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Evaluation of Antifungal Activity of *Moringa oleifera* Extracts as Natural Fungicide against Some Plant Pathogenic Fungi *In-vitro*

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Abstract Fungicidal effect of *Morienga oleifera* plant parts extract such as roots, leaves and pod coats was investigated *in vitro* against seven phytopathogenic fungi i.e., *Fusarium oxysporum, Fusarium solani, Alternaria solani , Alternaria alternata , Rhizoctonia solani , Sclerotium rolfsii and Macrophomina phaseolina*. Roots, leaves and pod coats extracts of *Moringa oleifera* significantly reduced radial growth, spore germination and dry mycelia yield of all tested pathogens. *M. oleifera* extracts had different degrees of antifungal activity against tested pathogens. Reduction effect on test pathogens was increased by the increase concentrations of *M. oleifera* extracts. The highest reduction records on radial growth, spore/sclerotia and dry mycelia weight of all tested pathogens were at 20%, 20 % and 50% concentrations of roots, leaves and pod coats extracts respectively. *F. oxysporum , F. solani* and *A. solani , A. alternate* were highly affected by *M. oleifera* extracts than *R. solani , S. rolfsii and M. phaseolina* . Roots and leaves extracts of moringa *oleifera* may be recommended as a potent bio-fungicide. This is a preliminary study on the use of *M. oleifera* extracts as natural fungicide against plant pathogens in Egypt. Extensive studies should be undertaken for the root and leaves extracts of *Moringa oleifera* as strong antifungal agents against fungal plant diseases in future studies.

Keywords: *Moringa oleifera* , phytopathogenic fungi , Antifungal activity , spore germination.

Introduction

Since fungicides are very expensive and cause serious environmental pollution, control strategies are today directed towards replacing the use of hazardous chemical fungicides by environmentally friendly natural products (Mamdouh and Eweis, 2007) .The plant world is a rich store house of natural chemicals that could be exploited for use as pesticides (Satish *et al.*, 2009).

Botanicals are now emerging as safer and more compatible approach to control phytopathogens. Higher plants are known to express fungitoxicity

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against spore germination and mycelial growth of phytopathogenic fungi (Talreia, 2010; El-Mohamedy *et al*., 2013) .Fungal infections cause significant loss in many economic crops. Crop losses are estimated to be about 14% worldwide (Agrios, 2005).

Chemical control may be available to effectively and extensively reduce the effects of most fungal disease but field application of these chemical fungicides may not always be desirable. Excessive and improper use of these fungicides presents a danger to the health of humans, animals, and the environment. Therefore, extensive searches for biofugicides that are environmentally safe and easily biodegradable have been carried out during the last two decades (Gnanamanickam, 2002).The investigation of plants containing natural antimicrobial metabolites for plant protection has been identified as a desirable method of disease control (Rai and Carpinella, 2006; Seema *et al.,* 2011; Seint and Masera, 2011; Dwivsdi and Neeta, 2012). The use of plant extracts and biocontrol agents have been seen as a viable method for controlling plant diseases. Various plant products like plant extracts, essential oils, gums, resins *etc.* were shown to exert biological activity *in vitro* and *in vivo* and are used as bio-fungicidal compounds (Fawzi *et al*., 2009; Al-Askar and Rashad, 2010).

Moringa oleifera Lam. (Family: Moringaceae) has gained much importance in the recent days due to its multiple used and benefits to agriculture and industry (Ashfag *et al*., 2012). Regarded as a miracle plant, all the parts of moringa plant are used for medicinal and other purposes. Roots ,flowers , bark ,stem ,leaves and seeds of moringa possess antimicrobial properties (Anjorin *et al.,* 2010, Dwivedi and Enespa, 2012).Recently, the roles of aqueous extracts of various parts in enhancing plant growth and productivity have been explored, making it even more valuable plant species (Fahey, 2005).

The fungicidal effect of Moringa extracts on some soil-borne fungi such as *Rhizoctonia, Pythium* and *Fusarium* was recorded by many investigators (Moyo *et al*., 2012). Dwivedi and Enespa (2012) indicate that *Moringa oleifera* extracts (leaves, bark and seeds) 75 % (v/v) showed significant inhibition in the mycelial growth of *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* . Leaves of *M. oleifera* are rich in zeatin, a cytokinin in addition to other growth enhancing compounds like ascorbates, phenolic and minerals like Ca, K, and Fe that makes it an excellent crop growth enhancer. *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitsterol, caffeoylquinic acid and kaempferol which have antifungal and antibacterial activities (Anjorin *et al.,* 2010). Moringa leaf extract is best used as plant growth enhancer, insect repellent and fungicide (Phiri and Mbewe, 2010). Adandonon *et al*. (2006) tested the aqueous Moringa Seed Extracts (AMSE) as a bio fungicide to replace

the synthetic ones which are currently being used to devise an organic approach. They also noted that AMSE with Apron Plus, and distilled water (medium for extraction of moringa seeds) were more effective as bio fungicide on groundnut seed pathogens Moyo *et al*. (2012) reported that acetone extract of *M. oleifera leaves* have antibacterial activity against some bacterial strains whereas both acetone and aqueous extracts have not exhibited any antifungal activity against *Candida albicans, Penicillium notatum, Aspergillus flavus* and *A. niger* even at 10 mg/ml concentration . Jamil *et al*., (2010) tested the efficacy of plants extract including *M. oleifera* and reported that selected plant extracts showed antifungal activity against *Mucar mucedo* and *Aspergillus niger* more strongly than *Aspergillus tamari* and *Rhizoctonia solani.* .on the other hands Seeama *et al*. (2011) noted that aqueous extract of *Piper betel* can be recommended to the farmers for the control of *Rhizoctonia solani.* Siva *et al*. (2008) reported that *Ocimum sanctum* (water extract method) showed 96% inhibition against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L .*Moringa oliefera* leaves extracts have been successfully used as seed treatment against some soil borne fungi (Talreia, 2010; Yasmeen *et al*., 2011; Foidle, *et al.*, 2001). The fruit extract of *Moringa oleifera* showed a broad-spectrum antibacterial *Staphylococcus aureus, Bacillus subtilis, Vibrio cholera, Bacillus cereus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Klebsiella species* and *Proteus species* and antifungal activity against pathogenic fungi- *Alternaria* sp, *Colletotrichum* sp, *Curvularia* spp and *Fusarium* sp. activity and antifungal activity (Mohammed *et al.,* 2012). The aim of this work was to investigate the antifungal activity of different extracts of *Moringia olelfera in vitr* on the growth and sporulation of some important phytopathogenic *Fusarium oxysporum , Fusarium solani , Alternaria solani , Alternaria alternata, Rhizoctonia solani , Sclerotium rolfsii , and Macrophomina phaseolina.*

Materials and methods

Plant pathogenic fungi

This study was carried out at the Department of Plant Pathology , National Research Center , Egypt . Seven plant pathogenic fungi i.e., *Fusarium oxysporum , Fusarium solani , Alternaria solani , Alternaria alternata, Rhizoctonia solani , Sclerotium rolfsii* and *Macrophomina phaseolina* were maintained and grown on potato dextrose agar medium.. These pathogens were isolated and identified at Plant Pathology Department. National Research Center , Cairo , Egypt , the pathogenicty of each fungi were tested and recorded in previous studies (El-Mohamedy *et al*., 2013).

Plant materials

The source of *Moringa oleifera* leaves , roots and pud coats were kindly obtained from Egyptian Scientific Society of Moringa (ESSM) ,National Research Center , Dokki , Cairo , Egypt.

Preparation of moringa extracts

The collected plant parts (leaves ,roots and pod coats) of *Moringa oleifera* were washed under tap water followed by distilled water and dried in shade . Dried samples were powdered in mixer/grinder. Powders of leaves ,roots and pod coats was mixed individually with distilled water in a ratio of 1:1 (w/v) and left overnight to allow the constituents to get dissolved in water, then filtered through muslin cloth and 100% plant extract solution was prepared. The extract obtained was subjected to the vacuum filtration followed by shaking. The processed 100 % extracts were poured in the Erlenmeyer flasks, plugged with cotton separately and heated at 50^{o}C for 15 minutes to avoid contamination (Madavi *et al*., 2005). The extracts were further diluted to different concentrations by adding distilled sterile water for further use in the experiment.

Antifungal activity assay

Antifungal activity of aqueous extracts of *Moringa oliefera* plant parts such as roots, leaves and pod coats against seven plant pathogenic fungi was carried out by poison food technique (Nene and Thaplyal, 1979).

Effect of moringa extracts against mycelia growth

Antifungal activity of moringa roots, leaves and pod coats extracts were performed by the agar medium assay. Potato dextrose agar (PDA) medium with different concentrations i.e., 5 , 10 ,15 , 20 , 25 % of moringa root extract ,10 , 20 , 30 , 40 and 50 % concentrations of moringa leaves and pod coats extracts were prepared by adding appropriate quantity of moringa each extract to melted medium . About 20 ml of the supplemented medium with each moringa extract was poured into glass Petri-dishes (9 cm x 1.5 cm) . A 6 mm diameter agar disk bearing hyphae of either *Fusarium oxysporum , Fusarium solani , Alternaria solani ,Alternaria alternata, Rhizoctonia solani , Sclerotium rolfsii* or *Macrophomina phaseolina* from 7-days-old colonies grown on PDA medium

was transferred at the centre of each Petri-dish. Positive control (without moringa extracts) plates were inoculated following the same procedure. Plates were incubated at 25 ℃ for 8 days and the colony diameter was recorded each day. Minimal inhibitory concentration (MIC) was defined as the lowest concentration of moringa oil in which no growth occurred. The MGI (Mycelia Growth Inhibition) percentage was calculated and expressed as percentage of reduction. The fungal toxicity of the extracts in terms of % inhibition of mycelial growth was calculated using the following formula:

% inhibition = dc – dt /dc x 100, where dc = average increase in mycelial growth in control, dt = average increase in mycelia growth in treatment.

Effect of moringa extracts against mycelia dry mass

The aqueous extracts of *Moringa oliefera* plant parts such as roots, leaves and pod coats were mixed aseptically with sterilized Potato broth medium to produce concentrations 0.5 ,1.0 ,1.5 ,2.0 ,2.5 % and 5 , 10 , 15 , 20 , 25 % of oil and seed extract respectively and dispensed in 50 mL aliquots into 250 mL Erlenmeyer flask. A 6 mm diameter agar disk bearing hyphae of either *F. oxysporum , F. solani , A. solani , A. alternate , R. solani , S. rolfsii* or *M. phaseolina* from 7-days-old colonies grown on PDA medium was transferred to each flask and incubated at 27±1 ℃ for 10 days. Five flasks were prepared for each treatment .The mycelia were harvested, dried to constant weight at 80±1 ℃, the dry mass yield was recorded and the percentage of reduction in mass production was calculated

Effect of moringa roots, leaves and pod coats extracts against spore and sclerotia germination

Sclerotia of *R. solani, S. rolfsii and M. phaseolina* produced on potato dextrose agar (PDA) were collected and surface disinfected by soaking them for 5min in 1:400 (w/v) bromine in water to kill hyphal extension, washed thoroughly with distilled water and dried. Ten sclerotia /Petri dish for either pathogen were plated on the surface of tap water agar (1.5% w/v) supplemented with different concentrations of oil and/or seed extract of moringa maintained above. The dishes were incubated at 27±1 ℃ for 24 h the percentage of germinated sclerotia were determined and five plates were prepared for each treatment.

For *F. oxysporum, F. solani , A. solani , A. alternate*, microscope slides were covered, each with 1 mL of spores suspension of each pathogen in aqueous solution of the desired oil and/or seed extract concentrations in Petri

dishes and then incubated at 27±1 ℃ for 8 h in complete darkness. Five plates were preparewd for each treatment and the means were compared.

Statistical analyses

All data were subjected to analysis of variance using the statistical analysis software (Co Stat, 2005). Comparisons among means were made using Duncan's multiple range test (Duncan, 1955).

Results

Fungicidal effect of *Morienga oleifera* plant parts extract such as roots , leaves and pod coats against mycelia radial growth, spore /sclerotia germination and dry weight mycelia yield on seven pathogenic plant fungi i.e., *Fusarium oxysporum, Fusarium solani, Alternaria solani, Alternaria alternata, Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* was observed at different concentrations of *M. oleifera* extracts in *vitro* .

Effect of Moringa oleifera roots extract(MRE)

Efficiency of *M. oleifera* roots extract (MRE) at different concentrations (5 ,10 , 15 , 20 , 25% v/v) in decrease linear growth, spore / sclerotia germination and dry weight mycelia yield of tested pathogenic plant fungi was investigated.

Results in Table (1) showed that fungal mycelia growth gradually decreased with increase in concentration of moringa roots extract (MRE).

These results confirmed that moringa roots extract at all tested concentration had antifungal activity against the most root rot and foliar disease pathogens under this investigation. Moringa roots extract at 25% was completely inhibit the linear growth of *F. oxysporum* ; *F. solani* ; *A. solani* and *A. alternata* .Meanwhile , *R. solani* , *S. rolfsii* and *M. phaseolina* showed growth reduction reach to 94.2 , 90.0 , and 87.4 % respectively at 25% MRE concentration . Moringa roots extracts at 15 and 20 % cause reduction in mycelia radial growth of all tested pathogens reach to 56.8 – 78.2 % and 71.8 – 90.8 %. Whereas, at low concentrations of MRE , least reduction records of mycelia radial growth were noticed at 5 % (20.0 – 48.8 %) and 43.2 – 63.4 % at 10 % concentration.

While, MRE at 5 % and 10 % concentrations had the minimum inhibitory effect against all tested pathogens especially on mycelial growth of *S. rolfsii* (21.8 % and 44.4 %) and *M. phaseolina* (20.0 % and 43.2 %). Meanwhile, MRE at 15 % concentration cause considerable inhibitory effect on all tested pathogens, as the reduction effect ranged from 56.8 % to 78.2 %. Mycelial

growth of *F. oxysporum, F. solani , A. solani , A. alternata* showed more sensitivity to MRE than *R. solani , S. rolfsii* and *M. phaseolina.*

Table 1. Efficacy of *M. oleifera* roots extract against some phtyopathogenic fungi on PDA medium

Pathogenic fungi	*M. oleifera* roots extract concentration %											
	Control		5%		10%		15%		20%		25%	
	D	I	D	I	D	I	D	I	D	I	D	I
Fusarium oxysporum	90a	0.0	46b	48.8	33c	63.4	20d	78.2	8e	90.8	0e	100
Fusarium solani	90a	0.0	48b	46.2	36c	60.2	22d	75.0	9e	90.0	0e	100
Alternaria solani	90a	0.0	59b	33.6	40c	55.8	27d	70.4	12e	86.6	0f	100
Alternaria alternata	90a	0.0	66b	26.7	44c	51.4	31d	65.0	16e	82.2	0f	100
Rhizoctonia solani	90a	0.0	69b	23.6	48c	46.2	35d	60.8	21e	76.4	6f	94.2
Sclerotium rolfsii	90a	0.0	70b	21.8	50c	44.4	38d	57.4	24e	72.8	9f	90.0
Macrophomina phaseolina	90a	0.0	72b	20.0	51c	43.2	37d	56.8	25e	71.8	11f	87.4

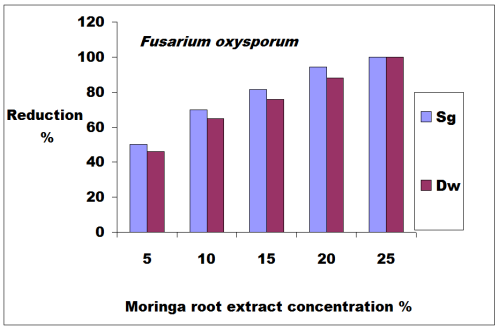
D= colony diameter (mm) I = Inhibition percentage (%) as compared to the control.
Means followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

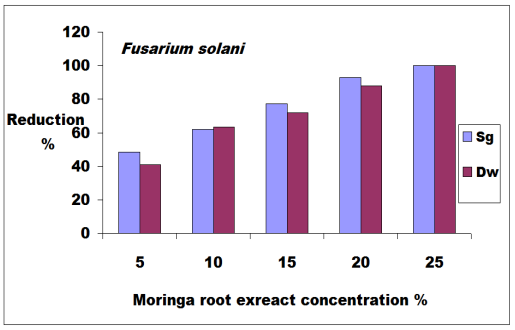
Studies on mycelia yield and spore germination represent and integrate part of the ecological studies of the pathogenic fungi, as spores are the specialized structures capable of initiating new growth. Once germination had occurred, the ensuring mycelia growth rate may be of prime importance in determining the degree of virulence of the fungus concerned. In our studies the antifungal efficacy of moringa oil towards spore germination of seven phytopathogenic fungi was studied *in vitro* and the results are presented in Figure (1).

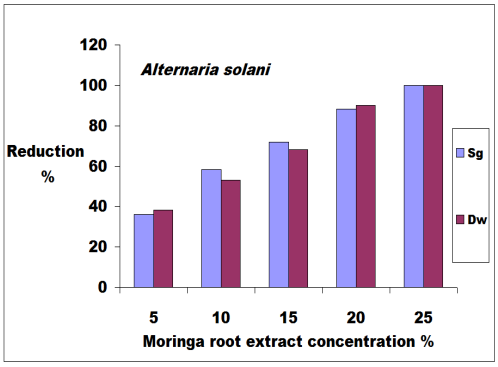
Studies on mycelia yield and spore germination represent and integrate part of the ecological studies of the pathogenic fungi .Different concentrations of moringa roots extract were tested against spore/sclerotia germination and dry mycelia weight of the same seven plant pathogenic fungi *in vitro* . A similar result was also noticed with spore/sclerotia germination and dry weight mycelia yield of all tested pathogens Figure (1).

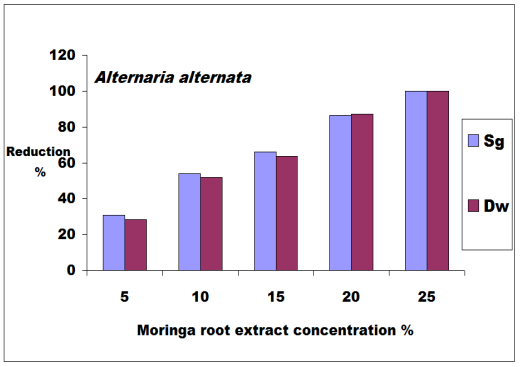
Figure (1) clear show that all concentrations of moringa oil inhibit spore germination of *F. oxysporum , A. solani* and *A. alternata* as well as sclertia germination of *R. solani , S. rolfsii* and *M. phaseolina* with different values .Spore/sclerotia germination and dry mycelia weight were gradually decreased with increase in concentration of *Moringa oliefera* roots extract . Maximum reduction was noticed with 25 % of root extract, as the inhibitory reduction in spore/sclerotia germination and dry mycelia weight of

F.oxysporum, F. solani, A. solani and *A. alternate* was 100 % , but ranged from 83.8 % to 90.0 % and 88.0 % to 92.8 % of spore/sclerotia germination and dry mycelia weight of *R. solani* , *S. rolfsii* and *M. phaseolina* .The extract of moringa roots extract at 15% and 20% were the most effective in decrease spore/sclerotia germination and dry mycelia weight of all tested pathogens .The inhibitory reduction effect were ranged from 53.4% to 81.4% and 67.0% to 94.2% of spore/sclerotia germination at these concentrations, and from 53.6% to 75.0% and 69.2% to 88.8 % of dry mycelia weight . Meanwhile, moringa roots extract at 10% showed the moderate inhibitory effect (39.4% to 70.0% and 40.2% to 64.8%) on both spore/sclerotia germination and dry mycelia weight of all tested pathogens . At the lowest concentration 5% of moringa roots extract , minimum reduction records of spore/sclerotia germination and dry mycelia weight were noticed by *R solani* (25.4% and 22.0%), *S rolfsii* (20.4% and 24.6%) and *M. phaseolina* (19.4% and 22.4%). The most affected pathogens by all concentrations of moringa roots extract were *F. oxysporum* , *F. solani* followed by *A. solani* and *A. alternata, but R. solani* , *S. rolfsii* and *M. phaseolina* showed less sensitivity. Spores germination of *F. oxysporum, F. solani* , *A. solani* and *A. alternata* showed more sensitivity to morigna seed extract than *R. solani* , *S. rolfsii* and *M. phaseolina*.









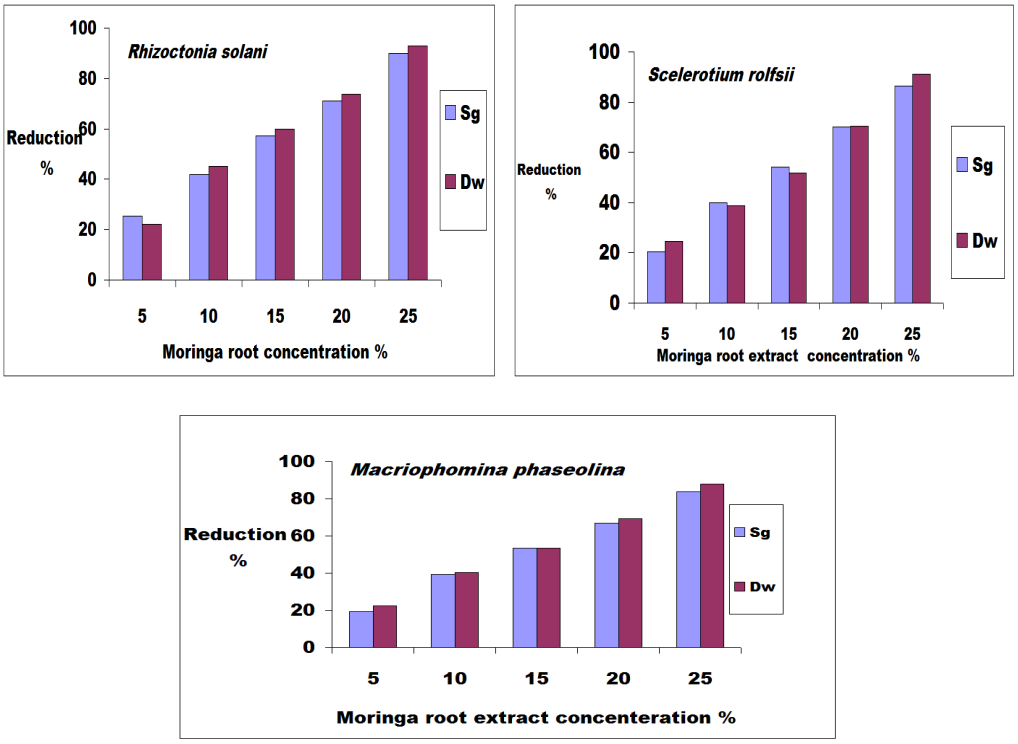


Fig. 1. Reduction % in spore/sclerotia germination (SG) and dry weigh mycelia growth (DW) of seven pathogenic plant fungi as affected by different concentrations of *Moringa oliefera* roots extract *in vitro*.

Effect of M. oleifera leaves extract

Efficiency of *M. oleifera* leaves extract (MLE) at different concentrations i.e., 10 ,20 , 30 , 40 , 20% (v/v) in decrease linear growth, spore / sclerotia germination and dry mycelia weight of the tested pathogenic plant fungi was studied using poisoned food technique.

Results in Table (2) show that all fungal mycelial growth gradually decreased with increase in concentration of moringa leaves extract (MLE). Moringa leaves extract at 50 % was completely inhibit the linear growth of *F. oxysporum* and *F. solani*, and reduced linear growth of *A. solani* , *A. alternata*, *R. solani* , *S. rolfsii* and *M. phaseolina* by 94.8, 91.8 , 90.2 , 88.8. 82.2 % respectively. MLE at 30 % and 40 % concentrations were the most effective causing reduction in mycelia growth ranged from 43.2% to 70.0 % and 62.8% to 88.8% respectively of all tested pathogens. While, the least effect of MLE was noticed at 10 % concentration as inhibitory reduction was ranged from 17.8 to 43.4 % of all tested pathogens. Whereas, moringa leaves extract at 20% concentration showed considerable inhibitory effect against all tested pathogens

(22.0 % to 56.4 %). It is clear to state that moringa leaves extract at all tested concentration had antifungal activity against the most root rot and foliar disease pathogens under this investigation. Mycelial growth of *F. oxysporum, F. solani, A. solani , A. alternata* showed more sensitivity to MRE than *R. solani , S. rolfsii* and *M. phaseolina* .

Table 2. Efficacy of *M. oleifera* leaves extract against some phtyopathogenic fungi on PDA medium

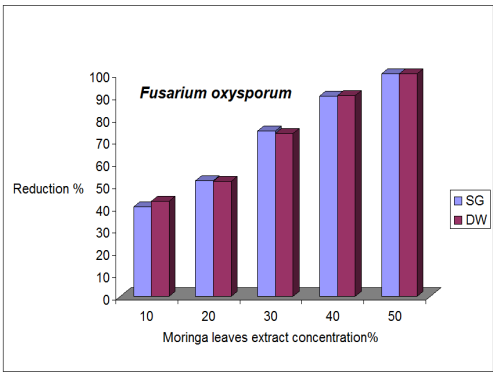
Pathogenic fungi	*M. oleifera* leaves extract concentration %											
	Control		10%		20%		30%		40%		50%	
	D	I	D	I	D	I	D	I	D	I	D	I
Fusarium oxysporum	90a	0.0	51b	43.4	39c	56.4	27d	70.0	10e	88.8	0e	100
Fusarium solani	90a	0.0	53b	40.6	46c	49.2	31d	65.8	11e	88.0	0e	100
Alternaria solani	90a	0.0	62b	30.4	53c	40.8	35d	60.8	15e	82.8	4e	94.8
Alternaria alternata	90a	0.0	68b	24.0	56c	37.6	34d	62.0	17e	80.6	7e	91.8
Rhizoctonia solani	90a	0.0	69b	23.6	62c	30.8	45d	50.0	28e	69.2	9f	90.2
Sclerotium rolfsii	90a	0.0	71b	20.8	65c	27.2	47d	47.2	30e	66.2	10f	88.8
Macrophomina phaseolina	90a	0.0	74b	17.8	70c	22.0	51d	43.2	33e	62.8	16f	82.2

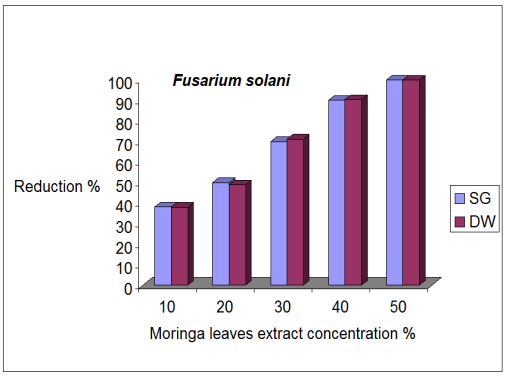
D= colony diameter (mm) I = Inhibition percentage (%) as compared to the control.
Means followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

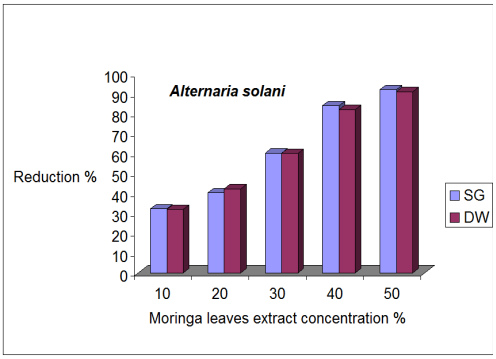
Concerning the effect of *Moringa oliefera* leaves extract (MLE) on spore/sclerotia germination and dry mycelia weight of the same seven plant pathogenic fungi ,different concentrations (10 , 20 , 30 , 40 , 50 %) of MLE were tested . A similar result was also noticed with all tested pathogens Figure (2).

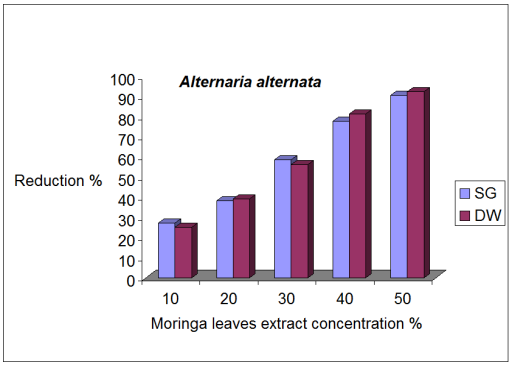
Figure (2) clearly showed that spore/sclerotia germination and dry mycelia weight of all tested pathogens were gradually decreased with increase in concentration of *Moringa oliefera* leaves extract (MLE). MLE at 50 % concentration inhibit spore germination and dry mycelia weight of *F. oxysporum* and *F. solani* by 100 %, *A. solani* by 92.0 % and 90.4 %, *A. alternate* by 91.0 % and 92.2 % respectively. Meanwhile, sclertia germination and dry mycelia weight of *R. solani , S. rolfsii* and *M. phaseolina* decreased by 85.0% , 85.0% , 80.8% and 86.4 % , 85.2% , 80.0 % respectively . While , MLE at 10 % and 20 % concentrations had the minimum inhibitory effect on spore germination and dry mycelia weight of all tested pathogens , especially with *R. solani* (21.2 % and 22.0%), *S. rolfsii* (20.8% and 21.2%) and *M. phaseolina* (16.2% and 19.2%) . The extract of moringa leaves extract at 30 % and 40 % concentrations were the most effective in decrease spore/sclerotia

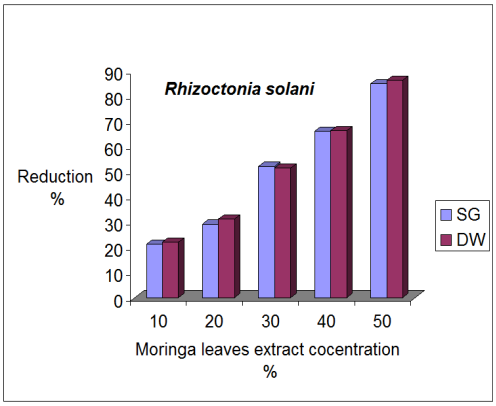
germination and dry mycelia weight of all tested pathogens .The inhibitory reduction effect were ranged from 41.2 % to 74.2 % and 61.0 % to 90.0 % of spore/sclerotia germination at these concentrations, and from 56.0 % to 73.2 % and 60.2 % to 90.2 % of dry mycelia weight .The most affected pathogens by all concentrations of moringa leaves extract were *F. oxysporum* , *F. solani* followed by *A. solani* and *A. alternata, but R. solani* , *S. rolfsii* and *M. phaseolina* showed less sensitivity.

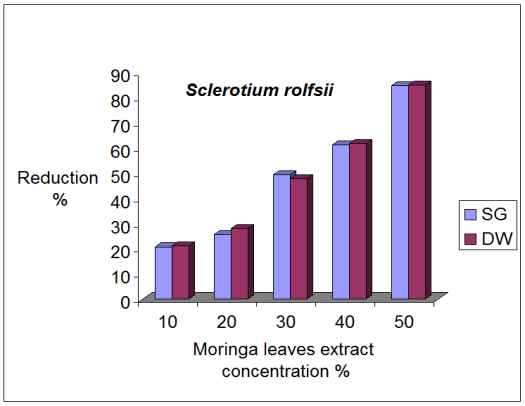












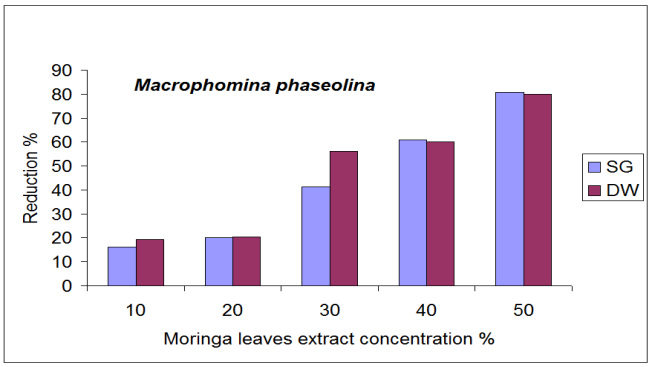


Fig. 2. Reduction % in spore/sclerotia germination (SG) and dry weigh mycelia growth (DW) of seven pathogenic plant fungi as affected by different concentrations of *Moringa oliefera* leaves extract *in vitro.*

Effect of pod coat extract

Efficiency of *M. oleifera* pod coat extract (MBCE) at different concentrations i.e., 10, 20, 30, 40, 50% (v/v) in decrease linear mycelia growth, dry mycelia weight and spore and/or sclerotia germination of the tested pathogenic plant fungi was studied using poisoned food technique.

Results in Table (3) show that all fungal mycelial growth gradually decreased with increase in concentration of moringa pod coat extract (MBCE) . MBCE at 50 % was completely inhibit the linear growth of *F. oxysporum* and *F. solani*, and reduced linear growth of *A. solani, A. alternata, R. solani, S. rolfsii* and *M. phaseolina* by 91.2, 88.2, 88.8, 85.4. 82.2% respectively.

Whereas, the highest records of reduction in mycelia growth of all tested pathogens were noticed with MBCE at 30% and 40% concentrations, as the inhibitory effect in mycelia growth was ranged from 40.8% to 65.4 % and 58.2% to 83.8% respectively of all tested pathogens. The least antifungal effect was noticed at 10% and 20% concentrations of MBCE, as inhibitory reduction was ranged from 13.8 to 36.0% and 21.0% to 42.5% of all tested pathogens, respectively. *F. oxysporum, F. solani* followed by *A. solani* and *A. alternate* showed more sensitivity to MBCE, but *R. solani, S. rolfsii* and *M. phaseolina* were less affected. *M. oleifera* pod coat extract had antifungal activity against the root rot and foliar disease pathogens under this investigation.

Table 3. Efficacy of *M. oleifera* pod coat extract against linear growth (mm) of some phtyopathogenic fungi on PDA medium

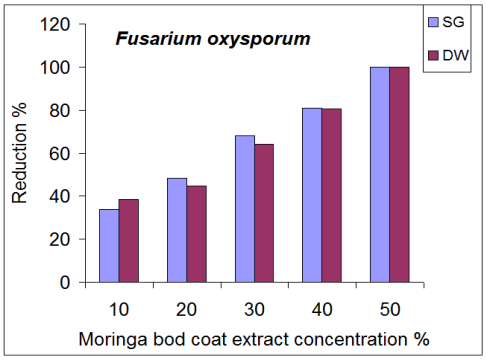
Pathogenic fungi	Concentration %											
	Control		10%		20%		30%		40%		50%	
	D	I	D	I	D	I	D	I	D	I	D	I
Fusarium oxysporum	90a	0.0	58b	36.0	52c	42.5	31d	65.4	15e	83.8	0f	100
Fusarium solani	90a	0.0	61b	32.2	58c	35.8	36d	60.4	17e	80.8	0f	100
Alternaria solani	90a	0.0	64b	28.4	60c	32.8	38d	58.2	21e	76.4	8f	91.2
Alternaria alternata	90a	0.0	65b	28.2	61c	32.0	43d	52.2	20e	77.0	11f	88.2
Rhizoctonia solani	90a	0.0	72b	19.8	66c	26.8	49d	46.0	28e	68.4	10f	88.8
Sclerotium rolfsii	90a	0.0	75b	16.6	68c	24.0	51d	43.2	35e	60.8	13f	85.4
Macrophomina phaseolina	90a	0.0	78b	13.8	71c	21.0	53d	40.8	39e	58.2	16f	82.2

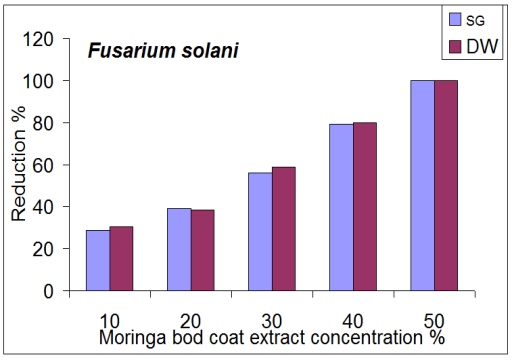
D= colony diameter (mm) I = Inhibition percentage (%) as compared to the control.
Means followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

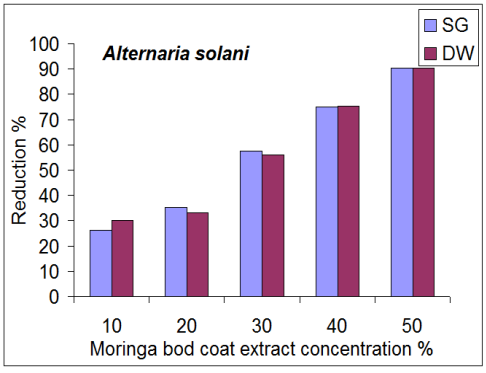
Results in Figure(3) demonstrated that all concentrations of *M. oleifera* pod coat extract (MBCE) hampered spore germination of *F. oxysporum* , *F. solani* , *A. solani* and *A. alternate* as well as sclertia germination of *R. solani* , *S. rolfsii* and *M. phaseolina* with different values . MBCE at 40% and 50 % cause complete (100%) inhibition of spore germination of *F. oxysporum* and *F. solani* .Meanwhile at the same concentration spore germination of *A. solani* and *A. alternate* decreased by 90.4 and 86.0%, sclerotia germination of *R. solani, S. rolfsii* and *M. phaseolina* decreased by 85.0, 82.4 and 77.8% respectively . At low concentrations of MBCE (5% and 10%) minimum inhibitory effect and least records of reduction were noticed, as the germinated spores and/or sclerotia varies from 14.0% to 33.8% and 21.0% to 48.2% of all tested pathogens respectively . Spores germination of *F. oxysporum, F. solani* followed by *A. solani* and *A. alternata* were more sensitivity to moringa pod coat extract than *R. solani , S. rolfsii* and *M. phaseolina*.

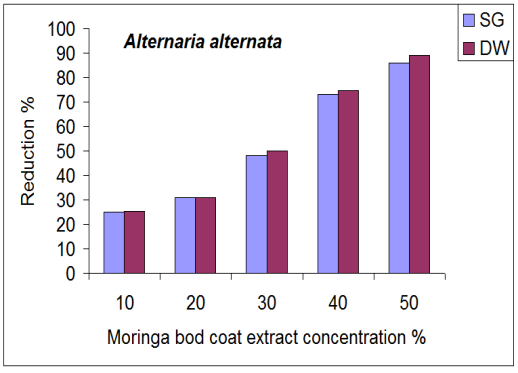
Concerning the effect of moringa pod coat extract (MBCE) on dry mycelia weight, the same trend of result was observed also in Figure(3). MBCE at 40 % and 50 % were the most effective in decreasing dry mycelia weight of *F. oxysporum* (80.4% and 100%), *F. solani* (80.0 % and 100%, *A. solani* (75.4 % and 90.4 %) , *A. alternata* (74.8% and 89.2%) *R. solani* (66.8% and 88.0%) *S. rolfsii* (60.0 % and 85.0 %) and *M. phaseolina* (57.2% and 80.0%). The least records in spore /sclerotia germination were noticed with 10 % and 15 % concentrations of moringa pod coat extract , as the reduction in dry mycelia weight of all tested pathogens was ranged from 15.4 % to 38.4 % and 20.4 % to

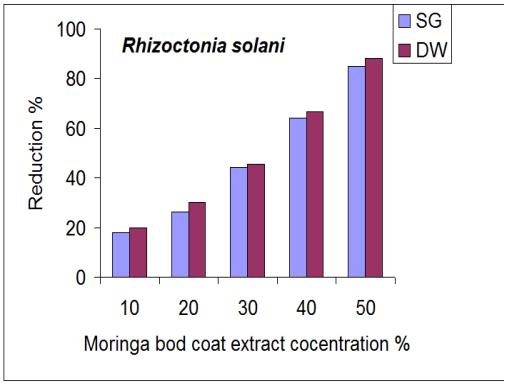
44.8 % respectively .Minimum inhibition of dry growth amount as recoded with *R. solani, S. rolfsii* and *M. phaseolina* .The most affected pathogens by all concentrations of moringa pod coat extract were *F. oxysporum , F. solani* followed by *A. solani* and *A. alternata, but R. solani , S. rolfsii* and *M. phaseolina* showed less sensitivity compeered with other pathogens.

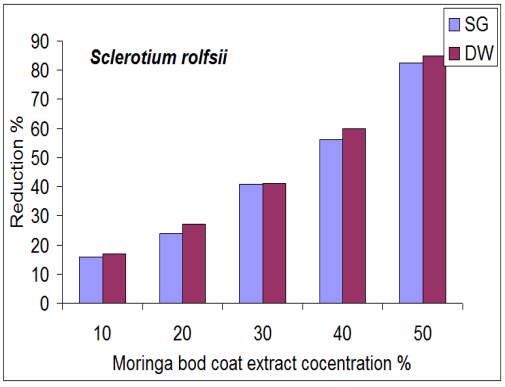












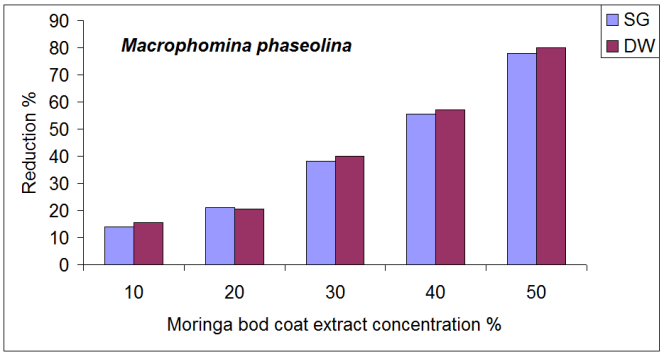


Fig. 3. Reduction % in spore/sclerotia germination (SG) and dry weigh mycelia growth (DW) of seven pathogenic plant fungi as affected by different concentrations of *Moringa oliefera* pud coats extract *in vitro.*

Discussion

The uses of plant derived products as diseases control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance. To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant diseases, the interest on essential oils and plant extracts has been increased (Price, 2000; Aba AlKhail, 2005; Mamdouh and Ewies, 2007; Dwivedi and Neeta, 2012, El-Mohamedy *et al.*, 2013).

Moringa oleifera Lam. (Family Moringaceae) is commonly known as horseradish tree or drumstick used as phytomedicine such as antioxidant, antimicrobial, anti-inflammatory, antipyretic, antidiabetic, antifertility, antiulcer and antitumor (Fahey, 2005; Foidle *et al.*, 2001; Anwar *et al.*, 2007). In this study, we investigated the antifungal activities of *Moringa oleifera* plant parts such as roots, leaves and pod coats extracts against seven plant pathogenic fungi i.e., *F. oxysporum, F. solani , A. solani , A. alternata, R. solani , S. rolfsii and M. phaseolina* in vitro . Our results clearly show that *Moringa oleifera* plant parts such as roots, leaves and pod coats at all tested concentration had antifungal activity against the most root rot and foliar disease pathogens under this investigation. Antifungal activities of the tested moringa extracts were increase by increase of concentration. Extracts of Moringa roots, leaves and pod coats at 25%, 50 % and 50 % concentrations were the most effective in decrease linear growth ,spore germination and dry mycelia weight of all tested pathogens . As, the maximum inhibition effect was noticed , the highest recodes of reduction in linear growth , spore germination and growth mass were observed with *F. oxysporum* , *F. solani* , *A. solani* and *A. alternata* followed by *R. solani* , *S. rolfsii* and *M. phaseolina* . Meanwhile, the least effect of

moringa extracts were at lowest concentrations especially in the case of leaves and pod coats extracts. These results are consistent with those obtained by other investigators who found an antifungal activity of moringa plant extracts against several pathogens (Adandonon *et al*., 2006; Al-Asker and Rashed 2010 ; Abdulmoneim *et al.,* 2011 ; Moyo *et al*., 2012; Talreia, 2010; Seint and Masara, 2011). Secondary compounds, considered as final products of plant metabolism or metabolite refuses, have important ecological functions for the plant which synthesize them. One of these functions is to protect the plants against infection by pathogens (Price, 2000; Anwar and Rashed, 2007).

Therefore, much plant extracts exhibited inhibitory properties in challenge tests against microorganisms. These extracts, however, contained specific component that can inhibit the growth of certain microorganisms (Bowers and Locke, 2000; Dubey *et al*., 2009; Jamil *et al*., 2010; Moyo *et al.,* 2013 ; Mohamedd *et al*., 2012).

Our results demonstrated that The most affected pathogens by all concentrations of moringa extracts were *F. oxysporum , F. solani* followed by *A. solani* and *A. alternate, but R. solani, S. rolfsii* and *M. phaseolina* showed less sensitivity especially at lowest concentrations of leaves and pod coats extracts . Similar studies have been carried out by different researcher on antifungal activity of extracts of many plants (Satish *et al*., 2007; Jamil *et al*., 2010; Anwar and Rashid, 2007). The fungicidal effect of Moringa extracts on some soil-borne fungi such as *Rhizoctonia, Pythium* and *Fusarium* was recorded by many investigators (Chuang *et al*., 2007; Al-Asker and Rashed 2010; Raj *et al*., 2011; Moyo *et al.,* 2012) .Dwivedi and Enespa (2012) indicate that *Moringa oleifera* extracts (leaves, bark and seeds) 75 % (v/v) showed significant inhibition in the mycelial growth of *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* . Leaves of *M. oleifera* are rich in zeatin, a cytokinin in addition to other growth enhancing compounds like ascorbates, phenolic and minerals like Ca, K, and Fe that makes it an excellent crop growth enhancer. *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitsterol, caffeoylquinic acid and kaempferol which have antifungal and antibacterial activities (Nikkon, 2003; Anjorin *et al.,* 2010; Ashfaq *et al*., 2012).

Many plants extracts have been found to be potent fungitoxic agents against many plant pathogens (Siripornvisal and Ngamchawee, 2010; El-Mohamedy *et al*., 2013; El-Shazly, 2000; Gujar and Talwamkar, 2012; Hadi and Kashefi, 2013). However, the harmful effects on fungi were restricted in (a) partial or complete inhibition on spore germination, sporulation or mycelia growth and (b) alternation in physiology and biochemistry activities of the fungal cells. Studies on spore germination represent and integral part of the

ecological studies of the pathogenic fungi, as spores are the specialized structures capable of initiating new growth. Our studies also show that show that Spore/sclerotia germination and dry mycelia weight of all tested pathogens were gradually decreased with increase in concentration of *Moringa oliefera* leaves extract (MLE). MLE at 50% concentration inhibit spore germination and dry mycelia weight of *F. oxysporum* and *F. solani* by 100%, *A. solani* by 92.0 % and 90.4 %, *A. alternate* by 91.0% and 92.2% respectively. Meanwhile , sclertia germination and dry mycelia weight of *R. solani , S. rolfsii* and *M. phaseolina* decreased by 85.0% , 85.0% , 80.8 % and 86.4 % , 85.2% , 80.0 % respectively . Roots and leaves extracts of moringa were the most effective in decreasing growth and germination of all tested pathogen ,meanwhile, pod coat extract show less effect . In this respect, Raj *et at*. (2011) demonstrated that aqueous extract of *Moringa oleifera* (Lam.) Root showed maximum number of inhibition against many pathogenic fungi and bacteria in vitro. They also noted that the phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, erpenoids, steroids, tannins, cardioglycosides, aminoacids and proteins which have antifungal and antibacterial activities.

Our results confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi and thus reduce the dependence on the synthetic fungicides. Roots and leaves extracts of moringa found to be the most efficient and it might be a promising materials for controlling these fungi. Finally, this study is only a preliminary one in Egypt. More studies are still needed in the future to test the antifungal activities of moringa extracts on other different fungal plant disease in *vitro* and under field conditions.

Conclusion

The main purpose of using plant extracts was to study their antifungal activity against the plant pathogens. In the present study, we use *Moringa oliefera* extracts as natural fungicides and as eco-friendly means to control fugal plant diseases . as most of the plant extracts are readily available, environmentally safe, less risky for developing resistance in pests, and pest resurgence, has less adverse effect on plant growth, less harmful to seed viability and quality, and above all, less expensive. All these observations and findings bring further evidence that the *Moringa oliefera* plants extracts such as oil, seeds, roots, leaves and pod coats, have the potential of becoming powerful and safe alternative means of disease control instead of the harmful fungicides .

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