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EFFECT OF ENVIRONMENTAL FACTORS ON SEED GERMINATION AND EARLY SEEDLING EMERGENCE OF CAROLINA GERANIUM (*Geranium carolinianum*)

*Efeito de Fatores Ambientais sobre a Germinação de Sementes e Emergência Precoce de Plântulas de Carolina geranium (*Geranium carolinianum*)*

ABSTRACT - Carolina geranium (*Geranium carolinianum*) is an emerging invasive weed in rape and wheat fields in China. A better understanding of its germination and emergence ecology will enable the development of integrated weed control strategies. In this study, we investigated the effects of temperature, photoperiod, soil water content, salinity, and burial depth, on germination and emergence of Carolina geranium. Germination percentages were over 74% under 15/20 and 20/25 °C night/day temperature regimes. Germination rate was independent of light/dark regime. Increasing salinity reduced germination of Carolina geranium from 81.1% at 0 mM to 0% at 160 mM NaCl. Seeds germination was peaked at 50% soil moisture, but was completely inhibited at < 20% and > 90%. The seedling emergence above 82.2% was observed when seeds were placed at a depth from 0 to 1 cm, and no seedlings emerged from seeds placed at a depth of 7 cm. Current work provide the basic information to effectively prevent and control this invasive weed in Chinese rape and wheat fields.

Keywords: temperature, photoperiod, salinity stress, soil water content, burial depth.

RESUMO - Carolina geranium (*Geranium carolinianum*) é uma planta invasora emergente nos campos de colza e trigo na China. Portanto, uma melhor compreensão da ecologia da germinação e emergência da espécie permitirá o desenvolvimento de estratégias de controle integrado de plantas daninhas. Neste estudo, foram investigados os efeitos de temperatura, fotoperíodo, umidade do solo, salinidade e profundidade de enterramento na germinação e emergência de Carolina geranium. Os resultados mostraram taxas de germinação superiores a 74% sob regimes de temperatura de 15/20 °C e 20/25 °C noite/dia. Não houve diferença significativa na germinação das sementes entre a exposição à luz e ao escuro. O aumento da salinidade reduziu a germinação de Carolina geranium de 81,1% a 0 mM para 0% a 160 mM NaCl. A germinação de sementes foi favorecida com 50% de umidade do solo, tendo sido completamente inibida pelo teor de água no solo abaixo de 20% e superior a 90%. Observou-se emergência das plântulas acima de 82,2% quando as sementes foram colocadas a uma profundidade de 0 a 1 cm, e houve germinação das sementes colocadas a uma profundidade de 7 cm. Este trabalho mostra que a influência dos fatores ambientais sobre a germinação de sementes de Carolina geranium poderia fornecer a base para a prevenção e controle eficazes da ocorrência de plantas daninhas e da infestação em campos de colza e trigo na China.

Palavras-chave: temperatura, fotoperíodo, estresse salino, teor de água no solo, profundidade de enterramento.

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INTRODUCTION

Carolina geranium is native to eastern North America and usually grows in disturbed habitats, including roadsides, old fields, and waste lands (Gama-Arachchige et al., 2011). In the 1940s, Carolina geranium was discovered in eastern China and was documented as a naturalized weed (Peng, 1978). This species has distinctive mericarps and can produce more than 500 seeds per plant (Flora of China, 2008). As a winter weed, Carolina geranium seeds germinate in autumn, flower in the following April to July and ripen from May to September. Its life cycle coincides with the growing season of typical Chinese winter crops, including winter rape and winter wheat. Carolina geranium seeds are spread by wind and irrigation in the field, and the species has gradually become a major weed in rape and wheat fields in China. Currently, Carolina geranium has caused serious damage to rape and wheat production in several Chinese provinces (Wang et al., 1999; Song et al., 2006; Li et al., 2011; Chen et al., 2013). It is reported that the density of Carolina geranium has already reached above 1,500 plants m^{-2} in rape fields in the Jianghuai region (Song et al., 2006) and 500 plants m^{-2} in wheat fields in Jianhu county, in Jiangsu, causing significant yield losses (Wang et al., 1999). Therefore, it is crucial to effectively prevent and control this species.

To date, herbicide application is highly effective in controlling weed species in crop fields. However, effective herbicides to control Carolina geranium are limited. An important reason is that the whole plant of this species is covered with trichomes and there is a high degree of lignification, which can make the herbicides difficult to permeate into the tissues. Moreover, this species tends to develop herbicide resistance during chemical control in China. According to our survey, Carolina geranium has developed tolerance or resistance to multiple herbicides, including isoproturon, tribenuron-methyl and bensulfuron, and hence there are control failures. Thus, developing other integrated systems for control of this invasive weed is one of the most important strategies. Because Carolina geranium reproduces and spreads predominately through seed production, seed germination and seedling emergence are critical steps in its lifecycle. It has been reported that several environmental factors, including temperature, water content, climate, soil and photoperiod determine the time and length of germination and emergence processes in most weed species (Penny and Neal, 2003; Nakamura and Hossain, 2009; Boddy et al., 2012; Yin et al., 2013; Dekker and Gilbert, 2014; Wang et al., 2016). Therefore, a comprehensive understanding of the environmental factors governing Carolina geranium germination is crucial for sustainable management of this invasive weed.

Washitani (1985) found a positive correlation between germination percentage and temperature, with optimal temperature between 28 and 30 °C, suggesting that temperature is one of the major factors contributing to emergence in the field. Gama-Arachchige et al. (2010, 2012, 2013) reported that the matured seeds of Carolina geranium had a water-impermeable seed coat and a physiologically dormant embryo. Carolina geranium seeds are sensitive to dormancy break during summer, but they do not imbibe. When sensitive seeds are exposed to low temperatures (<20 °C) in autumn, the water gap on the seed coat opens, after which the seed imbibes and germinates. Subsequently, they determined the physical dormancy (PY) break processes involving two temperature-dependent steps. Based on the above-mentioned results, they developed a thermal-temporal model to predict the sensitivity induction in Carolina geranium seeds. As for seed dormancy in Carolina geranium, Baskin and Baskin (1974) also reported that embryos of freshly-matured seeds of Carolina geranium are conditionally dormant. They found that freshly-matured seeds have hard coats and will not water unless scarified. Without scarification, the seed coat becomes permeable unless seeds were kept either 4.5 months of dry-laboratory storage or alternately wet and dry at relatively high temperatures of 25, 30/15 and 35/20 °C (day/night) during the summer dormancy period. However, there has been relatively little other detailed research on the biology and ecology of Carolina geranium.

The purpose of this research was to investigate the effect of temperature, photoperiod, soil moisture and salinity, and burial depth on seed germination and emergence of Carolina geranium. A better understanding of environmental factors governing the germination and rapid spread of Carolina geranium will shed light on the sustainable management of this invasive weed.

MATERIALS AND METHODS

Seeds

Carolina geranium seeds were collected from rape fields in Yuanjiang (28.42°-29.40°N, 112.14°-112.63°E), Hunan province, P. R. China, from May to June 2013. Soils in this region are alluvial paddy soils, with a pH range of 6.5-7.5 and organic matter content of 29.2 (± 0.46) g kg⁻¹. Through our investigation, each plant was found to produce 470 \pm 35 seeds on average, and 1000 seed weight is 2 to 3 g. After air drying for 7d, the Carolina geranium seeds were stored in a seed storage tank (HZ/HZMs/HZDs-1600II, Beijing Biofuture Institute of Bioscience & Biotechnology Development) with 5% relative humidity (RH) and 24h darkness at 5 °C. All germination tests were carried out in a temperature and light-controlled growth chamber (PRX-450B, Ningbo Safe Experimental Instrument Factory) between October and November, 2014 and replicated twice. Prior to seed germination and emergence experiments an imbibition test was conducted to assess the level of PY, and a seed viability test was carried out to evaluate the capacity of a seed to germinate. The objectives of the two tests were to ensure that all experimental seeds can be permeable and produce normal seedlings under favorable conditions.

Seed imbibition test

The imbibition test was performed at 20 \pm 2 °C for 3d. Three replicates of 90 intact and ungerminated seeds were placed on moist filter paper in Petri dishes and watered daily with tap water. Imbibed seeds were determined by a visible change in their size/volume ratio.

Seed viability

Seed viability was determined by using the tetrazolium test (Brewer 1949; Da Silva et al., 2012; Cui et al., 2014). Firstly, three replicates of 90 seeds were soaked for 24 h at room temperature. Then, the seeds were cut in half through the embryo axis one-by-one by hand with a sharp razor blade. For each subsample, one half of each seed section was immersed in a 2,3,5-triphenyl tetrazolium chloride (TTC) (Sigma Chemical Co.) solution in a 50 mL centrifuge tube in the dark at 20 °C. Seed halves were examined every hour. Those seeds whose embryo and endosperm had been completely stained red were classified as viable. Seeds with spotty or light-colored staining were classified as inviable. Percentage viability was computed as the number staining positive as a proportion of the total number tested (Hu et al., 2009).

Petri dish-based germination bioassay

The temperature, photoperiod and salinity stress experiments were conducted by evenly placing 30 seeds in a 9 cm diameter Petri dish. A double layer of filter paper was placed at the bottom of each dish, which were moistened with 6.5 mL deionized water. Petri dishes were sealed with Parafilm to prevent water-loss, and deionized water was added to ensure saturation of the filter paper throughout the entire experiment. All dishes were placed in a growth chamber with a light intensity of 12,000 lx, and 80% RH. Germination was monitored every other day for 14d because 70% of the seeds germinated during the first half of the duration based on the preparatory experiments. Seed germination was determined by approximately 2 mm radical protrusion through the seed coat (Hosseini et al., 2002). Percentage of germination was recorded daily as the proportion of germinated seeds from the total number of seeds in a single Petri dish.

Temperature

To investigate the effect of temperature on germination, Carolina geranium seeds were placed in a growth chamber under a series of day/night alternating temperature setting, including 5/10, 10/15, 15/20, 20/25 and 25/30 °C night/day. These temperature regimes were designed with a basis on the mean air temperature record for previous 5 years in Yuanjiang during the

fall (TianQi, 2014). All the treatments were under a 12/12h light/dark photoperiod with light intensity of 12,000 lx, and 80% RH.

Photoperiod

To determine the effect of photoperiod on germination, Carolina geranium seeds were subjected to 24/0, 12/12 and 0/24 h light/dark regimes per 24 h cycle at a 12/12 h alternating temperature setting of 20/25 °C night/day. For the darkness treatment, the dishes were covered by double layers of aluminum foil to prevent light penetration. The investigation for the darkness treatment was conducted after moving the dishes from the faint light in the darkness condition.

Salinity stress

During our survey, we found that the density of Carolina geranium is lower in saline fields. Thus, we speculated that salinity stress may reduce germination. To test the effect of salinity stress on germination, Carolina geranium seeds were incubated in 0, 10, 20, 40, 80, and 160 mM of sodium chloride (NaCl) solutions under a 12/12 h light/dark photoperiod, 20/25 °C night/day alternating temperature setting, and 80% RH.

Pot-based emergence bioassay

The soil water content and burial depth experiments were conducted by evenly sowing 30 seeds in a plastic pot (13 x 8 x 5 cm) filled with 200 g soil. Soil was collected from rape fields in the Research and Teaching Base at Hunan Agricultural University, Hunan province, P. R. China. Soils in this region are alluvial, with the contents of soil organic matter of 0-20 cm of 10 mg kg⁻¹ and pH range of 4.5-6.5. Before use, all of the soil was screened through a 5 mm sieve. All seedling emergence experiments were carried out in a light-controlled growth chamber. After sowing, the appearance of cotyledons was considered as seedling emergence.

Soil water content

Thirty seeds were buried in a plastic pot and then covered with 1 cm soil. Soil water content ranged from 10 to 90%, with a 10% increment. Pots were placed in a separate growth chamber according to different soil moisture levels with a 12/12h light/dark photoperiod with light intensity of 12,000 lx, 20/25 °C night/day alternating temperature setting. Soil water content was determined by the ratio of water to soil dry weight, which was measured before and after drying at 105 °C for 24h and was adjusted daily by weighing the pots and then by adding deionized water onto the soil surface to make sure that the claimed soil moisture was maintained throughout the experiment. Emergence was monitored every other day for 21d.

Burial depth

Thirty seeds were sown in each pot and then covered with soil at a depth of 0 (soil surface), 1, 2, 3, 4, 5, 6 and 7 cm. The pots were placed under a 12/12h light/dark photoperiod, 20/25 °C night/day alternating temperature setting, and 80% RH. Soil water content was adjusted daily by adding tap water onto the soil surface. The number of germinated seeds was measured every other day during the 21d assay period.

Data analysis

All experiments were carried out in a completely randomized design with three replications, and each experiment was conducted twice independently. To assess the effects of temperature, photoperiod, soil water content, salinity, and burial depth on germination and emergence of Carolina geranium, the experimental data were subjected to one-way ANOVA to assess all main

effects with the data processing system software SPSS version 20.0 for Windows. Treatment differences were evaluated by using the least significant difference (LSD) test at the level of 0.05.

RESULTS AND DISCUSSION

Imbibition and viability of seeds

The results of the imbibition test showed that the seeds were permeable. All the seeds were imbibitional at 20 °C for 3d. The imbibed seeds were easily distinguished by visible changes in their size/volume ratio. The tetrazolium test showed that seed viability was very high with 97% cumulative percentage. The pattern of high seed viability at seed maturity indicates that primary Carolina geranium seed dormancy is likely to be absent. High summer temperatures may have induced secondary dormancy (not assessed), but it would be caused by environmental factors after dispersal (Bewley et al., 2012). These results also implied that the seeds could break PY and became sensitive during the storage period and had good imbibition and excellent capacity to germinate. By the time we initiated the germination tests, all experimental seeds had been stored for 16 months in a seed storage tank under 5% RH, and 24h darkness at 5 °C. Such long storage can make seeds permeable. This is in agreement with a previous report of Baskin and Baskin (1974) that the nonscarified seeds of Carolina geranium became germinable after 4.5 months of dry-laboratory storage and had high percentages of germination in darkness and light at 25 °C.

Effect of temperature on Carolina geranium seed germination

When subjected to an array of alternating temperature settings, germination percentage increased from 57.8 to 82.2% as the night/day temperature raised from 5/10 to 20/25 °C. At the 15/20 °C and 20/25 °C temperature regimes, the germination percentages were significantly higher, although germination almost ceased at 25/30 °C (Table 1). The most suitable temperature for Carolina geranium germination was 20/25 °C (night/day), which corresponds to average daily minimum and maximum temperatures of 20/27 °C in September, based on the recent 5 year-record in Yuanjiang, Hunan province (TianQi, 2014). This most suitable temperature may explain the abundance of Carolina geranium in this region because seeds can mainly germinate from mid-September to mid-October after the overwintering rosette is formed (Baskin and Baskin, 1974). Moreover, Washitani (1985) found an optimal temperature of 28 to 30 °C for Carolina geranium germination.

Table 1 - Influence of temperature on seed germination of Carolina geranium seeds incubated in a 12 h photoperiod for 14 d

Night/day temperature (°C)	Germination ercentage (%) ⁽¹⁾
5/10	57.8 ± 1.1 c 6.9 ± 1.6 a
10/15	65.6 ± 2.9 bc 6.8 ± 1.3 a
15/20	74.4 ± 1.1 ab 7.0 ± 1.3 a
20/25	82.2 ± 2.9 a 6.0 ± 1.1 b
25/30	10.0 ± 1.9 d

⁽¹⁾ Values are mean ± standard error (SE) of three replications. Means within a column followed by the same letters are not significantly different between treatments at the 5% level according to Fisher's protected LSD test.

Effect of photoperiod on Carolina geranium seed germination

Light is an important factor, responsible for germination of many seeds (Jarvis and Moore, 2008; Yin et al., 2013). However, it had no apparent impact on Carolina geranium germination (Table 2). Throughout all the light/dark regimes, germination percentage ranged from 70 to

Table 2 - Effect of light on germination of Carolina geranium seeds incubated at alternate temperature day/night 25/20 °C for 14 d

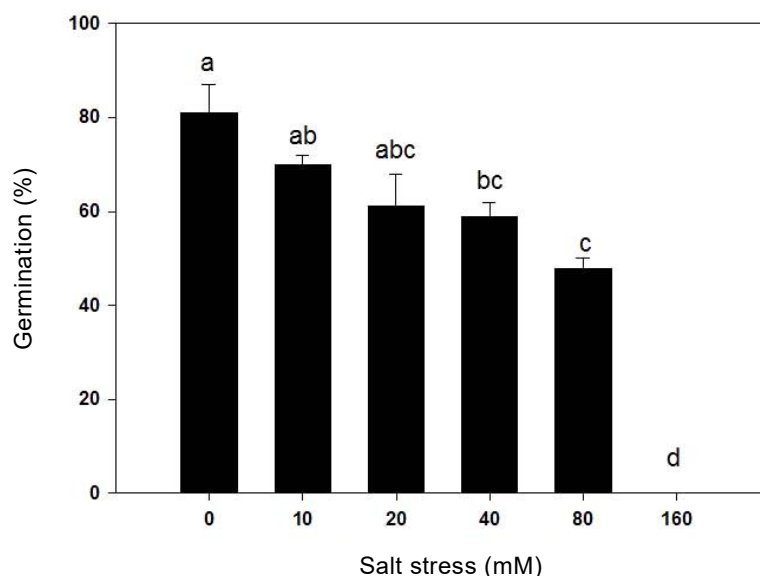
Light/darkness(h)	Germination percentage (%) ⁽¹⁾
24/0	75.6 ± 2.2 a
12/12	70.0 ± 19.0 a 6.8 ± 1.3 a
0/24	76.7 ± 1.9 a

⁽¹⁾ Values are mean ± standard error (SE) of three replications. Means within a column followed by the same letters are not significantly different between treatments at the 5% level according to Fisher's protected LSD test.

77% at 20/25 °C night/day. There were no significant differences between treatments, which was consistent with the results determined by Baskin and Baskin (1974) for germination of Carolina geranium seeds. Also, no difference was found in the germination of Stinkwort (*Dittrichia graveolens*) in the presence or absence of light (Brownsey et al., 2013). On the other hand, light did not affect the germination of Carolina geranium seeds, suggesting that the hard seed coats have no physical role in cutting off light transmission with seed germination.

Effect of salinity on Carolina geranium seed germination

Contrary to the effect of temperature, germination percentage was negatively influenced by salinity in Carolina geranium seeds (Figure 1). The control group had significantly higher germination percentages, followed by the lowest NaCl concentration (10 mM). The percentage was reduced to 47.8% when NaCl concentration reached 80 mM, and germination was completely ceased at 160 mM. This is consistent with previous reports on the impact of salinity (Zia and Khan, 2004; Kaydan and Yagmur, 2008; Luque et al., 2013). High osmotic pressure and toxicity of Na⁺ can potentially cause negative effects on seed germination (Akbarimoghaddam et al., 2011; Wang et al., 2016). Salinity, sometimes, can control weed because excessive accumulation of sodium in soil prevents seeds from germination.



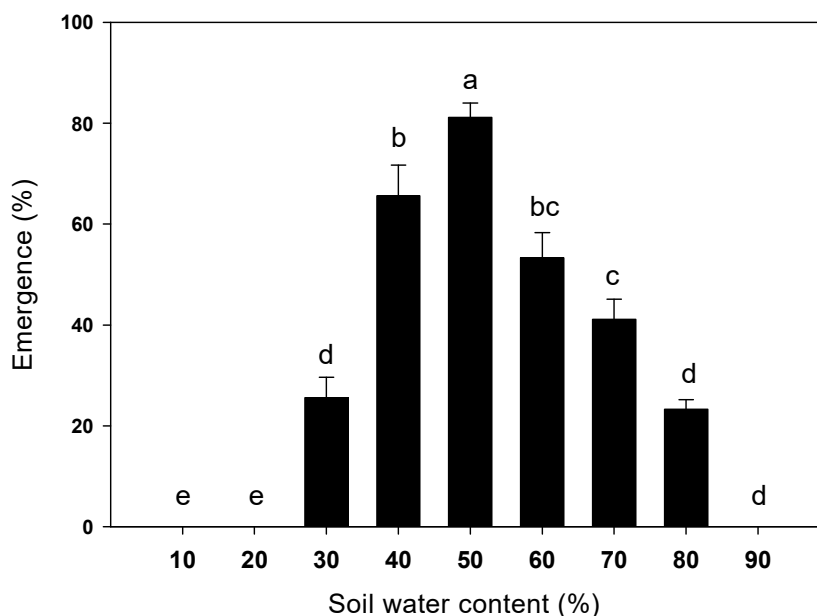
The same letters at the top of the columns indicate that values are not significantly different at the 5% level according to Fisher's protected LSD test.

Figure 1 - Effect of salt stress on germination of Carolina geranium seeds incubated at alternate temperature day/night 25/20 °C in a 12 h photoperiod for 14 d.

Effect of soil water content on Carolina geranium seedling emergence

The cumulative seedling emergence percentage increased from 25.6 to 81.1% when soil water content increased from 30 to 50%; thereafter, emergence percentage started to decrease

as soil water content increased. Germination ceased when soil water content was <20% or >90% (Figure 2). At the 50% soil water content level, emergence percentage was significantly higher. According to our survey, in Yuanjiang, Hunan province, P.R. China, local farmers rotate wetland crop rice with dryland crop rape in this region, and the intermediate soil moisture was maintained in rape fields. This explains the abundance of Carolina geranium in this region. It is at this point that water management may be a feasible strategy as an agricultural management practice. Three to 5 cm deep water for 7 to 10 days could reduce germination of this species before the cultivation of crops because high soil moisture (>80%) prevents seedling emergence. In a similar study in paddy fields, it was found that, after transplanting, a 10-20 cm deep-water layer maintenance could reduce infestation of barnyardgrass (*Echinochloa crusgalli*) (Zhang, 2003). Additionally, altering the choice of crops and rotational practices may reduce germination and seedling emergence of Carolina geranium because when the soil is too wet or too dry, it provides an unsuitable environment for germination (i.e. rotation of dryland crop rape with dryland crop wheat in the following year). Therefore, soil water content adjustment integrated with rotation management will greatly limit the occurrence and infestation of Carolina geranium.

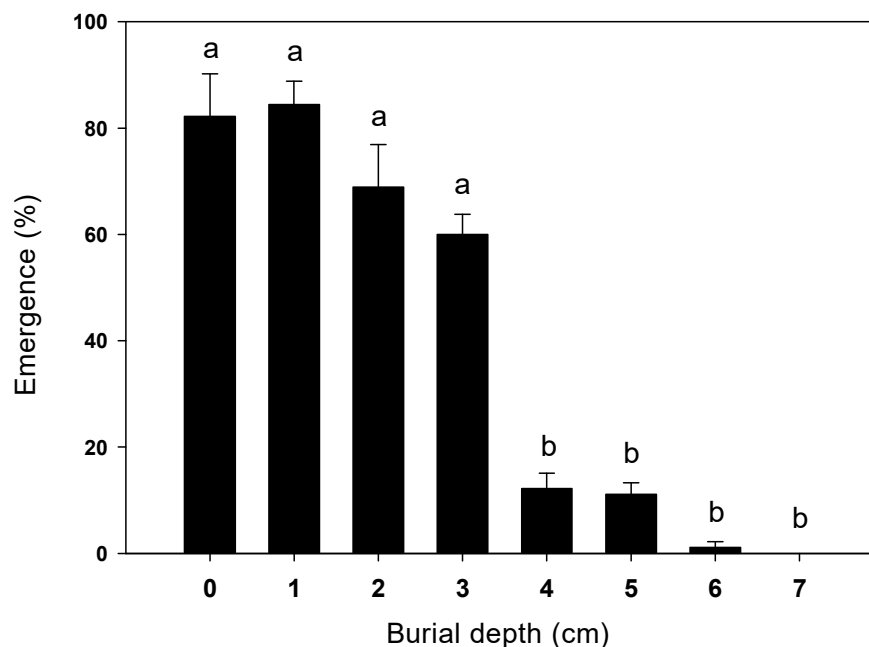


The same letters at the top of the columns indicate that values are not significantly different at the 5% level according to Fisher's protected LSD test.

Figure 2 - Effect of soil water content on seedling emergence of Carolina geranium seeds incubated at alternate temperature night/day 20/25 °C in a 12 h photoperiod for 21 d.

Effect of burial depth on Carolina geranium seedling emergence

Burial depth significantly affected Carolina geranium germination (Figure 3). The highest emergence percentage was found at a burial depth of 0 to 1 cm; thereafter, emergence decreased as the burial depth increased. Emergence percentage decreased significantly with an increase in burial depth of 4 cm. There was no emergence of the seeds placed at a depth of 7 cm. Similar results were reported for seedbox (*Ludwigia hyssopifolia*) (Chauhan and Johnson, 2009), hairy nightshade (*Solanum sarrachoides*) (Zhou et al., 2005), and Asia minor bluegrass (*Polypogon fugax*) (Wang et al., 2016). Shallow soil depths facilitate germination and emergence of seeds. This factor might lead to high density of Carolina geranium in rape fields with no-tillage or minimum tillage cultivation, which make seeds remain typically near the soil surface (Huang, 2007). In this sense, intermittent tillage may play the key role in agricultural practice by reducing seed germination of *Carolina geranium*. It is known that intermittent tillage is based on no-tillage or minimum tillage integrated with deep ploughing over a period of several years. This tillage effectively avoids the disadvantage of continuous no-tillage and deep ploughing every year; as a



The same letters at the top of the columns indicate that values are not significantly different at the 5% level according to Fisher's protected LSD test.

Figure 3 - Effect of burial depth on seedling emergence of Carolina geranium seeds incubated at alternate temperature night/day 20/25 °C in a 12 h photoperiod for 21d.

result, seeds remain on the soil surface and the buried seeds are brought to soil surface, respectively. For example, Zhang (2003) reported that winter plowing to a depth of 20 cm reduced distinct pondweed by approximately 60% in comparison with a control in a paddy field trial. In a cotton field trial, Zhang (2003) reported that when winter plowing was performed to a depth of 20-25 cm, density of purple nutsedge plants was reduced by 85.9% compared with unplowed plots. Thus, seed burial by tillage is an important strategy for management of Carolina geranium.

In summary, germination and emergence of Carolina geranium are influenced by temperature, salinity, soil moisture, and seed burial depth. This species germinated within the alternating temperature of 5/10 to 25/30 °C (night/day), with an optimal temperature of 20/25 °C. The seeds were able to germinate independent of lights. The germination percentages were negatively influenced by salinity and decreased as NaCl concentration increased from 0 to 160 mM. Seedling emergence was able to occur over a wide range of soil water content (30-80%), with a peak at 50%. The seedlings were higher at a burial depth of 0-1 cm with germination percentage over 80%. In comparison, seeds placed below a 6-7 cm soil depth, the germination percentage was below 1%. Given the results of our study and the limited herbicides available, we can reduce this species from germination and emergence by modulating environmental factors. Seed burial by tillage and soil water content by flooding or rotation can be a part of the integrated managing system to provide sustainable control of Carolina geranium.

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