Seed Germination and Seedling Growth of *Nigella Sativa* L. A High Value Medical Plant

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ABSTRACT

This work aims to study the germination conditions and the seedling growth of *Nigella sativa L.* This plant is renowned for its medicinal purposes used for centuries in Asia, Middle East and in Africa.

The germination requirements of *the* black cumin were studied under control conditions in the laboratory. The treatments included four light levels (0:24); (6:18); (12:12), and (24:0) hours of light: dark period. Seven salinity concentrations (0, 2, 4, 6, 8, 10 and 12 g/l NaCl), as well as seven temperature regimes (5°, 10°, 17°, 15°, 20°, 25° and 30° C), using a completely randomized block design.were tested in the present work.

This work shows that the germination of the black cumin was very sensitive to temperature with an optimum of 20°C. Germination stops below 10°C and above 25°C. The progressive increase of salinity until 8g/l has no significant effect on the germination (p<0.001). A significant inhibition of germination was obvious only after 10 g/l (p<0.001). The absence of light shows the best rate of germination that decreased when light period increases. Seedling monitoring shows that germination was achieved after 6 days, whereas the beginning of flowering was observed after 50 days from germination.

Keywords: *nigella sativa*, seed germination, seedling, salinity, light, temperature.

1. INTRODUCTION

Nigella sativa Linn., family *Ranunculaceae*, is an annual flowering plant that is grown and cultivated in North Hijaz (Fig. 1), representing the western part of Saudi Arabia (Migahid 1987). Black cumin is widely cultivated throughout South Europe, Syria, Egypt, Saudi Arabia, Iran, Pakistan, India and Turkey (Riaz ***et al***. 1996, Özlem and Süleyman 2004).

Black cumin (*Nigella sativa* L.) is used frequently in traditional and industrial pharmacology (D'Antuno ***et al***. 2002). It is announced that intact black cumin seeds or their extracts contain anti-diabetic, antihistaminic, antihypertensive, anti-inflammatory, anti-microbial, antitumour, galactagogue and insect repellent effects (Riaz ***et al***. 1996, Siddiqui and Sharma 1996, Worthen ***et al***. 1998).

In Saudi Arabia, this species was found in, its habitats, in low density. People consider it like a sensitive species that can exist in very restricted regions. One of the fundamental factors that limits the dispersion of this species is its difficulty to germinate.

The environmental factors such as temperature, salinity, light, and soil moisture simultaneously influence the germination process (Ungar 1995, El-Keblawy and Al-Rawai 2005). The establishment of plants in arid regions is often limited by the temperature even though the conditions of humidity are favorable (Mseddi ***et al***. 2002, Visser ***et al***. 2008). Knowledge of temperature effects on germination may be useful to evaluate the germination characteristics or the establishment potential among range species (Mseddi ***et al***. 2003, Mnif ***et al***. 2005, Kharrat ***et al***. 2011). Tolerance to salinity during germination is critical for the establishment of plants growing in saline soil of arid regions (Ungar 1995).

This work, which was conducted in the laboratory of Botany and Plant Biology at the college of Sciences in Hail, aims to study the effects of temperature, salinity and light on the germination of *Nigella sativa.* After germination, the second part of this study was reserved for the monitoring of seedling growth up to the flowering stage of this plant species.

2. MATERIALS AND METHODS

The fruits (capsules) of *Nigella sativa* used in this experiment were harvested in 2010 from its natural populations within the areas of Kassim and the region of El Hijaz in Saudi Arabia (Fig. 1). The seeds were removed from follicles, air dried and stored in paper bags at room temperature (25-30˚C) and capsules. After weighting, the seeds are ready to experiments.

Fig. 1. Distribution map of *Nigella sativa* in Saudi Arabia

Only mature and intact seeds were used in the experiment. By the use of optic lens damaged and very small seeds were eliminated. Then seeds were divided into groups each with 100 seeds. Every group was photographed and weighted, and then the average of 1000 seeds weight was calculated.

Germination trials were conducted in 9-cm sterile Petri dishes lined with two Whatman No. 1 filter papers and moistened with sterile distilled water to ensure adequate moisture for the seeds. To avoid fungus attack, seeds were surface sterilized in 0.1% mercuric chloride solution for 1 min, subsequently washed with distilled water and air-dried before being used in the germination experiments. Treatments were arranged in a factorial experiment (randomized complete block) with three replicates of 50 seeds each. Seed treatments included, 0/24 (continuous darkness), 8/16 and 16/8 h light/dark photoperiod.

The effect of temperature was determined by placing the Petri dishes in a germination chamber (incubator) for 20 days at constant temperatures of 5° C, 10° C, 15° C, 17° C, 20° C, 25° C and 30° C.

Seven salinity concentrations (0 g/l, 2 g/l, 4 g/l, 6 g/l, 8 g/l, 10 g/l and 12 g/l NaCl) were used based on a preliminary test for salt tolerance of the species.

For all attempts, the seeds were irrigated, counted and examined daily and considered germinated when the radicle was visible. At the end of the trials, data were subjected to analysis of variance procedures and mean separation using SPSS statistical packages.

To follow seedling phenology, small seedlings were transferred into growth tanks. Tanks were placed in a field with controlled conditions (temperature and light). To eliminate the competition, seedlings were separated by a distance of 5 cm.

3. RESULTS

Nigella sativa seeds were black, trigonal-shaped and have a small and short size (between 1-2 mm long). The average of the weight of 1000 seeds was 3.12±0.01 g.

In distilled water (0 g/l of salt) and continuous darkness, seeds of *Nigella sativa* were able to germinate at temperatures between 15° C and 25° C and the optimal temperature corresponds to 20 °C with percentage of germination of 72.4 %. At 10˚ C and 25˚ C, rates of germination were very low with respectively only 3.5 % and 4.5%. At 5˚ C and 30˚ C no germination were observed (Table 1).

Table 1. Percentage of germination seeds (rate of germination, %) of *Nigella sativa* at different experimental conditions of temperature and salinity. At the temperature 5˚C and 30˚C, as so as in salt concentration 12 g/l, no germination were recorded.

NaCl (g/l)	Rate of germination (%)						
	5˚C	10˚C	15˚C	17˚C	20˚C	25˚C	30˚C
0	0	3.5 ± 1.5	25 ± 3	63.5 ± 3.5	72.4 ± 4.5	4.5 ± 0	0
2	0	2.5 ± 1.5	21 ± 3.5	53.4 ± 1.6	64.5 ± 5.5	2.1 ± 1.5	0
4	0	2.5 ± 1.5	20.5 ± 4.5	42 ± 3.5	66.2 ± 3.8	2.1 ± 1.5	0
6	0	2.5 ± 1.5	15.5 ± 3.5	51.0 ± 4.0	63.5 ± 4.5	2.1 ± 1.5	0
8	0	0 ± 0	2.5 ± 1.5	11.5 ± 1.5	39.5 ± 5.5	0 ± 0	0
10	0	0 ± 0	0 ± 0	0 ± 0	4.2 ± 2.8	0 ± 0	0
12	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0
Salt effect			F = 49.213		$p <$ .001		
T˚ effect			F = 429.461		$p <$ .001		
Salt*T˚			F interaction = 12.554		$p <$ .001		

At this optimal temperature (20˚C), the incubation time (or number of days before germination) was estimated at three days. However, the estimated delay of full germination was about seven days starting from the beginning of the experiment.

Temperature, salinity and their interaction significantly (p<0.0001) affected the final percentage of germination of *Nigella sativa* (Table 1). Seed germination was the highest in distilled water and germination percentages decreased slowly with the increase in salinity from 72.4% in distilled water to 63.5% in salt concentration of 6 g/l (Table 1). In all experimental temperature, differences were not significantly at the salt concentrations ranging from 2 to 6 g/l. Germination start to be affected only at 8 g/l of NaCl with 39.5 % then decrease to 4.2 % at 10 g/l of NaCl. No germination was recorded (0 %) at 12 g/l of NaCl.

At optimal temperature 20°C, seeds started germination after three days (Incubation Period) and was stopped after 6 to 7 days (delay germination). The same observation was recorded at 17°C. Seeds need a long latent time at 15°C and 25°C when seeds start to germinate only after 9-10 days, but it takes only one day to germinate with a very low rate of germination. Increase of salinity from 0 g/l NaCl (distilled water) to 8 g/l NaCl have no effect (p<0.001) on the incubation period (also three days) and delay germination (6 to 7 days). These parameters change gradually from a concentration of 10 g/l NaCl with an incubation period of 4 to 5 days and delay germination of only 2 days (Table 2).

Table 2. Incubation Period (IP: number of days before germination) and delay of germination (DG) of *Nigella sativa* seeds, at different temperatures and different salinity concentrations. NG: No germination observed.

NaCl (g/l)	Temperature									
	10˚C		15˚C		17˚C		20˚C		25˚C	
	IP	DG	IP	DG	IP	DG	IP	DG	IP	DG
0	9	1	7	2	3	6	3	7	8	1
2	10	1	8	1	3	7	3	7	9	1
4	10	1	10	1	3	6	3	6	10	1
6	NG	NG	10	10	3	7	3	6	NG	NG
8	NG	NG	NG	NG	3	7	3	7	NG	NG
10	NG	NG	NG	NG	5	2	4	2	NG	NG

Germination in continuous darkness showed the highest rate (72.40%). It decreases thereafter according to light exposure. It is almost zero at 18 hour of light exposure and continuous light (Fig. 2). This shows that darkness is a key factor for the germination of the black cumin seeds.

Black cumin, variety Kassim, cycle started in March and finished in June- July by seed dispersion. In this study, the phenology evolution of this variety was followed from germination to the flowering stage (Table 3). Germination was achieved in 6 days by the apparition of the first spatula-shaped leaves. At 32 days, total length of seedling is about 10 cm with four pairs of lower spatula leaves and one to three upper dissected leaves. After 50 days approximately, it's the beginning of the flowering by the apparition of the first flower buttons, a long leaf petiole and the drop of the first lower spatula-leaves. All seedling growth steps are reported in Table 3.

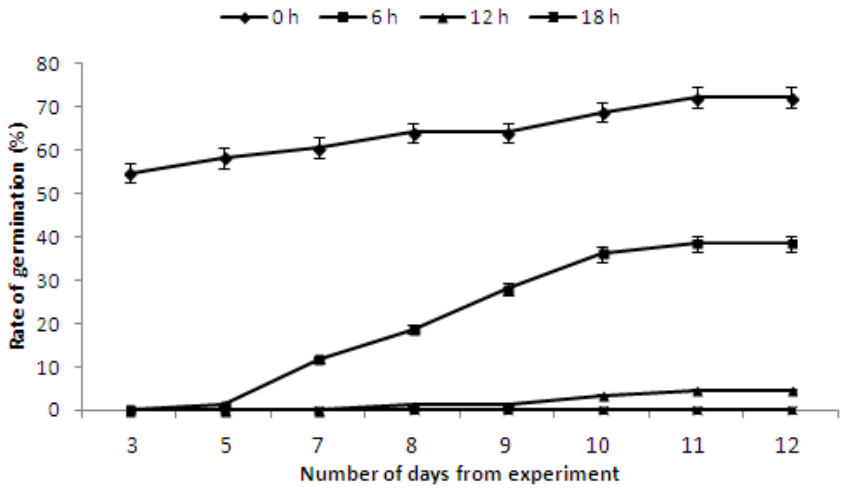

Fig. 2. Light effect on germination seeds of *Nigella sativa*
0h: 0h light/24h darkness; 6h: 6h light/18h dark; 12h: 12h light/12h darkness; 18h: 18h light/6h darkness

Table 3. Seedling Phenology and growth steps after seed germination of *Nigella sativa.*

Days after germination	Total length (cm)	Steps of seedling growth
6	1.0 ± 0.5	- yang stem of 1 cm approximately - apparition of two linked, small (0.5±0.2 cm), spatula-shaped leaves
15	5.2 ± 3.1	- elongation of stem to 4.1±2.3 cm - growth of leaves with same form : spatula-shaped leaves
20	6.1 ± 3.6	- stem keep approximately the same length with 5.3±1.2 cm - 3 to 4 pairs of linked leaves
25	8.8 ± 2.7	- stem at 6.5±1.5 cm - 3 to 4 pair of opposite leaves - elongation of petiole for leaves 2-3 cm
32	10.1 ± 3.4	- stem at 8.4±2.7 cm - 3 to 4 pairs of lower, opposite, spatula-shaped leaves - 1 to 3 upper, dissected leaves (finely divided)
50	15.7 ± 4.3	- stem at 10.7±3.5 cm - long petiole up to 5 cm some times - drop/fall of the first lower spatula-shaped leaves - apparition of button flowers and the beginning of the flowering

4. DISCUSSION

Temperature is the limited factor to the establishment of plants in arid regions when humidity conditions are favorable (Evans and Etherington 1990). This study shows that the germination of *Nigella sativa* was very sensitive to temperature. Optimum temperature for germination was about 20˚ C and the low decrease or increase of temperature may affect the rate of germination that will be only 2% at 25˚C and 15˚C. This result was approved for Mediterranean plants with optimal temperatures ranging between 15° and 20°C (Baskin and Baskin 1998). According to Baskin and Baskin (1998), temperature requirements for shrubs in hot semi-deserts, where *Nigella sativa* can exist, to achieve 60–100% germination, range from 15 to 35°C, with temperatures of about 20–25°C being suitable for most species. Temperature changes may affect a number of processes controlling seed vigor, including membrane permeability and the activity of membrane-bound and cytosolic enzymes (Gul and Weber 1999).

This work shows that seeds of *Nigella sativa* can germinate at high concentration of salinity. Increase of salinity from 0 to 6g/l of salt have no significant effect on germination rate which pass from 72.4% to 63.5%. Germination is slowly decreased starting from salinity of 8 g/l. These results may lead to the suggestion that, black cumin is a salt tolerant plant and may be considered as a halophyte. The same result was shown by Hajar ***et al***. (1996). Some studies have shown that black cumin is able to tolerate moderate levels of water stress (Mozzafari ***et al***. 2000). Although black cumin is cultivated in many countries, it is widely grown in arid and semi-arid regions where soils contain high levels of salts (Ashraf 2002).

However, high salinity can also completely inhibit seed germination at concentrations beyond the tolerance limits of the species (Khan ***et al***. 2001). In this work seed germination was completely inhibited at 12 g/l of salt.

Temperature can interact with salinity affecting rate of germination (El-Keblawy and Al-Rawai 2005). For *Nigella sativa*, no germinations were recorded at 10° C and 15° C and in a salt concentration of 8 g/l.

Germination of *Nigella sativa* was higher in darkness (24D: 0L) with a rate of 72.3%. Extension of light period decrease the rate of germination to 38.5% in 18D: 6L, then 4.7% in regime 12D:12L and finally completed inhibited (0%) in continuous light (0D: 24L). This result suggests that germination of *Nigella sativa* was light sensitive. This process explains that *Nigella sativ*a cannot exist in open area and must be protected under trees or mountains. Ajmal and Salman (2003), working on four perennial grasses that grow in open area (dark sensitive), reported that absence of light (darkness) can inhibited the germination. In addition, light requirement for germination may vary with temperature (El-Keblawy and Al-Rawai 2005).

The phenology monitoring of *Nigella sativa*, Kassim variety, shows that germination was achieved in 6 days by the apparition of the first spatula-shaped leaves. After 50 days approximately, it's the beginning of the flowering by the apparition of the first flower buttons, a long leaf petiole and the drop of the first lower spatula-leaves. Many factors can influence seedling growth. Differences in seedling growths could be attributed to the size of seeds (Upadhaya ***et al***. 2007). However, seedling and root lengths were also highly decreased for the seeds of the same species collected from the extremely polluted area as compared to the control (Shafiq and Iqbal 2007).

5. CONCLUSIONS

The germination of the black cumin was very sensitive to temperature with an optimum of 20°C. Germination stops below 10°C and above 25°C. However, black cumin seeds can tolerate salinity up to until 8g/l. The absence of light shows the best rate of germination that decreased when light period increases. Seedling monitoring shows that germination was achieved after 6 days, whereas the beginning of flowering was observed after 50 days from germination.

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REFERENCES

AJMAL, K.M. & SALMAN, G. (2003) Light, salinity, and temperature effects on the seed germination of perennial grasses. Amer. J. Bot., 90, 131-134.

ASHRAF, M. (2002) Salt tolerance of cotton, some new advances. Crit. Rev. Plant. Sc., 21 (1), 1-30.

BASKIN, C.C. & Baskin, J.M. (1998) Seeds. *Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego, USA.

D'ANTUNO, L.F., MORETTI, F. & LOVATO, F.S. (2002) Seed yield, yield components, oil content and essential oil content and composition of *Nigella sativa* L. and *Nigella damascena* L., Ind. Crops Prod. 15, 59-69.

EL-KEBLAWY, A. & AL-RAWAI, A. (2005) Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw) D.C. J. Arid Env., 6, 555-565.

EVANS, C. & ETHERINGTON, J.R. (1990) The effect of soil water potential on seed germination of some British plants. New Phytol., 115, 539-548.

GUL, B. & WEBER, D.J. (1999) Effects of salinity, light and temperature on germination in *Allenrolfea occidentalis*. Can. J. Bot., 77, 240-246.

HAJAR, A.S., ZIDAN, M.A. & AL-ZAHRANI, H.S. (1996) Effect of salinity stress on the germination, growth and some physiological activities of black cumin (*Nigella sativa* L.). Arab Gulf J. Sci. Res., 14, 445-454.

KHAN, M.A., GUL, B. & WEBER, D.J. (2001) Seed germination characteristics of *Halogeton glomeratus*. Can. J. Bot., 79, 1189-1194.

KHARRAT, A., BAUMEL, A., MSEDDI, K., TORRE, F. & CHAIEB, M. (2011) Polymorphism of *Cenchrus ciliaris* L. a Perennial Grass of Arid Zones. Afr. J. Ecol., 49(2), 209-220.

MIGAHID, A.M. 1987. *Flora of Saudi Arabia*. Fourth Edition, King Saud University – Riyadh.

MNIF, L., MSSEDI, K. & CHAIEB, M. (2005) *Cenchrus ciliaris* L. in the arid area of Tunisia: chromosome numbers, germinative and reproductive faculties of some populations. Acta Bot. Gal., 152, 45-56.

MOZZAFARI, F.S., GHORBANLI, M., BABAI, A. & FARAMARZI, M. (2000) The effect of water stress on the seed oil of *Nigella sativa*. J. Ess. Oil Res., 12, 36-38.

MSEDDI, K., MNIF, L., CHAIEB, M., NEFFATI. M. & ROUX, M. (2003) Aboveground phytomass productivity and morphologic variability of Tunisian accessions of *Cenchrus ciliaris*. Afr. J. Range Forage Sci., 21(1), 49-55.

MSEDDI, K., VISSER, M., NEFFATI, M., REHEUL, D. & CHAIEB, M. (2002) Seed and spike traits from remnant populations of *Cenchrus ciliaris* L., in South Tunisia: high distinctiveness, no ecotypes. J. Arid Envir., 50, 309-324.

ÖZLEM, T. & SÜLEYMAN, K. 2004. Effect of Seed Rate on Agronomic and Technologic Characters of *Nigella sativa* L. Int. J. Agr. Biol., 6(3), 529-532.

PROBERT, R.J. 1992. *The role of temperature in germination ecophysiology*. In: M. Fenner, Editor, Seeds: The Ecology of Regeneration in Plant Communities, CAB International, Wallingford, pp. 327-348.

RIAZ, M., SYED, M. & CHAUDHARY, F.M. 1996. Chemistry of the medicinal plants of the genus *Nigella*. Hamdard Medicus, 39(2), 40-45.

SHAFIQ, M. & IQBAL, M.Z. (2007) Germination and Seedling Behaviours of Seeds of *Peltophorum pterocarpum* D.C. Baker Ex K. Heyne Growing under Motor Vehicle Emission. Turk. J. Bot., 31, 565-570.

SIDDIQUI, A.A. & SHARMA, P.K.R. (1996) Clinical importance of Nigella *sativa* L. a review. Hamdard Medicus, 39 (2), 23-26.

UNGAR, I.A. (1995) *Seed germination and seed-bank ecology of halophytes*. In: J. Kigel and G. Galili, Editors, Seed Development and Germination, Marcel Dekker, New York, pp. 599-627.

UPADHAYA, K., PANDEY, H.N., PYNSAKHIAT, S.L. (2007) The Effect of Seed Mass on Germination, Seedling Survival and Growth in *Prunus jenkinsii* Hook. f. and Thoms. Turk. J. Bot., 31, 31-36**.**

VISSER, M., MSEDDI, K., CHAIEB, M. & NEFFATI, M. (2008) Assessing yield and yield stability of remnant populations of *Cenchrus ciliaris* L. in arid Tunisia: developing a blueprint for initiating native seed production. Grass Forage Sci., 63, 301-313.

WORTHEN, D.R., GHOSHEH, O.A. & CROOKS, P.A. (1998) The in vitro anti-tumor activity of some crude and purified components of black seed, *Nigella sativa* L. Anticancer Res., 18, 1527-1532.