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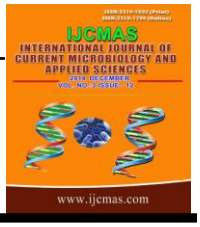

Original Research Article

Preliminary studies on response of *Moringa oleifera* plants to infection with some soil borne plant pathogenic fungi

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Keywords

Moringa oleifera, Root rot, Wilt, Soil borne pathogenic fungi

A B S T R A C T

Moringa oleifera seed germinates poorly and most seedlings die during early establishment. To solve this problem, the effects of soil and seed sterilization as well as the effect and susceptibility of *M. oleifera* to soil borne fungi during seed emergence and early seedling establishment were evaluated. The following fungi *Rhizoctonia* spp., *Rhizopus* spp., *Trichoderma* spp., *Fusarium solani, Fusarium oxysporum, Fusarium spp., Macrophomina spp., Helminthosporium spp., Pencicillium spp., Asperigullus niger, Asperigillus flavus* were isolated from *Moringa* seeds. Thus, seeds were sterilized prior to planting to eliminate any pathogen from the surface of the seed. Sterilized soil and seeds gave the highest emergence of *Moringa* seedlings .Observations indicated *F. solani, F. oxysporum, R. solani, S. rolfsii* and *M. phaseolina* infection in the seed that had failed to germinate, whilst those in the sterilized seeds showed no evidence of fungal attack. After 30 days of emergence, no seedlings showed any infection by *F. solani, F. oxysporum , R. solani , S. rolfsii* and *M. phaseolina* . This is the first record on resistance of *Moringa oliefera* plants against root rot and wilt diseases caused by *F solani , F oxysporum , R solani , S. rolfsii* and *M. phaseolina* .There is no clearly effect of the tested pathogens on vegetative growth parameter such as plant height , fresh and dry weight of shoots and roots of *Moringa* . It could be suggested that seeds and media used for *Moringa* ought to be sterilized with hypochlorite or another material or procedure to ensure good seed emergence and control seed and soil borne pathogenic fungi.

Introduction

Moringa oleifera Lamarck (*Moringa*) family *Moringa*ceae constitutes one of the multipurpose trees most demanded by the world population in recent years, mainly due to the wide variety of products of excellent nutritional quality it contributes, to its exceptional medicinal properties and to its use in animal and human feeding. It is also

used as flocculent in water treatment and as living fence and windbreak. In addition, it is utilized to produce biodiesel, ethanol, oil and gums; as well as in the control of vectors and infections caused by microorganisms and as biopesticide (Pérez *et al*., 2010; Ashfaq and Ashfaq, 2012; Martín *et al*., 2013). Roots, flowers, bark, stem, leaves and seeds of *Moringa* possess antimicrobial properties (Anjorin and Mohammed, 2009, El-Mohamedy and Abdalla, 2014 a, b)

In Egypt, the sowing and establishment of *Moringa* has increased remarkably, according to the scientific strategy followed at international level with such plant. This has increased the uncertainty regarding its health, because the available information about the topic is insufficient. In addition, the continuous introduction of new provenances widens the possibility of appearance of new pathogen agents, such as fungi; which have caused the following diseases: seedling wilting and root (*Diplodia* sp.) and fruit rot (*Cochliobolus hawaiiensis*), according to the reports by Ramachandran *et a*l. (1980), Kshirsagar and D'Souza (1989), Palada and Chang (2003).

Fungi cause a very wide range of disease in plants. Many soil borne fungal plant pathogens cause diseases of the roots or stems, thus disrupting the uptake and translocation of water and nutrients from the soil. This may result in appearance of symptoms similar to drought and nutrient deficiencies, which include wilting, yellowing, stunted growth and plant death. The fungi, which commonly cause seedling death, include *Pythium* spp., *Phytopthora* spp., *Rhizoctonia* spp., *Sclerotium* spp and *Fusarium* spp (Agrios, 1988). Reports of diseases that affect *Moringa* after emergence are few (Mandhokhot *et al*., 1994).

Moringa oleifera is relatively unaffected by disease problems. It's tolerant of occasional outbreaks of pest because of how vigorous it grows. This makes it possible for pesticide intervention to be generally unnecessary. Aphids and Cabbage Worms have been seen on *M. oleifera* , but it appears as if they cause no direct harm to the tree. Because this is such a versatile plant can grow in various places, many of the organisms that negatively affect *Moringa oleifera* are dependent on where the tree is located. In India, the tree is unaffected by diseases, but is impacted by root rot associated with poor drainage caused by *Diplodia sp* .*Moringa* growers indicate that there are cases of diseases similar to fungal wilts and damping off in a significant number of *Moringa* nurseries (Lezcano *et al*., 2014) . However, no research has been done to confirm or refute these claims.

Taking the above-explained facts into consideration, the objective of this study was to evaluate susceptibility of *M. oleifera* to infection by some soil borne plant pathogens as well as the effects of such pathogens on vegetative growth of *M. oleifera* .

Material and Methods

Plant material:

The present work was carried out at the Department of Plant Pathology, National Research Center, Egypt. The source of *Moringa oleifera* seeds kindly obtained from Egyptian Scientific Society of *Moringa* (ESSM), National Research Center, Dokki, Cairo, Egypt.

Pathogenic fungi

Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii and *Macrophomina phaseolina* were the most soil borne plant pathogens were used in this

study. These pathogens were isolated; identified and the pathogenic ability of each fungi were tested and recorded in previous studies by the author (El-Mohamedy *et al*., 2014a, b).

Isolation of *Moringa* Seed borne fungi

Laboratory analysis was done to examine *Moringa* seeds for seed borne pathogens before sowing (ISTA, 1976). The seeds were washed with sterile water and dipped in a 1:5 solution of hypochlorite (sterilized seeds) and water (non sterilized seeds) for 10 minutes. Thereafter, the seeds were incubated at a temperature of 26°C for three days. Then seeds were placed in blotter method and placed in incubation at 22 ± 2ºC for growth of fungi. After 10 days platting of seeds the growth of seed borne fungi on the seeds were recorded. Then the infected seeds are marked and placing in the Potato Dextrose Agar (PDA) medium. After that, the inocula of fungi are growing in the PDA medium. Lastly, the individual inoculum was isolated and set in PDA medium for pure culture. After 5 days of inoculation, the individual pathogenic fungi were identified by Stereomicroscope with the specific characters of mycelia and conidia. After incubation percentages of seed infection were recorded in each sterilized and non sterilized seeds. The isolated *Fusarium* sp. was maintained on PCNB peptone medium (Nelson *et al.* 1983), whereas other fungi were maintained on Potato Dextrose Agar (PDA) medium. The fungi were identified after reference to Booth (1977), Barnett and Hunter (1972), and Nelson *et al.* (1983).

Effect of seed sterilization on damping-off disease incidence:

To conform the role of seed borne fungi associated with *Moringa* seeds on damping - off disease incidence of the seeds and seedlings of *Moringa*. Five sterilized and non sterilized *Moringa* seeds were planted in each polythene bag of 25 cm diameter. Each seed was sowed at a depth of 1 cm, ten bags were used as replicated for each kind of seed. The percentages of damping –off disease incidence at pre-and post emergence damping -off were receded after 15 and 30 days of seed sowing.

Inoculums Preparation and pathogencity test

Pathogenic ability of five pathogenic fungi i.e., *Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* to induce damping –off, root rot and wilt disease on *Moringa oleifera* plants were evaluated in pot experiment under greenhouse conditions.

The experiment was carried out in autoclaved (121°C for two hours) sandy barley medium clay loamy soil (50% sand, 40% clay and 10% silt) artificially infested with the inoculums of each pathogen. Pathogen inoculum was obtained by growing the tested pathogenic fungi on sandy barley medium. This natural medium was prepared by mixing sand and barley (1:1 w:w and 40% water) then the mixture distributed in glass bottles was plugged with cotton plugs and sterilized three times (1 hr each time) at 131°C. The autoclaved medium was then inoculated individually with a 5- mm disk of each fungal pathogen and incubated at 25°C for two weeks. Soils were infested individually at ratio of 5% (w:w) with tested pathogenic fungi, then filled in plastic pots (25 cm-diam.) and irrigated for 1 week before sowing. A set of pots were also amended with the same rate of sand barley medium free pathogen. Sterilized and non sterilized *Moringa oleifera* seeds were sown in each pot, five

seeds/pot, and ten pots were used as replicate for each particular pathogenic fungi. Percentages of damping-off at pre- and post-emergence stages were calculated after 15 and 30 days of sowing. Root rot and wilt diseases were observed and recorded up to 60 days of the experimental period.

Pre-emergence (%) was based on the number of un-emerged seeds in relation to the number of sown seeds, while Post-emergence (%) was based on the number of plants showing disease symptoms in relation to the number of emerged seedlings.

Plant growth measurements:

A representative sample of 10 plants was taken by random 45 days after sowing from each experimental treatment for measuring the plant growth characters, as follows:
Plant height from soil surface to the highest point of the plant, total fresh weight and dry weight of plant (determined at 65ºC for 72 hours using the standard methods as illustrated by A.O.A.C. (1990).

Statistical analysis

Tukey test for multiple comparisons among means was utilized (Neler *et al.*, 1985).

Result and Discussion

Seed borne fungi of *Moringa*

Laboratory analysis was done to examine two lots A and B of *Moringa* seeds (A - *Moringa* seeds collected one year ago (2013) and B- *Moringa* seeds collected one month ago (June, 2014) for seed borne pathogens. Results in Table 1 show that sterilized *Moringa* seeds (immersed seed in sodium hypochlorite for 10 minutes) produced least percentages of fungi if compared with non sterilized seeds. The following fungi *Rhizoctonia* spp., *Rhizopus* spp., *Trichoderma* spp., *Fusarium solani, Fusarium oxysporum, Fusarium spp., Macrophomina spp., Helminthosporium spp., Penicillium spp., Aspergillus niger, Asperigillus flavus* were isolated from sterilized and non sterilized *Moringa* seeds. Seed and soil borne pathogens such as *Fusarium solani, Fusarium oxysporum* and *Fusarium* spp were attacked 7.0%, 3.5%, 3.5 % and 2.5 %, 0.5%, 2.0 % of non sterilized and sterilized *Moringa* seeds, respectively. *Rhizoctonia solani and Macrophomina spp* attacked seeds by 1.5 %, 2.5% and 0.5%, 0.5%, respectively. Meanwhile, *Alternaria spp* and *Helminthosporium spp* attacked seeds by 2.0%, 1.5% and 0.5%, 0.0% respectively .Sterilization of seeds reduced the number of contaminated fungi associated with *Moringa* seeds, where the high percentage of non contaminated seed (90.0%) compared with (59.5%) of infected seed in non sterilized seeds was recorded.

One the other hand, non sterilized *Moringa* seeds produced high percentages of *Rhizopus spp., Trichoderma spp., Penicillium spp., Aspergillus niger* and *Aspergillus flavus* if compared with sterilized seeds .According to the available literature, this is the first report of survey and isolation of seed born fungi associated with *Moringa* seeds. Some investigators noted that some fungal pathogens were isolated from decay and died seedlings of *Moringa* (Lezcano et al., 2014). Reports of diseases that affect *Moringa* after emergence are few (Mandhokhot et al., 1994). Kumar *et al*., 2013 found that *Moringa oleifera* , a new host record of *Cercospora apii s. lat.* from Uttar Pradesh in India. In general, seed-borne pathogens have significant influence on seed production and food industry because they: (i) can affect germination, growth and crop productivity (ii) cause seed and seedling diseases

resulting in the development of systemic or local infections (Bhattacharya and Raha, 2002; Lezcano et al., 2014)

Effect of seed sterilization on damping-off incidence on *Moringa* seedlings

Moringa seeds germination poorly and most seedlings die during early establishment. To study this problems the effect of sterilization of seeds and soil before sowing on susceptibility of *Moringa* seedlings to fungal diseases during emergence and early establishment were studied. Viability was tested prior to planting and the seed lots of *Moringa* were found to have up to 90% viability. Sterilization of *Moringa* seeds decreased percentage of contaminated and infected seeds by fungi from 80% compared to 19% of non sterilized seeds (seeds washed with sterilized water only). The high records of infected and contaminated seeds were found in old *Moringa* seed lot A if compared with recent harvest seeds lot B. Results in Table 2 indicate that sterilization of *Moringa* seeds as well as planting soil prior to sowing significantly reduce damping-off disease at both pre-and post-emergence damming –off on *Moringa* seedlings if compared with non sterilized seeds or soil media planting. Damages such as seed death, seedling and plant abnormalities or decreased seed vigour caused by seed-borne pathogens are not always recognized by users.

Table.1 Percentages % of *Moringa oleifera* seeds naturally infected with seed borne fungi

Type of seed	Isolated fungi	Non Sterilized seeds		Mean %	Sterilized seeds		Mean %
		A	B		A	B	
Infested seeds	*Fusarium solani*	8	6	7.0	3	2	2.5
	Fusarium oxysporum	3	2	3.5	1	0	0.5
	Fusarium spp	4	2	3.0	2	2	2.0
	Rhizoctonia solani	3	0	1.5	1	2	0.5
	Macrophomina spp	3	2	2.5	1	0	0.5
	Alternaria spp	2	2	2.0	1	0	0.5
	Helminthosporium spp	1	2	1.5	0	0	0.0
	Rhizopus spp	6	4	5.0	0	0	0.0
	Trichoderma spp	5	5	5.0	0	0	0.0
	Penicillium spp	4	5	4.5	2	0	1.0
	Asperigillis niger	6	5	5.5	1	2	1.5
	Asperigillis flavus	3	4	3.5	0	0	0.0
Infected seeds %		48	33	40.5	12	8	10
Non Infected seeds %		52	67	59.5	88	92	90.0

A = lot of *Moringa* seeds collected one year ago B = lot of *Moringa* seeds collected one month ago

Table.2 Damping -off disease incidence of *Moringa oleifera* plants sowing in naturally soil in greenhouse

Treatment		Pre-emergence - damping off after 10 day	Post emergence - damping off	
			After 20 day	After 30 day
Non-sterilized soil	Non-sterilized seed	21	3	0
	Sterilized seed	7	2	0
Sterilized soil	Non-sterilized seed	12	2	0
	Sterilized seed	4	0	0

Table.3 Response of *Moringa oleifera* plants to infection with different soil borne pathogenic fungi under artificially infested soil in green house

Pathogenic fungi	Damping –off disease incidence				Root rot /Wilt disease			
	Sterilized seeds		Non Sterilized seeds		Sterilized seeds		Non Sterilized seeds	
	Pre – emergence after 15 day	Post – emergence after 30 day	Pre – emergence after 15 day	Post – emergence after 30 day	After 30 day	After 60 day	After 30 day	After 60 day
Fusarium solani	8c	0.0a	19c	0.0a	0.0a	0.0a	0.0a	0.0a
Fusarium oxysporum	2a	0.0a	11b	0.0a	0.0a	0.0a	0.0a	0.0a
Rhizoctonia solani	6b	0.0a	12b	0.0a	0.0a	0.0a	0.0a	0.0a
Sclerotium rolfsii	5b	0.0a	14bc	0.0a	0.0a	0.0a	0.0a	0.0a
Macrophomina phaseolina	5b	0.0a	9b	0.0a	0.0a	0.0a	0.0a	0.0a
Control	3a	0.0a	7a	0.0a	0.0a	0.0a	0.0a	0.0a

Figures with the same letters in each column are not significantly differed (P≤0.05)

Table.4 Growth parameter of *Moringa oleifera* plants affected with different soil borne pathogenic fungi

Pathogenic fungi	Plant height (cm)	Fresh weight (gm)		Dry weight (gm)	
		Shoots	Roots	Shoots	Roots
Fusarium solani	55	18a	7a	7a	3a
Fusarium oxysporum	54	14ab	8a	6a	3a
Rhizoctonia solani	52	12b	6a	6a	2a
Sclerotium rolfsii	46b	13b	5a	5a	2a
Macrophomina phaseolina	55a	15a	5a	5a	2a
Control	58a	20a	8a	8a	3a

Figures with the same letters in each column are not significantly differed (P≤0.05)

A **B** **C** D **E** F

Figure.1 Pathogenicity of some soil borne pathogenic fungi on *Moringa oliefera.* A –control non infested soil, B - *Fusarium solani,* C - *Fusarium oxysporum,* D - *Macrophomina phaseolina,* E - *Rhizoctonia solani,* F - *Sclerotium rolfsii*.

Effect on Vegetative growth parameters

The effect of five soil born pathogenic fungi on growth of *Moringa oleifera planted* in artificially infested soil was investigated (Table 3). Results in Table 4 show that there is no clear effect of the tested pathogens on vegetative growth parameters such as plant height, fresh and dry weight of shoots and roots of *Moringa*. Plant height character showed a slight increase with *Fusarium solani* treatment as compared to *Fusarium oxysporum* and/or *Rhizoctonia solani*. Plant height was lowest with *Sclerotium rolfsii* treatment. However, differences among treatments were insignificant. Fresh weight of shoots and/or roots gave the same trend as plant height parameters being heights with *Fusarium solani* treatment and lowest with *Sclerotium rolfsii* treatment, with no significant difference among treatments.

Data in Table 4 also showed that dry weight of shoots and/or roots was not significantly affected by different treatments, although it was highest with *Fusarium solani* and lowest with *Sclerotium rolfsii* treatment.

Soil borne fungal pathogens which infect seeds and roots are a serious constraint to nursery production as they affect seedling establishment leading to poor emergence and delayed development of the seedlings. *Moringa* emergence and initial seedling growth rate is influenced mainly by soil and seed borne pathogenic fungi which causes seed rots and seedlings die after emergence. Sterilisation of *Moringa* seeds appeared adequate in dealing with this pathogen. The use of a commercial disease free growing medium ought to be promoted. If natural soil is required, then clay, which has to be sterilized, could be used. *Moringa oleifera was* not susceptible to infection by *F. solani*, *F. oxysporum, R. solani, S. rolfsii* and *M. phaseolina*, as there is no fungal diseases incidence after 30 days of seed sowing in artificially infested (Fig. 1).

References

Agrios, G. 1988. Plant pathology, 3rd edn. Academic Press Inc, New York.

A.O.A.C., 1990. Official Methods of Analysis of Association of Official Agricultural Chemists. 15th: 1045–1106.

Anjorin S.T., Mohammed, M. 2009. Effects of seed-borne fungi on germination and seedling growth of water melon (*Citrullus lanatus*). *J. Agric. Soc. Sci.,* 5: 77–80.

Ashfaq, M., Ashfaq, U. 2012. Evaluation of mosquitocidal activity of water extract of *Moringa oleifera* seeds against *Culex quinquefasciatus* (Diptera: Culicidae) in Pakistan. *Pak. Entomol*., 34(1): 21–26.

Bhattacharya, K., Raha, S. 2002. Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. *Mycopathologia*, 155: 135–141.

Barnett, H.L., Hunter, B.B. 1992. *Illustrated Genera of Imperfecti Fungi* Minneapolis: Burgess publishing Co. 241 Pp.

Booth, C. 1977. *Fusarium*: laboratory guide to the identification of the major species. Surrey, Commonwealth Mycological Institute, England.

El-Mohamady, R.S.R., Nehal S. El-Mougy, Abdel-Kader, M.M., Mejda Daami-Remadi, 2014. Survey of root and foliar fungal diseases of grown tomato at different locations in Egypt. *JEIT.*, 4(1): 269–276.

Fahey, J.W. 2005. *Moringa oleifera* : A Review of the medical evidence for its nutritional, therapeutic, and prophylactic properties, Part 1. *Phytochem.*, 47: 123–157.

Gilman, J.C. 1957. A manual of soil fungi. Iowa State University Press, Ames, Iowa, U.S.A. 450 Pp.

Goss, M., Tendai Shiri, A., Upenyu, M. 2013. The effects of pH and growing medium type on the susceptibility of *Moringa oleifera* to fungal diseases during seedling emergence. *Afr. J. Plant Sci.*, 7(12): 593–596.

Goss, 2007. A study into the initial establishment and growth of multi-purpose *Moringa oleifera* (L) with focus on population density, media type and pH when grown as a vegetable. 2007. Msc Thesis, University of Zimbabwe.

International Seed Testing Association (ISTA) 1976. International Rules for Seed Testing . *Seed Sci.Technol.,* 4: 51–177

Kshirsagar, C.R., D'Souza, T.F. 1989. A new disease of drumstick. *J. Maharashtra Agricult. Univ.,* 14(2): 241–242.

Kumar, S., Singh, R., Saini, D.C. 2013. *Moringa oleifera* , a new host record of *Cercospora apii s. lat.* from Uttar Pradesh, India. Plant Pathol. Quarantine 3(1), 12–13. doi 10.5943/ppq/3/1/2.

Lezcano, J.C., Alonso, O., Marialys, T, Martínez, E. 2014. Fungal agents associated to disease symptoms in seedlings of *Moringa oleifera. Lamarck Pastos y Forrajes,* 37(2): 228–232.

Machowicz, Z. 2010. Occurrence and characterization of *Colletotrichum dematium* (Fr.) Grove. *Pol. J. Microbiol.,* 59(3): 191–200.

Martín, C., Martín, G., García, A., Fernández, T., Hernández, E., Puls, J. 2013. Potencialidades y aplicaciones de *Moringa oleifera.* Una revisión crítica. Pastos y Forrajes, 36(2): 137–149.

Mandhokhot, A.M., Fugro, P.A., Gonkhalekar, S.B. 1994. A new disease of *Moringa oleifera* in India. *Indian Phytopathol.,* 47(4): 443.

Neler, J., Wasserman, W., Kutuner, M.H. 1985. Applied linear statistical models. In: Regression analysis of varlannce and experimential design, 2nd edn. Richard, D. Irwin Inc. Hame Wood Ilinols.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O. 1983. *Fusarium* species. An illustrated manual of identification. The Pennsylvania State University Press, University Park, Pennsylvania, USA, 203 Pp.

Rajangam, J., Azahakia Manavalan, R.S., Thangaraj, T., Vijayakumar, A., Muthukrishan, N. 2001. Status of production and utilization of *Moringa* in Southern India. www.*Moringa*news.org/actes/rajangam_en.doc. Accessed 4 June 2012.

Radovich, T. 2011. Farm and forestry production and marketing profile for *Moringa* (*Moringa oleifera*). In: C. R. Elevivitch, ed. *Specialty crops for Pacific island agroforestry*. Holualoa, Hawaii: Permanent Agriculture Resources (PAR), 2011.

Ramachandran, D., Peter, K.V., Gopalakrishnan, P.K. 1980. Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. *Econ. Bot.,* 34: 276–283.

El–Mohamedy, R.S.R., Abdalla, A.M. 2014a. Evaluation of antifungal activity of *Moringa oleifera* extracts as natural fungicide against some plant pathogenic fungi *In-vitro*. *J. Agricult. Technol.,* 10(4): 963–982.

El–Mohamedy, R.S.R., Aboelfetoh, M. Abdallah (2014b)Antifungal activity of *Moringa oleifera* oil and seed extract against some plant pathogenic fungi. *Middle East J. Agricult. Res.,* 3(2): 242–249.

Palada, M.C., Chang, L.C. (2003). Suggested cultural practices for *Moringa*. AVRDC International Cooperators Guide. Shanhua, Taiwan, AVRDC Pub., Pp. 03–545.

Pérez, A., Sánchez, T., Nayda, A., Reyes, F. 2010. Características y potencialidades de *Moringa oleifera,* Lamarck. Una alternativa para la alimentación animal. *Pastos y Forrajes,* 33(4): 349–362.