

DORMANCY AND GERMINATION OF VELVETLEAF
(*ABUTILON THEOPHRASTI* MEDIK.) AND REDROOT PIGWEED
(*AMARANTHUS RETROFLEXUS* L.) SEEDS

**Marija Ravlić, Renata Baličević, Pavo Lucić,
Petra Mazur*, Aleksandar Lazić***

Faculty of Agriculture in Osijek, Josip Juraj Strossmayer University of Osijek, Kralja
Petra Svačića 1d, 31000 Osijek, Croatia, corresponding author: mravlic@pfos.hr

*Student, Undergraduate study

Abstract

The aim of the study was to determine the influence of different methods on breaking seed dormancy of weed species velvetleaf (*Abutilon theophrasti* Medik.) and redroot pigweed (*Amaranthus retroflexus* L.). The influence of seed sowing depth on emergence and growth of weeds was also evaluated. The results showed that the best treatment for breaking seed dormancy of velvetleaf was immersion of seeds in hot water (60°C) for 1 hour, while immersion in distilled water and 0.2% KNO₃ solution had no significant effect. Seed germination of redroot pigweed was significantly increased in all treatments, however 2% KNO₃ solution and cold stratification at 5°C for 12 days had the greatest effect. Treatments had different influence on mean germination time, seed germination dynamics and seedling length of weeds. The highest percentage of emerged velvetleaf seedlings was recorded when seeds were sown at 1 to 4 cm depths and ranged from 55.6 to 67.9%. With the increase of sowing depth the emergence percentage decreased, however one quarter and one fifth of the seedlings emerged from 7 and 9 cm depths. Both seedling length and fresh weight were greater at lower sowing depths. Emergence of redroot pigweed was not significantly influenced by sowing depth, however seedlings length and fresh weight were greater at 5 cm depth than at 1 cm.

Keywords: seed dormancy, germination, weeds, sowing depth

Introduction

Weed species have a great ability to adapt to adverse environment conditions with seed physiology and morphology, such as seed size, development of special structures for seed dispersal and seed dormancy (Gal-

loway, 2001). Seed dormancy represents an inability of seed to germinate over a period of time under favourable environmental conditions (Bewley, 1997). It is also a mechanism that prevents seed germination under conditions when establishment and reproduction of plants are unfavourable (Gardarin and Colbach, 2015). Seed dormancy can be classified as primary dormancy, when the mechanisms occur on mother plants and it often exists for some length of time even after seeds leave the mother plant, and secondary dormancy when the mechanisms causing dormancy occur after seed dispersion (Vivian *et al.*, 2008). Various methods can be applied to break seed dormancy such as seed scarification, treatments with water, seed stratification and different chemical treatments (Tang *et al.*, 2010, Đikić *et al.*, 2011, Lemić *et al.*, 2014, Podrug *et al.*, 2014).

Seed dormancy is a major factor affecting emergence and time of appearance of weeds and constant weediness of soils from year to year (Bradford *et al.*, 2002, Podrug *et al.*, 2014). Knowledge on weed biology and ecology, seed dormancy and longevity can be used to predict weed infestations better and to evaluate sustainable management strategies (Bhowmik, 1997, Bradford, 2002).

The ability of weeds to produce huge amount of seeds results in build-up of weed seed bank on the surface and in the soil profile. Since the majority of the weed seeds is in the upper 10 cm of the soil, the study of the emergence of weeds from different soil depths and their appearance are important for their effective control and use of herbicides (Konstantinović *et al.*, 2011).

Velvetleaf (*Abutilon theophrasti* Medik.) is an invasive species from Malvaceae family and competitive weed in maize, soybean, sugar beet, potato, sunflower and onion. An important feature of velvetleaf is a high production of seeds and their longevity and dormancy (Knežević, 2006, Novak, 2007, Nikolić, 2014). Velvetleaf seed production ranges from 100 and up to 18 000 seeds per m² (Cardina *et al.*, 1995), while over 80% of seeds at harvest can be dormant (Cardina and Sparow, 1997). Redroot pigweed (*Amaranthus retroflexus* L.) is a significant weed species on nitrogen rich soils and occurs in row crops and on ruderal habitats (Knežević, 2006, Šarić *et al.*, 2011). Freshly developed seeds of redroot pigweed possess a certain degree of primary dormancy (Frost, 1971).

The aim of the research was to investigate (I) effect of different methods on breaking seed dormancy and (II) effect of different sowing depth on emergence and growth of velvetleaf and redroot pigweed.

Materials and methods

Two sets of experiments were conducted during 2015 in the Laboratory of Phytopharmacy at the Faculty of Agriculture in Osijek in order to examine different dormancy breaking treatments and different seed sowing depths on germination and seedling growth of weeds.

Seeds of velvetleaf and redroot pigweed were collected in 2014 and 2013 in the production areas in the Osijek-Baranja County. The collected seeds were cleaned and stored in paper bags. Prior to each experiment, the seed surface were disinfected for 20 minutes in 1% solution of NaOCl and rinsed three times with distilled water (Siddiqui *et al.*, 2009).

Petri dish bioassay: Treatments for breaking seed dormancy of velvetleaf were as follows: 1) control (untreated seeds); 2) immersion in distilled water for 24 h; 3) immersion in 0.2% solution of KNO₃ for 24 h; 4) hot water at 60°C for 1 h. Treatments for redroot pigweed included: 1) control (untreated seeds); 2) immersion in distilled water for 24 h; 3) immersion in 0.2% solution of KNO₃ for 24 h; 4) immersion in 1% KNO₃ solution for 24 h; 5) immersion in 2% KNO₃ solution for 24 h; 6) cold seed stratification at 5°C for 12 days.

After applying different treatments, seeds were germinated in Petri dishes. In each dish (90 mm) 30 weed seeds were placed on filter paper moistened with distilled water. Petri dishes were kept at room temperature at 20 ± 2°C for 9 days. Germinated seeds were recorded daily. At the end of experiment, germination (%), seedling root length (cm), shoot length (cm) and fresh weight (mg) were determined.

Pot experiment: The effect of different sowing depths was examined in pot experiments. Commercial substrate (NPK 210:120:260 mg/l, pH 5.6) was used. In plastic pots 30 velvetleaf seeds were sown at depths of 1, 2, 3, 4, 5, 7 and 9 cm, while 30 redroot pigweed seeds were sown at depths of 1, 3 and 5 cm. The bottom and upper layers of the soil were levelled and then pressured before and after seed sowing at various depths to increase the contact between soil and weed seeds.

The experiment lasted 10 days for velvetleaf and 9 for redroot pigweed. At the end of experiment, emergence (%), length (cm) and fresh weight (mg) of seedlings were determined.

Data collection and analysis: All experiments were set up as completely randomized design, each treatment had three replications and all experiments were conducted twice. Germination percentage was calculated for each replication using the formula: $G = (\text{Germinated seed} / \text{Total seed}) \times 100$, while percentage of emergence was calculated as $E = (\text{Emerg}$

seed/Total seed) x 100. Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981): $MGT = \sum (Dn) / \sum n$, where n is the number of seeds that germinated on day D, and D is number of days counted from the beginning of germination. Mean emergence time (MET) was calculated according to formula for MGT. The collected data were analysed statistically with ANOVA and differences between treatment means were compared using the LSD-test at probability level of 0.05.

Results and discussion

Petri dish bioassay: The effect of treatments on breaking seed dormancy of velvetleaf is represented in Figure 1. Seed germination in control was up to 10.1% indicating that the large proportion of seeds was dormant. The highest germination was observed in treatment with hot water at 60°C and was 23.8%. Đikić *et al.* (2011) reported that germination of velvetleaf increased up to 62.5% when seeds were immersed in hot water (80°C) compared to the control which was 3.3%. Immersion of seeds in hot or boiling water as method for breaking seed dormancy of velvetleaf was reported by others (Nurse and DiTommaso, 2005, Hosseini *et al.*, 2010, Sadeghloo *et al.*, 2013).

Immersion of seeds in distilled water and 0.2% KNO₃ solution had no significant effect on breaking seed dormancy and germination was 5.3% and 4.7%, respectively. Đikić *et al.* (2011) also stated that KNO₃ showed no significant effect, however in their experiment immersion of seeds in distilled water significantly improved germination. According to Horowitz and Taylorson (1985) abrasion, percussion, sodium hypochlorite, organic solvents and even pesticides had no effect on hard seeds of velvetleaf.

Impermeability of seed coat to water causes primary physical dormancy in velvetleaf (Cardina and Sparrow, 1997). Lignified, cutinized palisade layer accounts for more than half the thickness of the seed coat and is the principal cause of seed impermeability. The palisade layer is discontinuous at chalazal slit (Winter, 1960), the water gap where water entry is permitted, which is blocked by the chalazal plug in dormant seeds (Baskin and Baskin, 1998). Breaking physical dormancy involves disruption or dislodgment of water gaps which allows water entry and germination (Baskin and Baskin, 1998, Baskin *et al.*, 2000). Among artificial methods, the most effective for breaking seed dormancy in laboratory include hot water, drying and scarification which damage palisade layer, induce seed coat cracking and seed permeability (La Croix and Staniforth, 1964, Horowitz and Taylorson, 1985, De Souza and Marcos-Filho, 2001).

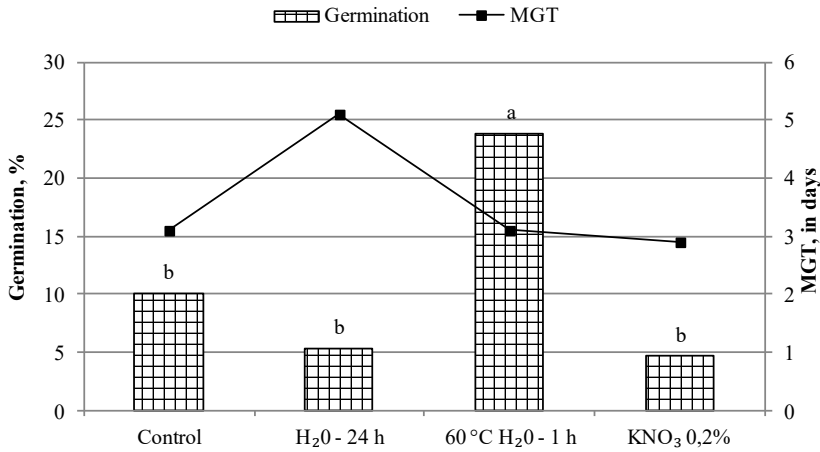


Figure 1. Effect of dormancy breaking treatments on germination and mean germination time of *A. theophrasti*

Mean germination time of velvetleaf seeds only differed slightly among treatments (Figure 1). Seeds germinated faster in treatments with KNO₃, control and hot water and mean germination time ranged from 2.9 to 3.1 days. In contrary, in treatment with seed immersion in distilled water mean germination time was 5.1 days.

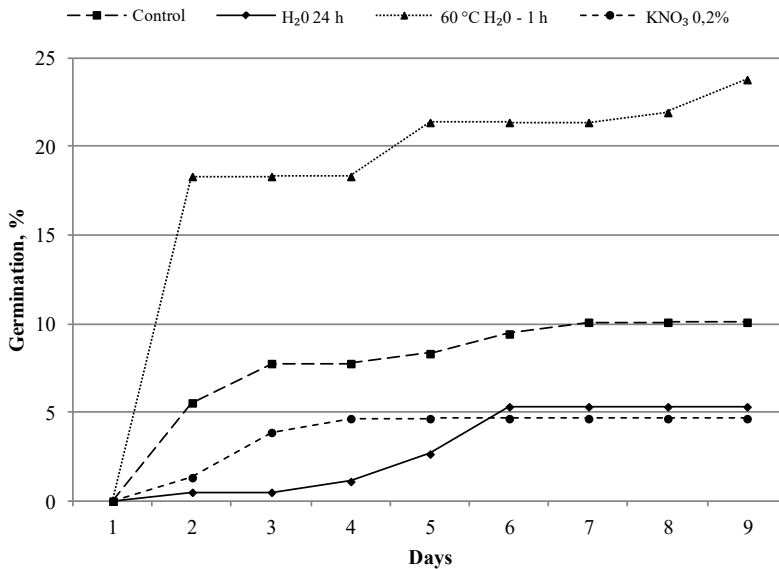


Figure 2. Germination dynamics of *A. theophrasti* seeds

Germination dynamics of velvetleaf seeds (Figure 2) indicated that the largest proportion of seeds germinated between the second and fourth day of the experiment. The exception was observed in treatment with seed immersion where majority of the seeds germinated on the fifth day. Baloch *et al.* (2001) reported that about 75% of velvetleaf seeds germinated in the first seven days of the experiment.

Treatments had no effect on root length and fresh weight of velvetleaf seedlings (Table 1). However, shoot length was significantly lower (by 28%) in treatment with KNO_3 , as compared to the control.

Table 1. Effect of dormancy breaking treatments on seedling growth of *A. theophrasti*

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (mg)
Control	2.5 a	5.0 a	60.9 a
H ₂ O – 24 h	2.8 a	4.7 ab	55.9 a
60°C H ₂ O – 1h	2.8 a	5.6 a	52.6 a
KNO ₃ 0.2%	2.6 a	3.6 b	46.2 a

Means followed by the same letter within the column are not significantly different at $P < 0.05$.

All treatments showed significant effect on breaking seed dormancy of redroot pigweed (Figure 3). In control germination was only 4.5%, while the highest germination was recorded in treatments with 2% KNO_3 solution (31.7%). Lower concentrations of KNO_3 solution also increased seed germination. According to Tang *et al.* (2010), besides KNO_3 , different nitrogen containing compounds i.e. ammonium chloride, ammonium nitrate and sodium nitrite significantly improve germination of redroot pigweed.

Cold stratification of pigweed seeds increased germination up to 28.3%. Similarly, Holik (2013) found that seed stratification at 4°C for 7 days resulted in germination up to 97.0%. Low temperature is a factor that breaks dormancy in thermophilic weeds (Noronha *et al.*, 2007). Conversely, Šarić and Vidović (1989) state that cold stratification had no effect on redroot pigweed germination.

Mean germination time of redroot pigweed seeds was shorter in all treatments as compared to control (Figure 3). Among treatments, seeds immersed in distilled water germinated fastest, while seeds subjected to cold stratification germinated slower. Lemić *et al.* (2014) stated that cold stratification delays germination of lambsquarters seeds compare to other dormancy breaking methods.

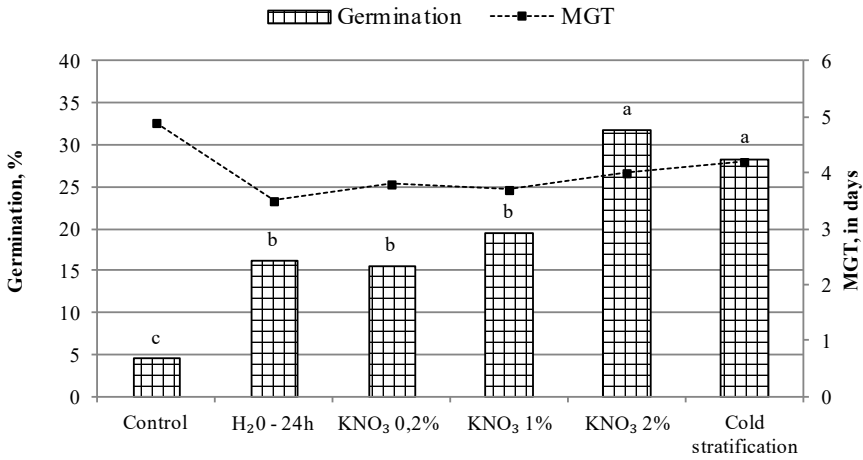


Figure 3. Effect of dormancy breaking treatments on germination and mean germination time of *A. retroflexus*

Germination dynamics of redroot pigweed seeds shows that in all treatments majority of the seeds germinated between third and fourth day of the experiment, while full germination was achieved on seventh or eighth day (Figure 4).

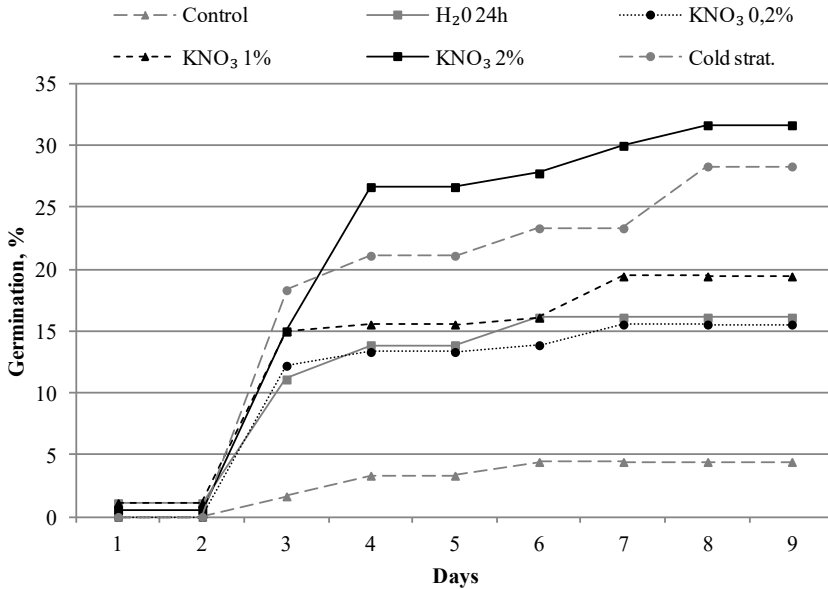


Figure 4. Germination dynamics of *A. retroflexus* seeds

Treatments showed significant effect on root and shoot length and fresh weight of redroot pigweed seedlings (Table 2). Except in treatment with seed immersion in distilled water, all treatments significantly increased root and shoot length of seedlings up to 57.8 and 81.3% as compared to control. Increase in root length of pigweed was reported by Soomarin *et al.* (2010) in treatments with sulphuric acid.

Table 2. Effect of dormancy breaking treatments on seedling growth of *A. retroflexus*

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (mg)
Control	1,4 c	1,6 c	4,0 c
H ₂ O – 24 h	1,6 bc	1,8 c	4,2 c
KNO ₃ 0,2%	2,1 ab	2,4 b	5,1 bc
KNO ₃ 1%	2,4 a	2,7 ab	5,7 ab
KNO ₃ 2%	2,1 ab	2,7 ab	6,3 ab
Cold stratification	2,0 ab	2,9 a	6,6 a

Means followed by the same letter within the column are not significantly different at $P < 0.05$.

Pot experiment: Seed sowing depth had significant effect on emergence of velvetleaf (Figure 5). The highest emergence percentage was observed when seeds were sown at 1 to 4 cm depths and ranged from 55.6 to 67.9%. With the increase of depth the emergence percentage decreased. However, 25.9 and 18.2% of seeds emerged from 7 and 9 cm depths.

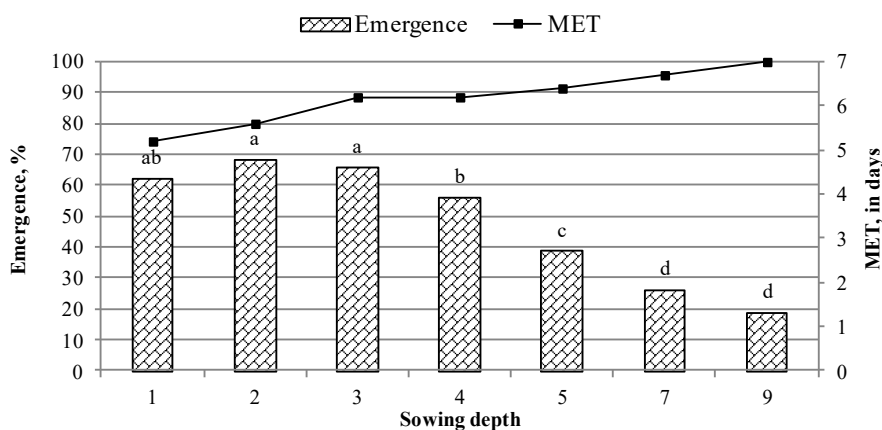


Figure 5. Effect of seed sowing depth on emergence percentage and mean emergence time of *A. theophrasti*

Sadeghloo *et al.* (2013) also stated that emergence of velvetleaf slightly decreased as sowing depth increased from 1 to 4 cm but decreased sharply when seeds were sown deeper than 4 cm. Lower seedling emergence at greater sowing depths could be associated with limited food reserves in the seeds (Mennan and Ngouajio, 2006). In contrary, according to Rakoš (2013) velvetleaf seedlings emerge equally well at all depths from 3 to 9 cm.

Mean emergence time was affected by sowing depth and seeds from depths of 1 and 2 cm emerged earlier, while seeds sown at 7 and 9 cm emerged slower, for 6.7 and 7 days (Figure 5).

Emergence dynamics of velvetleaf showed that in the first three days of the experiment none of the seedlings emerged regardless of the sowing depth (Figure 6). Furthermore, emergence of seeds sown at 7 and 9 cm depths was delayed and seedlings started emerging on the fifth day of the experiment. Majority of the seeds sown at lower depths emerged during the fifth and sixth day of the experiment, while emergence was completed in all treatments on the ninth day.

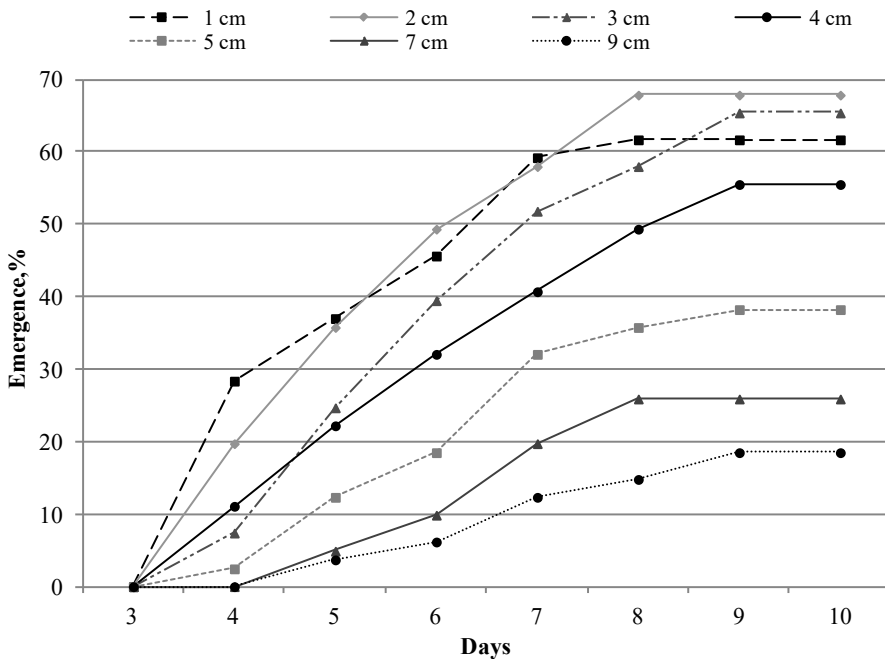


Figure 6. Emergence dynamics of *A. theophrasti* seeds

Sowing depth influenced total seedling length and fresh weight of velvetleaf (Table 3), however significant effect was recorded only at depth of 7 and 9 cm where both seedlings length and fresh weight were increased. Podrug *et al.* (2014) also stated that with the increase of sowing depth the length of the seedlings of johnsongrass increased, but on contrary their fresh weight decreased.

Table 3. Effect of seed sowing depth on seedling growth of *A. theophrasti*

Sowing depth	Seedling length (cm)	Fresh weight (mg)
1 cm	12.6 b	100.9 c
2 cm	12.4 b	107.1 c
3 cm	13.3 b	116.1 c
4 cm	12.6 b	118.4 c
5 cm	12.8 b	110.4 c
7 cm	15.5 a	146.3 b
9 cm	15.2 a	179.3 a

Means followed by the same letter within the column are not significantly different at $P < 0.05$.

Emergence percentage of redroot pigweed seeds decreased with the increase in seed sowing depth, however no significant differences were observed among treatments (Figure 7). According to Šarić and Vidović (1989) sowing depth significantly affected emergence of redroot pigweed and the highest percentage of emerged seedlings (40.0%) were recorded at 1 cm depth, while at depth of 5 cm only 10.0% of seedlings emerged.

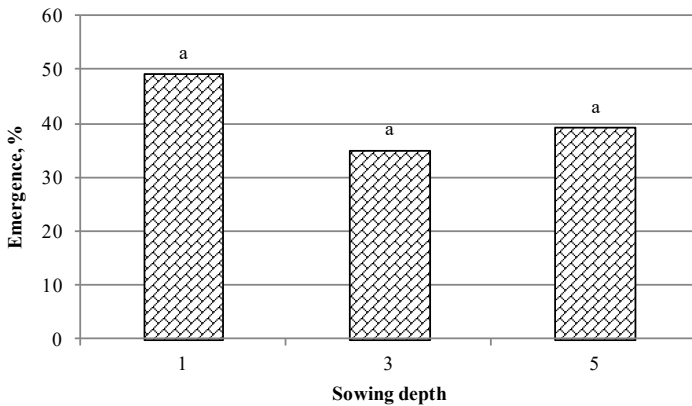


Figure 7. Effect of seed sowing depth on emergence percentage of *A. retroflexus*

Seed sowing depth also showed influence on seedling growth of redroot pigweed seedlings and both length and fresh weight of seedlings increased with depth (Table 4).

Table 4. Effect of seed sowing depth on seedling growth of *A. retroflexus*

Sowing depth	Seedling length (cm)	Fresh weight (mg)
1 cm	6.4 a	7.4 a
3 cm	7.7 b	9.9 b
5 cm	8.9 c	10.1 b

Means followed by the same letter within the column are not significantly different at $P < 0.05$.

Conclusions

The results of the study showed that different methods could be used in breaking seed dormancy of weeds. The best method for breaking seed dormancy of velvetleaf proved the application of hot water, while dormancy of redroot pigweed was successfully broken with KNO_3 solution and cold stratification. Pot experiment showed that both weed species have good emergence within 5 cm depth of soil. Understanding weed biology and ecology is important for development of environmentally friendly weed management strategies.

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