (3-phenyl-l, 4-benzonpyrone), 4-phenylcoumarine (4-phenyl-l, 2-benzopyrone) and catechins (e.g. Flavan-3-ols) which possess antimicrobial properties [7]. As a result of these antimicrobial bioactive compounds present in the Moringa extracts, they were able to inhibit the growth of the test pathogens, albeit at different levels of efficacy. This difference might be explained by presence of higher amounts of Moringa oil in the Moringa seed extracts compared to the leaf extracts. Moringa oil is used extensively in the cosmetic industry for perfumery and skin products because it has a very good propensity to bind with other molecules (making it a good perfume base), and as such makes it a very good moisturizing agent as well [33]. These properties of Moringa oil are enhanced by their stability due to the presence of linoleic acid, its non-sticking ability, which are further enhanced by its ability to resist rancidity [34]. The higher ability of the Moringa seed extracts to inhibit pathogen growth, can therefore, be attributed to the seed extract being able to have enhanced and prolonged contact with the test pathogens within the PDA since it could easily be absorbed, enabling it to spread faster and more easily within the infected media due to its ability to bind readily with other molecules. This fact then improves its antifungal efficacy due to its more fluid viscosity, and longer more effective spread and contact time with the test pathogens. The presence of saponins in the Moringa extracts might also explain their ability to inhibit fungal growth. Studies done by [35] revealed plant saponins to exhibit direct cytotoxic effects, a fact which might have been employed as an antifungal mechanism within the test pathogen cells. Furthermore, the presence of alkaloids such as the isoquinones, indoles, pyridines and benzyl-lisoquinolines present in both leaf and seed extracts, are known to infer inhibitory activity against several pathogens [36]. In this current study, Moringa seed extract exhibited strong antifungal action against *R. solani* and *F. solani,* these are not entirely supported by work done by [37] whose findings indicated very strong activity against *Botrytis cinerea,* yet very weak activity against *R. solani, C. orbiculare* and *S. sclerotiorum* using a bioactive secondary metabolite isolated from the Moringa roots.

Moringa oleifera is a multipurpose tree with a whole wide range of applications such as industrial, medicinal, nutritional, traditional and ayurverdic [38,39]. However, despite all the research which is being focused on utilizations of Moringa, there are so many areas which are yet to be scientifically validated. For instance the greater percentage of Moringa research is focused on its nutritive, medicinal and health value to humans and livestock, with its phytochemical screening and analysis targeting these particular areas of interest, which then leaves a void in terms of how the nature and composition of Moringa can also be effectively applied to improving agricultural productivity, bearing in mind the need to use sustainable and environmentally friendly farming practices.

4. CONCLUSION

Moringa leaf and seed extracts can be effectively utilized as antifungal biological agents against *R. solani* and *F. solani*. However, these *in vitro* studies need to be validated in field conditions to enable assessment of their efficacy within natural elements in the open fields.

For Moringa to be used effectively as a natural biopesticide, many aspects of its phytochemistry,

mode of action upon crop pathogens once it has been applied to crops, cost-effective extraction methods which take cogniscence of how easily Moringa denatures once exposed to harsh solvent and extraction processes, need to be taken into consideration. Research has to shy away from isolating, characterizing and multiplying these phytochemicals aimed at reproducing them synthetically, thus altering the very code of these elements, and instead aim to study this plant in a bid to minimise modifying it and its natural properties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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