

## Effects of Some Environmental Factors on Seed Germination and Seedling Growth of *Heliotropium europaeum* L.

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### Abstract

The typical plant heliotrope (*Heliotropium europaeum* L.) is an extremely competitive weed that has recently become an invasive weed problem in many areas of the world. If the species is not handled and produces a lot of little seeds, it will provide a remarkable colonizing and invasive capacity. Therefore, this study aimed to investigate some environmental aspects that may enhance or prohibit the seed germination and emergence, with the purpose of guiding the creation of long-term controlling plans to eradicate this plant. For this investigation, seeds were gathered from a variety of mature plants in various locations. The effects of temperature regimes, light, water stress, salinity, and burial depth on seed germination and seedling emergence of *H. europaeum* were examined. The results showed that seeds of *H. europaeum* could germinate in a wide range of temperatures. Light clearly promoted seed germination, demonstrating that concealed seed will remain inactive until distressed. Water stress significantly decreased germination of *H. europaeum* seeds. Germination was reduced significantly to less than 20 % at water potentials (-1 MPa). Over 80% of *H. europaeum* seeds germinate at low salinity (100 mM NaCl), while reasonable germination (75%) occurs at 200 mM NaCl, indicating that the seeds are fairly tolerant to salinity. In the burial depth treatment, the species' maximum appearance rates were 64.5 percent and 63

percent, at a depth of 0.5 cm and surface treatments. The findings of this research might be used to help create long- term controlling for this harmful plant.

**Key words:** Germination, water stress, high temperature, light and salinity.

## تأثير بعض العوامل البيئية على أنبات البذور ونمو الشتلات *Heliotropium europaeum* L

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الخلاصة

النبات (*Heliotropium europaeum* L) هو عشب تنافسي للغاية أصبح مؤخراً مشكلة أعشاب غازية في العديد من مناطق العالم. إذا لم تتم إدارته ، والعدد الكبير من البذور الصغيرة المنتجة لكل نبات ، يوفر قدرة استعمارية هائلة . لذلك هدف هذه الدراسة هو دراسة بعض الجوانب البيئية التي قد تعزز أو تحظر إنبات البذور وظهورها، من أجل الاسترشاد بها في تطوير استراتيجيات الإدارة طويلة الأجل لمكافحة هذه الحشائش. تم جمع بذور هذه الدراسة من العديد من مناطق مختلفة. تم التحقيق في النتيجة المترتبة على إنبات البذور وظهور الشتلات من *H. europaeum* من أنظمة درجات الحرارة مختلفة، والفترة الضوئية ، والإجهاد المائي ، والملوحة ، وعمق الدفن. أشارت النتائج إلى أن بذور *H. europaeum* كانت قادرة على الإنبات عبر مجموعة واسعة من درجات الحرارة. و تفضيل إنبات البذور بشكل إيجابي من قبل الضوء ، مما يشير إلى أن البذور المدفونة ستبقى في حالة نائمة حتى يتم ظروف الملائمة. الإجهاد المائي قلل بشكل كبير من إنبات بذور *H. europaeum* (65% من البذور الموجودة في التجارب ذات الإمكانات الأسموزية أقل من -0.65 MPa). تم تقليل الإنبات بشكل كبير إلى أقل من 20 % في إمكانات المياه (-1 MPa). بذور *H. europaeum* متسامحة بشكل معتدل مع الملوحة ، حيث ينبت أكثر من 80% من البذور عند مستويات منخفضة من الملوحة (100 mM NaCl) ، ويحدث إنبات معتدل (75%) عند 200 mM NaCl. في معاملة عمق الدفن ، وجدت أعلى ظهور بنسبة 64.5% و 63% على التوالي على عمق 0.5 سم ومعاملة سطحية. قد تساعد نتائج هذه الدراسة في تطوير استراتيجيات للإدارة طويلة الأجل لهذه الأعشاب الضارة .

**الكلمات المفتاحية:** الإنبات ، الإجهاد المائي ، الحموضة ، ارتفاع درجة الحرارة ، الضوء والملوحة.

## 1. Introduction

The genus *Heliotropium* is a member of the flowering plant family *Boraginaceae*. They are more usually referred to as European Heliotropes and are native to Europe, North Africa, and western Asia (Kandemir *et al.*, 2020; Craven , 2005).

The herb European heliotrope is grows in swamps and open tropical areas, where it is often found in the understory. It can be found growing in acidic and saline environments (Adams *et al.*, 2009). It is an annual herbaceous plant growing to up one meters in height, with a slender stem and opposite leaves, often arranged in pairs, which are lance-shaped, with a short petiole, and entire margins. The flowers are small and purple, with four petals and eight stamens. They are borne in small umbels which are spherical in shape, with a diameter of about 0.5 meters, and are arranged in a spiral pattern (Humphries *et al.*, 2018).

*H. europaeum* is a rangeland weed that can quickly take over an area if left unchecked. In order to combat this, ranchers have to manage their rangelands carefully in order to promote the growth of desirable plants such as grasses and forbs, while keeping the weed under control (Demiray *et al.*, 2013). One of the most effective management strategies used to control European heliotrope is to control its emergence. Ranchers can control the emergence of European heliotrope by grazing their livestock in areas where the weed is currently present, so that the plant is not able to germinate in the first place (Hunt *et al.*, 2009). Seeds of *H. europaeum* are small and sticky, and are usually washed out by rain before they germinate. To germinate, the seeds must be exposed to light for a certain length of time. The length of time range from a few hours to a few months, depending on seed type. The maximum germination time is about 4 months or more (Aliloo, 2013; Benvenuti, 2022).

The European heliotrope has resting buds at the base of its leaves that will later open to reveal the flowers; it also develops many small flowers in early summer, and seeds from those flowers are dispersed to grow more plants. It has a long period of dormancy when young, but once they reach reproductive maturity, they begin to flower at the beginning of the rainy season (Darabinejad, 2013). European heliotrope is a very competitive weed. It likes to live near the water, can grow quickly, and is difficult to tell where a heliotrope is, unless it has a flag or is already flowering.

European heliotrope is an herbaceous that can grow in a large range of habitats, including prairies, grasslands, savannahs, and forests. It can be weedy or invasive in many areas, and is often found near surface water, such as a lake or a stream (Benvenuti, 2022). European heliotrope is a weed problem in many parts of the world. If left unchecked, it can rapidly take over wasteland, roadsides, vacant lots and other open spaces. For example, it is an invasive species in California, Florida, Hawaii, South Carolina, Puerto Rico, and Taiwan. However, it is often the first indication that a problem exists; removal of plant heliotropes will therefore reduce the impact of the weed (Baskin and Baskin, 1998; Chauhan *et al.*, 2008).

Different types of seeds have different reactions to temperature when it comes to germination in the European heliotrope. For example, the hard-seeded heliotrope seeds will germinate better at cooler temperatures, while the soft-seeded heliotrope seeds will germinate better at warmer temperatures. The maximum germination temperature for hard-seeded heliotrope is around 30 °C, while the maximum germination temperature for soft-seeded heliotrope is around 35 °C. The minimum germination temperature for hard-seeded heliotrope is around 20 °C, while the minimum germination temperature for soft-seeded heliotrope is around 25 °C (Chauhan *et al.*, 2008; Humphries *et al.*, 2018; Benvenuti, 2022).

Numerous studies have also been conducted on how light affects the germination of European heliotrope seeds. These research' findings indicated that the majority of European heliotrope seeds do not sprout in darkness, with only a small percentage of seeds developing when they are planted in darkness. The majority of seeds will germinate when exposed to light, with the majority of seeds being able to germinate when exposed to light between 0 and 10 hours per day. (Humphries *et al.*, 2018; Benvenuti, 2022).

Other environmental conditions may be related to seed germination as well. For instance, inadequate moisture availability can prolong dormancy because seedlings may not be able to imbibe enough water or emerge competitively due to low soil moisture levels (Bittencourt *et al.*, 2017). In salty situations, many weed species' seed germination has been found to be greatly reduced by increased salinity (Rezvani and Zaefarian, 2016) While salinity and drought both cause an osmotic stress, the higher ion concentrations in salinity be able to take an extra significant inhibitory influence on seed growth.

The development, dormancy, and viability of seeds are similarly affected by the deepness of seed burial. It has been demonstrated that after a time of seed dry storage, which may happen when seeds are buried in dry soil, germination needs typically become less specific (El-Keblawy, 2014; Bebawi *et al.*, 2015). Additionally, the germination of seeds is significantly influenced by the nutrients available at diverse soil depths. Since only seeds that are near to the soil surface are often capable to sprout and emerge, Grundy *et al.* (2003) pointed out the importance of parameters related to seed burying and survival.

Understanding how European heliotrope germinates and emerges in response to different environmental factors may help to illuminate this weed among several plant crops. Additionally, thorough information of the ecological requirements for seed sprouting and seedling appearance in soil is a crucial requirement for the creation of a combined and ecologically harmless weed controlling strategy. The capability to expect seed germination and emergence in reaction to ecological situations is important for efficiently timing the use of mechanical, biological, and other management alternatives (Ghorbani *et al.*, 1999). The purpose of this study was to find out how the European heliotrope seed germination and emergence were affected by temperature, light, osmotic stress, salt stress, and planting depth.

## **2. Materials and Methods**

### **2.1 Seed collection and processing**

From numerous mature plants in various growth environments, mature seeds of *H. europaeum* were obtained. When the capsule turned brown, the seeds were deemed to be mature. Uncleaned seeds were brought to the lab in a bag with a label and stored there between 16 and 27 °C and 30 and 50 percent relative humidity until usage. In order to conduct germination tests, 25 seeds were evenly distributed in a 10 cm diameter Petri dish lined with Whatman® No. 10 filter papers. The Petri dishes were then wet with 5 ml sterile distilled water or a treatment solution to provide the seeds with an acceptable amount of moisture. The Petri dishes with contain seeds were subsequently put into seed germination cabinets, which had cool-white fluorescent lighting with a photosynthetic photon flux of 40 mol m<sup>-2</sup>s<sup>-1</sup>. For 30 days after the date of seeding, daily observations of seed germination were made. For all bellow trials; experiment design was used based on completely randomized design (CRD) with one factor.

## 2.2 Influences of Temperature regimes and Photoperiods

To exam the impacts of temperature regimes and photoperiods on germination percentages of *H. europaeum*. Seeds were subjected into three temperature regimes (20, 25, as well as 30 °C) under two light conditions 12:12 hour light: dark and 24 hour of constant darkness. Each treatment's four times replicated included 25 seeds. These temperature regimes have been chosen to reflect temperature changes during the spring, summer, and fall seasons. Petri dishes were given the dark treatment by being wrapped in aluminum foil.

## 2.3 Influence of salt condition on seed germination percentages

NaCl treatment solution have been applied directly to 25 seeds on each Petri dish. About 10ml of the related salt solution was used to stifle the filter paper for: Control (no solution), 10 mM, 50mM, 100mM, 150 mM, 200 mM, and 250 mM concentrations. Petri dishes were put at 30 °C temperatures under a 12:12 h light: dark regime. Petri plates were changed every other day and the treatments were kept in a cabinet with a consistent temperature and four repetitions of each treatment. For a period of 30 days, the rate of germination of *H. europaeum* seeds was monitored.

## 2.4 Influence of osmotic stress on seed germination

Polyethylene glycol (PEG) with an average molecular weight of 8000 has been set to get osmotic potentials of 0, -0.1, -0.2, -0.4, -0.65, -0.85, and -1.0 MPa of polyethylene glycol 8000 (Sigma-Aldrich Co., 3050 Spruce St., Louis, MO 63103) in 1 L of distilled water (Michel, 1983). *H. europaeum* seeds were tested at each dose of PEG, 4 replication of 25 seeds have been kept at 30 °C with 12:12 hours of light and darkness. It took 30 days to assess whether seeds would germinate.

## 2.5 Influence of burial deepness on seedling emergence and development

To examine how planting seeds deep affect germination and ensuing seedling emergence, this trial was conducted. The soil used for this test was transported to a location where this species dominates and then sterilized to remove any possible seeds. Sterilized sandy soil was layered to a depth of 1 cm at the bottom of each plastic pot (10 cm x 6 cm x 6 cm), which was then filled with the sterilized field-collected soil. This was done to promote soil moisture flow and freshening. About fifty seeds of *H. europaeum* have been positioned at 0.0, 0.5, 1.0, 2.0, 3.0, as well as 4.0 cm deep of the sterilized field composed soil, using four replications for each depth in single plastic pots. To guarantee minimal disruption of the seed samples, plastic pots have been individually marked with the sample number and set onto big butcher trays (28 cm x 44 cm x 5.5 cm). Trays were frequently submerged in water to keep the soil wet but not swamped. A growing chamber with a temperature of 30 °C and 12/12 light and dark photoperiods was used to house all of the trays. When the radicle reached a length of 2 mm beyond the seed coat, a seed or seedling was deemed to have germinated or emerged (Mahmood *et al.*, 2016). The appearance of fresh seedlings was tracked daily, with final counts being recorded two months following the final emergence.

## 2.6 Data analysis

After these tests conducted, the following formula was used to get the final germination percentage %:

$$FG\% = \frac{SG}{TS} \times 100 \quad [1]$$

Where, *FG %* is *Final germination percentage*; *SG*: is the all number of seeds were germinated and *TS*: is the total number of seeds planted (Wang *et al.*, 2009; Mahmood *et al.*, 2016).

All experiments were conducted twice (except for the seed burial depth trial) and the row data have been joint from the two repeated trials. A one-way analysis of Variance (ANOVA) has been



conducted to analysis row data using Minitab 20 software program. The figures existing here are constant means. After that means have been compared with Turkey's comparison test.

### 3. Results and Discussion:

#### 3.1 Effects of temperature and light regimes

Table 1, shows the effectiveness of different temperature regimes and photoperiod regimes on final germination percentage of *H. europaeum* seeds. It is clear that the highest germination percentage occurred when seeds treated under 30 °C, and 25 °C respectively, however the lowest percentage of final germination were recorded from 20 °C which was only 54 % under 12 h dark and 12 light. On the other hand, in regarding the impacts of light condition , general data shows that seeds of *H. europaeum* germinate better when they explosion to 12houre dark and 12 hour light rather than 24 hours darkness . As shown in table 1, seeds germinated in higher percentage under 12L/12 D in all temperature regimes (98 %, 95 %, and 54%), respectively. The percent results can be illustrated that seeds of *H. europaeum* prefer to germinate in warmer conditions, temperature 30 °C as found the optimum temperature degree, however it can be also illustrated from the results that this species can germinate and grow in a wide range of temperature regimes from cooler conditions to warmer conditions. Previous studies on this species and another species showed similar outcomes, for example Mahmood *et al.*, (2016) found similar results on an invasive weed species *Galenia pubescens*. Moreover, the impacts of environmental factors such as temperature regimes and light conditions have been studied on *H. europaeum* by Humphries *et al.*, (2018) in Australia, they initiate that seeds of this species can germinate higher percentage in a hot weather condition rather than cold condition, in their study, the alternative temperature regimes (30°C/20 °C at 12h L/ 12 Dark) was the optimum conditions for recording the highest percentage of seed germination , which is also similar to this current findings. In regarding to light influences,

previous studies shown that the majority of European heliotrope seeds will germinate better in light condition when compared with darkness (Humphries *et al.*, 2018; Benvenuti, 2022).

**Table 1:** Influence of different temperatures regime and photoperiods on the seed germination percentage of *H. europeem* \*

Treatments		Final Germination %
Temperature (°C)	Light	
20 °C	12:12 L/D	54 bc
	24 D	47 c
25 °C	12:12 L/D	95 a
	24 D	83 ab
30 °C	12:12 L/D	98 a
	24 D	91 a

Values followed by same letters are not significantly ( $p>0.05$ ) different from each other. L:D = 12 hour light and 12: hour Dar;, D = 24, hour dark.

### 3.2 Effect of salinity stress on seed germination

Figure 1, illustrated the effectiveness of different concentration of salinity on seed germination of *H. europaeum*. Results showed that the maximum percentage of germination (98 %) happened in the control treatment (no- stress), however the germination percentage was non significant at lower concentration of NaCl from 10 mM to 100 mM respectively. Germination reduced to 81 % and 75 % by rising concentration of NaCl near 150 mM and 200 mM, correspondingly. The current results

is relatively similar to a pervious study on the same species (Atak *et al.*, 2006), they reported that the salinity stress not effect on seed germination especially at lower concentration of NaCl. The maximal germination of further aggressive species, including the gigantic sensitive plant (*Mimosa invisa*), was 50% inhibited at  $255 \pm 3.5$  mM NaCl (Chauhan & Johnson, 2008). Similar to Kleinkopf (1976), who noted in the USA that *G. pubescens*, a species related to heliotrops, has a high capacity to germinate in salt pressure. These findings imply that *H. europaeum* seeds may sprout even at height soil salinity levels, and these types of soil are widespread in various regions of Iraq and elsewhere.

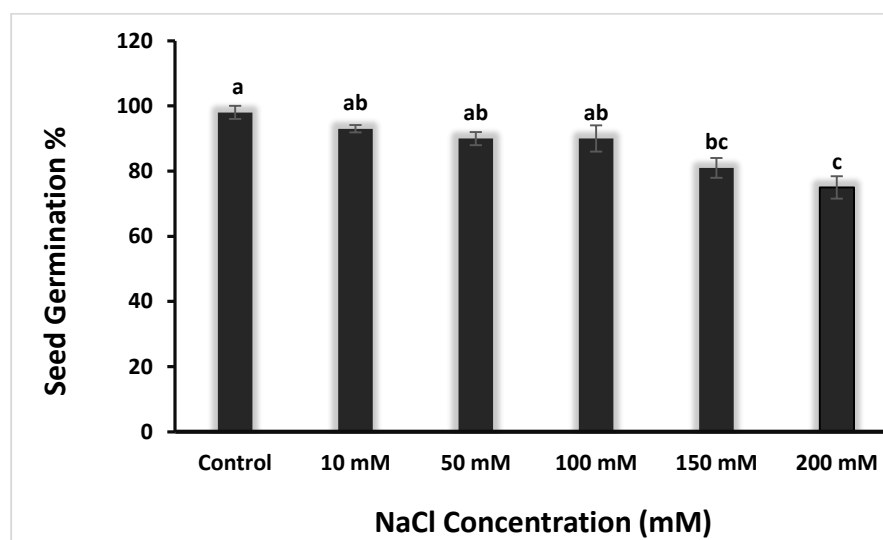
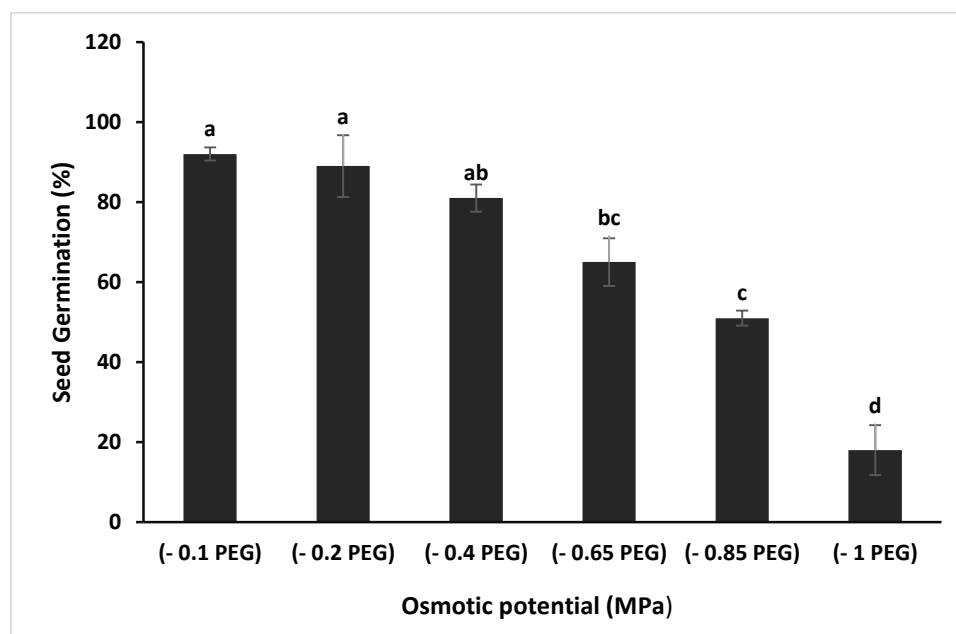


Figure 1: Effect of sodium chloride (NaCl) concentrations on germination of *Heliotropium europaeum* L seed. Erect bars signify  $\pm$  standard error of the mean Means with different letters within a column for each factor indicates significantly differences at  $p \leq 0.05$  of probability according to Tukey test.

### 3.3 Influence of water shortage on seed germination

Figure 2, shows the percentage of seed germination at different osmotic potentials. Germination of *H. europaeum* seeds were effected significantly with increasing water stress. It is clear that seed germination is very high at control treatment or no water stress, but the germination decreased gradually when seeds faced to higher water stress from (-0.4 PEG (81 %) to -1 PEG (18 % ) respectively. This results representative seeds of *H. europaeum* be able to germinate under borderline water pressure situations. A previous study reported that at -0.7 MPa, 50% of the feather lovegrass [*Eragrostis tenella* (L.) ex Roemer & J. A. Schultes] inhibited (Bajji *et al.*, 2002; Chauhan and Johnson2009). However, at an osmotic prospective of -0.6 MPa, the small-flowered mallow (*Malva parviflora* L.) exhibited no signs of germination (Chauhan *et al.*, 2006 b). Humphries *et al.* (2018) reported that this study's findings were similar in that they found that this weed germinated under mild osmotic stress, but that the untreated treatment had the maximum rates of germination (no osmotic stress). This study shows that *H. europaeum* prefers a wet environment and that drought is a significant influence preventive seed germination. The results of this investigation also showed that the species' germination decreased as osmotic stress increased.



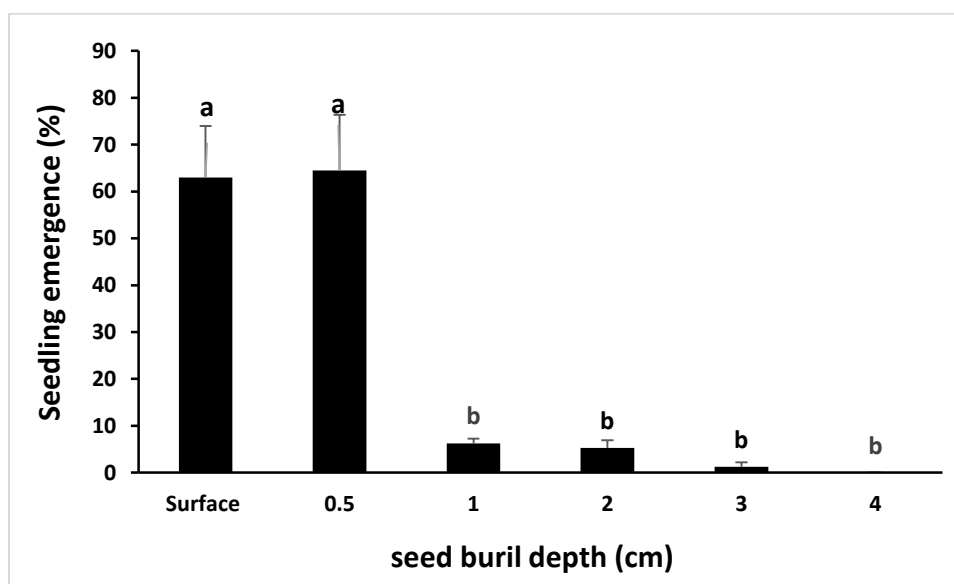
**Figure 2:** Effect of Osmotic potential (MPa) on germination of *Heliotropium europaeum* L. seed. Means with different letters within a column for each factor indicates significant differences at  $p \leq 0.05$  of probability according to Tukey test.

### 3.4 Influence of burial depths on seedling appearance

Appearance Seedlings of *H. europaeum* was highly significantly impacted by burying depth (as shown in figure: 3). One cm burial depth and surface treatments obtained the highest seedling emergences (64.5 % and 63%, respectively). Contrastingly, seedlings emerged of *H. europaeum* declined significantly with bigger burying planting seeds, seedlings emergence were reduced to (6.25 %, 5.25 %, and 1.25 %) when seeds placed in depth 1 cm, 2 cm, and 3 cm respectively. While in the 4 cm burial treatment, the emergence percentage had 0%.

Lower seedling emergence owing to increased depth of burial has been seen in numerous weeds, and advanced seedling appearance for seeds positioned on the soil surface is consistent with the beneficial effects of light on germination (Chauhan & Johnson, 2009; Tang et al., 2015). According to Woolley and Stoller (1978), light only reaches the top few millimeters of soil; seeds

buried farther down do not get light. Limited light penetration is thought to be the most likely explanation of the observed reduced emergence of buried seeds because of this species' need for light. However, some seeds (6 %) hidden at 1 cm, depth did appear (Figure 3), proposing that further reasons might be accountable for the absence of appearance from profoundly suppressed seeds. Gas circulation, which is contrariwise associated with burial depths, is one theory that might explain the phenomenon. The presence of CO<sub>2</sub> produced from soil biological activity could possibly be a factor (Benvenuti et al., 2001).



**Figure 3.** Effects of burial depth on seedling emergence of *Heliotropium europaeum* L. Vertical bars represent  $\pm$  standard error of the mean. Means with different letters within a column for each factor indicates significantly differences at  $p \leq 0.05$  of probability according to Tukey test.

## Conclusion

The outcomes of current research revealed clearly seeds of *H. europaeum* be able to germinate in numerous ecological surroundings. Seeds of *H. europaeum* had been capable to grow and develop in a wide range of temperature. While sprouting seeds of *H. europaeum* were not identically tolerating to salty and was sensitive to water stress. Light was observed to increase seed sprouting in *H. europaeum*, suggesting that buried seeds may likely to stay dormant up to disturbed. At a depth of 4 cm, seeds are unable to germinate, and seedling emergence is also impossible. In conclusion, present research has shown key flaws in the *H. europaeum* life cycle that land users might take advantage to deal a successfully control program to eradicate such an exotic species.

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