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Effect of *Moringa oleifera* Seed Oil, Root and Leave Extracts on Growth of Major Pathogenic Fungi of Tomato, Green Bean and Potato in Vitro

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Abstract Fungal plant diseases cause greet losses in yield and products of the important vegetable crops in Egypt. In this study, the antifungal activity of *Morienga oleifera* seed oil (MSO), roots extract (MRE) and leaves extract (MLE) against linear growth, spores and sclerotia germination of 18 pathogenic fungi incited tomato, green bean and potato plants was investigated. Crude extracts of roots, leaves as well as seed oil of *Moringa oleifera* significantly reduced radial growth, spores and sclerotia germination of all the tested fungi. *M. oleifera* seed oil and their extracts had different degrees of reduction in both growth and sporulation of the tested fungi. The complete reduction of radial growth, spores and sclerotia germination were at high concentrations of MLE (50%), MRE (20%) and MSO (3%) with all tested fungi. *Fusarium oxysporum , F. solani* and *Alternaria solani* were highly affected by MRE, MLE and MSO than *Rhizoctonia solani , Scleritium rolfsii , Scelerotina sclerotium and M. phaseolina* . It could be suggested that MRE, MLE and MSO of *Moringa oleifera* could be easily applied as a natural fungicide against fungal pathogens of many important crops.

Keywords: *Moringa oleifera*, phytopathogenic fungi, Antifungal activity, plant extract

Introduction

Fungal infections cause significant loss in many economic crops (Agrios 2005). Many plant pathogenic fungi incited tomato, potato and green bean ,such pathogens cause greet losses in yield and products during vegetative growth or after harvesting. Chemical control may be available to effectively and extensively reduce the negative effects of such fungal diseases, 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 natural and bio fungicides that are environmentally safe and easily biodegradable have been carried out during the last two decades

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(Gnanamanickam, 2002, Zaker, 2016; Seem *et al*., 2011; El-Mohamedy *et al*., 2017). 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). Many essential oils and plant extracts have been reported to have antifungal activities with no side effects on humans and animals (Rai and Carpinella, 2006; Tabassum and Vidyasagar, 2013). Essential oils may have a minimum adverse effect on the physiological processes of plants and have less environmental hazards compared to their synthetic alternatives. Since essential oils are plant products, they are easily convertible into a common organic material and eco-friendly (Gnanamanickam, 2002; Seema *et al*., 2011).

Moringa (*Moringa oleifera* Lam.) has gained much importance in the recent days due to its multiple used and benefits to agriculture and industry. Regarded as a miracle plant, all parts of moringa plant are used for medicinal and other purposes (Price, 2000; Anwar *et al*., 2007; Garima *et al*., 2011). In recent years, interests have been generated in the development of safer antifungal agents from natural plant products such as, essential oils and extracts to control fungal plant diseases. 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*. *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)

The aim of this work was to investigate the antifungal activity of *Moringa oleifera* seed oil (MSO), leaves extract (MLE) and roots extract (MRE) against 18 phytopathogenic fungi the causal agents of many fungal diseases tomato, green bean and potato *in vitro*.

Materials and methods

Plant materials

This study was carried out at the Department of Plant Pathology, National Research Center, Egypt. The source of *Moringa oleifera* seed oil (MSO), leaves extract (MLE) and roots extract (MRE) were kindly prepared and obtained

from Egyptian Scientific Society of Moringa (ESSM), National Research Center, Dokki, and Cairo, Egypt.

Pathogenic fungi

The 18 isolates of pathogenic fungi were investigated which belonging to eight fungal genera i.e., *Fusarium*, *Rhizoctonia*, *Alternaria*, *Phytophthora*, *Sclerotium*, *Sclerotina*, *Botrytis* and *Macrophomina* representing 11 species as follows : Seven isolates of tomato pathogenic fungi i.e., *Fusarium oxysporum* f. sp. *lycopersici* (Fol2), *F. oxysporum* f. *radicis* (Forl5), *F. solani* (Fs 5), *Rhizoctonia solani* (Rs 5), *Sclerotium rolfsii* (Sr 5), *Alternaria solani* (As 3) *and Phytophthora infestans* (Ph 3) & Seven isoltaes of pathogenic fungi of green bean *i.,e. Fusarium oxysporum* (Fox 1*), Fusarium solani* (Fs 5), *Rhizoctonia solani* (Rs 5), *Macrophomina phaseolin (*Mph 3), *Sclerotium rolfsii* (Sr 5), *Botrytis cinerea, Sclerotinia sclerotiorum* as well as four isolates of potato pathogenic fungi *i.e., Fusarium sembaticum, Rhizoctonia solani* (Rs 5), *Phytophthora infestans and Alternaria solani* were maintained and grown on potato dextrose agar medium. These fungal isolates were previously isolated and identified at Plant Pathology Department. National Research Center, Cairo, Egypt. The pathogenicity of each fungi was tested and recorded in previous studies by the author (El-Mohamedy *et al*., 2013).

Antifungal activity assay

Antifungal activity of *Moringa oleifera* seed oil (MSO), leaves extract (MLE) and roots extract (MRE) against the tested plant pathogenic fungi mentioned above was carried out using poison food technique (Nene and Thaplyal, 1979) and performed by the agar medium assay. Potato dextrose agar (PDA) medium with different concentrations of moringa leave extracts (MLE) i.e., 10, 20, 30, 40, 50 % and roots extract (MRE) i.e., 5,10,15,20 and 25% as well as different concentrations of moringa seed oil(MSO) i.e., 1.0, 1.5 , 2.0, 2.5 and 3.0 % were prepared by adding appropriate quantity of moringa seed oil to melted medium, followed by addition of Tween 80 (100 µL to 100 mL of medium) to disperse the oil in the medium . About 20 ml of the medium were poured into glass Petri-dishes (9 cm x 1.5 cm). A 5 mm diameter agar disk bearing hyphae 7-days-old colonies grown on PDA medium of each tested pathogenic fungi was transferred at the center of each Petri-dish. Positive control (without treatments) plates were inoculated following the same procedure. Plates were incubated at 25±2 ℃ for 8 days and the colony diameter was recorded each day. The MGI (Mycelia Growth Inhibition) percentage was

calculated and expressed as percentage of reduction. Sclerotia germination of *R. solani, S. rolfsii, M. phaseolina, Botrytis cienerea* and *Sclerotina sclerotiorum* produced on potato dextrose agar (PDA) in each treatment as maintained above were determined according to Manning *et al.* (1970).

Meanwhile, For *F. oxysporum, F. solani, F sembaticum, A. solani* and *Phytophthora infestans* microscope slides were covered with 1 mL of spore's suspension of each tested pathogen in aqueous solution of the desired moringa extracts or moringai seed oil concentrations in Petri dishes, then incubated at 27±1℃ for 8 h in complete darkness. The percentage of germination was assessed according to El-Abyad and Saleh (1917). Five plates were prepared for each treatment and the means were compared. Antifungal activity was calculated and expressed as percentage of reduction in both linear growth (LG) as well as on spores and sclerotia germination (SG and SCG) of each pathogenic fungi under investigations.

Statistical analyses

The data of this study 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

The antifungal activity of Moringa leaves extract (MLE) at five concentrations i.e., 10, 20, 30, 40 and 50 % (w/v), Moringa roots extract (MRE) at 5, 10, 15, 20 and 25 % (w/v) as well as *Moringa oleifera* seed oil (MSO) at 1.0, 1.5, 2.0, 2.0 and 3.0 % (w/v) concentrations against linear growth (LG), spores and sclerotia germination (SG and SCG) of 18 pathogenic fungal isolates, the causal agents of major fungal disease on tomato, green bean and potato crops was evaluated *in vitro.*

The antifungal activity of Moringa leaves extract (MLE)

The inhibitory effect of MLE at five concentrations i.e., 10, 20, 30, 40 and 50 % (w/v) against linear growth (LG) as well as spores and sclerotia germination (SG and SCG) of 18 isolates of pathogenic fungi of tomato, green bean and potato crops was investigated and the linear growth and their reduction as well as reduction in spores and scleriotia germination were illustrated in Tables (1 and 2).

Table 1. Reduction % in linear growth (LG) of pathogenic fungi on tomato, green bean and potato as affected by different concentrations of moringa leaves extract (MLE) on PDA medium

Isolate of Pathogenic fungi	Host plant	MLE concentration									
		10%		20%		30%		40%		50%	
		LG	R	LG	R	LG	R	LG	R	LG	R
Fusarium ox f. sp. *lycopersici* (Fol2)	**Tomato**	**63.0**	**30.0**	**52.0c**	**42.2**	36.0c	60.0	**19.8d**	**78.0**	**0.0b**	**100**
F. ox radicis. lycopersici (Forl5)	**Tomato**	**59.0**	**34.4**	**46.9d**	**47.8**	34.0c	62.2	**19.8d**	**78.0**	**0.0b**	**100**
Fusarium oxysporum (Fox 1)	**Green bean**	**53.0**	**41.1**	**46.2d**	**48.6**	**28.6d**	**68.2**	**0.0e**	**100**	**0.0b**	**100**
F. solani (Fs 5)	**Tomato**	**58.1**	**35.5**	**52.0c**	**42.2**	**34.0c**	**62.2**	**18.0d**	**80.0**	**0.0b**	**100**
F. solani (Fs 3)	**Green bean**	**50.0**	**44.4**	**36.0e**	**60.0**	**20.7d**	**77.0**	**0.0e**	**100**	**0.0b**	**100**
Fusarium sembaticum (Fse 2)	**Potato**	**58.1**	**35.5**	**46.8d**	**48.0**	**34.3c**	**61.8**	**25.0c**	**72.2**	**0.0b**	**100**
Alternaria solani (As 3)	**Tomato**	**61.0**	**32.2**	**54.0b**	**40.0**	**42.3b**	**53.0**	**26.8c**	**70.2**	**0.0b**	**100**
Alternaria solani (As 1)	**Potato**	**63.0**	**30.0**	**55.4b**	**38.4**	**39.7b**	**55.8**	**30.6b**	**66.0**	**0.0b**	**100**
Phytophthora infestans (Ph 3)	**Tomato**	**64.3**	**28.5**	**53.2b**	**40.8**	**41.4b**	**54.0**	**26.6c**	**70.4**	**0.0b**	**100**
Phytophthora infestans (Ph 1)	**Potato**	**62.1**	**31.0**	**50.4c**	**44.0**	**38.5c**	**57.2**	**21.6c**	**76.0**	**0.0b**	**100**
Rhizoctonia solani (Rs 5)	**Tomato**	**67.3**	**25.2**	**54.0c**	**40.0**	**44.6b**	**50.4**	**32.4b**	**64.0**	**0.0b**	**100**
Rhizoctonia solani (Rs 1)	**Green bean**	**63.0**	**30.0**	**50.0c**	**44.4**	**42.4b**	**52.8**	**30.2b**	**66.4**	**0.0b**	**100**
Rhizoctonia solani (Rs 2)	**Potato**	**65.7**	**27.0**	**54.0c**	**40.0**	**39.6b**	**56.0**	**27.0c**	**70.0**	**0.0b**	**100**
Sclerotium rolfsii (Sr 5)	**Potato**	**69.7**	**22.5**	**59.4b**	**34.4**	**43.3b**	**51.8**	**36.0b**	**60.0**	**0.0b**	**100**
Sclerotium rolfsii (Sr 3)	**Green bean**	**71.7**	**20.8**	**66.6b**	**26.0**	**42.4b**	**52.8**	**30.4b**	**66.2**	**0.0b**	**100**
Sclerotina. sclerotiorum (Sc 2)	**Green bean**	**70.2**	**22.0**	**57.6b**	**36.0**	**44.8b**	**50.2**	**32.0b**	**64.4**	**0.0b**	**100**
Botryties cinerea (Bc 3)	**Green bean**	**64.6**	**28.2**	**53.2c**	**40.8**	**41.2b**	**54.2**	**28.8c**	**68.0**	**0.0b**	**100**
Macrophomina phaseolina (Mph 3)	**Green bean**	**61.0**	**32.2**	**50.0c**	**44.4**	**37.8**	**58.0**	**20.7d**	**77.0**	**0.0b**	**100**
Control		**90.0a**	**0.0**	**90.0a**	**0.0**	**90.0a**	**0.0**	**90.0a**	**0.0**	**90.0a**	**0.0**

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

Table 2. Reduction % in spores and sclerotia germination of Pathogenic fungi on tomato, green bean and potato as affected by different concentration of MLE on PDA medium

Isolate of Pathogenic fungi	Host plant	MLE concentration				
		10%	**20%**	**30%**	**40%**	**50%**
Spores germination						
Fusarium ox f. sp. *lycopersici* (Fol2)	**Tomato**	**33.8**	**47.6**	**66.8**	**80.2**	**100**
F. ox radicis. lycopersici (Forl5)	**Tomato**	**38.0**	**42.6**	**67.0**	**81.8**	**100**
Fusarium oxyspoium (Fox 1)	**Green bean**	**42.4**	**48.6**	**78.0**	**100**	**100**
F. solani (Fs 5)	**Tomato**	**38.2**	**48.0**	**70.0**	**90.0**	**100**
F. solani (Fs 3)	**Green bean**	**48.0**	**53.2**	**78.2**	**100**	**100**
Fusarium sembaticum (Fse 2)	**Potato**	**38.2**	**50.4**	**68.0**	**100**	**100**
Alternaria solani (As 3)	**Tomato**	**34.8**	**44.8**	**58.2**	**74.2**	**100**
Alternaria solani (As 1)	**Potato**	**32.2**	**40.0**	**52.4**	**70.8**	**100**
Phytophthora infestans (Ph 3)	**Tomato**	**30.2**	**44.2**	**60.0**	**74.4**	**100**
Phytophthora infestans (Ph 1)	**Potato**	**33.8**	**50.2**	**64.2**	**77.4**	**100**
Sclerotia germination						
Rhizoctonia solani (Rs 5)	**Tomato**	**21.8**	**30.8**	**48.0**	**60.0**	**100**
Rhizoctonia solani (Rs 1)	**Green bean**	**24.6**	**32.0**	**46.2**	**62.0**	**100**
Rhizoctonia solani (Rs 2)	**Potato**	**24.4**	**36.0**	**51.0**	**66.4**	**100**
Sclerotium rolfsii (Sr 5)	**Potato**	**21.0**	**27.0**	**42.2**	**60.8**	**100**
Sclerotium rolfsii (Sr 3)	**Green bean**	**18.4**	**25.0**	**38.8**	**58.4**	**100**
Sclerotina. sclerotiorum (Sc 2)	**Green bean**	**20.0**	**32.0**	**44.4**	**61.2**	**100**
Botrytis cienerea (Bc 3)	**Green bean**	**23.4**	**34.2**	**48.0**	**60.0**	**100**
Macrophomina phaseolina (Mph 3)		**30.8**	**42.4**	**58.0**	**74.8**	**100**

All tested concentrations of MLE caused significant decrease in linear growth (mm) of all tested pathogenic fungi. The inhibitory of MLE increased by increasing its concentrations to reach to 100% reduction at 50 % concentration is demonstrated in Table 1. MLE at 40% concentration inhibited the linear growth by ranging from 100 – 60.0 %, however, at 30 % it causes reduction ranging from 77.2 -51.8%. Meanwhile MLE at 20 % and 10 % the least antifungal activity (<50% reduction) of all tested fungi. The highest records in linear growth reduction were with all isolates of *Fusarium, Phytophthora, Alternaria* followed by *Macrophomina, Rhizoctonia, Botrytis, Sclerotina* and *Sclerotium* in ascending order.

Results clearly showed that all tested concentrations of MLE had the ability to decrease the germination of both spores and sclerotia of the tested fungi with different values (Table 2). MLE at 50 % concentration caused complete reduction in spores and sclerotia germination at 40% concentration caused 100-70% and 74.8-58.4 % respectively in reduction of both spores and sclerotia germination. Meanwhile, at 20 % of MLE the moderate reduction (up to 53 of spores and up to 42.2% of sclerotia germination were recorded. The

least effect (< 50%) was at 10 % of MLE. Spores of all tested isolates of *Fusarium, Phytophthora* and *Alternaria* were highly affected with MLE than the sclerotia of *Macrophomina, Rhizoctonia, Botrytis, Sclerotina* and *Sclerotium.* It was observed that all tested isolates of *Fusarium, Phytophthora* and *Alternaria* were more sensitive to all concentartions of MLE than *Macrophomina* and *Rhizoctonia, meanwhile,* isolates of *Botryitis, Sclerotina* and *Sclerotium* were least affected by MLE (Tables 1 and 2).

The antifungal activity of Moringa root extract (MRE)

The inhibitory effect of MRE at five concentrations i.e., 5, 10, 15, 20 and 20 % (w/v) on linear growth (LG) as well as spores and sclerotia germination (SG and SCG) of 18 isolates of pathogenic fungi of tomato, green bean and potato crops was investigated and the linear growth and their reduction as well as reduction in spores and scleriotia germination were illustrated in Tables 3 and 4 .

Results demonstrated that MRE at all tested concentrations showed significantly inhibitory effects against the linear growth (LG) of all tested pathogenic fungi. The reduction in linear growth was increased by increasing the concentration of MRE (Table 3). All fungal isolates of *Fusarium, Phytophthora* and *Alternaria* were highly affected with all concentrations of MRE more than the other tested fungi. MRE at 25 % caused complete reduction of the linear growth (LG) of all tested fungi, however, at 20% the reduction percentages ranging from 100 – 79.0 % with *Fusarium, Phytophthora* and *Alternaria,* and by 80.0-66.0% with *Macrophomina, Rhizoctonia, Botrytis, Sclerotina* and *Sclerotium*. Meanwhile, at 15% concentration of MRE the reduction in linear growth of such fungi ranging from 85.0—62.0% and 71.8-48.0% respectively were recorded. MRE at 5% showed the least records of linear growth reduction with all tested fungi.

All tested concentrations of MLE could decrease germination of spores and/or sclerotia of the tested pathogenic fungi (Table 4). This decreasing in spores and/or sclerotia germination reached to 100% reduction of all tested fungi at 25% concentration of MRE, however at 20% concentration the reduction ranging 100-88.2 % and 90.0 - 65.2% of spores and sclerotia germination. Meanwhile MRE at 15 % caused reduction ranging from 94.4 - 70..6% and 82.2-60.0 % of spores and sclerotia germination respectively. However, the moderate effects on spores and sclerotia germination (78.4-56.6% and 66.4-37.8% reduction) were recorded at 10 % of MRE. The least effects on the germination of each spores and sclerotia of the tested fungi were at 5 % of MRE. Spores of *Fusarium, Phytophthora* and *Alternaria* were more affected

with MRE than sclerotia of *Macrophomina, Rhizoctonia, Botrytis, Sclerotina* and *Sclerotium*.

The antifungal activity of Moringa seed oil (MSO)

The inhibitory effect of MSO at five concentrations i.e., 1.0, 1.5, 2.0, 2.5 and 3.0 % (w/v) on linear growth (LG) as well as spores and sclerotia germination (SG and SCG) of 18 isolates of pathogenic fungi of tomato, green bean and potato crops was investigated and the linear growth and their reduction as well as reduction in spores and scleriotia germination were illustrated in Tables 5 and 6. Results showed that all tested concentrations of MSO were significantly inhibited the linear growth (LG) of all tested pathogenic fungi with different degrees of reduction. MSO at 3.0 % completely reduction in the linear growth (LG) of all tested fungi were recorded, but at 2.5 % the reduction reached to 100% of all isolates of *Fusarium, Phytpphthora* and *Alternaria*, but ranging from 88.0 - 62.0% of *Macrophomina, Rhizoctonian, Botrytis, Sclerotina* and *Sclerotium*. Meanwhile, MSO at 2.0 % caused linear growth reduction of all of *Fusarium, Phytophthora* and *Alternaria* tested isolates ranging from 84.2-62.2 % and by 62.0 - 42.0 % of *Macrophomina, Rhizoctonian, Botryties, Sclerotina* and *Sclerotium* were noted. However, the reduction reached 72.0 - 50.8% and 56.0 - 40.2% of the same pathogenic fungal isolates respectively at 1.5 % concentration.

Results demonstrated that MSO at all tested concentrations that resulted in decreasing spores and sclerotia germination of all tested fungi to reach to 100 % reduction at 3.0 % concentration of MSO (Table 6). Spores of *Fusarium, Phytophthora* and *Alternaria* was highly affected by all tested MSO concentration when compared with sclerotia of *Macrophomina, Rhizoctonian, Botrytis, Sclerotina* and *Sclerotium*. Meanwhile, at 2.5 % of MSO the reduction reached to 100% of spore germination with *Fusarium, Phytpphthora* and *Alternaria*, but ranging from 74.0 – 64.2 % of *Macrophomina, Rhizoctonia, Botrytis, Sclerotina* and *Sclerotium* that at 2.0% concentration the reduction reached to 90.0-68.2% and 70.0-55.0% of the same pathogenic fungi respectively. The moderate effect of MSO was at 1.5 % concentration. It decreased the spores and sclerotia germinaton of all tested fungi to reach 77.8-58.0% and 57.4 - 50.8% respectively. However, the reduction of spores and sclerotia germination ranging from 60.2-52.2% and 48.2-32.8% at 1.0% of MSO with all tested fungi were noted.

Table 3. Reduction % in linear growth (LG) of plant pathogenic fungi as affected by different concentrations of moringa leaves extract (MRE) on PDA medium

Isolate of Pathogenic fungi	Host plant	MRE concentration									
		5%		10%		15%		20%		25%	
		LG	R	LG	R	LG	R	LG	R	LG	R
Fusarium ox f. sp. *lycopersici* (Fol2)	**Tomato**	**52.4b**	**41.7**	**36.0d**	**60.0**	**27.0d**	**70.0**	**13.5c**	**85.0**	**0.0b**	**100**
F. ox radicis. *lycopersici* (Forl5)	**Tomato**	**50.4b**	**44.0**	**36.0d**	**60.0**	**23.2d**	**74.2**	**10.8c**	**88.0**	**0.0b**	**100**
Fusarium oxysporum (Fox 1)	**Green bean**	**52.1b**	**42.1**	**36.0d**	**60.6**	**23.1d**	**74.3**	**9.0d**	**90.0**	**0.0b**	**100**
F. solani (Fs 5)	**Tomato**	**46.8d**	**48.0**	**30.9e**	**65.6**	**17.1e**	**81.0**	**0.0d**	**100**	**0.0b**	**100**
F. solani (Fs 3)	**Green bean**	**49.0c**	**45.5**	**27.0e**	**70.0**	**13.5e**	**85.0**	**0.0d**	**100**	**0.0b**	**100**
Fusarium sembaticum (Fse 2)	**Potato**	**52.4b**	**41.7**	**36.0d**	**60.0**	**22.5d**	**75.0**	**5.4d**	**94.0**	**0.0b**	**100**
Alternaria solani (As 3)	**Tomato**	**50.0c**	**45.0**	**43.2c**	**52.4**	**27.0d**	**70.0**	**14.4c**	**84.0**	**0.0b**	**100**
Alternaria solani (As 1)	**Potato**	**55.8c**	**38.0**	**45.0c**	**50.0**	**34.2c**	**62.0**	**17.2c**	**80.8**	**0.0b**	**100**
Phytophthora infestans (Ph 3)	**Tomato**	**53.4c**	**40.6**	**39.6d**	**56.0**	**26.8d**	**70.2**	**27.0b**	**79.0**	**0.0b**	**100**
Phytophthora infestans (Ph 1)	**Potato**	**50.9c**	**43.4**	**35.2d**	**60.8**	**19.1e**	**78.8**	**14.4c**	**84.0**	**0.0b**	**100**
Rhizoctonia solani (Rs 5)	**Tomato**	**56.9c**	**36.7**	**43.2c**	**52.0**	**32.4c**	**64.0**	**25.2b**	**72.0**	**0.0b**	**100**
Rhizoctonia solani (Rs 1)	**Green bean**	**58.5b**	**35.0**	**45.0c**	**50.0**	**28.9dc**	**67.8**	**22.5b**	**75.0**	**0.0b**	**100**
Rhizoctonia solani (Rs 2)	**Potato**	**55.8c**	**38.0**	**40.5c**	**55.0**	**25.0d**	**72.2**	**18.0c**	**80.0**	**0.0b**	**100**
Sclerotium rolfsii (Sr 5)	**Potato**	**63.0b**	**30.0**	**50.0b**	**44.4**	**47.6b**	**58.2**	**27.0b**	**70.0**	**0.0b**	**100**
Sclerotium rolfsii (Sr 3)	**Green bean**	**64.9b**	**27.8**	**53.0b**	**30.0**	**46.8b**	**48.0**	**30.6b**	**66.0**	**0.0b**	**100**
Sclerotina sclerotiorum (Sc 2)	**Green bean**	**58.5b**	**35.0**	**46.6b**	**48.2**	**38.8c**	**56.8**	**18.0**	**80.0**	**0.0b**	**100**
Botrytis cinerea (Bc 3)	**Green bean**	**57.4b**	**36.2**	**46.0b**	**48.8**	**34.2c**	**62.2**	**23.4b**	**74.0**	**0.0b**	**100**
Macrophomina phaseolina (Mph 3)	**Green bean**	**53.8c**	**40.2**	**37.4**	**58.4**	**25.3d**	**71.8**	**18.0c**	**80.0**	**0.0b**	**100**
Control		**90.0a**	**0.0**	**90.0a**	**0.0**	**90.0a**	**0.0**	**90.0a**	**0.0**	**90.0a**	**0.0**

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

Table 4. Reduction % in spores and sclerotia germination of pathogenic fungi on tomato, green bean and potato as affected by different concentration of MRE on PDA medium

Isolate of Pathogenic fungi	Host plant	MRE concentration				
		5%	10%	15%	20%	25%
	Spores germination					
Fusarium ox f. sp. *lycopersici* (Fol2)	**Tomato**	**50.4**	**67.2**	**82.0**	**92.2**	**100**
F. ox radicis. lycopersici (Forl5)	**Tomato**	**52.2**	**68.2**	**88.2**	**90.0**	**100**
Fusarium oxysporium (Fox 1)	**Green bean**	**50.4**	**73.2**	**92.8**	**100**	**100**
F. solani (Fs 5)	**Tomato**	**58.8**	**74.8**	**90.0**	**100**	**100**
F. solani (Fs 3)	**Green bean**	**60.4**	**78.4**	**94.0**	**100**	**100**
Fusarium sembaticum (Fse 2)	**Potato**	**50.2**	**68.2**	**84.2**	**100**	**100**
Alternaria solani (As 3)	**Tomato**	**50.6**	**58.4**	**80.0**	**90.0**	**100**
Alternaria solani (As 1)	**Potato**	**44.8**	**56.6**	**70.0**	**88.2**	**100**
Phytophthora infestans (Ph 3)	**Tomato**	**46.0**	**60.4**	**77.0**	**88.0**	**100**
Phytophthora infestans (Ph 1)	**Potato**	**52.6**	**64.0**	**80.0**	**92.2**	**100**
	Sclerotia germination					
Rhizoctonia solani (Rs 5)	**Tomato**	**40.2**	**58.2**	**70.0**	**73.0**	**100**
Rhizoctonia solani (Rs 1)	**Green bean**	**42.0**	**53.8**	**68.2**	**80.0**	**100**
Rhizoctonia solani (Rs 2)	**Potato**	**46.2**	**58.4**	**80.2**	**84.4**	**100**
Sclerotium rolfsii (Sr 5)	**Potato**	**32.7**	**40.2**	**50.2**	**66.8**	**100**
Sclerotium rolfsii (Sr 3)	**Green bean**	**30.4**	**37.8**	**60.0**	**65.2**	**100**
Sclerotina. sclerotiorum (Sc 2)	**Green bean**	**33.6**	**52.8**	**74.0**	**80.0**	**100**
Botryties cinerea (Bc 3)	**Green bean**	**38.8**	**50.8**	**65.4**	**78.0**	**100**
Macrophomina phaseolina (Mph 3)		**48.8**	**66.4**	**82.4**	**90.0**	**100**

Table 5. Reduction % in linear growth (LG) of plant pathogenic fungi on tomato, green bean and potato as affected by different concentrations of moringa leaves extract (MSO) on PDA medium

Isolate of Pathogenic fungi	Host plant	MSO concentration									
		1.0%		1.5%		2.0%		2.5%		3.0%	
		LG	R	LG	R	LG	R	LG	R	LG	R
Fusarium ox f. sp. *lycopersici* (Fol2)	**Tomato**	**46.6e**	**48.2**	**31.5**	**65.0**	**27.0**	**70.0**	**0.0**	**100**	**0.0**	**100**
F. ox radicis. lycopersici (Forl5)	**Tomato**	**48.9e**	**45.6**	**35.1**	**61.0**	**28.1**	**68.8**	**0.0**	**100**	**0.0**	**100**
Fusarium oxysporum (Fox 1)	**Green bean**	**45.0e**	**50.0**	**32.4**	**64.0**	**25.0**	**72.2**	**0.0**	**100**	**0.0**	**100**
F. solani (Fs 5)	**Tomato**	**48.7e**	**54.8**	**25.2**	**72.0**	**14.2**	**84.2**	**0.0**	**100**	**0.0**	**100**
F. solani (Fs 3)	**Green bean**	**45.0e**	**50.0**	**27.0**	**70.0**	**16.2**	**82.0**	**0.0**	**100**	**0.0**	**100**
Fusarium sembaticum (Fse 2)	**Potato**	**49.6e**	**44.8**	**36.0**	**60.0**	**28.8**	**68.0**	**0.0**	**100**	**0.0**	**100**
Alternaria solani (As 3)	**Tomato**	**44.4e**	**50.6**	**37.8**	**58.0**	**36.6**	**66.0**	**0.0**	**100**	**0.0**	**100**
Alternaria solani (As 1)	**Potato**	**50.0d**	**44.0**	**44.2**	**50.8**	**34.0**	**62.2**	**0.0**	**100**	**0.0**	**100**
Phytophthora infestans (Ph 3)	**Tomato**	**45.0e**	**50.0**	**34.0**	**62.2**	**27.0**	**70.0**	**0.0**	**100**	**0.0**	**100**
Phytophthora infestans (Ph 1)	**Potato**	**48.2e**	**46.4**	**37.9**	**57.8**	**30.6**	**66.0**	**0.0**	**100**	**0.0**	**100**
Rhizoctonia solani (Rs 5)	**Tomato**	**53.8d**	**40.2**	**46.8**	**48.0**	**40.3**	**55.2**	**25.0**	**72.2**	**0.0**	**100**
Rhizoctonia solani (Rs 1)	**Green bean**	**52.2d**	**42.0**	**43.2**	**52.0**	**36.0**	**60.0**	**10.6**	**88.2**	**0.0**	**100**
Rhizoctonia solani (Rs 2)	**Potato**	**55.8d**	**38.0**	**49.8**	**44.6**	**41.4**	**54.0**	**27.0**	**70.0**	**0.0**	**100**
Sclerotium rolfsii (Sr 5)	**Potato**	**63.0b**	**30.0**	**57.6**	**36.0**	**52.2**	**42.0**	**34.2**	**62.0**	**0.0**	**100**
Sclerotium rolfsii (Sr 3)	**Green bean**	**61.0b**	**32.2**	**53.6**	**40.4**	**46.8**	**48.0**	**32.0**	**64.4**	**0.0**	**100**
Sclerotina. sclerotiorum (Sc 2)	**Green bean**	**58.5c**	**35.0**	**53.8**	**40.2**	**45.2**	**49.8**	**30.6**	**66.0**	**0.0**	**100**
Botryties cinerea (Bc 3)	**Green bean**	**57.7c**	**35.8**	**51.4**	**42.8**	**44.2**	**50.8**	**30.4**	**66.2**	**0.0**	**100**
Macrophomina phaseolina (Mph 3)	**Green bean**	**52.2d**	**42.0**	**39.6**	**56.0**	**34.2**	**62.0**	**10.8**	**88.0**	**0.0**	**100**
Control		**90.0a**	**0.0**	**90.0**	**0.0**	**90.0**	**0.0**	**90.0**	**0.0**	**90.0**	**0.0**

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

Table 6. Reduction % in spores and sclerotia germination of pathogenic fungi on tomato, green bean and potato as affected by different concentration of MSO on PDA medium

Isolate of Pathogenic fungi	Host plant	MSO concentration				
		1.0%	1.5%	2.0%	2.5%	3.0%
	Spores germination					
Fusarium ox f. sp. *lycopersici* (Fol2)	**Tomato**	51.6	70.0	74.0	100	100
F. ox radicis. lycopersici (Forl5)	**Tomato**	50.0	66.2	70.0	100	100
Fusarium oxysporum (Fox 1)	**Green bean**	55.2	73.4	80.0	100	100
F. solani (Fs 5)	**Tomato**	57.2	77.8	90.0	100	100
F. solani (Fs 3)	**Green bean**	54.4	74.4	88.2	100	100
Fusarium sembaticum (Fse 2)	**Potato**	42.2	60.0	68.2	100	100
Alternaria solani (As 3)	**Tomato**	**54.6**	**64.4**	**70.0**	**100**	**100**
Alternaria solani (As 1)	**Potato**	52.6	58.0	68.2	100	100
Phytophthora infestance (Ph 3)	**Tomato**	**55.2**	**64.2**	**72.2**	**100**	**100**
Phytophthora infestance (Ph 1)	**Potato**	53.8	60.2	75.0	100	100
	Sclerotia germination					
Rhizoctonia solani (Rs 5)	**Tomato**	**41.0**	**55.2**	**71.2**	**84.0**	**100**
Rhizoctonia solani (Rs 1)	**Green bean**	**44.4**	**51.4**	**71.0**	**80.2**	**100**
Rhizoctonia solani (Rs 2)	**Potato**	**40.4**	**54.2**	**70.0**	**77.4**	**100**
Sclerotium rolfsii (Sr 5)	**Potato**	35.6	50.8	58.0	64.2	100
Sclerotium rolfsii (Sr 3)	**Green bean**	32.8	52.4	64.4	70.0	100
Sclerotina. sclerotiorum (Sc 2)	**Green bean**	42.2	54.8	66.4	74.0	100
Botrytis cinerea (Bc 3)	**Green bean**	44.6	54.2	70.2	100	100
Macrophomina phaseolina (Mph 3)	**Green bean**	**48.2**	**57.4**	**74.0**	**90.0**	**100**

Discussion

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 the present study, the antifungal activities of *Moringa oleifera* extracts i.e., MLE, MRE and MSO against 18 plant pathogenic fungi, the causal agents of many root rot, wilt and foliar diseases of tomato, green bean and potato, was investigated in vitro. Our results demonstrated that Moringa *oleifera leaves* extracts (MLE), roots extracts (MRE) as well as moringa seed oil and (MSO) at all tested concentration had antifungal activity against all tested pathogenic fungi. Moringa seed oil (MSO) and MRE showed highly antifungal activities against all tested fungi, as the highest records in linear growth, spore and

sclerotia germination of all tested pathogens were observed with all concentrations of MSO and MRE. The highest recodes of reduction in linear growth, spores and sclerotia germination were observed with all tested isolates of *Fusarium*, *Phytophthora* and *Alternaria* ,as they were highly sensitive to MLE , MRE and MSO than all isolates of *Macrophomina*, *Rhizoctonia*, *Botrytis* and *Sclerotium* .

These results are agreement with those obtained by other investigators who found an antifungal activity of moringa plant extracts against several pathogens (Adandonon *et al*., 2006; Anwar and Rashid, 2007; Al-Asker and Rashed 2010; Abdulmoneim *et al.*, 2011; Moyo *et al*., 2012; Talreia, 2010; Seint and Masara, 2011, El-Mohamedy and Aboelfetoh, 2014 a,b). Jed and Fahey (2005) noted that antimicrobial activity of *Moringa oleifera* extracts may be attributed two main phytochemicals viz., pterygospermin and benzyl isocyanate which have strong antifungal and antimicrobial activity**.** In this respect, many essential oils and plant extracts have been found to be potent fungitoxic agents against many plant pathogens (Satish *et al*., 2007; Jamil *et al*., 2007; Anwar and Rashid, 2007; Sun *et al*., 2007, Siripornvisal and Ngamchawee, 2010; El-Mohamedy *et al*., 2013; Tabassum and Vidyasagar, 2013; Abd el-kader *et al*., 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. In additions, 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).

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; Hadi and Klefi, 2013; El-Mohamedy and Aboelfetoh, 2014 a, b). 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*. Raj *et at*. (2011) demonstrated that aqueous extract of *Moringa oleifera* (Lam.) Root showed maximum 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.

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 demonstrated that spore/sclerotia germination of all tested pathogens were gradually decreased to reach 100% reduction with increasing concentrations of MLE (50%), MRE(20%) and MSO (3%). Meanwhile, sclertia germination of *R. solani, S. rolfsii* and *M. phaseolina, S. sclerotiorum* and *B.cinerea* showed less sensitive against MLE, MRE and MSO in ascending order. These finding are in agreement with these reported by many investigators (Bowers and Locke, 2000; Dwivedi and Enespa, 2012; Abdulmoneim *et al*., 2011; Chuang *et al*., 2007, El-Mohamedy and Aboelfetoh, 2014 a, b)

Conclusion

The results of this study demonstrated that, Moringa leaves extract MLE. Moringa roots extract MRE and *Moringa* seed oil MSO were the most efficient and might be as promising natural compounds for controlling such plant pathogenic fungi. Therefore, it could be used as natural fungicide to compact pathogenic fungi and thus reduced the dependence on the synthetic fungicides. More studies are still needed in the future to test the antifungal activities of moringa extracts on other different fungal plant diseases in *vitro* and under field conditions.

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