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LIGHT, SALINITY AND TEMPERATURE EFFECTS ON THE SEED GERMINATION OF *NIGELLA SATIVA* L.

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Abstract

This work was carried out in the laboratory of Botany in the College of Sciences in the University of Hail, Saudi Arabia. It deals with the germination conditions of *Nigella sativa*, used for medicinal purposes for centuries, both as an herb and pressed into oil, in Asia, Middle East and Africa.

The germination requirements *Nigella sativa*, were studied under control conditions in the laboratory. Treatments included four light levels (0 :24) ; (6: 18) ; (12 : 12) then 24 :0) hour (light : dark) period, six salinity concentrations (0, 2, 4, 6, 8, and 10 gl/L NaCl), and four temperature regimes (15°, 17°, 20° and 25°), using a completely randomized block design.

This work shows that germination of *Nigella sativa* was very sensible to temperature with an optimum of 23°C. Germination will stop below 15°C and after 25°C. For salinity the best seed germination of all experiments was obtained in a distilled water control. The progressive increase in salinity, until 8g/l, have no significant effect on germination. The inhibition of germination was significant only after 10g/l. The absence of light give the best rate of germination that would be lower if light period increase and it will be inhibited completely at 18h of light regime. In this work optimal conditions for germination of *Nigella sativa* were determined.

Key Words: *Nigella sativa, germination, light, temperature, salinity*

Introduction

The seed of Nigella sativa, known as black seeds, has been shown to contain more than 30% of fixed oil and about 0.4 to 0.45% (w/w) of volatile oil (El-Alfy et al., 1975). Chemical investigation of the fixed and volatile oils from black seed has shown that thymoquinone (TQ) is a major component of the volatile oil, while the fixed oil contains mainly unsaturated fatty acids including the unusual (C 20:2) arachidic and eicosadienoic acids (Houghton et al., 1995).

Black cumin (Nigella sativa L.) is used widely in traditional and industrial pharmacology (Patel et al., 1996) and (D'Antuno et al., 2002). It is reported 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). Other medical effects like: Antiasthmatic (M.H. Boskabady, et al. 2010), protective toxicity (Ahmed M. Mohamadin et al. 2010), plasma antioxidant capacity (Maznah Ismail et al. 2010, Mahmoud et al., 2015), antifungal (Eugene A. Rogozhin et al. 2010), anti-nephrotoxicity (İhsan and Engin 2010), antiallergic (Soheila et al. 2010), retards the progression of atherosclerosis (Mohammad and Hedi, 2010) and many other diseases (G. Lupidi, et al. 2010, Osama and Soliman, 2010) were cited. This is a special offering of the East African form of Nigella, known to local inhabitants by the Arab name "habat soda." This is considered by many to be the most useful of herbs, being used to rid the body of infection and parasites. The fixed oil is used externally against any and all skin problems.

Nigella sativa Linn., family Ranunculaceae, is an annual flowering plant that grown and cultivated in North Hijaz, representing the western part of Saudi Arabia(Figure 1). It grows to 20–30 cm length, with finely divided linear leaves. The flowers are delicate, and usually colored light blue and white, with 5–10 petals. The fruit is a large and inflated capsule composed of 3–7 united follicles, each containing numerous seeds. The seed is used as a spice. Some studies have shown that black cumin (Mozzafari et al., 2000) is able to tolerate moderate levels of water stress. 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. However, salt affected soils can be used by growing salt tolerant crops because such crops would allow expansion of crop production to areas where conventional reclamation procedures are economically or technically limited. Since N. sativa is a potential medicinal plant, it could be grown on salt-affected lands if it can tolerate high degree of salt. The present study was conducted to assess the response of the germination to salt stress since the mechanism by which a plant tolerates salt is complex and it differs from species to another. High levels of salts affects plant growth at all stages of development and sensitivity to salinity varies from one growth stage to another. Diverse effects of salt stress on germination, seedling growth as well as some physiological activities of a number of cultivated plant species have been extensively investigated (Hussain, et al., 2008; Shah, et al., 1987; Ashraf, et al., 1994, Wei-Qing et al., 2015).

The establishment of plants in arid regions is often limited by temperature when moisture conditions are favorable. Knowledge of temperature effects on germination may be useful to evaluate the germination characteristics or the establishment potential among range species.

Different explanations have been offered for the germination of highly sensitive seeds, and several conditions have been shown to activate the germination of such seeds. The cut of costs increase water supply, variation of quantity and intensity of light. Reciprocal relation of heat and light have been suggested as factors affecting the germination of light-sensitive seeds.

This work, which was conducted in the laboratory of Botany and Plant Biology at the college of Sciences in Hail, aims at studying the effect of temperature, salinity and light on the germination of *Nigella sativa.*

Materials and Methods

Vegetal Material: seed collection

The fruits (capsules) of *Nigella sativa* used for this experiment were harvested last year from its natural populations within the areas of Kassim and the region of El Hijaz in Saudi Arabia (figure 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.

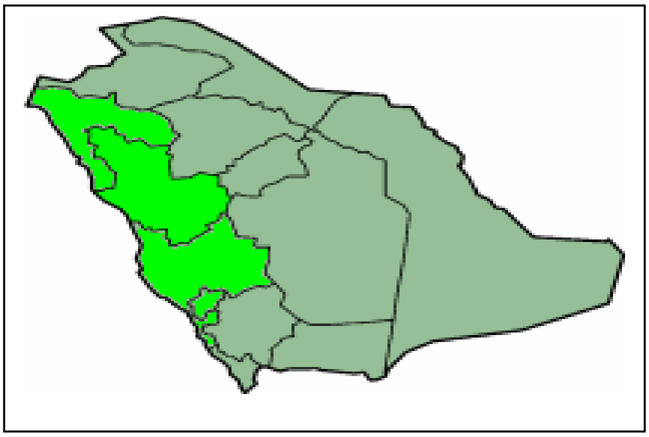

Figure 1: distribution map of *Nigella sativa* in saudi Arabia

Seeds preparation

Only mature and intact seeds were used in the experiment using optic lens, undamaged and the very small seeds were eliminated. The seeds were divided into groups each with 100-seeds. Every group was photographed and weighted; the average of 100 seeds weight was calculated.

Germination test

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. 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 15°C, 17°C, 20C° and 25C°. Six salinity concentrations (0 g/l, 2 g/l, 4 g/l, 6 g/l, 8 g/l and 10g/l NaCl) were used based on a preliminary test for salt tolerance of the species. Table 1 resume germination trials conducted in this work.

Table 1: Experimental conditions for germination of *Nigella sativa*

1- Temperature and salinity effect on germination of *Nigella sativa*

Oven Temperature	Salinity of water utilized for germination				
15˚C - 17˚C- 20˚C- 25˚C	Distilled water (0g NaCl/l)	Salt water at 2g NaCl/l	Salt water at 4g NaCl/l	Salt water at 6g NaCl/l	Salt water at 8g NaCl/l

2- Light effect on germination of *Nigella sativa*

Oven Temperature	Duration of light			
20 ˚C	0h (Darkness)	6h	12h	18h

For all trials, 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.

Results

1. Temperature effect

Temperature, salinity and their interaction significantly ($p<0.0001$) affected the final percentage of germination of *Nigella sativa* (Table 2). Seed germination was highest in distilled water and germination percentages decreased slowly with the increase in salinity (Table 2; Figure 2). Seeds of *Nigella sativa* were able to germinate at temperatures between 15 and 25 °C and the optimal temperature corresponds to 21 °C. At 10˚C and 25˚C, rates of germination were very low with respectively only 4% and 2%. Maximum rate of germination was observed at 20˚C with 20.7 seeds that corresponds to 41.40%.

Table 2. Number of germinated seeds of *Nigella sativa* at different experimental conditions.

NaCl (g/l)	Temperature					Light at 20°	
	10˚C	**15˚C**	**17˚C**	**20˚C**	**25˚C**		
0	2 ± 0.3	14 ± 2	18.0 ± 1.0	20.7 ± 4.3	1 ± 0	0h	20.6 ± 4.3
2	1 ± 0.3	12 ± 1	6.7 ± 0.3	13.3 ± 4.3	0.6 ± 0.3	6h	11 ± 5
4	1 ± 0.3	13 ± 1.3	12.0 ± 1.0	14.0 ± 3	0.6 ± 0.3	12h	1.3 ± 0.3
6	1 ± 0.3	9 ± 1	14.7 ± 2.3	19.3 ± 4.3	0.6 ± 0.3	18h	0 ± 0
8	0 ± 0	1 ± 0.3	3.3 ± 0.3	11.3 ± 4.3	0 ± 0		
Salt effect	F = 49.213		Prob < .001			**Light effect**	
T˚ effect	F = 429.461		Prob < .001			F=44.227	
Salt*T˚	F interaction = 12.554		Prob < .001			Prob < .001	

The pre-germination period in normal conditions was about 3 days**.** This period was observed at 17˚C and 20˚C in all salt concentrations. The number of days before germination increase when temperature becomes lower than 15˚C or more than 25˚C. The increase in light periods extend the pre- germination period (Table 3).

2. Salt effect

Seed germination decrease with the increase in NaCl concentrations at all temperatures (Table 2). But at 20 °C the decrease of germination with salinity increase was the lower. At 20˚C and salinity of 6g/l rate of germination was so higher with a value of 3.6%. This result shows that germination tolerates salinity, so it starts to decrease only from 8g / l NaCl. At salinity of 8g / l NaCl, no seeds germinated were observed at 10 °C and 25 °C (Figure 2). This can be explained by the presence of a positive interaction between temperature and salinity. Seeds germinated after 2-3 days at 17°C and 20°C, but they need a long latency time at 15°C and 25°C, the seeds germinated only after 9-10.

Table 3. Pre-germination Period: number of days before germination (NG: No Germination observed)

NaCl (g/l)	Temperature					Light at 20°	
	10˚C	**15˚C**	**17˚C**	**20˚C**	**25˚C**		
0	8	7	3	3	9	**0h**	3
2	8	7	3	3	10	**6h**	5
4	10	8	3	3	10	**12h**	7
6	10	9	3	3	10	**18h**	NG
8	NG	10	3	3	NG		

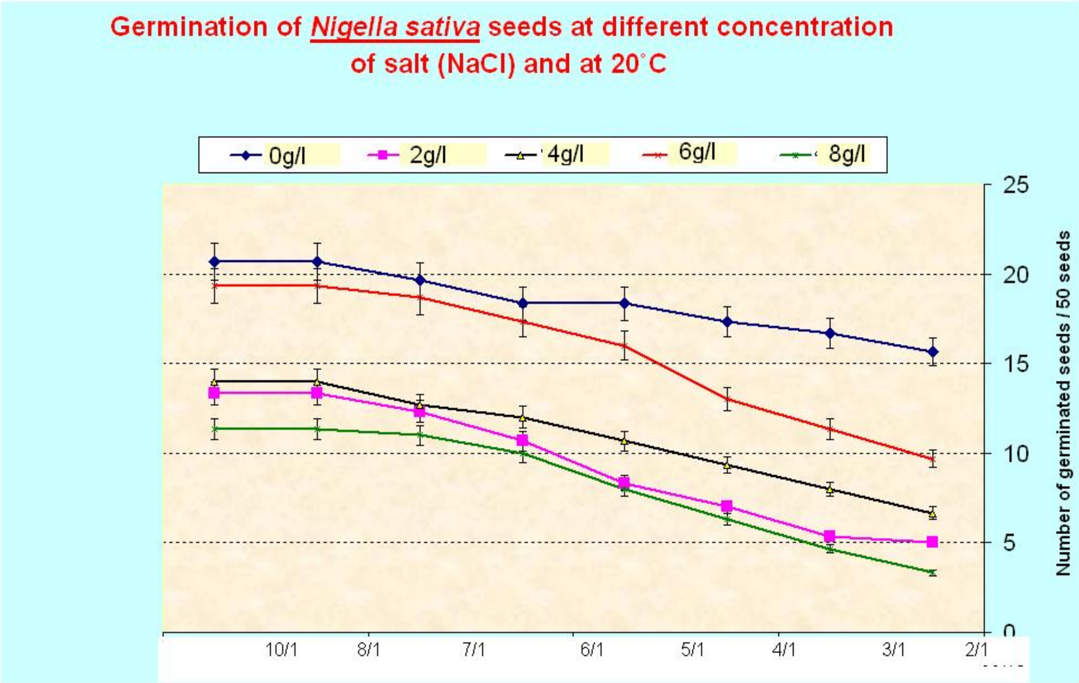

Figure 2. Germination of *Nigella sativa* seeds at different salt concentrations (NaCl) and 20˚C

The curves analysis of figure 3, that show seeds germination of *Nigella sativa* at different concentrations of salt and at 17˚C (Figure 3), indicates that the seeds start to germinate 3 days after incubation and at all salt concentrations. The maximum germination was observed when seeds were placed at distilled water with a germination rate of 18 seeds (36%).

From 2 to 6 g / l NaCl differences in the germination rates is not significant, with values ranging from 6.7 (13.4%) to 14.7 (29.4%) seeds. Only at 8g / l NaCl that germination was influenced by salt, represented with low germination rate of around 3.3 seeds (6.6%)

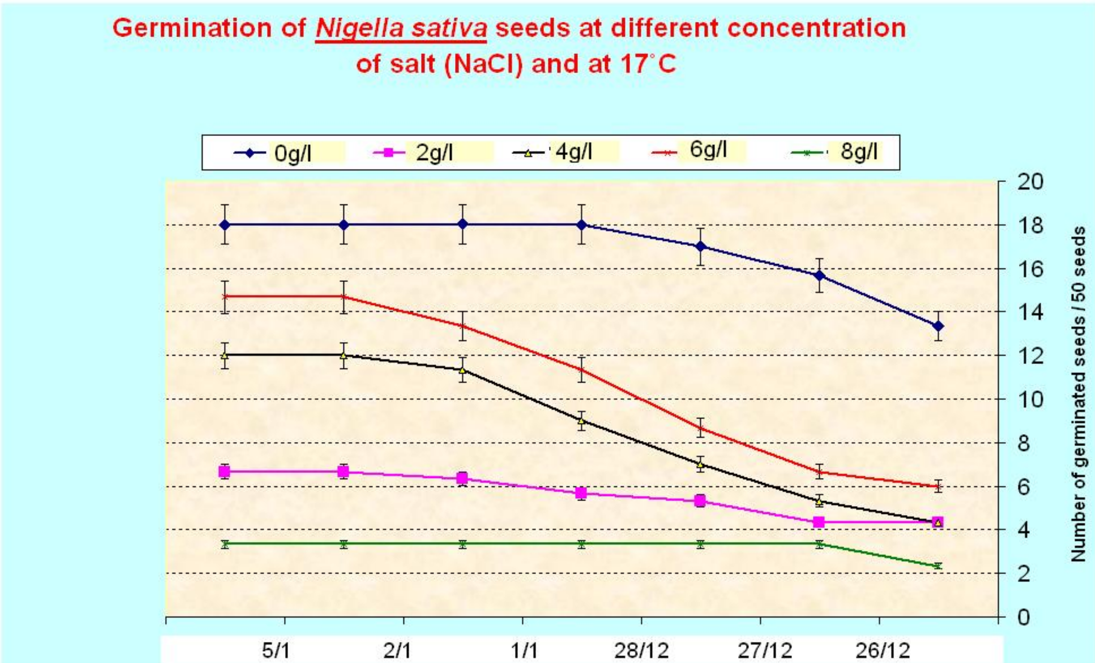

Figure 3. Germination of *Nigella sativa* seeds at different salt concentrations (NaCl) and 17˚C

At 25 ˚C, figure 4 shows that germination rates were very low, not exceeding 1 seed germinated out of 50 (2%). No germination observed at 8g/l.

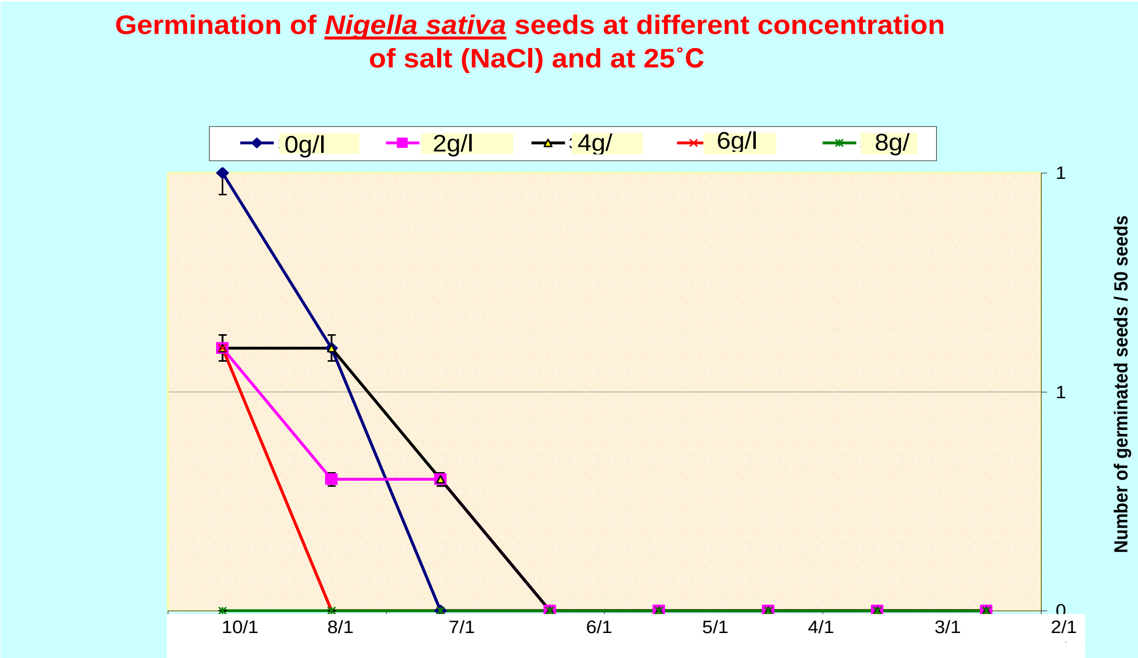

Figure 4. Germination of *Nigella sativa* seeds at different salt concentrations (NaCl) and 25˚C

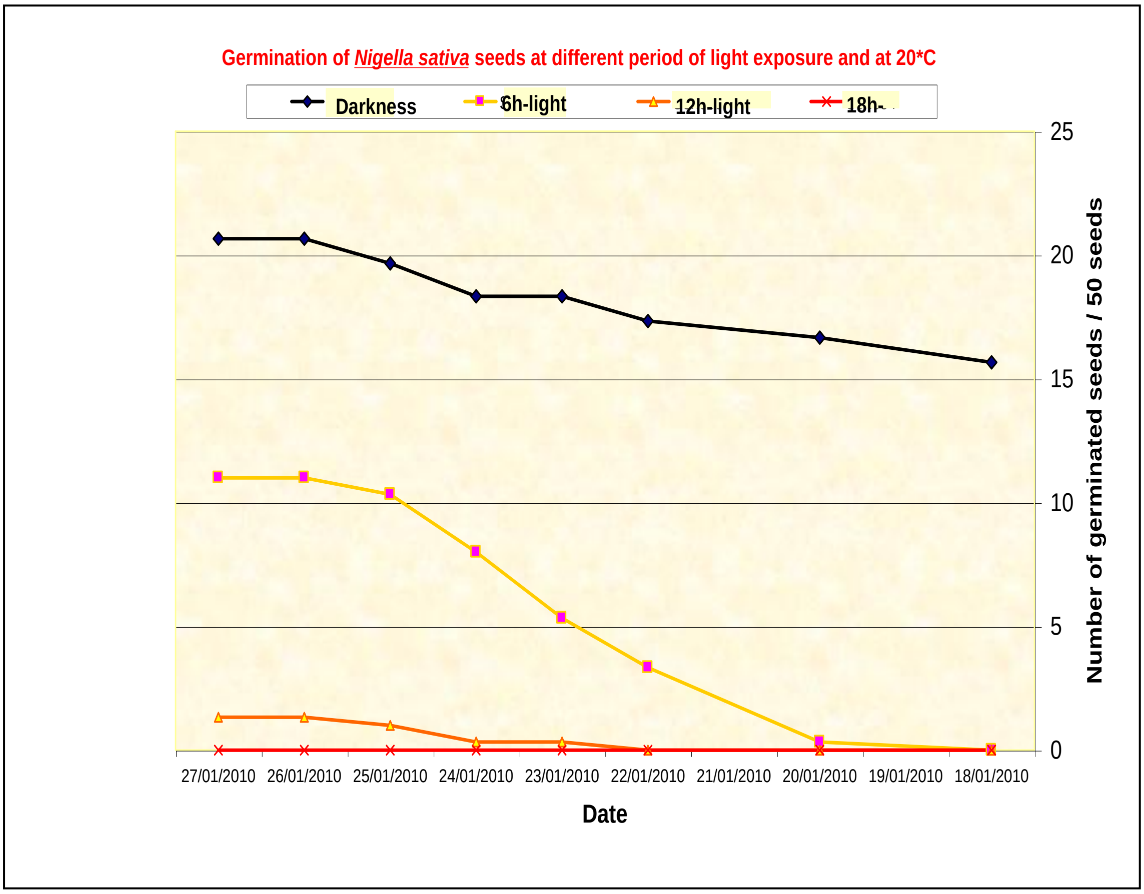

Figure 5. Germination of *Nigella sativa* seeds at different salt concentrations (NaCl) and 17˚C

3. Light effect

Seeds Germination of *Nigella sativa* at different periods of light were reported in figure 5. Germination in darkness represents the highest rate (41.32%). It decreases thereafter according to light exposure. It is almost zero at 18 hour of light exposure. This shows that darkness was a key factor for the germination of *Nigella sativa*.

Conclusion

This study allows to recognize the optimal conditions for seed germination of <u>*Nigella sativ*</u>a

-An optimum temperature of about 20˚C;

-A maximum rate of germination which is 41.32% at 20˚C, in distilled water and in darkness;

-A salinity tolerance, which can exceed 8 g / l of NaCl;

-Sensitivity to light that inhibits germination.

Discussion

<u>*Nigella sativa*</u> exhibit a small wide of temperature for germination that was comprised between 17˚C and 21˚C**.** This specie showed to be sensible to temperature**.** According to Baskin and Baskin (1998), temperature requirements for shrubs in hot semi-deserts and deserts to achieve 60–100% germination range from 15 to 35 °C, with temperatures of about 20–25 °C being suitable for most species.

This work shows that seeds of <u>*Nigella sativa*</u> can germinate at high concentration of salinity. 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 indicate that seeds of halophytes can remain viable for an extended period of exposure to salt stress and germinate when conditions are favorable (Khan & Ungar, 1997; Zia & Khan, 2004). In addition, high salinity can completely inhibit seed germination at concentrations beyond the tolerance limits of the species (Khan et al., 2001).

<u>*Nigella sativa*</u> was a sensible light species that preferred darkness to germinate. But light requirement for germination may vary with temperature (El-Keblawy and Al-Rawai, 2005).

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