Indian Journal of Weed Science **51**(4): 372–380, 2019

Print ISSN 0253-8040 Online ISSN 0974-8164

Effects of environmental factors and ageing on germination of golden crownbeard (*Verbesina encelioides*) - A wide spread weed of Northern India

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Article information

DOI: 10.5958/0974-8164.2019.00078.9

Type of article: Research article

Received : 6 August 2019
Revised : 26 September 2019
Accepted : 30 September 2019

Key words
Accelerated ageing
Burial depth
Germination
Light
Temperature
Salinity

ABSTRACT

Golden crownbeard (*Verbesina encelioides*), which is abundant along roadsides in Northern India, has started to invade field borders in the South West of Punjab. This study was conducted to find the effect of environmental factors and accelerated ageing on germination of this weed. It germinated over a wide range of temperatures (15/5-35/25°C) with optimum germination at 25/15°C. Light was not a pre-requisite for germination. Germination was completely inhibited at -0.6 MPa. The seeds germinated at 160 mM sodium chloride (13.3%), but no germination was observed at 180 mM NaCl. Germination of seeds was very low at pH less than 5 or more than 8. Germination was 95% when seeds were placed on the soil surface. No emergence was observed when seeds were buried to 6 cm or greater depth. Accelerated ageing of seeds for 20 or more days completely inhibited germination. Results indicate that this weed can emerge in multiple germination flushes. It also has the ability to invade drought affected areas, and can grow in soils that are moderately saline, slightly acidic, or alkaline. However, this weed is not expected to buildup persistent soil seed banks due to rapid loss of viability (time to 50% persistence = 4.11 days) under accelerated ageing.

INTRODUCTION

Verbesina encelioides (Cav.) Benth. and Hook. f. ex. Gray, commonly known as golden crownbeard, is a member of the Asteraceae family. It is native to tropical America, but has infested five continents, including America (Argentina, Arizona, Hawaii and Mexico), Africa (Algeria, Egypt and Morocco), Asia (India, Saudi Arabia and Yemen), Europe (Belgium, France and Spain) and Australia (CABI 2015). It was reported as a troublesome annual weed of many crops, *viz*., corn (*Zea mays* L.), rice (*Oryza sativa* L.), radish (*Raphanus sativus* L.), pearl millet (*Pennisetum glaucum* (L.) R.Br.), wheat (*Triticum aestivum* L.), chickpea (*Lens culinaris* M.), honeydew melon (*Cucumis melo* L.), rapeseed (*Brassica napus* L.) and peanuts (*Arachis hypogaea* L.) in Australia, Argentina and the United States (Kaul and Mangal 1987). In northern India, *V. encelioides* has not yet infested crops but it is a major roadside weed, especially in south west Punjab districts including Ferozepur, Fazilka, Bathinda, Barnala and Sangrur.

Golden crownbeard is an aggressive annual weed that grows very fast and produces large numbers of winged seeds, which disperse readily. The seed production potential of this weed is 300-350 seeds per capitulum and an average of 29-254 capitula per plant (Sayari *et al*. 2016). It is a poisonous plant, producing signs of galegine toxicity in livestock that were given water containing dried plant material (Lopez *et al*. 1996). The phytotoxic ability of floral and leaf extracts of *V. encelioides* is higher than root leachates and stem extracts (Goel 1987). It also possesses allelopathic properties as demonstrated by the presence of secondary metabolites, *viz*. flavonoids, terpenoids and sesquiterpenes, which have been reported to inhibit seed germination of plants like *Scaevola taccada* (Gaertn.) Roxb. and *Ipomoea pes-caprae* (L.) R. Br. The species is commonly found along roadsides and field boundaries and this weed serves as an alternate host of mealy bug which is a major insect pest of the cotton crop and a host for thrips which are responsible for spreading many viral diseases in cotton, chilli and onion *etc*.

Seed germination is the most critical event for the success of any weed as it is the first stage at which the weed can compete for an ecological niche where it encounters favourable environmental conditions such as temperature, light exposure, soil moisture, pH, soil salinity and burial depth (Chauhan and Johnson 2010). Light is an essential requirement for the germination of positively photoblastic seeds, promoting the germination of seeds located at or near the soil surface. Temperature is an important factor for seed germination, with some weeds germinating only within a narrow temperature range while others possess the ability to germinate at a wide temperature range. Salinity and moisture stress affects germination by decreasing the water uptake capacity of seeds. Sodium and chloride ions are mainly responsible for toxicity in plants (Yadhav *et al*. 2011). Sodium and chloride reduce germination rates as well as root growth of seedlings. Soil pH is also an important factor that affects seed germination and early seedling growth. Seed burial depth affects seedling emergence by influencing the availability of storage reserves, light, moisture and temperature (Shoab *et al*. 2012).

Studies of weed seed persistence under field conditions involve both biotic and abiotic environmental factors and are therefore useful but time-consuming, financially demanding and ineffective in large-scale studies (Ishikawa-Goto and Tsuyuzaki 2004). Artificial seed ageing under controlled conditions can be an important *ex-situ* tool for predicting the persistence of weed seeds in soil. Accelerated ageing helps to predict the long-term germination response of a particular weed species in a relatively short time span.

Information on different environmental factors affecting germination helps in understanding the invasion potential of weed species. Knowledge about weed seed persistence is key to understanding weed seed dynamics in the soil and can assist in improving weed management strategies. Limited information is available regarding the effect of environmental factors on germination of *V. encelioides*. The present study was conducted to study the effect of environmental factors *viz*., light, temperature, moisture, salinity, pH and accelerated ageing on germination of a north Indian population of *V. encelioides*.

MATERIALS AND METHODS

Collection of seeds

Golden crownbeard plant bears fruits in the acropetal succession with younger fruits developing at the apex and older fruits on basal positions of the shoot. Mature seeds of golden crownbeard were collected from older fruits at basal positions of plants growing along roadsides from Ludhiana (30°19'N latitude and 75°29'E longitude), Faridkot (30°34'N latitude and 76°24'E longitude), Bathinda (30°46'N latitude and 75°08'E longitude) and Ferozepur (30°17'N latitude and 74°46'E longitude) districts of Punjab, India, in October 2015. Seeds were bulked, cleaned and stored at room temperature in airtight plastic containers until used in experiments. The harvested seeds were non-dormant and were capable of germination immediately after harvesting.

Experimental sites

Experiments were performed in the Weed Physiology Laboratory, Department of Agronomy, Punjab Agricultural University, Ludhiana, India from November 2015 to November 2016. The monthly data of maximum, minimum and mean temperatures at Ludhiana in 2016 was recorded by the School of Climate Change and Agricultural Meteorology, Punjab Agricultural University, Ludhiana (**Table 1**). The burial depth experiment was done in pots placed under field conditions at the Punjab Agricultural University research farm (30°56' N latitude and 75°52' E longitude) in the months of December-January 2015-16, when environmental conditions are most favorable for the emergence of seedlings.

Germination protocol

Uniform sized seeds (visually selected) of golden crownbeard were surface sterilized with 0.1% mercuric chloride for two minutes to avoid any fungal infection, and then they were washed four times for two minutes each time with distilled water. Seed germination was tested by uniformly placing 30 seeds on Whatman No.1 filter paper in 9 cm

Table 1. Temperature at Ludhiana, Punjab, India during the year 2016

Month	Temperature		
	Maximum (°C)	Minimum (°C)	Mean (°C)
January	17.2	7.4	12.3
February	23.0	9.0	16.0
March	28.0	14.6	21.3
April	36.6	19.6	28.1
May	39.6	24.6	32.1
June	39.1	27.7	33.4
July	33.5	27.3	30.4
August	33.3	26.1	29.7
September	34.0	25.5	29.7
October	32.7	19.0	25.9
November	27.7	12.0	19.9
December	22.3	8.5	15.4

Petridishes. Either 5 ml of distilled water or a treatment solution was added to the Petridishes and they were incubated at 15 °C (optimum temperature for germination in both light and dark conditions) in an environmental chamber (Model MAC MSW-127, Delhi, India), unless otherwise specified.

Experimental treatments

Temperature

Seed germination was tested under both alternate day/night temperature regimes (12 h light/12 h dark), *viz*. 15/5, 20/10, 25/15, 30/20, 35/25 and 40/30°C; and constant temperatures (24 h light), *viz*. 10, 15, 20, 25, 30 and 35°C using distilled water.

Light

To study the effect of light on germination, seeds kept in Petridishes were incubated in three light regimes- 12 h light/12 h dark, 24 h light and 24 h dark at 15°C, which was found to be the optimum temperature for germination of this weed. To achieve complete darkness, Petridishes were wrapped with double layers of aluminum foil. Data on germination and seedling growth of dark-incubated dishes were recorded only on the 15th day after initiation of the experiment.

Salinity stress

The ability of seeds to germinate under different salt stress levels was examined by using sodium chloride (NaCl) solutions of 1, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 320 and 400 mM concentrations.

Moisture stress

The ability of seeds to germinate under different levels of moisture stress was tested using solutions of polyethylene glycol (PEG) 8000 having water potentials of 0, -0.1, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa (Michel and Kaufmann 1973).

pH

The effect of pH on germination of seeds was tested using buffered solutions with pH ranging from 3 to 10. Buffered solutions of pH 3 and 4 were prepared with 0.2 mM potassium hydrogen phthalate (Chachalis and Reddy 2000). A pH 5 and 6 buffer was prepared with 2 mM of 2-(N-morpholino) ethanesulfonic acid. A 2 mM solution of 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid was used for preparation of buffered solutions of pH 7 and 8. To prepare buffered solutions of pH 9 and 10, 2 mM of tricine was used. Final adjustments of each buffer solution were made using 0.1 M HCl or 0.1 N NaOH.

Burial depth

The effect of seed burial depth on seedling emergence of golden crownbeard was studied using plastic pots of 25 cm diameter with holes at the bottom (for drainage and aeration). Pots were filled with soil from a field with no previous infestation of golden crownbeard. The soil filled in the pots was loamy sand having 0.61% organic matter, 7.3 pH and 0.17 dSm^{-1} electrical conductivity. Fifty seeds were placed on the soil surface or buried at 0.5, 1, 2, 4, 6, 8 and 10 cm deep. The pots were irrigated with sprinkler as needed.

Seed ageing

Five grams of freshly harvested seeds (one week after collection) were weighed and accelerated ageing was done by keeping them at 45°C under humid storage (Biabani *et al*. 2011) for 20 days. The seeds were contained in a mesh bag which was placed on a sieve suspended over water contained in a desiccator held at 45°C in an oven. Ageing was followed by air drying of seeds at room temperature for restoration of their original weight. The contents of various biochemical reserves were estimated from control and aged seeds. These aged seeds were also tested for germination at the optimum temperature in an environmental chamber.

Biochemical parameters

The contents of various biochemical reserves, *viz*., total soluble sugars (Dubois *et al*. 1956), total soluble proteins (Lowry *et al*. 1951), total free amino acids (Lee and Takahashi 1966) and starch content (Clegg 1956) were determined from the control and accelerated aged seeds for 1,4, 7 and 10 days.

Observations recorded

Germination counts were made at 24-h intervals for 15 days after the start of the experiments, with the criterion for germination being visible protrusion of the radicle. Germination (%) was calculated as: (no. of seeds germinated /total number of seeds sown) × 100.

The speed of germination (germination index, GI) was calculated as described by the Association of Official Seed Analysts (AOSA), 1983:

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Mean germination time (MGT) was calculated as per the method given by Ellis and Roberts (1981):

MGT= ∑ (Dn) / ∑ n

Where n is the number of seeds germinated on day D and D is the number of days counted from the beginning of germination.

On the 15th day of each experiment, seedling length was measured with a centimeter scale. Seedling vigor index (SVI) was calculated as described by Abdul-Baki and Anderson (1973):

Seedling vigor index = seedling length (cm) x germination (%)

Statistical analyses

All experiments were conducted three times in a completely randomized design using three replications. There were no significant differences between the results of the repeated experiments, so data were pooled before being subjected to analysis of variance (ANOVA) using CPCS 1 software (the program that computes necessary statistics concerning design with equal or unequal numbers of replications), with means separated using least significant difference (LSD) at 0.05. Regression analysis was used for calculating 50% germination inhibition due to moisture stress, salinity, burial depth and ageing.

RESULTS AND DISCUSSION

Effect of temperature

The optimum day/night temperature regime for germination and seedling growth of golden crownbeard was 25/15°C (12 h light/12 h dark) (**Table 2**). The highest germination per cent and minimum germination time were observed at the 25/ 15°C day/night temperature regime. No seeds germinated at the alternate day/night temperature of 40/30°C. The maximum recorded seedling vigour index (SVI) occurred at the day/night temperature regime of 25/15°C, and it was decreased by about 86.5% at 35/25°C compared with 25/15°C. Germination of golden crownbeard was also tested at six constant temperatures under a 24 h dark period. The highest germination was observed at 15°C; however, the seeds were able to germinate over a wide temperature range from 15-30°C. The minimum germination time and mean germination time and the maximum speed of germination were recorded at 15°C. Temperatures above 15°C reduced the seedling growth of golden crownbeard (**Table 3**). As depicted in **Table 1**, temperatures at the experimental site in the months of October-March (except the month of January) were favorable for the emergence of golden

Table 2. Effect of day/night temperature regime on germination and seedling growth of golden crownbeard incubated with a 12 h/12 h photoperiod for 15 days

Temperature (°C) (12 h light/12 h dark)	Germination (%)	Time to start germination (days)	Mean germination time (days)	Germination speed	Seedling vigor index
15/5	80.0±2.52	2.0±0	5.50±0.02	4.88±0.04	230.9±25.5
20/10	76.7±1.20	2.0±0	5.53±0.15	4.63±0.03	198.7±25.5
25/15	94.4±2.94	2.0±0	4.63±0.09	7.87±0.18	400.9±16.8
30/20	61.1±2.94	2.0±0	5.69±0.09	4.20±0.17	101.0±5.18
35/25	45.6±2.94	3.0±0	6.81±0.07	2.82±0.04	31.1±3.79
40/30	0	0	0	0	0
LSD (α=0.05)	7.31	0.02	0.26	0.32	58.9

Data are mean ± standard error of three replicates

Table 3. Effect of constant temperature on germination and seedling growth of golden crownbeard incubated with a 24 h photoperiod for 15 days

Temperature (°C)	Germination (%)	Time to start germination (days)	Mean germination Time (days)	Germination Speed	Seedling vigor index
10	0	0	0	0	0
15	97.8±2.22	2.0±0	3.73±0.04	9.77±0.1	512.3±36.3
20	83.3±3.33	2.0±0	4.44±0.16	7.16±0.4	407.3±26.8
25	63.3±3.33	3.0±0	5.14±0.24	4.35±0.1	127.9±10.5
30	33.3±3.33	3.0±0	5.67±0.33	2.22±0.3	65.9±6.04
35	0	0	0	0	0
LSD (α=0.05)	7.78	0.59	0.55	0.66	58.8

Data are mean ± standard error of three replicates

crownbeard, thus favoring multiple flushes of this weed throughout the year. Lu *et al*. (2006) studied the germination of *Eupatorium adenophorum* Spreng., a medicinal plant (Chakravarty *et al*. 2011) and a weed of the Asteraceae family and reported germination over a wide temperature range of 10–30°C, with optimum germination at 25°C.

Effect of light

Seed germination was similar statistically under the three light regimes, that is, 12h light/12h dark circardian rhythm, continuous darkness and continuous light conditions (**Table 4).** These findings indicate that germination of golden crownbeard was independent of light. However, seedlings grown in the dark were etiolated, having chlorotic and elongated shoots and resulted in maximum SVI. However, some members of the Asteraceae family have positively photoblastic seeds, like *Bidens tripartite* L. which can germinate only in the presence of light (Benvenuti and Macchia 1997).

Table 4. Effect of light on germination and seedling growth of golden crownbeard incubated at 15°C for variable photoperiods for 15 days

Photoperiod (hours)	Germination (%)	Seedling vigor index
24	83.3±3.33	303.5±14.7
0	86.7±3.33	673.3±28.5
12/12	90.0±0	470±49.3
LSD (α=0.05)	NS	117.5

Data are mean ± standard error of three replicates

Effect of moisture stress

The seeds of golden crownbeard could tolerate moisture stress upto -0.4 MPa, but no germination was recored at -0.6, -0.8 and -1.0 MPa. The osmotic potential required for 50% inhibition of the maximum germination was -0.3 MPa (**Figure 1**). Mean germination time was increased by two folds at -0.4 MPa relative to the control. Seedling growth was adversely affected by moisture stress, with 8.64, 68.6 and 93.8% reductions in seedling vigor index at -0.1, -0.2 and -0.4 MPa as compared with the control. This indicates that *V. encelioides* can tolerate moderate degree of moisture stress.

Effect of salinity

The seeds of golden crownbeard exhibited a significant reduction in germination under salinity stress. The concentration of NaCl required for 50% inhibition of maximum germination was 91.8 mM (**Figure 2**). Germination was completely inhibited at 180 mM NaCl (**Table 6**). It can germinate under a moderate level of salinity, which is an important

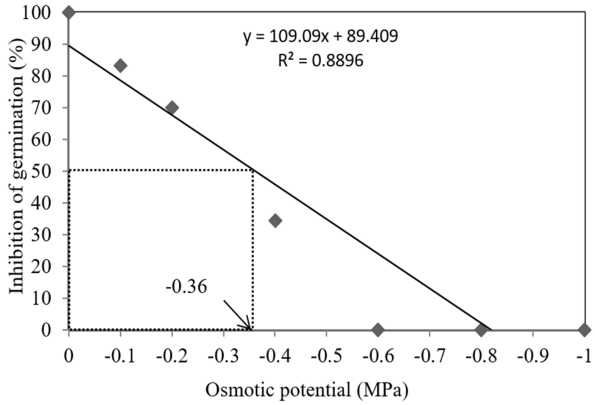

Figure 1. Effect of moisture stress on germination of golden crownbeard. Osmotic potential required for 50% inhibition of germination is shown by an arrow.

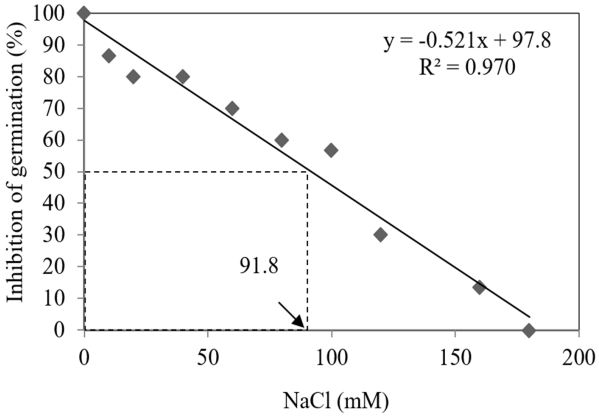

Figure 2. Effect of sodium chloride (NaCl) on germination of golden crownbeard incubated at 15°C with a 24-h photoperiod for 15 days. Sodium chloride concentration required for 50% inhibition of germination is shown by an arrow.

abiotic stress that limits the growth and development of plants. This species has already invaded field borders in some isolated patches in Bathinda district in Punjab (unpublished), where this weed may have a competitive advantage against crops in terms of emergence in salinity affected fields.

Effect of pH

Germination was very low when pH was ≤ 5 or > 8, but increasing pH from 5 to 7 enhanced the germination of *V. encelioides* (**Table 7**). The minimum germination was observed at pH 10, at which seeds also required the longest time to start of germination. Acidic pH condition also had detrimental effects on germination, with only 23-33% germination observed at pH 3-5. Maximum germination was recorded at pH 7, coupled with minimum mean germination time. The highest SVI

Table 5. Effect of moisture stress on germination and seedling growth of golden crownbeard incubated at 15°C with a 24-h photoperiod for 15 days

Osmotic potential (MPa)	Time to start germination (days)	Mean germination time (days)	Germination speed	Seedling vigor index
Control	2.33±0.33	4.03±0.13	8.83±0.61	578.7±49.0
-0.1	3.00±0	5.45±0.17	5.60±0.14	528.7±25.5
-0.2	3.00±0	6.42±0.21	4.09±0.46	181.8±10.1
-0.4	4.33±0	8.24±0.55	1.39±0.32	35.9±5.51
-0.6	0	0	0	0
-0.8	0	0	0	0
-1.0	0	0	0	0
LSD (α=0.05)	0.54	0.72	0.97	118.2

Data are mean ± standard error of three replicates

Table 6. Effect of sodium chloride (NaCl) on germination and seedling growth of golden crownbeard incubated at 15°C with a 24 h photoperiod for 15 days

NaCl (mM)	Time to start germination (days)	Mean germination time (days)	Germination speed	Seedling vigor index
Control	2.00±0	4.50±0.21	8.73±0.14	413.3±23.5
10	2.00±0	5.09±0.71	6.72±0.6	291.5±37.2
20	3.00±0	5.71±0.47	4.99±0.49	236.8±7.02
40	3.00±0	5.42±0.17	4.96±0.17	170.2±26.0
60	3.30±0.33	6.71±0.25	3.79±0.16	135.4±17.6
80	4.00±0	7.38±0.65	2.72±0.07	67.5±12.1
100	4.00±0	8.03±0.76	2.32±0.36	57.3±9.99
120	4.70±0.33	8.67±0.58	1.28±0.09	38.3±3.63
140	5.30±0.33	8.73±0.58	0.82±0.04	17.5±0.47
160	5.70±0.33	8.87±0.48	0.43±0	7.4±2.15
180	-	-	-	-
240	-	-	-	-
320	-	-	-	-
400	-	-	-	-
LSD (α=0.05)	0.52	1.17	0.70	65.4

Data are mean ± standard error of three replicates

Table 7. Effect of pH on germination and seedling growth of golden crownbeard incubated at 15°C with a 24-h photoperiod for 15 days

pH	Germination (%)	Time to start germination (days)	Mean germination Time (days)	Germination speed	Seedling vigor index
3	23.3±3.33	9.00±0	12.6±0.22	0.60±0.07	21.7±5.30
4	26.7±3.33	9.00±0	12.1±0.22	0.77±0.01	29.0±5.57
5	33.3±3.33	7.67±0.67	10.8±0.78	0.99±0.13	37.7±4.99
6	73.3±3.33	2.67±0.33	5.00±0.15	7.10±0.1	185.3±10.7
7	76.7±3.33	2.00±0	4.50±0.29	7.40±0.06	259.5±18.3
8	56.7±8.82	7.00±0	9.06±0.35	2.56±0.04	85.6±12.3
9	33.3±3.33	8.33±0.67	11.3±0.39	0.93±0.14	38.3±2.17
10	16.7±3.33	13.0±0	14.0±0	0.66±0	6.33±1.04
LSD (α=0.05)	12.3	0.99	1.06	0.39	38.5

Data are mean ± standard error of three replicates

was recorded at pH 7, followed by pH 6. Seed germination of golden crownbeard over a broad pH range (3-10) indicates that pH is not a limiting factor for germination of this weed. The pH of agricultural land in Punjab varies from 7 to 8. In this pH range, golden crownbeard exhibited 57 to 77% germination, indicating that pH is not likely to be a limiting factor for germination in most the Punjab soils. Unlike *V. encelioides*, *Bidens alba*, another wasteland weed found in northern India that belongs to the Asteraceae family, can only germinate over a narrow pH range of 5 to 7, indicating a lesser capacity of this weed to invade agricultural lands with slightly alkaline soils (Ramirez *et al.* 2012).

Table 8. Effect of accelerated ageing (at 45°C and 60% RH) on germination and seedling growth of golden crownbeard incubated at 15°C with a 24-h photoperiod for 15 days

Accelerated ageing (days)	Time to start germination (days)	Mean germination time (days)	Germination speed	Seedling vigor index
0	2.00±0	4.03±0.13	8.83±0.61	388.7±15.3
1	2.00±0	4.60±0.21	7.37±0.89	230.0±9.88
4	3.00±0	5.71±0.47	4.99±0.49	121.7±8.69
7	3.00±0	8.67±0.58	1.28±0.09	42.3±6.04
10	4.00±0	8.73±0.58	0.82±0.04	14.2±3.25
20	-	-	-	-
30	-	-	-	-
45	-	-	-	-
60	-	-	-	-
LSD (α=0.05)	0.13×10^{-5}	0.96	0.79	38.9

Data are mean ± standard error of three replicates

Effect of burial depth

Maximum germination (95%) of golden crownbeard seeds was recorded at the soil surface and emergence was reduced by 84% at 4 cm soil depth. The burial depth required for 50% inhibition of emergence of golden crownbeard was 2.1 cm (**Figure 3**). No seedling emergence was observed when seeds were buried in the soil profile deeper than 4 cm. Germination of golden crownbeard decreased with increased seed burial depth. Similar observation on decreased emergence of *S. oleraceus* with increased burial depth has also been reported (Chauhan *et al.* 2006). The highest emergence of *S. oleraceus* (77%) was recorded with seeds placed on the soil surface, whereas seedling emergence was reduced to 38, 22, 3 and 1% at burial depths of 1, 2, 3 and 4 cm, respectively. Seedling emergence was completely inhibited when seeds were buried at a depth of 5 cm. In contrast to *V. encelioides*, seeds of *Ambrosia artemisiifolia* L. and *Bidens alba* (weeds of the Asteraceae family) recorded 16.7 and 18% emergence when buried at the soil depth of 6 cm (Dinelli *et al.* 2013, Ramirez *et al.* 2012). However, the inability of golden crownbeard seeds to emerge from deep soil layers could be due to smaller seed size {1000 seed weight (without wings) = 1.38 g, 1000 seedweight (with wings) = 1.84 g}, and thus having limited food reserves. Therefore, farming practices such as no-till and minimum tillage may promote greater seedling emergence of *V. encelioides*.

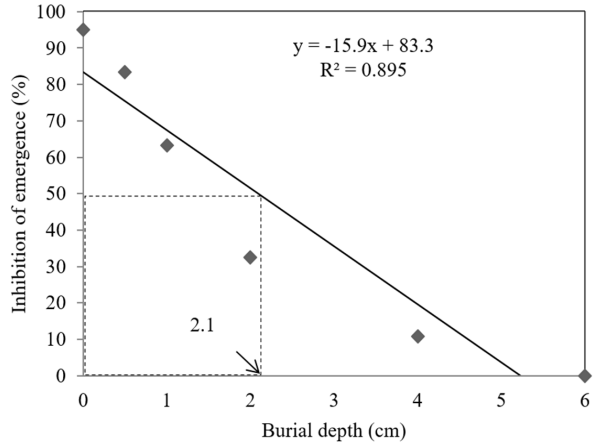

Figure 3. Effect of burial depth on seedling emergence of golden crownbeard. Burial depth required for 50% inhibition of emergence is shown by an arrow. Mean temperature during the months of December and January was 15.4 and 12.3°C, respectively.

Seed ageing

Accelerated ageing of the seeds caused considerable reduction in germination and seedling vigor of golden crownbeard. The P_{50} value for 50% inhibition of maximum germination due to accelerated ageing was 4.11 days. On the 10th day of ageing, germination of seeds was reduced to only 13% as compared to 86.7% germination of the control (**Figure 4**). The average germination time increased with a concomitant decrease in germination speed

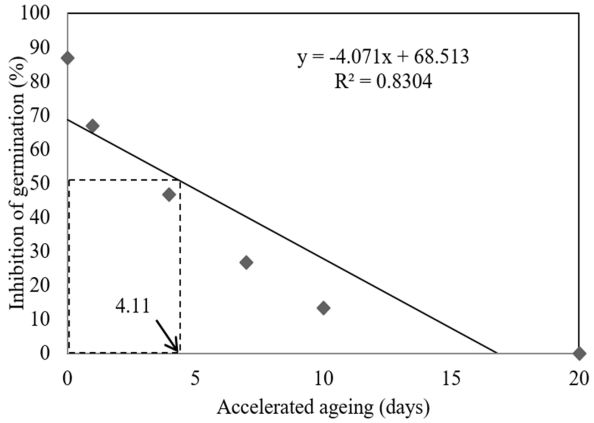

Figure 4. P_{50} value for 50% inhibition of maximum germination due to accelerated ageing of golden crownbeard is shown by an arrow.

due to accelerated ageing. Mean germination time increased almost two-fold on the 10th day of ageing, coupled with a 90.8% decrease in the speed of germination as compared with the control (**Table 8**). The process of seed ageing reduced golden crownbeard germination, which may be attributed to decreased starch and total soluble protein content of seeds, with a concomitant increase in the amount of total soluble sugars and total free amino acids (**Figure 5 and 6**).

Accelerated ageing is a process in which seeds are subjected to moist conditions and high temperatures under controlled conditions in the laboratory. No germination was observed when golden crownbeard seeds were aged for 20 days or more, indicating limited persistence of seeds. Based

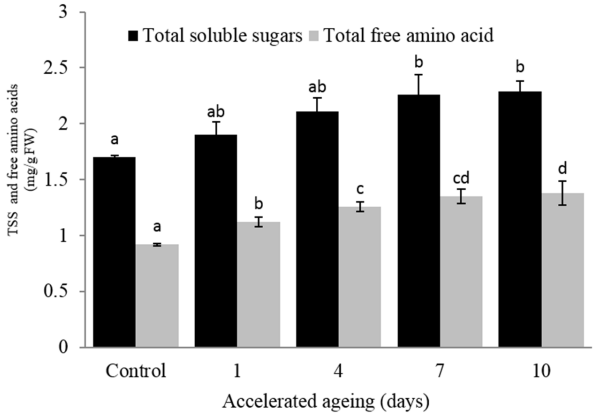

Figure 5. Effect of accelerated ageing on total soluble sugars and total free amino acids in golden crownbeard seeds. Vertical bars represent standard errors of the means. Means followed by common letters do not differ significantly at 5% level of significance

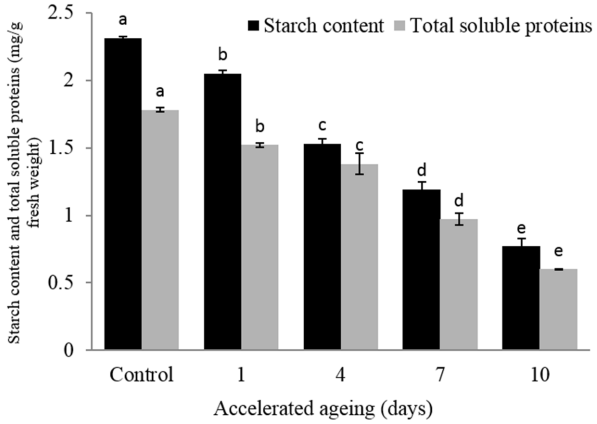

Figure 6. Effect of accelerated ageing on starch and total soluble protein content in golden crownbeard seeds. Vertical bars represent standard errors of the means. Means followed by common letters do not differ significantly at the 5% level of significance

on the controlled ageing test (CAT), 13 species of emerging and common weeds of Queensland were assessed for their seed longevity. The seed longevity data of these species was linked with field seed-persistence, based on which Long *et al*. (2008) reported a positive correlation between controlled ageing conditions and field seed persistence. They categorized the weed species into three types for describing seed bank persistence: transient (<20 days to reach P_{50} with field persistence < 1 year), short lived (P_{50} value of 20-50 days with 1-3 years field persistence) and extended persistence (P_{50} value of > 50 days with > 3 years field persistence). Our results indicate that *V. encelioides* is not expected to buildup persistent seed banks like other weeds, *viz*. *Chenopodium album* L. and *Capsella bursa-pastoris* (L.) Medicus, which can persist for more than 20 years (Gulden and Shirtliff 2009). The process of ageing reduced content of starch and total soluble proteins, with a concomitant increase in the amount of total soluble sugars and total free amino acids in the seeds of golden crownbeard. Similar results were reported by Ravikumar *et al*. (2002) for the effect of accelerated ageing on sugar, starch, protein and amino acid content of *Dendrocalamus strictus* (Munro) Kurzseeds.

The results of our studies showed the ability of this weed to germinate over a wide temperature range, favoring multiple flushes of this weed throughout the year. The study on accelerated ageing indicates that seeds of golden crownbeard may not have long-term persistence under field conditions. However, more studies under field conditions are needed to determine the fate and persistence of seeds of this weed.

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