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Article

Seed Longevity of Spotted Spurge and Wild Poinsettia Under Diverse Environmental Conditions

*Longevidade de Sementes de **Euphorbia** manchada e Leiteiro sob Diversas Condições Ambientais*

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ABSTRACT - Seed longevity under different environmental conditions is considered as one of the most important factors in the spread and persistence of an exotic species. The Experiments were conducted to determine seed persistence in soil, effects of submergence in water, flooding of the soil, and high temperatures on germination and viability of spotted spurge (*Chamaesyce maculata*) and wild poinsettia (*Euphorbia heterophylla*) as two exotic species in different regions of Golestan province. Spotted spurge seeds buried at depth of 10 cm maintained their viability above 95% after a year, while wild poinsettia seeds were destroyed completely after exhuming the soil. Seeds of both species were unable to germinate under submergence, but 92% of the spotted spurge seeds remained viable under this condition for 14 d. No germination was observed after 9 weeks submersion. Submersion duration drastically affected seed germination of wild poinsettia, so that no germination occurred after 6 d submersion. Twelve days after flooding, spotted spurge emergence decreased by 57% compared to the control. Ten percentage of wild poinsettia seedlings emerged when flooding was kept up to 12 d after sowing, while control had 96% emergence. Germination of spotted spurge seeds subjected to 140 ^{o}C for 5 min was 5%. Viability of wild poinsettia seed was completely lost at 120 and 140 C for 5 min. These results suggest that spotted spurge is capable of forming persistent seedbank. Seeds of spotted spurge were partially tolerant to submersion in water, but wild poinsettia seed are susceptible to submergence. The burning of crop residue could also prevent augmenting the soil seed bank of both species.

Keywords: *Chamaesyce maculata*, *Euphorbia heterophylla*, fire, flooding, seedbank, seed persistence.

*RESUMO - A longevidade de sementes sob diferentes condições ambientais é considerada um dos fatores mais importantes na propagação e persistência de espécies exóticas. Os experimentos foram conduzidos de forma a determinar a persistência das sementes no solo e os efeitos de submersão em água, inundação de solo e altas temperaturas na germinação e viabilidade de **Euphorbia** manchada (**Chamaesyce maculata**) e leiteiro (**Euphorbia heterophylla**) como duas espécies exóticas em diferentes regiões da província de Golestan. As sementes de Euphorbia manchada colocadas a uma profundidade de 10 cm mantiveram a sua viabilidade acima de 95% depois de um ano, enquanto as de leiteiro ficaram completamente destruídas depois da exumação do solo. Sementes de ambas as espécies foram incapazes de germinar após submersão, mas 92% das sementes de **Euphorbia** manchada mantiveram-se viáveis sob essa condição durante 14 dias. Não se observou germinação após nove semanas de submersão. A duração da submersão afetou drasticamente a germinação das sementes de leiteiro, uma vez que após seis*

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dias de submersão não ocorreu germinação. Doze dias após a inundação, a emergência de ***Euphorbia*** *manchada decresceu 57%, comparada com a do controle. Dez por cento das sementes de leiteiro emergiram quando a submersão foi mantida até 12 dias, enquanto no controle 96% germinaram. A germinação de sementes de Euphorbia manchada sujeitas a 140 °C durante cinco minutos foi de 5%. A viabilidade de sementes de leiteiro foi completamente perdida a 120 °C e 140 °C durante cinco minutos. Esses resultados sugerem que Euphorbia manchada é capaz de formar banco de sementes persistente. Sementes desta espécie foram parcialmente tolerantes à submersão em água, mas as de leiteiro mostraram-se suscetíveis a essa submersão. A queima de resíduos agrícolas também pode ajudar a prevenir o aumento do banco de sementes de ambas as espécies.*

Palavras-chave: *Chamaesyce maculata*, *Euphorbia heterophylla*, fogo, alagamento, banco de sementes, persistência de sementes.

INTRODUCTION

The seeds stored in the soil provide a major source of new weed infestations in coming years (Davis et al., 2005). The seed bank of exotic plant species may be considered as a source of propagules that enhance the probability of its establishment and persistence at a site (Gioria et al., 2012), especially during the introduction phase and for self-incompatible founder populations (Elam et al., 2007). The seed bank also allow to buffer the environmental changes that may occur over time (Gioria et al., 2012).

Dispersal of propagules is a key event in the invasion of alien plants into new geographical regions and in spreading alien species throughout the new region after initial introduction (Kowarik and Säumel, 2008). As well as, dispersal of sexual propagules may determine the level of gene flow within and among populations, thus affecting adaptation (Eminniyaz et al., 2013). Deposition of weed seeds in irrigation canals and rivers is one of the important and very rapid ways of dispersing seeds of some invasive plants (Thebaud and Debussche, 1991).

Beyond fecundity and dispersal capacities, the fate of seeds after reaching a new site is important in determining the outcome of invasion (Moravcova et al., 2006). The resilience of durable seed banks is a major difficulty to effective and sustainable management of invasive plants in many parts of the world (Hossain and Begum, 2015; Milakovic and Karrer, 2016). Therefore, measures that only control plants are insufficient to drive populations below tolerable density thresholds (Richardson and Kluge, 2008). An important factor in controlling invasive plant infestations is frequently the enhancement of the deterioration of their durable seed bank to reduce seedling re-establishment (Marques et al., 2014). The three most important loss factors affecting the seeds in the upper seed bank are fire-related mortality, germination and decomposition (Richardson and Kluge, 2008). Prescribed burning has primarily been used as a way for the control of invasive late-season annual broadleaf and grass species (Ditomaso et al., 2006). Fire is the best available control option for killing seeds in the leaf litter. The fire application is also more appropriate to habitats where the target seed bank is shallow (Richardson and Kluge, 2008). In addition to the temperature, the soil moisture and oxygen levels can affect the germination and decomposition of seeds in the soil as mentioned loss factors. High soil moisture resulting from flooding may decrease soil oxygen concentration or inhibit gaseous mobility within the soil (Boyd and van Acker, 2003); hence, it influences on weed seed viability (Begum et al., 2006; Dorji et al., 2013). At low oxygen concentrations and poor gas diffusion, anaerobic metabolites are built up around the seed and may inhibit seed germination (Benvenuti and Macchia, 1995). Overall, harnessing these factors to influence germination can help to improve weed management (Chauhan and Johnson, 2010).

Spotted Spurge (*Chamaesyce maculata* (L.) Small) and wild poinsettia (*Euphorbia heterophylla* L.) are two summer annual, exotic species of Golestan province, Iran. The species are annual ruderal weeds with a life cycle from spring to autumn, and both species produce prolific seed. They have a primary explosive dispersal system (autochory) (Frenedozo, 2004; Ohnishi and Suzuki, 2008). As it can be seen, they colonize spring crops, especially in soybean fields in Golestan province.

Since crop residue is burned after harvesting in some fields in Golestan province, and fields may also be periodically saturated with water due to rice culture in some part of this province, we attempted to investigate effects of soil flooding and high temperature pretreatment on viability of seeds. Furthermore, seed persistence in the soil was studied according to the crucial role of seed banks in invasiveness as well as the need to manage seeds to achieve sustainable control. As knowing the potential fate of seeds that drop in the irrigation canal is important for the study of potential of species distribution in the new region, seed longevity of spotted spurge and wild poinsettia in the water was also investigated.

MATERIALS AND METHODS

Seed collection

Spotted spurge seeds were harvested in the summer (August to September) of 2011 from plants growing in soybean fields in Kordkuy (36°79'N, 54°10'E) of Golestan province, Iran, and wild poinsettia seeds were collected from Marzan-Kalate (36°52'N, 54°35'E) region in Golestan province on September 2011. Spotted spurge and wild poinsettia seeds stored in paper bags and kept at 4 °C and room temperature (24±2 °C), respectively, based on our findings on the best conditions for maintaining their viability until used in experiments.

Seed persistence in soil

One hundred seeds of each species were wrapped separately in fine mesh polyester cloth bags (15 by 15 cm) and buried at depth of 10 cm in 20 cm diameter plastic pots with drainage holes which were filled with relatively equal amounts of loam soil and sand with 1.7% soil organic matter and pH 7.5. The pots were placed in outdoor on March 10, 2012 for one year. Seed samples were exhumed on the 10th day of each month until March 2013. Recovered seeds were placed on moistened filter paper in petri dishes and placed in a seed germinator for 14 d (14 h light at 35 °C). Then, percentage germination was determined by counting the number of seeds with 2 mm radicle during the 14 d period.

Influence of submergence on seed survival

For this experiment, 2000 seeds of each species were placed in two beakers and covered with tap water to a depth of 10 cm. Water in each beaker was replaced daily. One hundred seeds of spotted spurge were removed from the water every week until no germination was observed (Asgarpour et al., 2015). As preliminary experiment indicated that seeds of wild poinsettia lost completely their viability after 7 days, the withdrawal of the seeds of this species from water was daily. After treatment, 4 replicates of 25 seeds each were incubated in Petri dishes on a sheet of filter paper moistened with distilled water at temperature regime of 35 °C with 14 h photoperiod. Non-germinated seeds were tested for viability with a 1% tetrazolium chloride solution.

Effect of flooding on seedling emergence

Twenty-five seeds of spotted spurge and wild poinsettia were separately planted 0.5 and 1 cm deep in 10 cm diam pots. The pots were placed in buckets containing distilled water, so that the water was 5 cm above the soil. Pots were kept in the greenhouse at 32/23 °C day/night with a photoperiod of about 14 h using natural sunlight. Pots were removed from the buckets after 2, 4, 6, 8, and 12 d of flooding, and drained. Nonflooded seeds were considered as the control. Seedling emergence was recorded for 30 d.

Effect of high temperature pretreatment on seed germination

A study was performed to determine the effect of exposure time and high temperatures, as seeds might experience during the burning of crop residue or soil heating treatments, and seed moisture content on seed germination of spotted spurge and wild poinsettia. To that end, seeds were placed in petri dishes containing some distilled water for about 24 h, so that the seeds absorb water, but not submerged. Dry and soaked seeds were placed in an oven in open container at 80, 100, 120, and 140 °C temperatures for 1 and 5 min. The treated seeds were subsequently incubated at 35 °C in a 14 h photoperiod for 14 d. In the control treatment, seeds were kept at room temperature (24±2 °C) before being incubated.

At the end of all experiments (except for flooding treatment), viability of nongerminated seeds was tested using a 1% tetrazolium solution.

Statistical analyses

Normal distribution of data (Kolmogorov–Smirnov test) was tested before applying analysis of variance (ANOVA). The square root transformations were made when necessary to reach normality variance needed for the analyses.

Data of seed persistence in soil and results of seed viability for submerge treatment were analyzed using completely randomized design. The high temperature pretreatment data were analyzed as a completely randomized factorial design with three factors (temperature, exposure time, and soaking seeds in water for 24 h (imbibition)).

All experiments (except persistence in soil) were repeated twice. The experimental runs were not statistically significant; therefore, the data from the two runs were pooled before further analysis. All data were analyzed using PROC GLM in SAS (9.1), and means were compared using Fisher's Protected LSD test at the 0.05 level of significance. SigmaPlot software (v. 11.0) was used to plot figures.

RESULTS AND DISCUSSION

Seed persistence in the soil

Spotted spurge seed did not germinate in the bags during burial at depth of 10 cm and maintained their viability for 12 months, so that the percent germination of exhumed seeds in the Petri dishes was above 95% for all 12 moths (data not shown).

After exhuming the wild poinsettia seeds, it was observed that they were destroyed completely during one month.

Effect of submersion on germination (longevity in water)

Spotted spurge seeds had no germination during immersion in water, but most of the seeds remained viable under this condition for 2 weeks, as 92% of seeds germinated after their removal from water, and it did not have significant difference with control. Afterwards, germination had a decreasing trend, so that germination after 3 and 4 weeks submerging was 63 and 34%, respectively. After 9 weeks submersion, no germination was observed. Submersion of seeds for ≥ 3 weeks led to significant increase in the number of dead seeds at end of the test. Germination data of spotted spurge were fitted to a three-parameter sigmoid curve. Time required for 50% inhibition of the maximum germination, estimated from the fitted model, was 3.5 weeks (Figure 1A).

Wild poinsettia seeds were also unable to germinate under submergence. Submersion duration drastically affected seed germination. Loss of seed germination followed an inverse linear trend (Figure 1B). Germination percentage of the control and one day submersion in water was 81 and 66.3%, respectively, and no germination occurred after 6 days submersion or more (Figure 1B). The tetrazolium viability test showed that non-germinated seeds were not viable.

Effect of flooding on emergence

Seedling emergence of both species was influenced by flooding. After soil drainage, some seedlings started to emerge. The emergence of spotted spurge increased significantly after 4 d

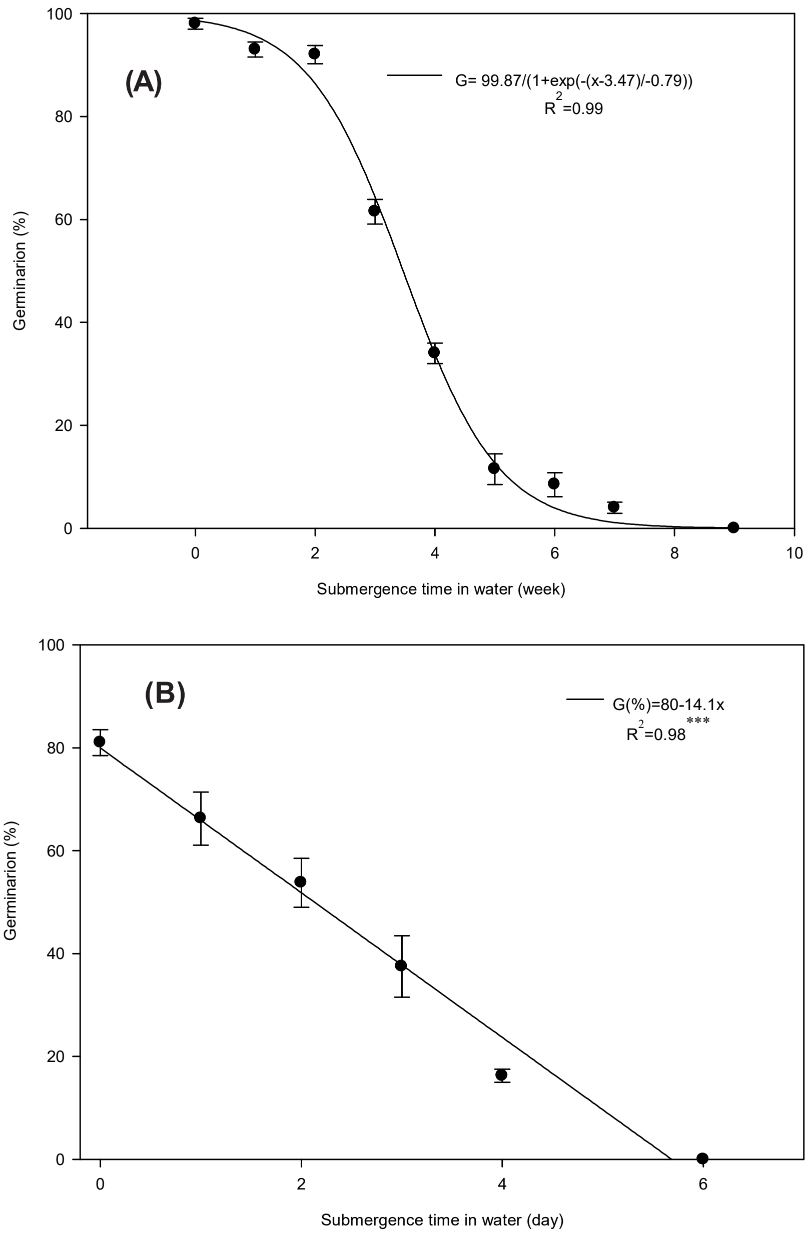

Figure 1 **-** Effect of submergence in water on germination of spotted spurge (A) and wild poinsettia (B).

flooding (42%) when compared with unflooded conditions (21%). Treatments of flooding for 6 and 8 days had nearly equal germination compared to the control. Emergence percentage declined to 9% after 12 d flooding, which had a significant difference with the control (Figure 2A).

Seedling emergence of wild poinsettia decreased sharply with an increase in duration of flooding. In comparison to control seeds, which had 96% emergence, 10% seedlings emerged when flooding was maintained up to 12 d after sowing. According to fitted three-parameter logistic model between flooding duration and seedling emergence, the time for emergence to decrease to 50% was estimated 4.3 d (Figure 2B).

Effect of pre-treatment high temperature on germination

Temperature, time, and the temperature × time interaction had significant effects on spotted spurge germination ($p<0.01$). Seed germination of wild poinsettia was significantly influenced ($p<0.01$) by the main effect of factors (High temperature, time exposure and soaking seed) and their interaction (except moist × time interaction).

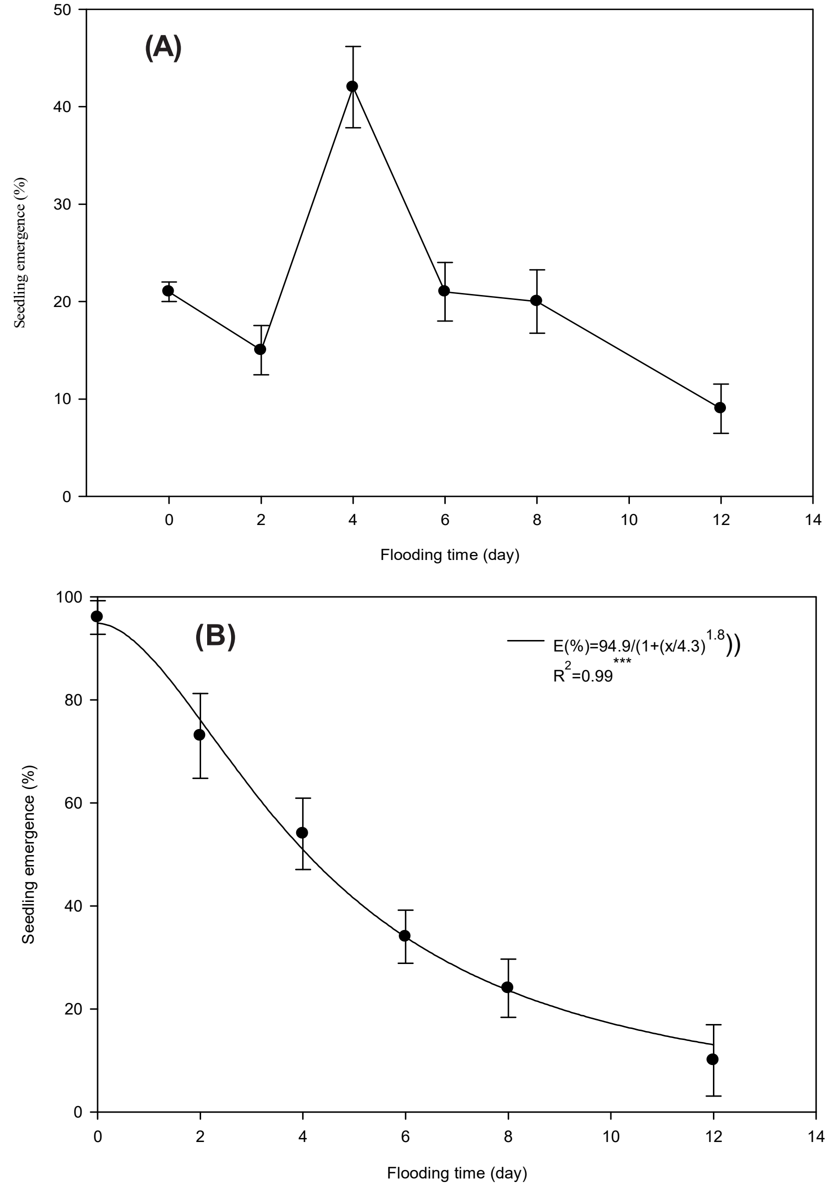

Figure 2 - Effect of flooding the soil on emergence of spotted spurge (A) and wild poinsettia (B).

Three-parameter sigmoid models described well the relationship between high temperatures and the germination percentage (Figure 3). Increase of temperature up to 100 °C did not have significant negative effect on spotted spurge germination, but thereafter, it was observed a decreasing trend with increasing temperature. The germination percentages of the wild poinsettia seeds following exposure to the different pre-treatments of high temperatures were statistically different to the control (Figure 3). Estimates from the model predicted exposure to 139 °C reduced germination of the spotted spurge to 50% of the maximum, while 95 °C had the same effect on wild poinsettia.

Seeds of spotted spurge and wild poinsettia were found to be highly susceptible to exposure time at temperatures above 120 and 100 °C, respectively (averaged on soaking seed treatment). The longer exposure with increasing temperature decreased germination percentages of both species and this was much more obvious in the case of wild poinsettia. Germination of spotted spurge seeds subjected to 140 °C for 1 and 5 min was 82 and 5%, respectively. Germination in the rest of the treatments was above 80%. Viability of wild poinsettia seed was completely lost at 120 and 140 °C for 5 min. Wild poinsettia seeds that were preheated at 80 °C for 1 and 5 min and 100 °C for 1 min germinated ≥ 38%, but it was significantly less than in the control (Table 1).

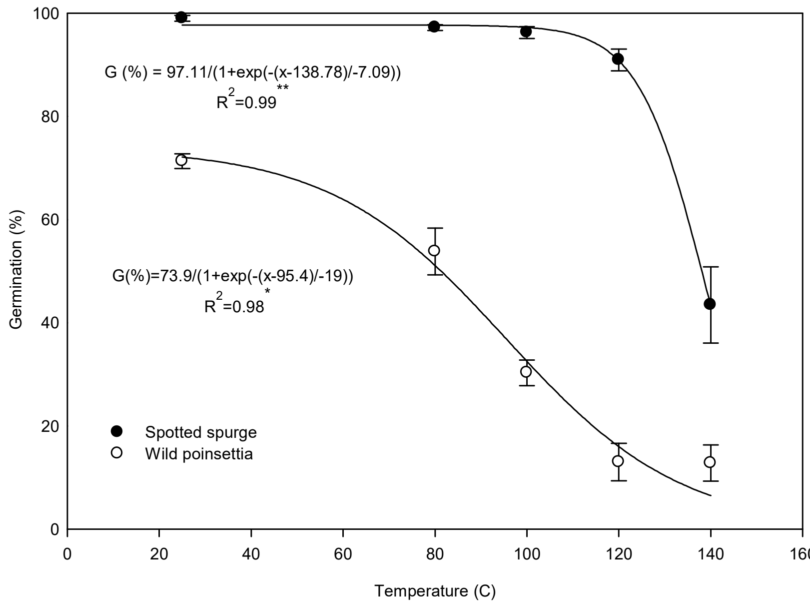

Figure 3 **-** Effect of pre-treatment of high temperature on germination of spotted spurge and wild poinsettia.

Table 1 **-** Effects of intensity and duration of high temperatures on germination of spotted spurge and wild poinsettia

Temperature (°C)	Spotted spurge		Wild poinsettia	
	time (min)		time (min)	
	1	5	1	5
25 (control)	98.5 a	98.5 a	71.3 a	71.3 a
80	97.5 a	97.5 a	54.0 b	53.5 b
100	96.3 a	93.4 a	38.5 c	22.0 d
120	98.8 a	83.1 b	26.0 d	0.0 e
140	84.4 b	4.4 c	25.5 d	0.0 e

For all test conditions, means with different letters for each species differ significantly ($p<0.01$).

There was no significant difference between germination percentage of dry and soaked seeds of spotted spurge at each temperature level. Unlike heating dry seeds at 80 °C that did not have any effect on seed viability of wild poinsettia, preheating soaked seeds at the same temperature reduced 33.8% in germination compared to the control (Table 2). Therefore, wild poinsettia seeds at lower temperatures that have absorbed water, experience greater thermal damage.

Spotted spurge produce abundant seeds which maintain their viability in the soil indicating this weed is capable of forming persistent seedbank and acting as the primary source of weed infestation in subsequent years that will guarantee the continuity of its populations (Hossain and Begum, 2015). Therefore, long-term management system is necessary for depleting the soil seedbank and controlling this invasive weed. The rapid loss of wild poinsettia seeds in the soil may be due to hypoxia conditions caused by rain and snow falling during one month after burial of seed in the soil. This was consistent with the results of our flooding experiment. According to Bannon et al. (1978), seeds of wild poinsettia rapidly lost their viability when seed stored at 10.8% moisture at 25 °C or 18.6% moisture at 5 or 25 °C.

Seed persistence is mainly a species-specific trait. Nevertheless, some evidence suggests that the soil and climate may affect the longevity of seeds (Fenner and Thompson, 2005). Two primary determinant environmental factors of seed longevity are temperature and moisture content (Davis et al., 2005). These variables directly influence the activity and efficiency of metabolic enzymes and repair antioxidants, thus affecting seed health and ability to persist (Walters et al., 2005). Also, the first critical step in the formation of a persistent soil seed bank is burial. Burying seeds, especially small seeds, reduces the germination or predation potential (Fenner and Thompson, 2005), and it has been shown that seeds are more likely to survive at

Table 2 - Effects of high temperatures and soaking seed on germination of spotted spurge and wild poinsettia

Temperature (°C)	Spotted spurge		Wild poinsettia	
	Dry seed	Soaked seed (24 h)	Dry seed	Soaked seed (24 h)
25 (control)	98.5 a	98.5 a	71.3 a	71.3 a
80	97.7 a	96.8 a	70.0 a	37.5 b
100	95.6 a	94.1 ab	33.5 bc	27.0 c
120	93.8 a	88.1 b	16.5 d	9.5 d
140	42.5 c	46.3 c	13.5 d	12 d

For all test conditions, Means with common letters for each species are not significantly different ($p<0.01$).

deeper soil layers (Mennan and Zandstra, 2006) as we can observe in our experiment on spotted spurge seeds which survive at deep burial (10 cm).

The results of present study suggest that longevity of spotted spurge seeds in water were much more than seed survival of wild poinsettia. A part of spotted spurge seeds germinated after 7 weeks submergence, while viability of wild poinsettia seeds lost less than a week. Lipids, around 60% of seed dry mass, are the main reserve composition stored in the endosperm of wild poinsettia (Suda and Gioggini, 2000). Lipid respiration consumes more oxygen than carbohydrate catabolism, and in seeds that are flooded, metabolism will be subjected to a greater hindrance from a lack of oxygen with a consequent loss in viability (Crawford, 2003). Therefore, oil-rich seeds of wild poinsettia are very susceptible to oxygen deprivation during the early stages of germination and it is not surprising that seeds were destroyed rapidly in anaerobic conditions. Therefore, spread possibility of spotted spurge seeds by water flow is more than wild poinsettia seeds. We can also conclude that water in the irrigation canals is one of dispersal agents for seeds of this invasive species and that the best way to control its spread is to prevent plants growing beside the canals from setting seed. However, longevity of spotted spurge seed in water was much shorter than that of Creeping bentgrass (*Agrostis stolonifera*), as its seed retained their viability for 17 weeks in water (Zapiola and Mallory-Smith, 2010).

The results obtained in our study indicated that some seeds of spotted spurge can tolerate short periods of flooding (about 12 days), while wild poinsettia seeds are susceptible to flooding conditions. Thus, this species is not capable to be established at flooded or low drained soils. According to viability of spotted spurge seed during several weeks in submersion in water, lack of emergence of some seeds at soil after flooding conditions may be due induction of secondary dormancy, and/or light requirement.

In flooded or saturated soil, the low oxygen exchange associated with respiration of aerobic organisms leads to hypoxia or anoxia (Bailey-Serres and Voesenek, 2008). Flood conditions also alter soil chemistry, such as increased concentrations of Ammonium and polyphenolic levels and decreased soil inorganic nitrogen (Unger et al., 2010). Floods cause deep changes in soil nitrate concentrations. Since seeds of some species require nitrates to germinate, floods can change germination rate of species (Mollard and Insausti, 2009). These conditions inhibit seed germination or may induce secondary dormancy and light, nitrate, and/or alternating temperature requirements for germination (Benvenuti and Macchia, 1995; Boyd and van Acker, 2003; Mollard et al., 2007; Unger et al., 2010). Flooding also influences decomposition by altering in soil microbial community structure and their associated metabolic processes (Unger et al., 2010), possibly cause exhaustion of respiratory substrates (Scarano et al., 2003) and/or producing toxic metabolites (Boyd and van Acker, 2003; Kolb and Joly, 2010), which can lead to cell death and loss of seed viability (Vidal et al., 2014).

Two species had a relatively different responses to the intensity and duration of high temperatures. Factors involved in determining seed survival following fire are a combination of temperature intensity and duration, soil moisture, seed thermotolerance and seed location in the vertical soil profile, and the maximum depth from which a seed can emerge (Cohen and Rubin, 2007). Heat generated by fire may reach soil surface temperatures above 500 °C (Bebawi and Campbell, 2002); though burning of crop residue may not increase the soil surface temperature to this extent (Bolfrey-Arku et al., 2011). Temperatures at 5 cm in the mineral soil rarely exceed 150 °C (DeBano, 2000). However, maximum temperature has shorter durations at the soil surface, while heat was maintained for a longer period at soil depths (Certini, 2005). Consequently, seeds of spotted spurge and wild poinsettia on the soil surface are destroyed completely, and also due to an almost complete eradication of spotted spurge seeds and wild poinsettia at 120 °C for 5 min and 140 °C for 5 minutes, respectively, expect a lot of seeds at a depth above 5 cm which lost their viability. Nevertheless, some buried seeds below 5 cm may escape from the effect of heat. It indicates that the some seeds, especially deep buried seeds, could still germinate after the passage of a fire.

Temperature duration in addition to maximum temperatures influenced the seed survival of two weed seeds. So, heating at moderate temperatures for a long duration may have a similar effect with high temperatures for a short time. Hence, soil solarization can be used rather than plant residue burning. Cohen et al. (2008) stated germination of *Acacia saligna* at 50 °C for 72 h was equivalent to an exposure 80 °C for 15 min. Soil solarization has been found to be a very effective strategy against sun spurge (*Euphorbia helioscopia*) (Holm et al., 1979). Narbona et al. (2006) observed that germination of *Euphorbia nicaeensis* was not affected by the high-temperature treatments of 100 °C for 1 and 5 min, while germination at temperatures of 120 °C for 5 min was significantly less than in the control batch. Intense heat treatments (15 min at 100 °C and % min at 120 °C) cause a drastic reduction in germination of *Euphorbia boetica* (Narbona et al., 2007).

Soaked seeds of two species showed different response to fire that it could be due to differences in water absorption rate. Wild poinsettia seeds germinated in control from the first day, while germination of spotted spurge seeds began on the second day. Thus, phase I, and phase II are longer in spotted spurge and probably seeds in soaking stage before exposure to heat treatment have absorbed less water. Germination of 12 species of *Lespedeza* spp. was reduced to below 5% by exposure to moist heat of 90 °C, while in the dry heat treatment, all species germinated over 50% (Cushwa et al., 1968).

In summary, these results suggest that spotted spurge is capable of forming persistent seedbank. Seeds of spotted spurge were partially tolerant to submersion in water, but wild poinsettia seed are susceptible to submergence. Thus, water flow can spread spotted spurge seeds. The burning of fallow vegetation could also prevent augmenting the soil seed bank of both species. These results indicate more persistent seedbank of spotted spurge and its higher ability to distribution compared to wild poinsettia. These were consistent with field observations in the province. Spotted spurge has more distribution than wild poinsettia in Golestan province, as it has now been dispersed in many counties of the province, but wild poinsettia is reported only in east of the province (Siahmarguee et al., 2018).

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