

Original article

## Effect of Soil on *Moringa oleifera* Lam. Seed Germination and Establishment

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### Abstract

This study investigated the germination and early growth performance of *Moringa oleifera* Lam. seeds collected from four Libyan populations (Benghazi, Zliten, Misurata, and Tripoli) when sown in four different soil types from the Benghazi region, beside sand and peatmoss media. Pod morphological traits varied significantly across sources, revealing a distinct trade-off in resource allocation. Benghazi produced the heaviest pods (18.4 g) with fewer and larger seeds (39.9 g/100 seeds), while pods from Misurata and Zliten were lighter with more numerous but smaller seeds. Despite these differences, total seed mass per pod remained conserved across populations. Germination success was primarily governed by soil type rather than seed origin, with Sidi Ali soil exhibiting significant inhibitory effects on germination. Seedling growth was influenced by complex interactions between seed source and soil type. The seedlings of seeds that were obtained from Zliten demonstrated superior vegetative vigour, producing the longest shoots and roots, while Benghazi seedlings showed the poorest initial growth despite originating from the largest seeds. Seeds sown in sand promoted optimal root development, whereas organic-rich media (peatmoss, Ganfouda) enhanced shoot biomass. Soil from the Quarsha site consistently limited all growth parameters. These findings highlight that selecting appropriate seed sources (particularly Zliten) and optimizing soil conditions are crucial for successful *Moringa* establishment in Libyan environments.

**Keywords.** *Moringa*, *Moringa Oleifera*, Libya, Seed Germination, Early Establishment.

### Introduction

*Moringa oleifera* Lam. (Family: Moringaceae), the miracle tree, thrives across nearly all tropical and subtropical regions worldwide, but it is believed to be native to Afghanistan, Bangladesh, India and Pakistan. Almost all the parts of the tree are utilized for their essential nutrients. *Moringa oleifera* leaves are rich in beta-carotene, minerals, calcium and potassium [1]. Dried leaves contain approximately 70% oleic acid, making them suitable for producing moisturizers. The bark of the tree is considered highly beneficial in treating various disorders, including ulcers, toothaches and hypertension. Roots, however, are known to aid in the treatment of toothache, helminthiasis and paralysis [2]. The flowers are used to treat ulcers and an enlarged spleen. The tree is believed to possess remarkable properties in combating malnutrition among infants and lactating mothers [3].

*Moringa* is widely distributed globally, but its indigenous origins are in India, Arabia and the East Indies. It is common in Asia, Africa, the Caribbean, Latin America, the Pacific Islands, Florida, Madagascar, Central America, Cuba, the Philippines, Ethiopia and Nigeria [4]. The history of the plant indicates that *M. oleifera* was introduced from India to Africa, Southeast Africa, and the Philippines in ancient times. It thrives in tropical and subtropical regions and grows at temperatures of about 25–35°C. *Moringa oleifera* is a deciduous tree typically cultivated in tropical and subtropical areas worldwide [5]. It grows best in indirect sunlight and without waterlogging, in soil that is slightly acidic to alkaline. The tree begins to bear fruit at 6 to 8 months of age. Commercial cultivation occurs in various places, such as many countries in Africa, Mexico, Hawaii, and South America, but due to diverse soil conditions, the nutrient content varies from one country to another [6, 7]. *Moringa oleifera* is a fast-growing, evergreen, deciduous perennial tree that reaches approximately 10 to 12 meters in height. The bark is whitish-grey and surrounded by thick cork. Young shoots have purplish or greenish-white bark. The flowers are yellowish, creamy white and fragrant. The mature fruit is a hanging capsule measuring 20-45 cm in length, containing 15-20 dark brown, globular seeds with a diameter of 1-1.2 cm [5]. It grows well in sandy or loamy soil with a pH of 6.5-7.5 and a rainfall of 250–300 cm [4]. The direct seeding method is generally preferred as it germinates profusely. The seeds are sown 2 cm deep in a well-prepared nursery, and germination occurs within 5-12 days. The thirty-centimetre-high seedlings are ready for transplanting into the open field, spaced 5 x 5 m apart. It can also be propagated from cuttings 1 m long and 4-5 cm in diameter, but due to its shallow root system, these plants tend to be sensitive to drought and wind [8].

In a previous study conducted in Nigeria to determine the soil type suitable for germination of *Moringa* seeds, four soil types were tested: Sandy soil, Sandy loam soil, Clay loam soil and Clay soil. Climatic conditions at the time of the study included a semi-arid climate with average daytime temperatures ranging from 15 to 48°C. The region suffers from soil degradation, making it a realistic environment for testing *Moringa* adaptability [8]. Sandy loam soil resulted in the highest and fastest germination with uniform seedling growth. Sandy soil showed early germination but lacked consistency and seedling vigour. For successful *Moringa* cultivation, opt for sandy loam soils when possible. This soil provides the best combination of

aeration, moisture retention, and nutrient availability. Clay and sandy soils are less ideal, although *Moringa* demonstrates resilience across various types [8].

However, India is the largest producer of *Moringa*, with an annual production of 1.1 to 1.3 million tons of tender fruits from an area of 380 km<sup>2</sup>. Among the states, Andhra Pradesh leads in both area and production (156.65 km<sup>2</sup>) followed by Karnataka (102.8 km<sup>2</sup>) and Tamil Nadu (74.08 km<sup>2</sup>). In other states, it occupies an area of 46.13 km<sup>2</sup>. It has varied genotypes from diverse geographical regions and introductions from Sri Lanka. *Moringa* seed contains 40% of oil. It may be obtained without cost as a by-product of oil extraction. Thus, *Moringa* seeds can first be used for oil extraction without reducing their effectiveness for water treatment. *Moringa* oil is of high quality and potentially has a high market value. The oil is of equal value for cooking and as the main ingredient in soap manufacture [9, 10].

*Moringa oleifera* has a variety of activities, including uses as a galactagogue, rubefacient, antiscorbutic, diuretic, stimulant, purgative, antimicrobial, antibacterial [11], anti-inflammatory, antitumour, antioxidant, anti-ageing agent, hypoglycaemic, antithyroid, anti-cellular [12], hypocholesterolaemic and antispasmodic. Moreover, it lowers circulatory strain, reduces cerebral pains and lessens headaches. Various therapeutic properties have been attributed to different parts of this highly regarded tree. Practically every part of this plant, such as bark, gum, root, fruit (pods), flowers, leaves, seeds and seed oil, has its own significance. They have been used in traditional folk medicine to treat a broad range of illnesses [13].

*Moringa oleifera* grows best in direct sunlight at altitudes below 500 m. The minimum annual rainfall requirement is around 250 mm. However, in waterlogged soil, the roots tend to rot. In areas with heavy rainfall, trees can be planted on small hills to encourage water run-off. The presence of a long taproot tuber makes it resistant to periods of drought. Trees can be easily grown from seed or from cuttings. Temperature ranges are 25–35°C, but the tree will tolerate up to 48°C in the shade and can survive light frosts. *Moringa* seeds have no dormancy period, so they can be planted as soon as they are mature and remain viable for up to one year. *Moringa* trees will flower and fruit annually, and in some regions, twice yearly. During its first year, a *Moringa* tree can grow up to five meters in height and produce flowers and fruit. If left unpruned, the tree can eventually reach 12 meters tall with a trunk 30 cm wide; however, it can be cut back annually to one meter from the ground. The tree will quickly recover and produce leaves and pods within easy reach. Within three years, a tree can yield 400-600 pods annually, with mature trees capable of producing up to 1,600 pods [14]. This study aims to assess the capability of *moringa* seeds collected from various regions across the territory to germinate and establish in different types of soils gathered from areas around Benghazi city.

## Methods

### Seed Sources

Seeds were collected from trees growing in four different cities in Libya, which are Benghazi, Zliten, Misurata and Tripoli. Seeds were packaged and mainly exported to the laboratory where they had been tested. The first three locations sent seeds in their pods, except Tripoli, where the seeds had already been removed from the pods.

### Source of Soils

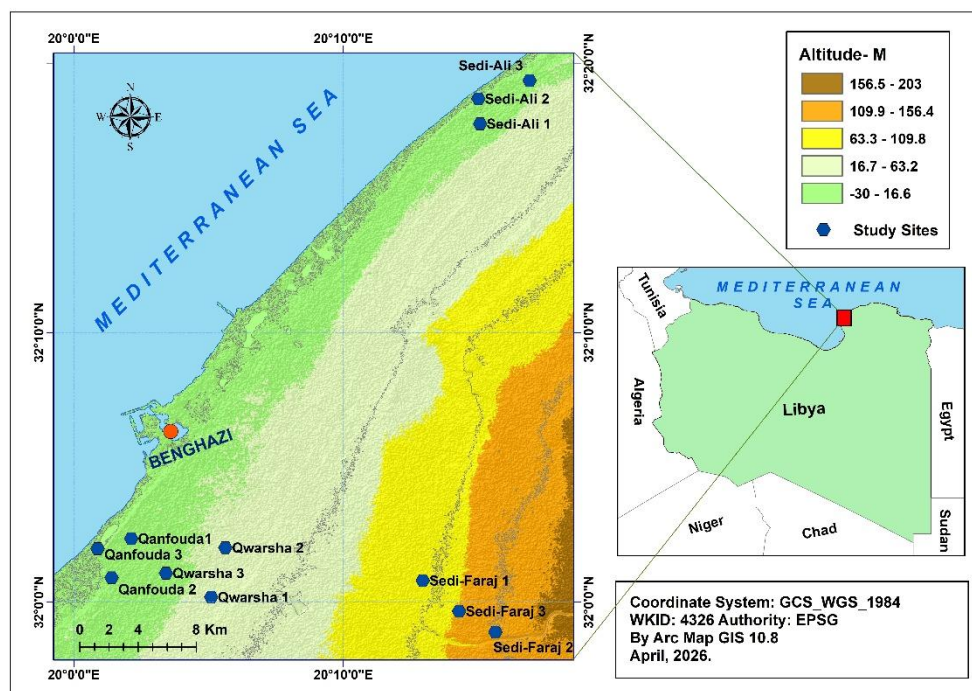
Soil was collected from four different areas around Benghazi city, with each area divided into three sites. The areas included Ganfoda, Alquarsh, Sidi Farg, and Sidi Ali. Additionally (Fig. 1), sand and peat moss were used as alternative soil types. Three replicates of pots were prepared for each site.

### Soil Profile

The soil profile and characterization were determined according to Burt (2004) [15].

### Laboratory Experiment

In this experiment, the length of the pod, weight and the number of seeds were measured using a tailor tape and seed mass was measured using an analytical balance. Seeds were sterilized by soaking the seeds in 2% sodium hypochlorite for 5 minutes. They were then placed in 20×2×6.5 cm containers, each containing 10 seeds, and arranged in two lines on two layers of paper towels, then covered with two additional layers of paper towels. The seeds in the containers were watered with 20 ml for the first time, and therefore with equal amounts of water (10 ml) every two days. The germination percentage and seedling performance were measured. Since the lengths of the shoot and root systems were measured with a ruler, and their fresh weights were measured with an analytical balance. The dry mass was obtained by oven-drying for 24h at 60°C [16].



**Figure 1. Locations from which the soils were collected (source: Dr Mohmood M. M. Soliman)**

### Glasshouse Experiment

In this experiment, pod and seed measurements were taken, as in the previous study; also, seed sterilization was performed. Empty pots were weighed, then filled with various types of soil (clay from three regions, sand, and peat moss), and their field capacity was measured. Soil was collected from three different areas around Benghazi city, with each area divided into three sites. The areas included Ganfoda, Alquarsh, Sidi Farg, and Sidi Ali (Fig. 1). Additionally, sand and peatmoss were used as alternative soil types. Three replicates of pots were prepared for each site. Five seeds were placed in each pot. The seeds were watered with equal amounts of water every two days. After 90 days, the seedlings were carefully removed, and their shoot and root lengths were measured with a ruler or measuring tape. Additionally, their fresh and dry weights were determined using an analytical balance.

## Results

### Soil Characteristics

All four samples occupy a spectrum from reddish-brown to yellowish-orange (Ochre). In soil science, these hues are diagnostic of specific mineral compositions:

#### Iron Oxides

The red/orange tint is oxidized iron. Samples from sites 1 & 2 (Redder): Likely contain Hematite ( $\text{Fe}_2\text{O}_3$ ), suggesting well-aerated, highly weathered conditions. Whereas, samples from sites 3 & 4 (Yellower): Likely contain Goethite ( $\text{FeO}(\text{OH})$ ), which often forms in slightly more moist or cooler environments than hematite (Table 1).

#### Organic Matter

The relatively bright chroma (intensity) suggests low to moderate organic matter content. Darker, duller browns would indicate higher humus levels (Table 1).

### Pod Length (cm)

The analysis of variance showed a highly significant effect of Pod Source on pod length ( $F_{(2, 181)} = 36.08$ ,  $p < 0.001$ ). This indicates that there are real, statistically detectable differences in mean pod length between at least two of the three source populations. Zliten site produced the longest pods (Mean = 39.26 cm, CI: 37.88 - 40.64). Pods from Benghazi were significantly shorter than pods from Zliten but longer than those from Misurata (Mean = 36.64 cm, CI: 35.36 - 37.93) (Plate 1, Table 2, Fig. 2). However, the geographic origin of pods in this study has a strong and significant influence on pod length. Zliten and Benghazi populations tend to produce longer pods than the Misurata population.

### Pod Mass

One-way ANOVA showed a highly significant effect of Pod Source on Pod Mass ( $F_{(2, 181)} = 37.32$ ,  $p < 0.001$ ). The average mass of pods varies significantly depending on their source. Where pods from Benghazi were the heaviest and formed a distinct group (Mean = 18.40 g, CI: 17.45 - 19.34). Whereas, pods from Zliten (Mean = 13.45 g, CI: 12.44 - 14.47) and Misurata (Mean = 12.91 g, CI: 11.85 - 13.96) pods were significantly

lighter than Benghazi pods and were not statistically different from each other in mass. Overall, the Benghazi *Moringa* population produces pods with a significantly greater biomass than the other two populations (Table 2, Fig. 2).

**Table 1. The visual structure, composition, texture, and structure classification, porosity, and scientific observations of soil collected from four different sites around the city of Benghazi: Sidi Ali, Sidi Farg, Alquarsh and Ganfoda. Burt (2004) [15].**

Site	Visual Structure	Composition	Texture & Structure Classification	Porosity	Scientific Observations
1 Sidi Ali	Subangular Blocky	A mix of fine earth and medium-sized aggregates (peds). It has a good balance of macropores (for drainage) and micropores (for water retention)	Clastic/Aggregated Structure	High (Macroporosity)	Contains distinct peds (soil aggregates). The presence of large voids between these aggregates facilitates rapid water infiltration and gas exchange.
2 Sidi Farg	Massive / Fine-grained	This sample appears highly disturbed or pulverized. It has a high surface area, suggesting a higher silt or fine sand content. It is prone to surface "crusting" when wet	Pulverized / Fine-Grained	Low to Moderate	The soil appears highly disturbed or sieved. While it has high total porosity, the effective porosity (permeability) is lower because the fine particles can easily compact and clog drainage paths.
3 Alquarsh	Granular / Fragmental	Contains larger, distinct mineral fragments and "crumb" structures. This indicates a coarse-textured soil (sandy loam or gravelly loam) with very high permeability.	Granular / Fragmental	Very High	Exhibits a "crumb" structure with a high proportion of coarse fragments. This structure is ideal for root penetration and prevents waterlogging due to high Hydraulic Conductivity.
4 Ganfoda	Cloddy / Angular Blocky	Large, heavy aggregates are visible. Strong indicator of high clay content. These clods form when clay-rich soil dries and shrinks.	Blocky / Composite Structure	Intermediate	Shows a mixture of fine "matrix" material and large "clods." This suggests a higher clay content



**Plate 1. Shapes and Sizes of Moringa pods for a) Benghazi, b) Misurata, c) Zliten sources**

### Seed Number per Pod

Also, the one-way ANOVA analysis showed a highly significant effect of Pod Source on the Number of Seeds per Pod ( $F_{(2, 181)} = 15.43$ ,  $p < 0.001$ ). The geographic origin of the pod significantly influences the number of seeds developed per pod. Pods obtained from Misurata contained the highest number of seeds (Mean = 18.06, CI: 17.03 - 19.08). Also, pods from Zliten had a high seed count (Mean = 16.48, CI: 15.50 - 17.47) and were not statistically different from Misurata. But, pods obtained from Benghazi contained significantly fewer seeds than the other two sources (Mean = 14.23, CI: 13.32 - 15.15) (Table 2, Fig. 2). There is a trade-off between pod mass and seed number. While, Benghazi pods are the heaviest (due to a larger pod wall), they contain fewer seeds. Conversely, Misurata and Zliten pods, while lighter, invest more in seed production per pod.

### Seed Mass per Pod

The analysis of variance found no significant effect of Pod Source on total seed mass per pod ( $F_{(2, 181)} = 2.16$ ,  $p = 0.118$ ). Despite differences in the number of seeds, the total dry mass of all seeds within a pod is statistically similar across all three populations. Tukey's paired-way test confirms that all three  $\pm$  belong to the same homogeneous group. There are no significant pairwise differences between Benghazi (5.70 g), Zliten (5.32 g), and Misurata (5.15 g) (Table 2, Fig. 2). This suggests that the total reproductive investment in terms of seed biomass per pod is conserved across these geographically distinct populations. The plants from different sources achieve a similar total seed mass through various strategies: Benghazi plants produce fewer but potentially larger/heavier individual seeds. In comparison, Misurata and Zliten plants achieve a similar total mass by producing a greater number of smaller/lighter seeds. The results of pod characteristics demonstrate clear genetic and/or phenotypic plasticity in pod and seed traits among the three populations. The most significant finding is the evidence of a resource allocation trade-off where Benghazi pods had fewer, larger seeds inside a heavy, robust pod. Whereas, Misurata/Zliten had more and smaller seeds inside a lighter pod.

**Table 2. Pod length and mass, along with seed number and seed mass from the three pod sources in the territory. Mean  $\pm$  StDev. The Tripoli site was excluded due to insufficient pods.**

Site	Pod Length (cm)	Pod Mass (g)	Seed Number/pod	Seed Mass/pod (g)
Benghazi	36.6 $\pm$ 6.57 <sup>b</sup>	18.4 $\pm$ 4.71 <sup>a</sup>	14.2 $\pm$ 4.39 <sup>a</sup>	5.7 $\pm$ 1.70 <sup>a</sup>
Zliten	39.3 $\pm$ 5.15 <sup>a</sup>	13.5 $\pm$ 3.83 <sup>b</sup>	16.5 $\pm$ 3.45 <sup>b</sup>	5.3 $\pm$ 1.41 <sup>a</sup>
Misurata	30.9 $\pm$ 3.82 <sup>c</sup>	12.9 $\pm$ 2.97 <sup>b</sup>	18.1 $\pm$ 3.55 <sup>b</sup>	5.1 $\pm$ 1.35 <sup>a</sup>
All Sites	35.8 $\pm$ 6.36	15.1 $\pm$ 4.69	16.1 $\pm$ 4.15	5.4 $\pm$ 1.52

\*Different letters mean statistical differences.

### Seed Mass

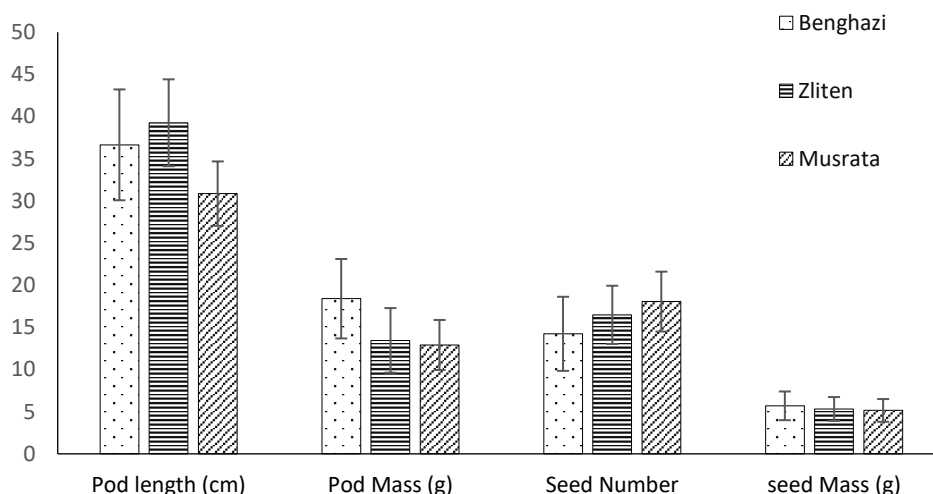
The one-way ANOVA revealed a highly significant effect of Seed Source on the Mass of 100 Seeds ( $F_{(3, 31)} = 79.08$ ,  $p < 0.001$ ). This indicates that there are extremely big, statistically detectable differences in the mean seed weight between at least some of the four geographic source populations (Benghazi, Misurata, Tripoli, and Zliten) (Fig. 3). The model explains a very high proportion of the variance in seed weight, with an R-sq(adj) value of 87.32%. This means that the geographic origin of the seed accounts for over 87% of the variability observed in seed mass, suggesting that this trait is heavily influenced by genetic and/or environmental factors specific to each location (Fig. 3). Seeds from Benghazi were significantly heavier than all other groups (Mean = 39.97 g per 100 seeds). They form a distinct and top-performing group. Seeds from Zliten (Mean = 32.30 g) were significantly lighter than Benghazi seeds but significantly heavier than those from Misurata and Tripoli. They form an intermediate group. Seeds from Misurata (Mean = 28.53 g) and Tripoli (Mean = 27.40 g) were the lightest and were not statistically different from each other. They form a distinct and lower-weight group (Fig. 3).

### Seed Germination

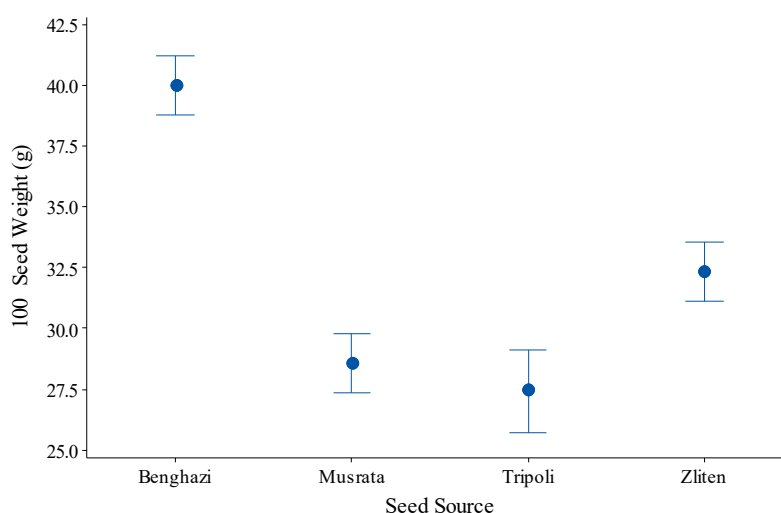
The results clearly demonstrate that soil source (growth medium) has a far greater and more consistent impact on seed germination success (number of germinated seedlings) than the geographic seed source. The effect of seed origin was statistically negligible, whereas the type of soil used for planting was a highly significant factor, though its impact varied across experimental batches (Plate 2).

### Effect of Seed Source on Germination

There was no significant effect of seed source on germination percentage ( $F_{(2, 135)} = 1.14$ ,  $p = 0.322$ ) (Fig. 4). The minor differences observed in the mean number of germinated seedlings between Benghazi (2.89), Misurata (3.02), and Zliten (3.37) are statistically insignificant and likely due to random chance.



**Figure 2. Means of pod length, mass, seed number and seed mass.**



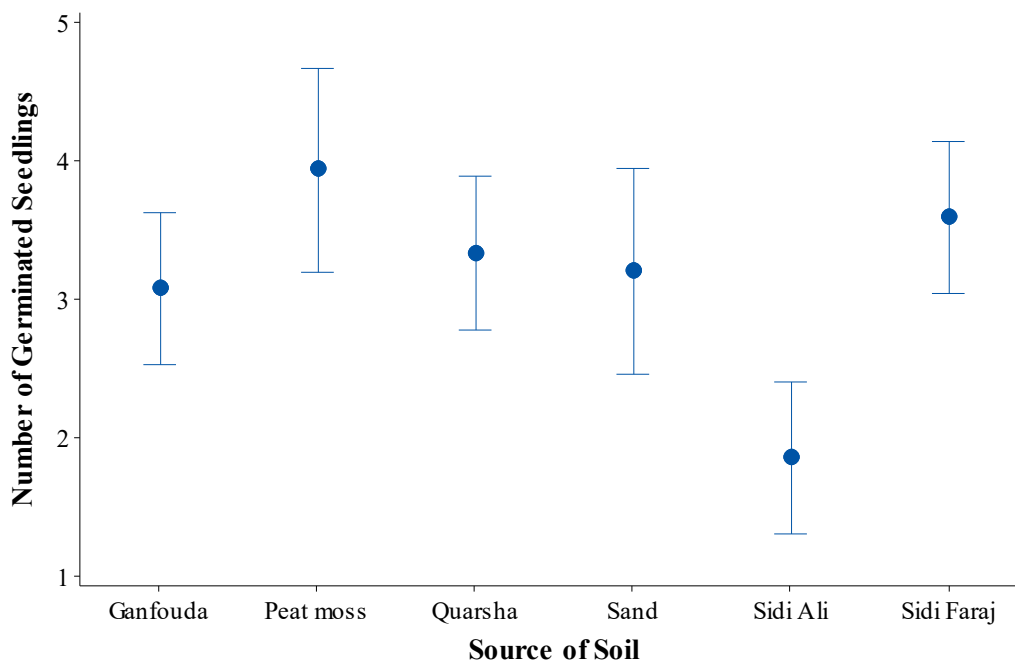
**Figure 3. Means of seed mass that were obtained from the four different sources. Mean  $\pm$  SE.**



**Plate 2. The growth of seedlings in different soil types. a) Zliten b) Misurata c) Benghazi.**

### **Effect of Soil Source on Germination**

The one-way ANOVA results showed a highly significant effect of soil region ( $F_{(5, 132)} = 5.78, p < 0.001$ ). Since Sidi Ali soil resulted in significantly lower germination (Mean = 1.85 seedlings) compared to all other soils, which formed a largely homogenous high-germination group (Means from 3.07 to 3.93). Peat moss, a standard nursery medium, performed very well (Mean = 3.93) (Fig. 4). In this study, the primary driver of germination success appears to be the growth medium (soil), not the origin of the seed. This highlights the critical importance of edaphic (soil-related) factors in the early establishment of this plant species.



**Figure 4. Means of germinated seeds in six different soil types. Mean  $\pm$  SE.**

### Laboratory Experiment

The results demonstrate significant and biologically meaningful intraspecific variation in early seedling growth and biomass allocation among seeds from different geographic sources. The geographic origin (Seed Source) is a significant determinant of seedling vigour and growth strategy under controlled laboratory conditions. The most pronounced differences are observed in root system development and biomass accumulation, with the Benghazi population consistently showing the least vigorous growth.

### Shoot Length

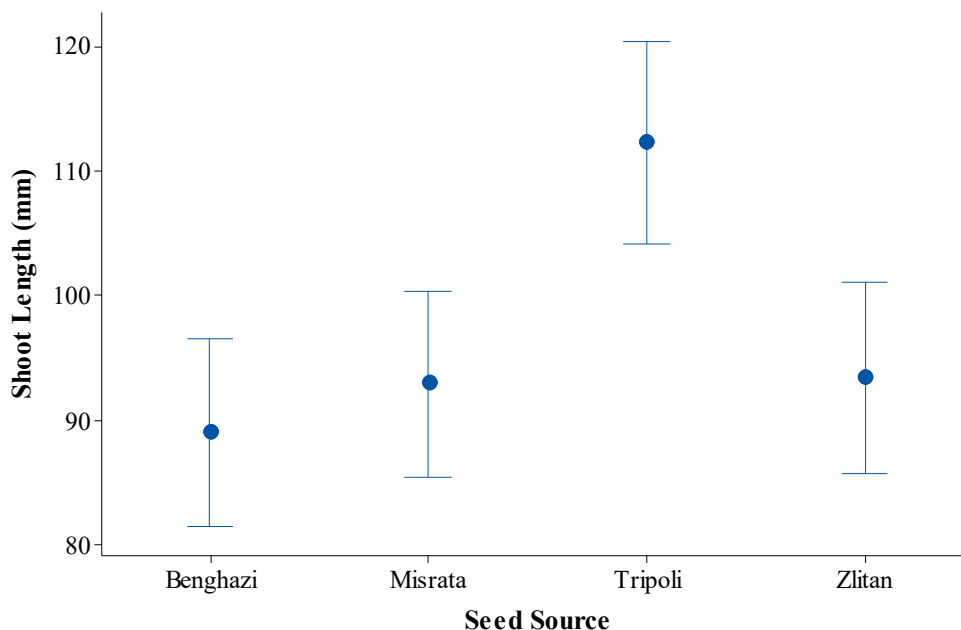
A significant effect of Seed Source was found ( $F_{(3, 114)} = 6.84(3, p < 0.001)$ ), accounting for 13.31% of the variation (adj.  $R^2$ ). Since seedlings from Tripoli developed significantly longer shoots (112.27 mm) than all other sources. Seedlings from Benghazi, Misurata and Zlitan formed a separate, statistically homogenous group with shorter shoots (89.00 - 93.38 mm). The Tripoli population has a genetic and/or maternal advantage for early shoot elongation under these controlled conditions (Fig. 5).

### Root Length

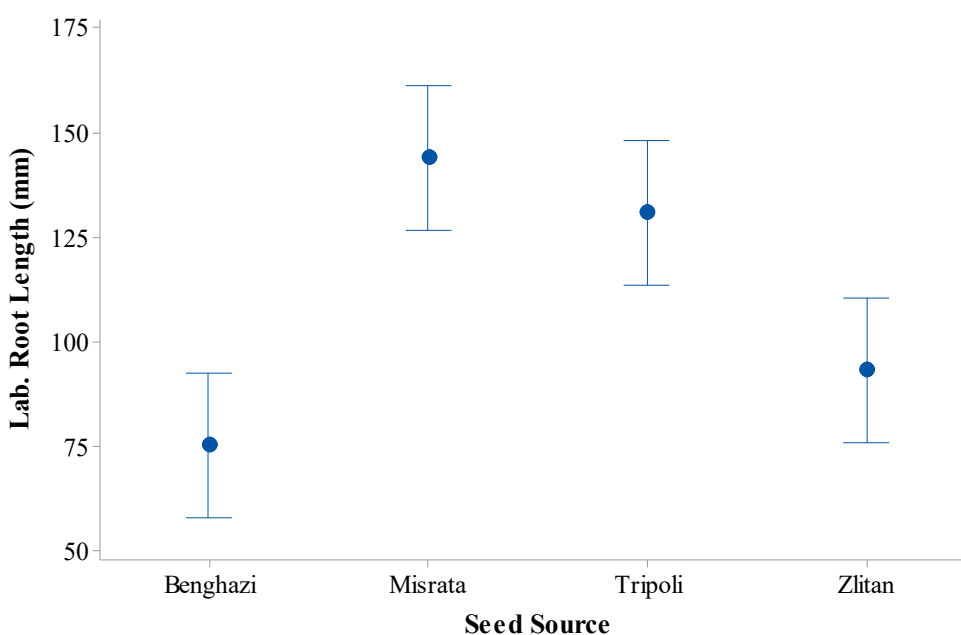
A highly significant and strong effect of Seed Source was found ( $F_{(3, 114)} = 24.19, p < 0.001$ ), explaining 37.89% of the variation (adj.  $R^2$ ). This was the most influential factor. However, seedlings from Tripoli and Misurata developed extensive root systems (~144-151 mm), forming a distinct group. In contrast, seedlings from Zlitan and Benghazi had significantly shorter root systems (~75-96 mm). It seemed that populations differ profoundly in their root foraging strategy. Tripoli and Misurata seeds produce seedlings primed for aggressive soil exploration, which could confer a significant advantage in nutrient and water acquisition (Fig. 6).

### Biomass Accumulation (Fresh & Dry Mass)

In terms of the Shoot Fresh Weight (g) and Shoot Dry Weight, both traits showed highly significant effects of Seed Source (Shoot Fresh:  $F_{(3, 114)} = 10.25, p < 0.001$ ; Shoot Dry:  $F = 10.92, p < 0.001$ ). The pattern is consistent for both measures. Seedlings from Zlitan, Tripoli and Misurata accumulated significantly more shoot biomass and were statistically similar. Seedlings from Benghazi had consistently and significantly lower shoot biomass (approx. 35-40% less fresh weight and 40-50% less dry matter than the others). The Benghazi population exhibits inherently lower vigour for above-ground biomass production. The higher dry matter content (calculated as Dry/Fresh weight ratio) suggests similar tissue density across sources; the difference is primarily in total mass. In the matter of Root Dry Weight, there was no significant effect of Seed Source on root dry weight ( $F_{(3, 114)} = 1.13, p = 0.342$ ). However, while Tripoli and Misurata seedlings developed longer, heavier (fresh weight) roots, the actual dry matter content of these roots was not significantly different from the shorter roots of Benghazi and Zlitan seedlings. This indicates a difference in root structure or hydration rather than carbon investment. The extensive root systems of Tripoli and Misurata may consist of finer roots or roots with higher water content, optimizing them for exploration rather than storage.



**Figure 5. Effect of seed source on shoot length in the laboratory experiment. Mean  $\pm$  SE.**



**Figure 6. Effect of seed source on root length in the laboratory experiment. Mean  $\pm$  SE.**

### **Glasshouse Experiment**

This experiment investigated the early growth performance of seedlings from three geographic seed sources (Benghazi, Misurata, Zlitan) grown in six different soil types. The results reveal that both seed source (genetic origin) and soil type (growth medium) are significant factors influencing seedling morphology and biomass, but their effects vary by specific trait (Plate 2).

### **The Effect of Seed Source (Genetic Origin)**

The origin of the seed had a highly significant ( $p < 0.001$ ) impact on almost all measured traits, indicating strong genetic and/or maternal effects on early seedling development. In terms of shoot and root length (Vegetative Vigour), there was a clear and consistent ranking: Zlitan > Misurata > Benghazi. Seedlings from Zlitan were the most vigorous, producing the longest shoots and roots, followed by Misurata. Benghazi seedlings showed significantly less vegetative growth. This suggests genetic adaptation or predisposition for

faster initial development in the Zlitan and Misurata populations. For the fresh mass (Water Content and Overall Size), Zlitan produced the heaviest shoots, Benghazi was intermediate, and Misurata was the lightest. The grouping (Zlitan A; Benghazi A B; Misurata B) indicates that Zlitan is superior, Benghazi is variable, and Misurata is consistently lower. Moreover, Zlitan seedlings developed a significantly larger root fresh mass compared to both Benghazi and Misurata, which were statistically similar. Regarding the dry mass (Biomass Accumulation), the effect was weaker but still significant at the threshold ( $p=0.05$ ). While Zlitan had the highest mean dry mass, post-hoc tests did not find significant differences between the three sources, suggesting more variability in actual biomass production than in size. Zlitan seedlings again outperformed the others in root dry mass, allocating significantly more biomass to their root systems than both Benghazi and Misurata. However, the Zlitan population consistently demonstrates superior early seedling vigour, particularly in root system development. The Benghazi population, despite originating from the heaviest seeds (as per seed mass data), showed the poorest initial vegetative growth in this controlled environment, indicating a potential trade-off between seed size and initial growth speed or different growth strategies.

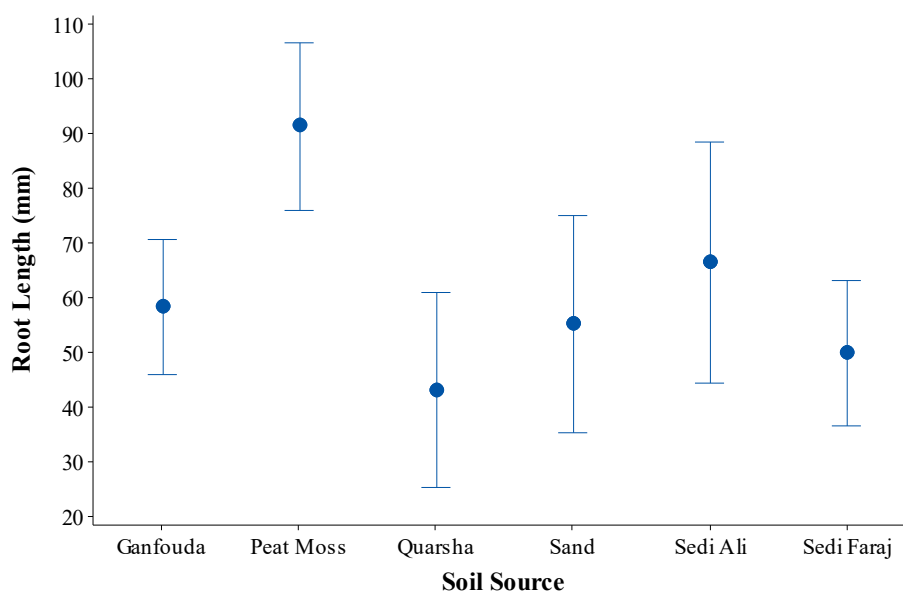
### **The Effect of Soil Type (Growth Medium)**

The soil type had a significant and often dramatic impact on seedling growth, sometimes explaining more variance than seed source (e.g., up to 23.27% for Root Fresh Mass vs. 7.24% for Root Length from seed source). Soil type had a marginally non-significant effect on the shoot length ( $P=0.071$ ), meaning the growth medium did not cause major differences in how tall the seedlings grew across all sources. Whereas, soil type has affected the root length, which was significant ( $P=0.001$ ). Peat moss growth medium promoted the longest roots, significantly outperforming poorer soils like Quarsha (Fig. 7). Sandy soil was the standout medium for root development, producing seedlings with the highest root fresh mass and root dry mass by a significant margin (Table 3). This suggests a well-aerated medium like sand encourages strong root proliferation. Whereas, soils like Peat moss and Ganfouda were the best media for shoot biomass production (Shoot Fresh and Dry Mass), likely due to better moisture retention and nutrient availability compared to other soils. However, Quarsha soil consistently resulted in the poorest performance across almost all biomass metrics (lowest shoot and root fresh/dry mass), indicating it is a highly limiting growth medium, likely due to poor physical structure or nutrient deficiency. Nevertheless, Sidi Ali and Sidi Faraj soils generally produced intermediate to poor results, often grouping with each other or with the low-performing Quarsha soil. The growth medium is a crucial factor for early seedling success, especially for biomass accumulation. Well-aerated soils (Sand) excel for root growth, while organic-rich soils (Peat moss, Ganfouda) support better shoot biomass. Infertile or compacted soils (Quarsha, Sidi Ali and Sidi Faraj soils) severely limit seedling development.

**Table 3. Effect of soil type on root length. Grouping information using the Tukey method and 95% confidence**

Soil Source	N	Mean	Grouping
Sand	32	0.7008	A
Peatmoss	54	0.4185	B
Ganfouda	83	0.3050	B C
Sidi Ali	26	0.1768	B C D
Sidi Faraj	72	0.1550	C D
Quarsha	40	0.0626	D

*\*Means that those that do not share a letter are significantly different.*



**Figure 7. Effect of soil type on root length in the glasshouse experiment. Mean  $\pm$  SE.**

## Discussion

This study provides a comprehensive analysis of the morphological variation in *Moringa oleifera* pods and seeds from different Libyan populations and a critical evaluation of how seed origin and soil type interact to influence germination and early seedling performance. The results reveal significant intraspecific variation and highlight the paramount importance of edaphic factors in the early establishment phase of this economically vital species. The significant morphological differences observed in pod length, pod mass, and seed number per pod among the Benghazi, Misurata, and Zliten populations are indicative of substantial intraspecific genetic diversity or phenotypic plasticity in response to local environmental conditions. This finding aligns with global observations of *M. Oleifera* variability, where nutrient content and morphological traits are known to differ from country to country due to diverse soil and climatic conditions [6, 7]. The most compelling finding is the evidence of a resource allocation trade-off. The Benghazi population invests in fewer, but larger seeds within a heavy, robust pod. In comparison, the Misurata and Zliten populations invest in a greater number of smaller seeds within a lighter pod.

Crucially, the total reproductive investment in seed mass per pod remains constant across all populations. This conservation of reproductive effort suggests a tightly regulated trait, possibly to ensure seedling viability, while allowing other characteristics to adapt to local pressures such as predation, dispersal mechanisms, or resource availability. The findings demonstrate profound intraspecific variation in seed size (a key fitness and yield trait) among populations from different geographic sources. Benghazi stands out as a source of significantly larger, heavier seeds. This could be due to superior genetic potential for seed filling, more favourable local growing conditions (e.g., soil fertility, water availability), or an interaction of both. The clear separation of Zliten into an intermediate group suggests a different genetic or environmental adaptation compared to both the high-weight (Benghazi) and low-weight (Misurata/Tripoli) sources. The fact that Misurata and Tripoli seeds are statistically similar but both significantly lighter than the others indicates that environmental or genetic factors limiting seed size may be shared or similar in these locations.

The profound variation in 100-seed weight, with Benghazi seeds being significantly heavier than those from other locations, further underscores this local adaptation. This trait is a key determinant of seedling vigour, as larger seeds contain more nutritional reserves to support initial growth [1]. The high explanatory power ( $R\text{-sq}(\text{adj}) = 87.32\%$ ) of seed source for this variation points toward strong genetic control or consistent local environmental effects (e.g., soil fertility, water availability) during seed development [4]. This result has direct implications for cultivation: selecting the Benghazi population could be advantageous for programs prioritising high-yielding seed production.

A central finding of this study is that germination success is primarily governed by the growth medium, not by the geographic origin of the seed. The lack of a significant effect of seed source on germination percentage ( $p=0.322$ ) stands in stark contrast to the highly significant effect of soil source. This demonstrates that the inherent genetic potential for germination is relatively uniform across these populations, but its expression is contingent upon edaphic conditions. The most robust finding across multiple batches is the consistently poor germination in Sidi Ali soil. This suggests that this soil may contain specific properties (e.g., high salinity, poor structure, nutrient deficiency, or the presence of allelopathic chemicals) that are inhibitory to seed germination. Soils that promote good germination (e.g., Peat moss, Sidi Faraj, Sand) are effective, but their ranking is not consistent across all experimental batches. This indicates that their efficacy may depend

on interactions with other environmental variables (e.g., temperature, watering regime) not fully controlled in this experiment. Peat moss, as a controlled, nutrient-rich, and well-draining medium, consistently performs well, validating its use as a standard. This finding corroborates the work of Wakawa & Usman (2016) [8], who also found that germination in *M. oleifera* is highly dependent on soil texture. They reported that sandy loam soil yielded the highest and most uniform germination, a pattern our study reflects. Soils like Peat moss and Sidi Faraj, which likely share good aeration and moisture retention properties with sandy loam, performed well. The consistently poor germination in Sidi Ali soil across experimental batches suggests it may possess inhibitory properties, such as high salinity, poor structure, or nutrient deficiency, which are known to suppress germination in many species [17, 18]. This highlights a critical practical insight: for successful *Moringa* cultivation, optimizing soil conditions is a more decisive initial step than selecting a specific seed source for germination rate alone.

The glasshouse experiment clearly demonstrates that subsequent seedling growth is influenced by a complex interaction between seed origin (genetics) and soil type (environment). The superior early seedling vigour of the Zlitan population, particularly in root system development (length and fresh mass), indicates a genetic predisposition for rapid establishment. This is a valuable trait for overcoming initial establishment challenges in reforestation or agroforestry systems. Interestingly, the Benghazi population, despite producing the largest seeds, showed the poorest initial vegetative growth. This suggests a potential trade-off where resources are allocated to seed size rather than initial growth speed, a strategy that might be advantageous in more competitive or resource-poor environments where a large seed reserve provides a buffer [19]. This experiment demonstrates that seedling performance is not determined by genetics or environment alone but by their interaction. A superior genotype (e.g., Zlitan) will still perform poorly in a limiting soil (e.g., Quarsha), and a poor genotype (e.g., Benghazi) can perform better in an optimal soil. The Zlitan population should be prioritised for programs aiming for rapid establishment and strong root development. Using a well-aerated, organic-rich growing medium is crucial. Amending poor soils like Quarsha would be necessary for successful seedling establishment.

Sand-based mixes are excellent for promoting a strong root system. Both seed origin and soil type are profound drivers of early seedling performance. Optimal seedling production would involve selecting vigorous genetic sources like Zlitan and cultivating them in favourable growth media like Peat moss, Ganfouda, or Sand, while avoiding impoverished soils like Quarsha. The soil type had a dramatic and often dominant effect on biomass accumulation. The results strongly support the principle that soil physical properties dictate root development. Sand, a well-aerated medium, excelled in promoting root biomass, consistent with findings that oxygen availability is crucial for root growth and function [20]. Conversely, organic-rich soils (Peat moss, Ganfouda), which typically offer better moisture and nutrient retention, supported greater shoot biomass production. This aligns with the nutrient requirements for photosynthetic tissue development [21]. The consistently poor performance across all metrics in Quarsha soil identifies it as a highly limiting growth medium. Its poor physical structure, likely leading to waterlogging or compaction, and/or severe nutrient deficiencies, severely stunts seedling development. This is a crucial consideration for large-scale planting efforts in Libya, suggesting that such soils would require significant amendment (e.g., with organic matter or sand) to support successful *Moringa* establishment.

## Conclusions

In conclusion, this study identifies a two-stage process for establishing *Moringa oleifera* in Libya. Firstly, germination depends heavily on soil conditions. Secondly, seedling growth results from an interaction between the genetic potential of the seed source and the soil quality. Therefore, for the best outcomes in nursery production and restoration projects, The Zlitan population should be prioritized due to its superior seedling vigour and root development. The Benghazi population remains valuable for its high pod mass and large seed size. A well-draining, aerated, and organic-rich medium is crucial. A mixture of sand (to promote root development) and peat moss or compost (to support shoot growth and nutrient retention) is ideal. Soils such as Quarsha and Sidi Ali should be avoided or heavily amended. Land should be assessed for soil type before planting. The genetic advantages of certain seed sources can only be fully realised if paired with suitable soil conditions.

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## Ethical Statement

The authors confirm that all experimental protocols were conducted in accordance with relevant institutional and national guidelines for plant research. The collection of *Moringa oleifera* seeds from various Libyan populations (Benghazi, Zlitan, Misurata and Tripoli) and the acquisition of soil samples from the Benghazi region were performed in compliance with local environmental regulations. This study did not involve any endangered or protected species, and no human or animal participants were involved in the research.

### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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### Author Contributions

All authors contributed to the conception of the study. Material preparation, data collection, and analysis were carried out by Nouria Shwayil and Esraa Hassan. Abdullah Bouhjr assisted with double-checking the data and figures. The experiment design for this study was organized by Tarek Mukassabi, who also wrote the first draft of the manuscript. All authors revised and annotated earlier drafts and read and approved the final manuscript.

### Data Availability

The datasets produced and examined in this study are available from the corresponding author upon reasonable request.

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