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The effects of pH and growing medium type on the susceptibility of *Moringa oleifera* to fungal diseases during seedling emergence

Maria Goss, Tendai. A. Shiri and Upenyu Mazarura*

Department of Crop Science, Faculty of Agriculture, University of Zimbabwe, Box MP167, Mount Pleasant, Harare, Zimbabwe.

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***Moringa oleifera* seed germinates poorly and most seedlings die during early establishment. To solve this problem, the effects of pH and growing medium type on the susceptibility of *M. oleifera* to fungal diseases during seed emergence and early seedling establishment were evaluated in a 3 x 6 factorial experiment arranged as a randomized complete block design with three replications in a greenhouse experiment. Six growing medium types (sandy soil, clay soil, pine bark, clay + sandy soil, clay + pine bark and sand + pine bark) were evaluated at pH 6.2, 8.2 and *in situ* pH for each growing medium type. The control was the sterilised media of *in situ* pH. Seed viability tests showed 90% viability. Analysis of the seed showed the presence of *Fusarium* spp., *Pythium* spp., *Dreschlera* spp., *Rhizopus* spp. and *Chactomium* spp. Thus, seed was sterilised prior to planting to eliminate any pathogen from the surface of the seed. Media and pH were found to have a significant effect on the emergence of seeds. *In situ,* sterilised pine bark and clay gave the highest emergence. After emergence, no seedlings showed any infection indicating that reported seedling deaths could be a result of seed borne diseases. Observations indicated *Fusarium* spp. infection in the seed that had failed to germinate, whilst those in the sterilised media showed no evidence of fungal attack. This means media used for *Moringa* ought to be sterilised with hypochlorite or another material or procedure to ensure good seed emergence.**

Key words: pH, susceptibility, viability, media, pathogens, emergence, sterilised, *Fusarium* spp.

INTRODUCTION

Moringa belongs to the family Moringaceae, a genus comprising of thirteen tree species that grow in the tropical and subtropical regions of the world. *Moringa oleifera* is a soft wood native to India with great potential in combating extreme hunger and poverty. These nutritious trees grow quickly in many environments and can feed people as well as livestock. Nutritional analyses has shown that *Moringa* leaves contain large amounts of several important nutrients such as vitamin A and C, calcium, potassium and surprisingly contains all essential amino acids and complete proteins, which is rare for a plant (Anonymous, n.d.).

The use of *Moringa* in Zimbabwe has been largely medicinal, especially in the management of HIV/AIDS (Monera and Maponga, 2011). This is not surprising because *Moringa* has been reported to have numerous clinical benefits (Fahey, 2005). In addition to this, *Moringa* is now an essential part of many individual nutritional gardens in Zimbabwe, though there is no commercial production. *Moringa* is a proven water purifier with remarkable nutritional value and happens to grow in places where bad water, poor diets and diseases cause high mortalities (Fritz, 2000).

Pilot studies have been done to determine the effects

*Corresponding author. E-mail: umazarura@agric.uz.ac.zw or umazarura@yahoo.com. Tel: +263773 788 853.

of different medium types, pH levels and fertilizer types on the general growth of the tree. In these studies as well as in some nurseries, it was also observed that a significant proportion of the seedlings succumbed to fungal diseases (Chimonyo, 2006; Goss, 2007). This can be a major setback in *Moringa* nurseries, and can result in reduced quantity and quality of *Moringa* seedlings. In spite of this, *Moringa* has received minimum attention. This is more so in relation to studies on diseases that affect the tree at various stages of development. At germination, it is likely that medium type and pH could be important.

Soils and soil conditions affect the growth of crops indirectly by their effect on disease, among many factors, whilst adverse soil conditions such as poor drainage greatly increase the chances of serious infection with root fungi (Davies et al., 1993). Soil pH affects pathogen development in the soil since soil is a natural reservoir of innoculum.

A gram of soil normally contains ten to a hundred meters of mycelia fragments (Foth, 1990). 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).

A disease caused by *Drechslera haraiiensis*, whose major symptom is the extensive rotting of pods has been reported (Rajangam et al., 2001). Zimbabwean *Moringa* growers indicate that there are cases of diseases similar to fungal wilts and damping off in a significant number of *Moringa* nurseries. However, no research has been done to confirm or refute these claims.

MATERIALS AND METHODS

Study site

The experiment was carried out at the University of Zimbabwe, Crop Science Department in a greenhouse.

Experimental design

The experiment was laid out as a 3 x 6 factorial, arranged in a randomized complete block design. Factor 1 was pH with 3 levels namely: *in situ* pH, pH 6.2 and 8.2 on the calcium chloride scale. Factor 2 was growing medium type with six levels namely: clay, sand, pine bark, sand + pine bark, clay + sand and clay + pine bark. Each treatment was replicated once in each of three blocks. The control was the *in situ* pH and was sterilised for each growing medium type.

pH amendment

The pH of each media was determined and the pH amended to the desired level using lime to increase pH and ferrous sulphate to lower pH. After amendments, the media were left to stabilize for about three weeks after which measurements were done to check whether the required pH levels had been obtained. The *in situ* pH of the respective media were as follows: clay pH 6.2, pine bark pH 4.2, sand pH 5.8, sand + pine bark pH 5.6, clay + pine bark pH 6.0 and 5.9 for clay + sand pH 5.9.

Seed tests, pathogen analysis and planting

Viability was tested prior to planting and the seed lots were found to have 90% viability. Laboratory analysis was done to examine seeds for seed borne pathogens before sowing and the following pathogens: *Rhizoctonia* spp*., Rhizopus* spp., *Pythium* spp., *Chactomium* spp. *and Dreschlera* spp. were identified. *Fusarium* spp. attacked 30% of the seedlings, *Chactomium* 10%, and *Rhizopus* spp. 12%. The seeds were then washed with sterile water and dipped in a 1:6 solution of hypochlorite and water. Thereafter, the seeds were incubated at a temperature of 26°C for ten days. The seeds were lastly immersed in hot water at 75°C for five minutes before planting.

Five seeds were planted in each polythene bag of 25 cm diameter. Each seed was sowed at a depth of 1 cm. Watering was done prior to planting the seed and 0.3 L of water was applied in each growing medium type twice a week.

Data collection and analysis

Disease severity and incidence were measured. Severity of any fungal disease was measured on a scale of 1 - 5, with 5 being the most severely infected count and 1 being the least infected. Incidence was obtained by counting the number of diseased plants. Assessment of the media was done to determine the presence of naturally occurring fungi in each growing medium, within each media using water based agar and microscopy for identification. In addition to this, slides were prepared using the tissue from diseased plants and seeds which had not germinated.

Results were analyzed using Genstat and Analysis of Variance (ANOVA) was carried out for emergence of seeds and disease incidence and severity. Fisher's Protected Least Significance Difference test (LSD) at 5% was used to separate means.

RESULTS

Effects of pH and medium type on emergence

Clay

The pH of 6.2 produced the least germination, which was significantly lower ($P<0.05$), than that of pH 8.2 or the *in situ* sterilised clay. The two, pH 8.2 and the sterile *in situ* clay, were not significantly different and both had high germination (Figure 1). The results were somewhat reversed in the case of the sand. Here, pH 6.2 and 8.2 significantly ($P < 0.05$) increased germination when compared with the sterile *in situ* sand. The sterile *in situ* sand had the lowest germination (Figure 1).

Germination from pine bark was not affected by pH

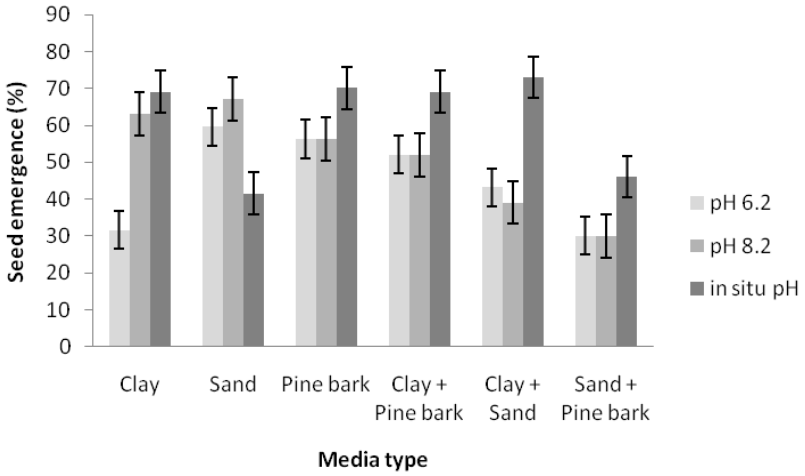

Figure 1. Effects of pH and growing medium type on seedling emergence. Error bars indicate LSD at 5%.

although the sterile pine bark tended to increase germination. Similar results were observed with the mixture of pine bark and clay, showing, perhaps the dominant pine bark effect (Figure 1). A similar trend was observed when pine bark was mixed with sand although this mixture produced very low germination. On the other hand, the clay and sand mixture was somewhat additive in the case of the sterile mixture but antagonistic with the other treatments.

In summary, the germination of *Moringa* was affected differently by different media but tended to be higher with the sterile media in all growing medium types except when pine bark was mixed with sand or when sand was alone.

Effect of growing medium type and pH on fungal infection

There was no incidence of fungal disease on the seedlings in the nursery after emergence during the duration of the experiment. The seeds which failed to geminate were dug up and analyzed for fungal infection. Identification procedures in the laboratory indicated the pathogen which had caused the rotting to be *Fusarium* spp. with no other fungi being present. However, the seed from the sterilised media showed no signs of infection upon analysis. The degree or severity of seed rotting however varied from treatment to treatment as indicated by the variation in colour and level of rotting observed in the seeds being analyzed (Table 1).

DISCUSSION

Seed viability tests carried out prior to planting indicated that the seed used had a viability of 90%. Growing medium type had a significant effect on emergence, affecting the rate of emergence, with all media except sand exhibiting high germination in the *in situ* pH growing medium. The sand at pH 8.2 showed the most pH-depended improvement over the *in situ* treatment. These results are supportive of the preliminary studies done (Goss, 2007) where the largest *Moringa* biomass production was realized in the sandy soil and these studies further indicated that *Moringa* performed well in pH ranges of 7.6 to 8.7 (calcium chloride scale).

This study shows that the use of good quality seed and sterilisation of media may solve the poor germination of *Moringa*. However, 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. There was no fungal infection associated with the pine bark media. This commercial material must therefore have been free from disease pathogen contamination. Use of composted pine bark in planting media has been reported to result in successful control of diseases caused by several soil borne fungi such as *Pythium* spp., *Phytopthora* spp. and *Rhizoctonia* spp. (Agrios 1988).

Laboratory seed analysis revealed that the seeds which failed to germinate had been infected by *Fusarium* spp. This is an interesting finding. It appears that despite reports that *Moringa* is highly resistant to diseases, it is in fact susceptible to *Fusarium* spp. resulting in seed rots and reduced germination. In this experiment, analysis of the seed which had failed to germinate suggested that poor germination may have been a result of seed borne diseases or soil borne pathogens. Whilst findings from this study are supportive of preliminary studies (Goss, 2007) which indicated high susceptibility of *Moringa* seed to fungal infections, there is need for further studies. Such studies could use artificial infection.

The poor germination, which was associated with the *in situ* sterilized sand, suggested that other factors affected

Table 1. Analysis of seed that failed to emergence for pathogen infection.

Treatment	Color observed	Type of fungi identified
Clay[1]*	Brownish	*Fusarium* spp.
Clay[2]	Creamish brown	*Fusarium* spp.
Sand[1]	Creamish brown	*Fusarium* spp.
Sand[2]	Brownish black	*Fusarium* spp.
Pinebark[1]	Brown	*Fusarium* spp.
Pinebark[2]	Creamish brown	*Fusarium* spp.
ClayPinebark[1]	Blackish, brown	*Fusarium* spp.
ClayPinebark[2]	Brownish, cream	*Fusarium* spp.
ClayaSandS[1]	Dark brown ,black	*Fusarium* spp.
ClaySandS[2]	Dark brown	*Fusarium* spp.
SandPinebark[1]	Brownish	*Fusarium* spp.
SandPinebark[2]	Creamish brown	*Fusarium* spp.
ClayPinebark[3]	Creamish	No fungi identified
Clay[3]	Creamish	*Fusarium* spp.
Sand[3]	Creamish	No fungi found
Claysand[3]	Creamish brownish	Fusarium spp.
SandPinebark[3]	Creamish	No fungi found
Claysand[3]	Creamish	No fungi found

*1: pH 6.2, 2: pH 8.2, 3: *in situ* pH + sterilized.

germination in this growing medium. It is conceivable that the good drainage associated with sand could have led to poor moisture availability for the germinating seed. However, despite the presence of *Fusarium* spp. in sand at pH 6.2 and 8.2, germination was good, showing perhaps that the good drainage could have somewhat led to the leaching of the pathogen.

Lastly, contrary to expectations, there were no incidences of fungal diseases in the field after emergence of the seedlings. This suggests that seed borne diseases may account for the reported poor germination of *Moringa* and not diseases coming after emergence.

Conclusion

Moringa emergence and initial seedling growth rate is influenced mainly by medium type and infection by *Fusarium* spp. which causes seed rots. Sterilisation of media appeared adequate in dealing with this pathogen. The use of a commercial disease free growing medium such as pine bark ought to be promoted. If natural soil is required, then clay, which has to be sterilised, could be used.

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