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Effect of temperature on in vitro seed germination and mean germination time of endemic medicinal plant *Nepeta viscida* Boiss. (Lamiaceae)

Yelda Emek
Biology Department, Science & Art Faculty, Adnan Menderes University, Kepez-Aydın, Turkey

Abstract
Nepeta viscida Boiss. is an endemic plant belongs to the family Labiatae. It has medicinal properties. There is no study on seed germination of this plant. Therefore, study of germination of this plant is essential. Effect of temperature on *in vitro* seed germination and mean germination time of the plant were determinated. The seeds of the medicinal plant were exposed to germination temperature treatments ranging from of 10 to 30°C. The highest germination obtained at 25 °C (60 %). The lowest germination obtained at 10 °C. The mean germination time has been affected by temperature. This is the first report on effect of temperature on *in vitro* seed germination of *Nepeta viscida* Boiss.

Keywords: *nepeta viscida boiss.* medicinal, endemic, *in vitro*, germination, temperature

1. Introduction
Because of its geopraphical location, ecological properties and vegetation history, Turkey has a extremely rich flora. Although Turkey has one fifteenth of total land covered by European countries, it has an overwhelming number of endemic species (34.5 %) [1] The number of plants used for medicinal purpose are 1,000-2,000 [2]. Turkey is a gene center for *Lamiaceae* family, which comprises a lot of aromatic plants. The family has high endemic plant ratio of 44.2% [2, 3]. Genus *Nepeta* is presented by 33 species in the Flora of Turkey of which are endemic [4]. *Nepeta* species contain monoterpenes, sesquiterpenes, cyclopentanoid iridoids derivatives and nepetalactones. *Nepeta* species are commonly used in Turkish folk medicine as diuretic, antiseptic, antispasmotic, antitussive, anti-asthmatic and anti-inflammatory [5]. They have also different biological activity such as anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activity [6, 7]. Most of the *Nepeta* species contain nepetalactone and 1,8 cineol / linalool [8, 9, 10].
N.viscida Boiss. is an endemic plant which spread in Aydın, İzmir, Manisa, Muğla and Uşak (TÜBİVES). It contains forty-one compounds such as α-terpineol (31.57%), terpinen-4-ol+β-caryophyllene (7.52 %), γ-elemene (6.23%), 1,8-cineole (6.02 %), β-pinene (5.08 %) [11]. In addition, Oztürk and his colleagues have reported that *N.viscida* essential oils have high antimicrobial activity [12].
Seed germination studies of rare and endemic species are of great importance in the identification of conservation strategies [13]. First, seed germination is the only way to protect the genetic diversity of populations [14]. Secondly, these species are under the risk of extinction in many cases. Therefore, accurate and precise understanding of the germination abilities of these taxa is of importance in the preservation and continuation of generations [15, 16].
Germination is the first step in the life of most flowering plants. It starts with the uptake of water (imbibition) is in the resting phase seed and generally ends with the emergence of a radicle [17]. Germination depends on existence on a desirable level of the significant environmental factors (water, temperature, oxygen and light), which change to seed characteristics and special requirement of different plant species and cultivars.
Seeds of each plant type need a minimum temperature for germination. Sometimes, some seed types germinate at low temperatures such as zero or close to zero degrees, but these temperatures may prevent germination of other seed types [18]. Copeland and McDonald were reported that for the most plant the optimum and maximum germination temperatures are 15-30 and 30-40°C, respectively [19].
Many endangered species can be quickly propagated and preserved for a minimum of plant material, rendering low impact on wild populations by using *in vitro* techniques [20]. In recent years, there has been an increased interest in *in vitro* techniques offer powerful tools for germplasm conservation and the mass multiplication of many threatened plant species [21]. Seed is preferred as starting material for *in vitro* studies due to its representative of the genetic structure and diversity [22].
Due to medicinal properties the use of the plant has increased. Therefore there is now an urgency to conserve of wild population of *N. viscida* for further uses. Germination is the first step for plant life. The aim of study is to determine the effect of temperature on *in vitro* seed germination of *N.viscida*.

2. Materials and Methods
2.1 Seed collection, viability and sterilisation
Seeds of *N. viscida* were collected from Ödemiş, İzmir, Turkey. Seeds were stored in paper bags at room temperature (20-25 ˚C).
Seed vigour was determined by Tetrazolium test [23]. For this aim, halved seeds were treated in tetrazolium solution (TTC,

0.1%) for 2 hour at room temperature and red staining embryos were evaluated as alive. The seed viability was repeated at 6-month intervals.

Seeds were washed under running tap water for an hour. Seeds were surface-sterilized in 70 % EtOH for 3 min, then in 2.25 % NaOCl (including 3-4 drops tween 20) for 5 min followed by 3 rinses for 5 min each with sterile distilled water. Due to the seed dormancy, germination experiments were carried out at least 6-8 months after seed collection [24]. The sterilised seeds were cultured in 190 cc jars containing 50 mL of distilled water (DW). The pH was adjusted to 5.8 before sterilisation by autoclaving at 105 kPa and 121 ˚C for 15 min. Paper bridges and glass beads were used to provide physical support for the seeds. To determine the effects of temperature on the germination of *N.viscida* seeds, cultures were maintained at different temperature (10, 15, 20, 25 and 30˚C) with a 16-h light period.

Seeds were evaulated germinated when the radicula emergence. Germinated seeds were counted weekly. The experiments were continued for 8 weeks. After this period germination percentage and mean germination time (MGT) were determined according to Ellis and Roberts [25]. MGT = Σ (n. w)/N, where n is the number of seeds germinated end of the week, w is the number of weeks from the beginning of the test, and N is a total number of seeds germinated at the end of the experiment. The optimum temperature for germination was calculated on the basis of constant temperature as: To =Σ tp/Σ p, where p is the percentage germination at temperature t [26].

Statistical analysis

The experiments were conducted using five replicate of 20 seeds for each treatment. All experiments were repeated three times. Data were subjected to x´ = arcsine √(x/100) transformation and differences were analyzed by analysis of variance and the means seperated using Duncan's multiple range test at p< 0.05.

3. Results and Discussion

Seed viability dependent on time is shown in Table 1. As you seen seed viability determined 80% and time has no effect on seed viability.

Table 1: Seed viability of *N.viscida* seeds six months intervals.

Time (month)	0	6	12	18
Seed Viability (%)	85	80	80	80

Temperature is found an effective factor on seed germination of *N. viscida*. The germination percentege is shown in figure 1 and the mean germination time is shown figure 2. Seeds incubated at 25 °C showed the highest germination percentage (60 %- figure 3), whereas the germination rate below and above at this temperature showed a decreasing tendency. Although the germination percentage obtained at 20 °C is lower than 25 °C, there is not any difference statistically. The optimum temperature for germination is calculated as 21.7 °C. Besides, the mean germination time is affected by temperature.

Bannayan *et al.* have reported that temperature is an effective factor on germination of some Labiate species [27]. While seeds of *Nepeta binaludensis, N. glomerulosa and N.crassifolia* showed the highest germination percentage at 20 ˚C respectively 75.2%, 54.9%, 75%, *Thymus kotschyanus, Rubia tinctorum* showed the highest germination percentage at 15 ˚C respectively 79 %, 47.6%. Researchers studied the seed germination of *Origanum dictamnus L., Sideritis syriaca L. subsp. syriaca, Salvia pomifera L. subsp.* pomifera and *Salvia fruticosa Miller* were found that 15, 20, and 25 °C were the most ideal temperatures[28]. However, Erdağ and her colleagues have reported that seeds of *Dorystoechas hastata* Boiss. & Heldr. ex Bentham showed maximum germination percentage at 25 ˚C [29]. At the same time they observed a decrease in germination percentage at lower and higher temperatures.

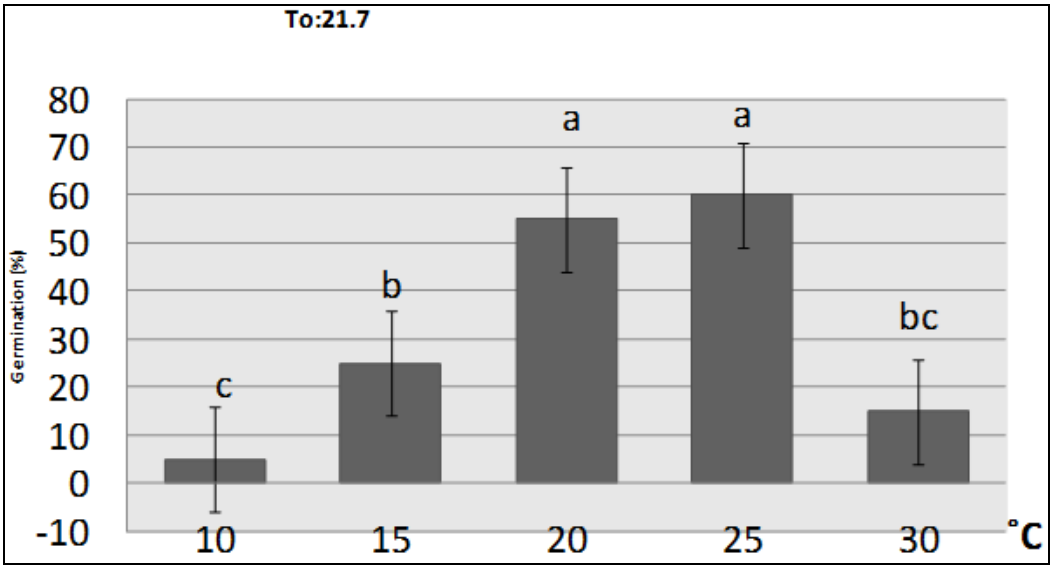

Fig 1: Germination percentage of *Nepeta viscida* seeds at different temperatures. SE bars with different letter (s) are significantly different by Duncan's multiple range test (p< 0.05). To: calculated optimum temperature.

Temperature were shortened the mean germination time (figure 2). While seeds incubated at 20 and 25 °C were germinated earlier than other temperatures, seeds incubated higher temperature than these value were germinated slowly. However, seeds cultured at 15 and 10 °C were showed the slowest germination time. Mehmet Demir Kaya and his colleagues have reported that different treatments were effected on the mean germination time [30]. In addition, Gairola *et al.* were obtained that the mean germination time in seed at different temperatures varied from the minimum at 35 °C to the maximum at 20 °C [31]. All of these results is paralelled with results of my study.

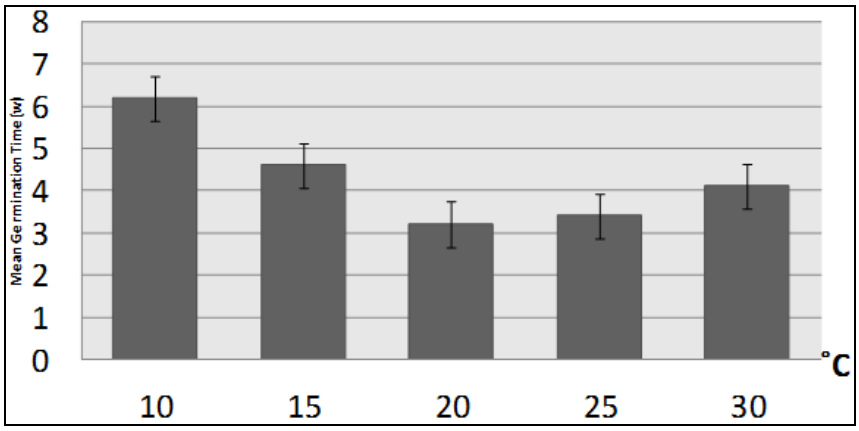

Fig 2: Mean germination time of *Nepeta viscida* seeds at different temperatures.

Fig 3: Germinated *Nepeta viscida* seeds at 25 ºC.

Temperature has been determined as an effective factor on the seed germination of the endemic *N.viscida* Boiss. This is the first study on the effect of temperature on *in vitro* germination of species. The results of this study will form the basis for further studies on *in vitro* propagation of that plant, with enormous potential as a medicinal plant.

4. Conclusion

This study is about the effect of temperature on *Nepeta viscida* Boiss. seeds. Results obtained from studies indicate that seeds of *Nepeta viscida* reguire of 21.7 ºC for optimum germination. Findings obtained can be further used to establish *in vitro* cultures. So that it can respond to medicinal uses by using less plant material without damaging the plants in the nature

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6. References

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