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Full Length Article

Evaluation of Seed Germination and Growth Characteristics of *Moringa oleifera* and *M. peregrina* under Laboratory, Greenhouse and Field Conditions

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Abstract

This work aimed to evaluate production of *Moringa peregrina* and *M. oleifera*, in the central region of Saudi Arabia. Seed germination using seven temperature regimes in laboratory showed that 20°C is the optimum temperature for both species allowing the germination of 87–90% of cultivated seeds within 5 days, mainly from the fourth to seventh days of sowing. Satisfied germination (80%) was also obtained with 15 and 25°C for *M. oleifera* and *M. peregrina*, respectively. No germination occurred under 10 and 40°C for both species. The high temperature increased germination speed but deceased the germination percentage. The low temperature delayed the germination time. Under the optimum temperatures, both species had good germination time and rate but *M. oleifera* showed higher germination index. Under greenhouse, mid October was better than mid-February for seed germination and seedling growth of both species. Germination efficiency under greenhouse was different from that of laboratory where seed germination percentage of *M. peregrina* was higher than that of *M. oleifera*; 80% versus 53%, respectively. *M. peregrina* also showed better seedling growth specially in number of leaves. However, *M. oleifera* showed highly better growth under field conditions regarding height, spread, leaves number, leaf area and leaf dry weight. It also flowered 6 months after transfer to field or 8 months after sowing. The reported results could be beneficial for commercial production of such important crops in the central region of Saudi Arabia and similar regions. The reported results could be also important at fundamental level regarding the effects of temperature and growth trends.

Keywords: Life tree; *Moringaceae*; Sowing; Temperature; Comparison

Introduction

Moringa is one of the most economically important multipurpose tree species widely cultivated in the great part of the world. Among the thirteen species of *Moringaceae* family, *M. oleifera* and *M. peregrina* are the most important on agricultural, nutritional and medicinal levels (Leone *et al*., 2015). All parts of *Moringa* tree are edible and consumed by humans in dry tropics for nutrition, disease treatment and prevention purposes (Fahey, 2005; Olson and Fahey, 2011). The alimentary, pharmaceutical and medicinal uses of *M. oleifera* were reviewed (Leone *et al*., 2015; Gopalakrishnan *et al*., 2016). *M. oleifera* also represents an important crop in regions injured by desertification and water deficit and an important commercial species for agro-pharmaceutical industry (Araújo *et al*., 2016). *M. peregrina* is also important in preventing the progression of many diseases where its leaves are rich in effective phenolic compounds. The young leaves of *M. peregrina* are also rich in vitamins, minerals, proteins and can be eaten as a vegetable (Al-Owaisi *et al*., 2014). The nutritional and medicinal properties of *M. peregrina* and its economic impact as bio-fuel was reviewed (Robiansyah *et al*., 2014). *Moringa* can grow under wide range of environment in all kinds of lands including poor soils of arid and semi-arid regions. It is also a highly drought tolerant plant, resistant to most pests and diseases and can adapt to the warm, frost and tropical dry conditions (Mridha, 2015).

Moringa species are actually growing as native or introduced trees in more than 60 countries of Asia (20), Africa (18), North America (3), Central America and Caribbean (14), South America (3) and Oceania (4) (Muhammad *et al*., 2016). *M. oleifera* is native to south Asia (India and Pakistan) and introduced to many tropical and subtropical countries where it became the most widely cultivated species throughout the world (Nouman *et al*., 2014). *M. peregrina* is native to Saudi Arabia and locally grown in many areas of Northeastern Africa, Madagascar and Arabia (Migahid, 1978; Robiansyah *et al*., 2014).

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Mridha (2015) reviewed the distribution of *M. peregrina* in Saudi Arabia and reported that it is mainly distributed in South and North Hijaz along the Red Sea coast. He suggested the extension of plant to other parts as drought tolerant plant to save water, vegetable crop for human consumption and economic tree to minimize desertification and improve the environmental conditions.

There is a growing interest in *Moringa* because of its multiple uses, adaptation under wide range of climatic conditions and tolerance to biotic and abiotic stresses. Seed germination of *M. oleifera* was studied under greenhouse conditions where the higher temperature 20/30ºC was found better than 10/20ºC (Muhl *et al*., 2011; Batool et al., 2016). The larger seeds of *M. peregrina* were also better for decreasing germination time and increasing seedling growth rate (Gomaa and Xavier, 2011). Germination of *M. peregrina* at alternating temperature 25/35ºC was found to be higher than that achieved at constant temperature 25ºC (Alatar, 2011). To the best of our knowledge, no work compared production, growth or yield of the two species, *M. oleifera* and *M. peregrina*, and there is no information about their growth and yield under greenhouse or field conditions. The evaluation of such economic species in countries with limited water resources as Saudi Arabia is also very essential. In the aim of the enrichment of such arid region by valuable trees, we assessed germination and growth of some ornamentally and economically important trees for the production under arid zones conditions (Al-Soqeer, 2010; Hassanein, 2010a; 2015). The present study aimed to evaluate production of the introduced *M. oleifera* and the native *M. peregrina* under central Saudi Arabia conditions. Seed germination of both species were studied under laboratory and greenhouse conditions, and their growth were assessed under both greenhouse and field conditions.

Materials and Methods

Plant Materials and Study

The study was carried out on two *Moringa* species at Qassim region, the center of Saudi Arabia. The studied species were the introduced *M. oleifera* and the native *M. peregrina*. Seeds of *M. oleifera* were imported from Whatcom Seed Company, Oregon, USA however, those of *M. peregrina* were collected from locally grown trees. Experiments were conducted in the Horticulture laboratory, greenhouse and floriculture experimental farm of Agriculture and Veterinary Medicine College, Qassim University, Saudi Arabia during 2015 and 2016.

Seed Germination and the Optimum Temperature for Germination in Laboratory

Germination of *M. oleifera* and *M. peregrina* was firstly studied under laboratory conditions using the two way Grant's thermogradient plate. Seeds of both species were subjected to seven constant temperature regimes including 10, 15, 20, 25, 30, 35 and 40ºC. Experiment was arranged in a randomized complete split blot design with two factors and three replicates. Seeds were placed in plastic Petri dishes of 90 mm diameter covered with Whatman filter paper, and moistened whenever necessary. Five healthy seeds were sown per Petri dish and 15 seeds were designed per treatment. Petri dishes representing the two species, the seven temperature degrees and the three replicates were arranged on the thermogradient plate given forty two experimental units. Data on germination were recorded daily starting from the first germination until no further germination. The germination criterion was two millimeter radical protrusion. The following germination characteristics were recorded for all studied treatments. Germination percentage (G %) was calculated as the percent of germinating seeds related to the total cultivated seeds. The mean germination time (MGT) was calculated for each lot using the following formula ($MGT= \Sigma(n.D)/\Sigma n$) cited by Ellis and Roberts (1980), where n= number of seeds newly germinated at time D, D= days from the beginning of germination test, Σn= total number of seeds germinated at the end of the test. Germination rate (GR) was calculated as the reciprocal of the mean germination time (Matthews and Hosseeini, 2006). The germination index (GI) was calculated as described by the association of official seed analysis (AOSA, 1983) using the equation:

$$GI = \frac{\text{Number of germinating seeds}}{\text{Days of first count}} + \cdots + \frac{\text{Number of germinating seeds}}{\text{Days of last count}}$$

The imbibition period was also recorded as the number of days after sowing till the first germination (IP-F) and last germination (IP-L).

Seed Germination and Seedling Growth under Greenhouse Conditions

Seed germination and seedling growth of *M. oleifera* and *M. peregrina* were studied under greenhouse conditions during two different seasons, mid October 2015 and mid February 2016. For each season, seeds of both species were sown in pots of 25 cm diameter filled by a mixture (2: 1) of sand and peat. Forty seeds were cultivated in twenty pots, two seeds per pot, for each species. All pots were maintained under greenhouse conditions until the end of experiments, two months after sowing. Irrigation was done similarly for all pots at 24 h interval till the beginning of germination then at 48 h intervals till the end of experiments. After the germination stop, two weeks after sowing, the percent of germinating seeds was recorded in relation to the total number of cultivated seeds to record the germination percentage (G %). Pots were thinned to one seedling per pot or transplanted by similar seedlings where twenty seedlings were prepared per species to study seedling growth. Seedlings were maintained under the same greenhouse

where stem length (cm) and number of leaves per seedling were recorded every two weeks starting from the second week till the eighth week after sowing. Chlorophyll content in leaves was determined using a SPAD-501 Chlorophyll Meter (Konica Minolta, Co. Ltd., Japan).

Plant Growth and Flowering under Field Conditions

To study growth and yield of *M. oleifera* and *M. peregrina* under open field conditions, seedlings of two months old were transferred to an experimental field, at the Agricultural Researches Station of Qassim University, equipped by drip irrigation network in mid April 2016. Twenty seedlings were cultivated at five meters culture spacing from each species, and all agricultural practices were carried out similarly for both species. Plant height and stem diameter was recorded every two months starting from the second to eighth month after transferring to field. Furthermore, several growth and flowering parameters were recorded on all plants of both species at the end of experiment; the eight month of transferring or the tenth month of sowing. Tree spread or the covering area was calculated by the multiplication of branches expansion from north to south by their expansion from east to west. Several leaf characteristics including leaf length, leaflets and pinnates number per leaf and pinnate area were measured for all the cultivated trees of both species using three leaves per tree. The whole leaf area was calculated by the multiplication of pinnate area by the number of pinnates per leaf. Leaf dry weight was measured after drying of leaves at 80°C for 72 hours. Flowering and fruiting were also recorded.

Statistical Analysis

For germination in laboratory, germination percentages were transformed using (x + 0.5 then Arcsine scale), and other germination parameters were transformed using (x + 0.5 then square root) to normalize the distribution (Zar, 1996). The transformed data were statistically analyzed according to analysis of variance technique (ANOVA) by a two-factor (temperature regimes and species) randomized complete block design (RCBD) to determine significant differences for both factors and their interaction. For significant differences, comparison of means was performed using Duncan's multiple range test. All analyses were achieved using MSTATC microcomputer program (MSTATC, 1990). The other data on growth characteristics under greenhouse and field conditions were statistically analyzed according to *t*-test using Excel Program 2007.

Results

Seed Germination and the Optimum Temperature for Germination in Laboratory

The study of seed germination of *M. oleifera* and *M. peregrina* under seven different temperature regimes showed highly significant differences for the studied factors and their interaction (Table 1). No seed germination occurred under neither the lowest temperature 10°C nor the highest one 40°C whatever *Moringa* species. The temperature regime affected significantly all germination characteristics regardless *Moringa* species. The germination percentage increased with increasing temperature till 20°C where the maximum germination was recorded. However, germination decreased at higher temperatures and it was fully inhibited at 40°C. The mean germination time (MGT), the imbibition period of first and last germination (IP, F & L) were also the highest at 15 and 20°C then decreased with increasing temperature. Furthermore, the temperature 20°C allowed high germination index (GI) with intermediate germination rate (GR) compared to other studied regimes. Generally, 41.0% of *M. peregrina* seeds germinated starting from the day 2.5 after sowing with mean germination time of 2.9 and germination index of 0.44. However, 47.6% of *M. oleifera* seeds started germination 2.4 days after sowing with mean germination time of 2.8 and germination index of 0.71. The results showed that the two *Moringa* species had similar germination time and imbibition periods for first and last germination. However, *M. oleifera* showed significantly higher germination percentage, rate and subsequently higher germination index compared to *M. peregrina*. Regarding the interaction, the temperature degree of 20°C was the best for the germination of both *Moringa* species. Under this temperature, germination percentage reached to 93% and 87% for *M. oleifera* and *M. peregrina*, respectively. However, similar good germination results were obtained from *M. oleifera* with 15°C and *M. peregrina* with 25°C. The optimum temperatures also showed the highest germination time and imbibition periods with intermediate germination rate and index (Table 1).

Seed Germination and Seedling Growth under Greenhouse Conditions

Results obtained on the germination of *M. oleifera* and *M. peregrina* under greenhouse conditions at two different seasons are shown in Table (2). The first seed germination occurred seven days after sowing, and stopped one week later where no further germination was observed (Fig. 1). Germination in October was better than that performed in February specially for *M. peregrina* which showed higher germination percentage compared to *M. oleifera* for both seasons (Table 2). Seedlings growth, expressed as stem length and number of leaves, was also better at the first season, for both species, compared to the second one. *Moringa* species showed different responses in stem length where *M. oleifera* was better at the first season, in the contrary of the second one. However, significantly higher leaves were produced by *M. peregrina* seedlings at both seasons. Furthermore, leaves of *M. peregrina* seedlings were distributed along the whole stem as they were unipinnate and dark in color versus bipinnate and light leaves of *M. oleifera* (Fig. 1).

Table 1: Germination of *M. oleifera* and *M. peregrina* seeds incubated at different temperature degrees on thermogradient plate under laboratory conditions

Temperature	*Moringa* species	G %	MGT	GR	GI	IP (F)	IP (L)
10°C	-	0.00 e	0.00 f	0.00 e	0.00 d	0.00 e	0.00 f
15°C	-	76.67 b	7.21 a	0.14 d	0.54 b	6.33 a	8.00 a
20°C	-	90.00 a	5.02 b	0.20 c	0.96 a	4.33 b	6.67 b
25°C	-	73.33 b	3.76 c	0.28 b	1.07 a	3.00 c	5.17 c
30°C	-	53.33 c	2.92 d	0.37 a	1.01 a	2.67 c	3.67 d
35°C	-	16.67 d	1.00 e	0.25 c	0.42 c	1.00 d	1.50 e
40°C	-	0.00 e	0.00 f	0.00 e	0.00 d	0.00 e	0.00 f
P value (F-test) for temperature		0.000	0.000	0.000	0.000	0.000	0.000
-	*M. peregrina* (P)	40.95 b	2.90 a	0.12 b	0.44 b	2.52 a	3.52 a
-	*M. oleifera* (O)	47.62 a	2.79 a	0.23 a	0.71 a	2.43 a	3.62 a
P value (F-test) for species		0.003	-	0.000	0.000	-	0.054
10°C	P	0.00 g	0.00 g	0.00 f	0.00 f	0.00 g	0.00 f
	O	0.00 g	0.00 g	0.00 f	0.00 f	0.00 g	0.00 f
15°C	P	73.33 cd	7.25 a	0.14 e	0.51 e	6.33 a	8.00 a
	O	80.00 bc	7.17 a	0.14 e	0.57 de	6.33 a	8.00 a
20°C	P	86.67 ab	5.13 b	0.20 d	0.91 bc	4.67 b	6.67 b
	O	93.33 a	4.90 bc	0.21 d	1.02 b	4.00 c	6.67 b
25°C	P	80.00 bc	4.25 cd	0.24 c	1.00 b	3.33 d	5.67 c
	O	66.67 cd	3.28 e	0.32 b	1.14 ab	2.67 e	4.67 d
30°C	P	46.67 ef	3.67 de	0.28 bc	0.64 cde	3.33 d	4.33 d
	O	60.00 de	2.17 f	0.47 a	1.39 a	2.00 f	3.00 e
35°C	P	0.00 g	0.00 g	0.00 f	0.00 f	0.00 g	0.00 f
	O	33.33 f	2.00 f	0.50 a	0.83 bcd	2.00 f	3.00 e
40°C	P	0.00 g	0.00 g	0.00 f	0.00 f	0.00 g	0.00 f
	O	0.00 g	0.00 g	0.00 f	0.00 f	0.00 g	0.00 f
P value (F-test) for interaction		0.001	0.000	0.000	0.001	0.000	0.000

G%, Germination percentage; MGT, Mean germination time; GR, Germination rate; GI, Germination index or speed; IP, Imbibition period for first (F) or last (L) germination. Means with similar letter at the same partition are not significantly different at $\alpha = 0.05$

Fig. 1: Seed germination, seedling growth under greenhouse conditions, and plant growth under field conditions for *M. oleifera* (Mo) and *M. peregrina* (Mp). (a) Seeds of Mo, (b) Seeds of Mp, (c) Seed germination of both species two weeks after sowing, (d) Seedling of Mo, (e) Seedling of Mp, (f) Leaves of Mo seedling, (g) Leaves of Mp seedling, (h, j) Growth and leaf characteristics of Mo plants under field conditions, (i, k) Growth and leaf characteristics of Mp plants under field conditions, (l, m) Flowering and fruiting of Mo plant

The determination of chlorophyll justified this observation where leaves of *M. peregrina* showed significantly higher chlorophyll content in both seasons (Table 2). Following the development of stem and leaves of studied species at the first season showed the superiority of *M. oleifera* in stem length starting from the fourth week, and the superiority of *M. peregrina* in leaves development starting from the second week (Fig. 2). Therefore, *M. peregrina* gave significantly better seed germination and seedling growth under greenhouse conditions. It can be clearly noticed that germination results under greenhouse conditions were different from those obtained under laboratory conditions where *M. peregrina* showed better germination percentage under greenhouse compared to *M. oleifera*, which was better under laboratory.

Plant Growth and Flowering under Field Conditions

Growth of the two *Moringa* species 8 months after transferring to field are illustrated in Fig. (1). *M. oleifera* plants showed higher and rapid growth under field conditions compared to those of *M. peregrina*. Morphological characteristics of *M. peregrina* plants differed under field conditions where trees grew vertically with no branching, and their leaves were bipinnate with small leaflets. However, *M. oleifera* trees showed vigor growth with branching and their leaves were tripinnate with bigger leaflets. Flowering and fruiting were observed on all

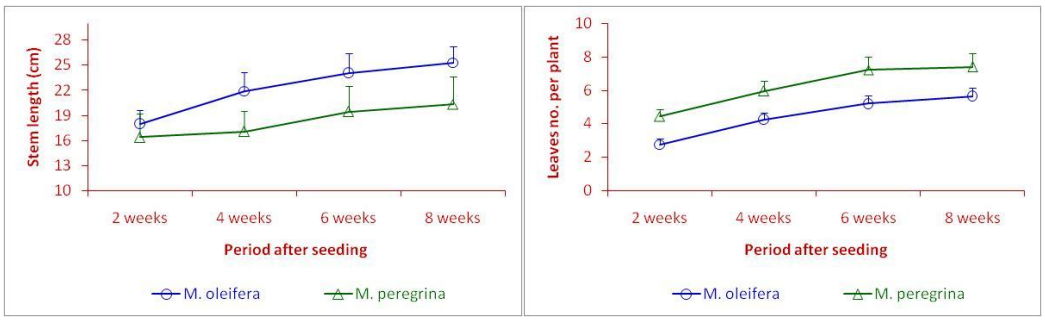

Fig. 2: Growth of *M. oleifera* and *M. peregrina* seedlings during the first eight weeks after sowing on mid October under greenhouse conditions, Means of 20 seedlings with confidence interval at significance level α= 0.05

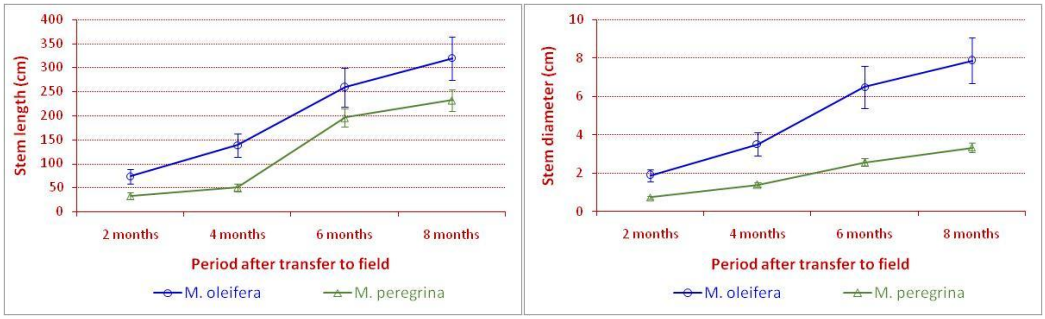

Fig. 3: Growth of *M. oleifera* and *M. peregrina* trees during the first eight months after transferring to the field, Means of 20 seedlings with confidence interval at significance level α= 0.05

M. oleifera trees however no flowers were obtained from any *M. peregrina* tree till the end of experiment. Data recorded on the growth of both *Moringa* species under field conditions are presented in Table (3). *M. oleifera* showed significantly higher stem length, stem diameter and tree spread compared to *M. peregrina*. Despite the similar leaf length of both species, *M. peregrina* had higher leaflets number per leaf however *M. oleifera* had more pinnates number and pinnate area. *M. oleifera* also showed bigger leaf area and leaf dry weight. Monitoring the development of stem length and diameter during the 8 months under field conditions showed better development for *M. oleifera* starting from the first two months (Fig. 3). Both species grew rapidly under field but the growth rate of *M. oleifera* was higher than that of *M. peregrina* specially in stem diameter. Results obtained at field showed better growth for *M. oleifera* compared to *M. peregrine,* which is different from the results obtained at greenhouse.

Discussion

The study of seed germination under laboratory conditions showed a vital role of temperature in the germination process, where it was fully inhibited under the lowest and highest degrees; 10 and 40°C. The high temperature prevented the germination of many species including tropical medicinal plants (Kumar *et al*., 2011). The extremely high and low temperatures may affected negatively the viability of embryos and the availability of water. Results showed that the high temperature increased the germination speed and deceased the germination percentage however the low temperature delayed the germination time. The high temperature may be caused a stress inducing a fast germination and subsequently low germination percentage in such short time. However, the optimum conditions maintained high germination percentage at intermediate rate. It is well known that the higher germination index denotes higher percentage and rate of germination. The difference between the two studied species may be explained by the genetic variation and the different origin (Mridha, 2015; Muhammad *et al*., 2016). The optimum temperatures reported on *Moringa* under greenhouse conditions ranged from 20 to 35ºC (Alatar, 2011; Muhl *et al*., 2011). According to these reports, the high temperature was better than the low one, which is not in agreement with our results because of the different conditions between greenhouse and laboratory. However, the optimum temperature for *M. peregrina* 25/35°C (Alatar, 2011) was higher than that reported on *M. oleifera* 20/30°C (Muhl *et al*., 2011), which justify our comparison.

Results on seed germination under greenhouse showed the preference of October compared to February. It could be explained by the climatic changes between the two seasons.

Table 2: Germination percentage, two weeks after sowing, and growth characteristics of seedlings, eight weeks after sowing, of *M. oleifera* and *M. peregrina* under greenhouse conditions

Germination and seedling growth	First season (October 2015)			Second season (February 2016)		
	M. oleifera	*M. peregrina*	*P value (T-test)*	*M. oleifera*	*M. peregrina*	*P value (T-test)*
Germination (%)	52.5 %	80.0 %	0.000**	50.0 %	66.7 %	0.000**
Stem length (cm)	25.3	20.3	0.009**	10.6	11.7	0.034*
No. of leaves	5.7	7.4	0.000**	5.1	6.1	0.007**
Chlorophyll content	47.5	68.0	0.000**	50.2	69.0	0.000**

Means of 40 seeds or 20 seedlings per species, * significant, ** high significant

Table 3: Growth characteristics of *M. oleifera* and *M. peregrina* trees under field conditions, 8 months after transferring

Growth characteristics	*M. oleifera*	*M. peregrina*	P value (T-test)
Stem length (m)	3.19	2.32	0.034*
Stem diameter (cm)	7.87	3.33	0.000**
Tree spread (m^2)	7.07	0.86	0.005**
Leaf length (cm)	47.43	52.71	0.099
Leaflets number per leaf	8.24	11.19	0.000**
Pinnates number per leaf	399.05	254.57	0.026*
Pinnate area (cm^2)	2.57	0.66	0.039*
Whole leaf area (cm^2)	586.63	160.49	0.002**
Leaf dry weight (gm)	3.63	2.10	0.004**
Flowering (%)	100 %	---	---

Means of 20 trees per species and three leaves per tree, * significant, ** high significant

It was previously reported that *Moringa* production is dependent on the season and climatic conditions (Palada *et al*., 2007). The different responses of *Moringa* species under different seasons could be related to the different environmental conditions, which probably were more suitable to one species than the other. Germination trend under greenhouse was different from that of laboratory which was clearly noticed from the superiority of *M. peregrina* under greenhouse compared to that of *M. oleifera* under laboratory. It could be explained by the different media, culture and environmental conditions. We previously found different trend in the drought tolerance of pelargonium under *in vitro* and greenhouse conditions (Hassanein and Dorion, 2006; Hassanein, 2010b).

Both *Moringa* species showed extremely rapid growth and development under field conditions, which can be explained by the availability of more nutrients and rooting area compared to the restricted root volume in pots under greenhouse conditions. Several works on woody species reported better growth under field compared to pot cultivation as reviewed by Kawaletz *et al*. (2014). The planting density may be another factor affecting growth as recently reported by Zheng *et al*. (2016). Growth trend under field was also different from that obtained under greenhouse. The more rapid growth and flowering of *M. oleifera* under field may be related to its nature and ability to adapt with such conditions (Araújo *et al*., 2016).

Conclusion

Seed germination of *M. oleifera* and *M. peregrina* in laboratory showed that 20°C is the optimum temperature degree for both species. Good germination was also recorded at 15 and 25°C for *M. oleifera* and *M. peregrina*, respectively. No germination was obtained from any species under 10 and 40°C. Under the optimum temperatures, both species showed high germination percentages and good germination rate in standard time; 87–90% in five days mainly from the fourth to seventh days. Under greenhouse, mid October was better than mid February for seed germination and seedling growth of both species. *M. peregrina* showed better seed germination and seedling growth, specially number of producing leaves, under greenhouse compared to *M. oleifera*. In the contrary, growth of *M. oleifera* was highly better under field conditions because of its nature and adaptation ability. Both species grew rapidly at field but *M. oleifera* trees were superior in height and spread, as it had higher leaves number of greater area and heavier dry weight. *M. oleifera* also flowered 6 months after transfer or 8 months after sowing but no flowers were observed on *M. peregrina* plants. The reported results provide valuable information on the production of the most important *Moringa* species in three different stages under different conditions including laboratory, greenhouse and field, which could help in their diffusion commercially as important medicinal and nutritional crops. The comparison between the two species indicated the possibility of their successful production under aid zones conditions. The native *M. peregrina* could be commercially produced in the central region of Saudi Arabia and similar regions as alternative to the introduced *M. oleifera*, for more water use efficiency and drought tolerance. The effects of temperature, the variation in germination efficiency between laboratory and greenhouse, and the different growth trend under greenhouse and field could be also beneficial at fundamental level for better understanding these phenomena.

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