

## EFFECTS OF ARTIFICIAL ACCELERATED AGING ON GERMINATION BEHAVIOUR OF THE COSMOPOLITAN WEED *CHENOPODIUM ALBUM*: IMPLICATIONS FOR WEED CONTROL

Ali El-Keblawy<sup>1</sup>

### ABSTRACT

Accelerated aging is a physical stress commonly used in order to obtain information on seed vigor within a short period of time. The present study aimed to assess the effects of temperature and duration of accelerated aging on the germination behaviour, expressed as final germination percentage and germination rate, of seeds of the cosmopolitan weed *Chenopodium album*. Seeds were aged by incubating them inside sealed boxes with distilled water at 45, 55 and 65 °C for periods of time ranged between one and six days. Seed germination was tested at three incubators. Accelerated aging significantly reduced both final germination percentages and germination speed. The overall germinations after one, two, three and six days of aging decreased by 46%, 88.7%, 85% and 83%, respectively, from that of unaged seeds. Germination was completely inhibited after aging of the seeds at 65 °C. The results discussed in the light of employing accelerated aging technique in obtaining information about the most effective temperature and duration that are required to deteriorate seeds of weeds during the soil solarization.

**Key words:** *Chenopodium album*, accelerated aging, seed germination, soil solarization, weed control.

### INTRODUCTION

Mature seeds gradually lose viability during storage. Generally, natural seed aging decreases germination percentage and slows germination speed (Palma *et al.*, 1995). In addition to natural aging, seed aging could be accelerated artificially. Accelerated aging is a physiological stress using high temperatures and relative humidity that permit controlled deterioration of the seeds (Delouche & Baskin, 1973; Copeland & McDonald, 2001). This technique is commonly used in order to obtain information on seed vigor in a timely manner, through which changes in the seeds at the cellular level during long-term storage can be simulated within a comparatively short period of time (Abdul Baki, 1969; Thapliyal & Connor, 1997). Significant differences existed also between unaged and artificially aged seeds, with lower germination percentage and slower germination speed in the latter (Coin *et al.*, 1995; Chiu *et al.*, 1995; Bailly *et al.*, 1996; Rehman *et al.*, 1999).

The development of proper weed control methods in agriculture depends largely on the understanding of weed population dynamics. The possibility of increasing the efficiency of control methods on both a short-term and a long-term basis is related to our capacity for identifying the stages and process that are most critical for the regulation of the population (Benecch-Arnold & Sanchez, 1995). Seedling stage has been considered as the most vulnerable one in the life cycle of a plant (Fenner, 1987) and consequently, it is a common target of weed control methods. In addition, manipulation of soil seed bank was considered as a successful method for weed management in agroecosystems (Kremer, 1993).

<sup>1</sup> Biology Department, Faculty of Science, UAE University, P.O. Box 17551 Al-Ain, UAE

E-mail: [a.keblawy@uaeu.ac.ae](mailto:a.keblawy@uaeu.ac.ae)

Permanent address: Faculty of Education, Suez Canal University, Al-Arish, United Arab Emirates

Under field conditions, seed aging of weeds is also accelerated through the soil solarization, which is a physical technique used for weed and plant disease control in regions receiving high level of solar radiation. Solarization is usually recognized through mulching of the soil with transparent polyethylene sheets with 100–150  $\mu\text{m}$  thickness (Almasoom *et al.*, 1993; Chase *et al.*, 1999; Habeeburrahman & Hosmani, 1996; Mudalaginyappa & Ramachandrappa, 1999; Marengo & Lustosa, 2000). The effect of solarization on the deterioration of weed seeds was usually attributed to the elevated soil temperatures that result from mulching the soils with polyethylene sheets. For example, soil temperature at 5-cm depth was 10 °C warmer in solarized than in control plots in Brazil and this was high enough to reduce weed biomass and density in about 50% of weed species (Marengo & Lustosa, 2000). Also, soil solarization under conditions of tropical India raised the temperature by 8.7–11.0 °C which resulted in the decrease of the seed emergence of four weedy species by over 90% and weed densities were even lower than that result from normal practice of weed control (Habeeburrahman & Hosmani, 1996). The importance of high humidity during soil solarization was emphasized by several researchers (Arora & Yaduraju, 1998; Ahmad *et al.*, 1996).

Despite the common use of both solarization and accelerated age techniques no attempt has been made to explain the deterioration of soil seeds through solarization in the light of artificial seed accelerated aging. Actually, both of the two techniques depend on the high temperature and relative humidity to cause cell membrane damage and increase ion leakage from it (Bailly *et al.*, 1996). Accelerated aging technique could provide us with valuable information, during relatively short time, about the status of seed vigor after exposing weed seeds for different periods and levels of temperature and humidity during the process of solarization. Such information would determine the most effective temperature level and duration and humidity level that must be provided by solarization to control every kind of weeds. The present study aimed at using accelerated aging technique in determining the lethal temperature and most effective duration of temperature to deteriorate of seeds of the serious cosmopolitan *Chenopodium album* L. weed. Because the time of weed seedling emergence determines the strength of competition between weeds and crops the speed of germination will also be assessed in the present study.

## MATERIALS AND METHODS

*Chenopodium album* seeds were collected during April, 2000 from several places including disturbed sites and farms cultivated with various kinds of vegetables from Al-Ain Area, UAE. Seeds were collected from 40–50 individuals, separated, stored at room temperature in brown paper bags.

After six months of storage (October, 2000) seeds of *C. album* were divided into 12 groups, each with about 500 seeds. Each group was tied in muslin cloth, moistened with water and placed on wire mesh platforms in one of 12 sealed 1000-ml boxes (10 cm x 17 cm x 6 cm) containing 50 ml of distilled water. The distance between the platform and water surface was 1.5 cm. Seeds were misted daily in the muslin cloth to ensue maximum possible relative humidity during the treatment. To achieve accelerated aging, four boxes were incubated (hereafter referred aged) at each of three incubators adjusted at 45, 55, and 65 °C. The four boxes were opened after one, two, three and six days and the seeds were air dried at room temperature. Aged and unaged (control) seeds were germinated in 9 mm plastic Petri-dishes with one Whatman No. 1 filter paper moistened with distilled water. Germination was conducted in three incubators adjusted at 15, 20 and 25 °C with three replications of 25 seeds for each treatment. Radical emergence was the criterion for germination to be recorded. Germinated seedlings were counted and removed every alternative day for 12 days post-seed sowing.

The rate of germination was estimated using a modified Timson index of germination velocity =  $\Delta G/t$ , where G is the percentage of seed germination at 2d intervals and t is the total germination period (Khan & Ungar, 1984). The maximum value using this index in the instant studies was  $600/12=50$ . The higher the value, the more rapid is the germination.

Three way analyses of variances (ANOVAs) were carried out to demonstrate the effects of the main factors (aging period, aging duration and temperature of germination and their interactions) on the final germination percentage and rate of germination as dependent variables. The germination percentage was arcsine transformed and germination rate was log transformed to meet the assumptions of ANOVA. This transformation improved normality of the distribution of the data. Tukey least significant range (LSR) test was used to determine if the differences among mean. Paired t-tests were carried out to compare between germination parameters of unaged seeds with those aged for one, two, three, and six days. All the statistical methods were performed using SYSTAT, version 10.0.

## RESULTS AND DISCUSSION

### Effects on germination percentage

Three-way ANOVA indicated that there are highly significant effects for both aging period ( $F_{4,73}=38.6$ ,  $P<0.001$ ) and aging temperature ( $F_{2,73}=263.0$ ,  $P<0.001$ ), but not the temperature of incubation during seed germination ( $F_{2,73}=0.4$ ,  $P>0.05$ ) on the final germination. In order to evaluate the interactions between the examined factors the germination results of unaged seeds were excluded from the three way ANOVA model, as there are no variations in aging temperature and aging period in this group. The result of this analysis showed the same levels of significance that mentioned above for the main factors. The overall germination after one, two, three and six days of aging decreased by 46.88, 7, 85 and 83%, respectively, from that of unaged seeds (control). The LSR-tests indicated that the germination percentages after the different aging periods were significantly lower than that of the control. The same test showed non-significant differences between the germination of seeds aged for two, three and six days, but all of them were significantly lower than that of seeds aged for one day (Table 1).

Regarding the aging temperature, the overall germination for seeds aged at 45°C was significantly greater than that of seeds aged at 55°C, but both of them were significantly lower than the germination of unaged seeds. Germination of seeds aged at 45°C (53.3%) and 55°C (19.1%) decreased by 42.1% and 79.2%, respectively than that of unaged seed (92%, Table 1). Germination was completely inhibited after aging of seeds at 65°C, so the results are not shown and excluded from the analysis.

None of the interactions between the main factors had significant effect on final germination percentage ( $P>0.05$ ), except the interaction between aging period and aging temperature ( $P<0.001$ , Table 2). Small fractions of the seeds germinated after one day of aging at 55°C (2.2%), but none of them germinated after aging for two, three or six days at the same temperature (Fig. 1). Seeds aged at 45°C germinated to 95.1% after one day of treatment, but decreased to 20.4% after two days and, unexpectedly, increased again to 27.6% and 31.1% after three and six days of aging respectively. According to t-tests, the germination of unaged seeds was significantly greater than that of seeds aged for one day at 45°C, and both of them were significantly greater than germination of seeds aged for two, three and six days at the same temperature. It is interesting to note that t-test showed significant increase in germination of seeds aged for three and six days at 45°C compared to those aged for only two days at the same temperature. The germination of seeds aged for three and six days was 32.0% and 48.8% greater than that of seeds aged for two days at 45°C (Fig. 1).

### Effects on germination rate

Three way ANOVA showed that the effects on rate of germination were significant for aging duration ( $F_{3,73}=75.4$ ,  $P<0.001$ ), aging temperature ( $F_{2,73}=689.3$ ,  $P<0.001$ ), but not for temperature of germination ( $F_{2,73}=1.44$ ,  $P>0.05$ ). Compared to control, the germination speed decreased by about 50% after seed aging for only one day and by about 90% or more for longer periods of aging. The germination was faster for unaged seeds than those aged for all periods, but the difference in germination speeds between seeds aged for two, three and six days were insignificant (Table 2).

The germination speed was significantly faster for seeds aged at 45 °C than those aged at 55 °C and both of them were significantly slower than that of unaged seeds. Generally, germination was faster for seeds germinated at 25 °C than those germinated at both 15 and 20 °C, but the differences were non-significant by Tukey's LSR test (Table 1).

The three way ANOVA, after the exclusion of unaged seeds, showed significant effects for the main factors and the interactions between aging temperature and both of aging period ( $P < 0.001$ ) and temperature of germination ( $P < 0.05$ ) on germination rate (Table 2). For seeds aged at 45 °C, t-tests showed that the germination rate of unaged seeds (40.1) do not differ significantly from that of seeds aged for one day (38.9), but both of them exceeded significantly the rates of seeds aged for 2, 3 and 6 days. At the same temperature, the germination rate differed significantly between all pairs of days, except between the rates after 2 and 3 days of aging (Fig. 2). Similar to the trend observed in final germination percentages, the germination rate for seeds aged at 45 °C decreased from 39 after one day of aging to 5.4 after two days and then increased again to 8.7 and 8.4 after 3 and 6 day of aging, respectively (Fig. 2).

**Table 1. Effects of period and temperature of accelerated aging and temperature of germination on final germination percentage and germination rate (mean  $\pm$  SE) of *C. album* seeds.**

Factor		Final germination*	Rate of germination
Aging	0	92.0 <sup>a</sup> $\pm$ 1.6	40.1 <sup>a</sup> $\pm$ 0.7
Period (day)	1	49.3 <sup>b</sup> $\pm$ 11.1	20.0 <sup>b</sup> $\pm$ 4.6
	2	10.4 <sup>c</sup> $\pm$ 2.6	2.7 <sup>c</sup> $\pm$ 0.7
	3	13.8 <sup>c</sup> $\pm$ 3.5	4.3 <sup>c</sup> $\pm$ 1.1
	6	15.6 <sup>c</sup> $\pm$ 3.9	4.2 <sup>c</sup> $\pm$ 1.0
	45	53.3 <sup>a</sup> $\pm$ 5.0	20.3 <sup>a</sup> $\pm$ 2.4
Aging Temperature (°C)	55	19.1 <sup>d</sup> $\pm$ 5.5	8.2 <sup>b</sup> $\pm$ 2.4
Germination Temperature (°C)	15	37.3 <sup>d</sup> $\pm$ 7.6	13.8 <sup>a</sup> $\pm$ 3.2
	20	36.3 <sup>d</sup> $\pm$ 7.1	13.8 <sup>a</sup> $\pm$ 3.0
	25	35.1 <sup>d</sup> $\pm$ 7.0	15.2 <sup>a</sup> $\pm$ 3.3

\* Means with the same letter in each category are not significantly different at  $P = 0.05$ , according to Tukey's LSR test.

**Table 2. Three-way analysis of variance test for the effects of period and temperature of accelerated aging and temperature of germination on final germination percentages and germination rate of *C. album* seeds. ns= non significant at  $P = 0.05$ .**

Source of Variation	df	Final germination		Rate of germination	
		F-values	P	F-values	P
Aging period (P)	3	255.98	<0.001	202.2	<0.001
Aging temperature (T)	1	1958.37	<0.001	3346.2	<0.001
Germination temperature (GT)	2	1.46	ns	4.1	<0.05
P*T	3	117.85	<0.001	38.0	<0.001
P*GT	6	1.99	ns	1.9	ns
T*GT	2	1.28	ns	4.5	<0.05
P*T*GT	6	2.21	ns	1.9	ns
Error	48				

The aging of the seed is generally indicated by reduction in germination level and speed (Coin *et al.*, 1995; Rehman *et al.*, 1999). In the present study, the accelerated aging greatly reduced both final germination percentage and germination rate of *Chenopodium album* seeds. The final germination declined from 92% for the unaged seeds to 49.3%, 10.4%, 13.8% and 15.6% for seeds aged for one, two, three and six days, respectively. A similar result had been obtained by Chiu *et al.* (1995), who concluded that accelerated aging decreased the germination percentage and increased the mean germination time in watermelon seeds aged at 45 °C and 79% relative humidity for 6 days. French bean seeds were artificially aged after exposing to 42 °C and 100% relative humidity during one to 16 days. The germination percentage decreased from 100% at 0-4 days of accelerated aging to 22% at six days and finally to 0% after 16 days of aging (Begnami & Cortelazzo, 1996).

While most of the studies on accelerated aging showed a decrease in germination level with the increase in aging duration or aging temperature, the effect on germination rate was not consistent in different studies. Accelerated aging reduce germination rate in several species (e.g., watermelon, Chiu *et al.*, 1995; lettuce, Bradford *et al.*, 1993; cauliflower, Thornton & Powell, 1995), but decreased it in several other species (e.g., bahiagrass, Mullahey *et al.*, 1996; two *Acacia* species, Rehman *et al.*, 1999). In the present study, while germination rate of seeds aged for one day didn't differ from that of unaged seeds, it significantly decreased when seeds aged for longer time (two, three and six days). The germination rate for seeds aged at 45 °C decreased from 39.0 after one day of aging to 5.4 after two days, but unexpectedly increased again to 8.7 and 8.4 after 3 and 6 day of aging, respectively (Fig. 2). It seems that the duration of aging could be the important factor that responsible for the conflicting results between the different studies.

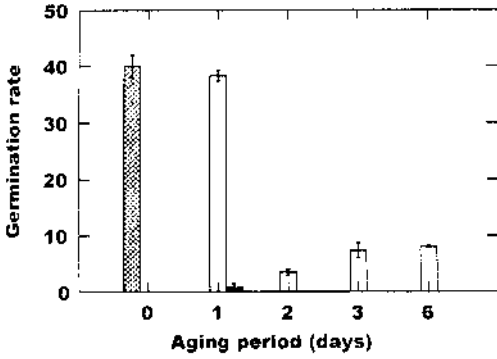
The present study showed a significant increase in germination of seeds aged for three days and six days at 45°C compared to those aged for only two days at the same temperature. Unexpectedly, the germination of seeds aged at 45°C for three and six days was 32.0% and 48.8%, respectively, greater than that of seeds aged for two days (Fig. 1). Veselova *et al.* (1999) arrived to a similar result when they subjected *Pisum sativum* seeds to accelerated aging for 16 days at 40 °C and 80% relative humidity. After 5-7 days, seed germination decreased from 80 to 72%. After 8-10 days the germination unexpectedly increased to 92%, but then decreased on further aging. Veselova *et al.* (1999) attributed this change to an increase in seed heterogeneity upon aging, which resulted in increase in the proportion of abnormal seeds in an initially uniform seed lot. It might be possible that the earlier periods of aging stimulated the formation of some kinds of heat shock proteins (HSP) in some seeds that increased their resistance to higher temperatures and humidity at later periods of aging. In another study for accelerated aging on the seeds of two *Acacia* species, aging seeds significantly reduced the final germination at 55 than at 80°C (Rehman *et al.*, 1999) They attributed the reduction of the germination at 55°C to the heavy fungal infection of the seeds at that temperature, whereas the higher temperature at 80°C killed or restricted the growth of microorganisms.

Zhang and Macdonald (1997) showed that the decline in relative humidity of the accelerating aging chamber led to decrease the seed moisture content and this resulted in less deterioration for the small-seeded plants. This emphasizes the importance of the availability of high relative humidity during soil solarization for weed control. Arora and Yaduraju (1998) showed that solarization significantly reduced the weed density in irrigated than un-irrigated soils. Ahmad *et al.* (1996) also indicated that seven weeks solarization of irrigated soils raised its temperature by 11.5°C over non-solarized soil at 10-cm depth and this effectively controlled 98.5% of the weeds. These findings further support the importance of relative humidity during soil solarization.

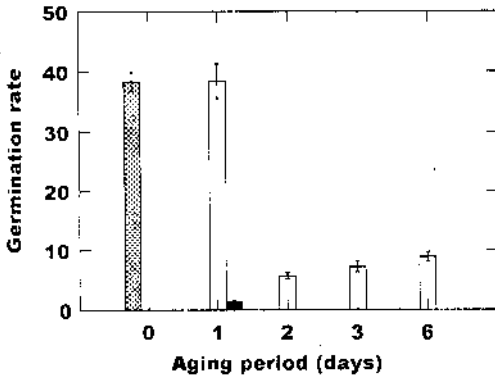
Germination of initially misted seeds of 10 common arable weeds, after heating at several temperatures and durations, was prevented by temperature of 75°C or higher for periods of 0.5 days or longer (Thompson *et al.*, 1997). These authors showed that the incubation of initially moistened

seeds of *Chenopodium album* for 16 days reduced their germination by 56% at 56 °C. In the present study, however, seed aging for two days at 55 °C resulted in complete deterioration of *C. album* seeds. This, again, support the importance of presence of high relative humidity for seed deterioration during the soil solarization. Further studies are important to determine the maximum temperatures and durations of aging that is required to prevent the germination of seeds of the different weedy species. These studies would determine the duration of solarization in irrigated soil that is required for controlling different weeds.

A: 15 °C



B: 20 °C



C: 25 °C

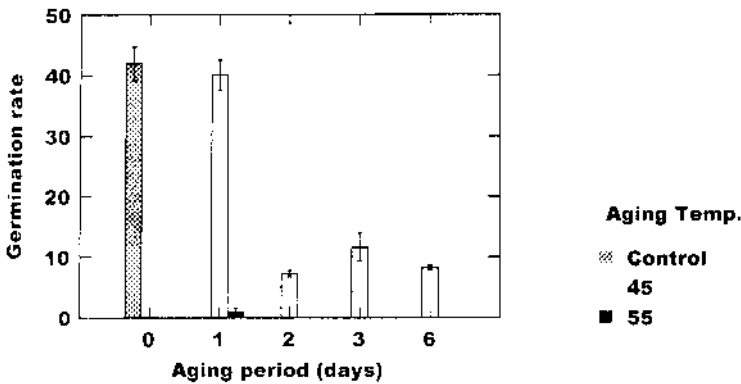


Fig. 1. Effects of period and temperature of accelerated aging and temperature of germination (A = 15, B = 20 and C = 25 °C) on final germination percentages of *Chenopodium album* seeds.

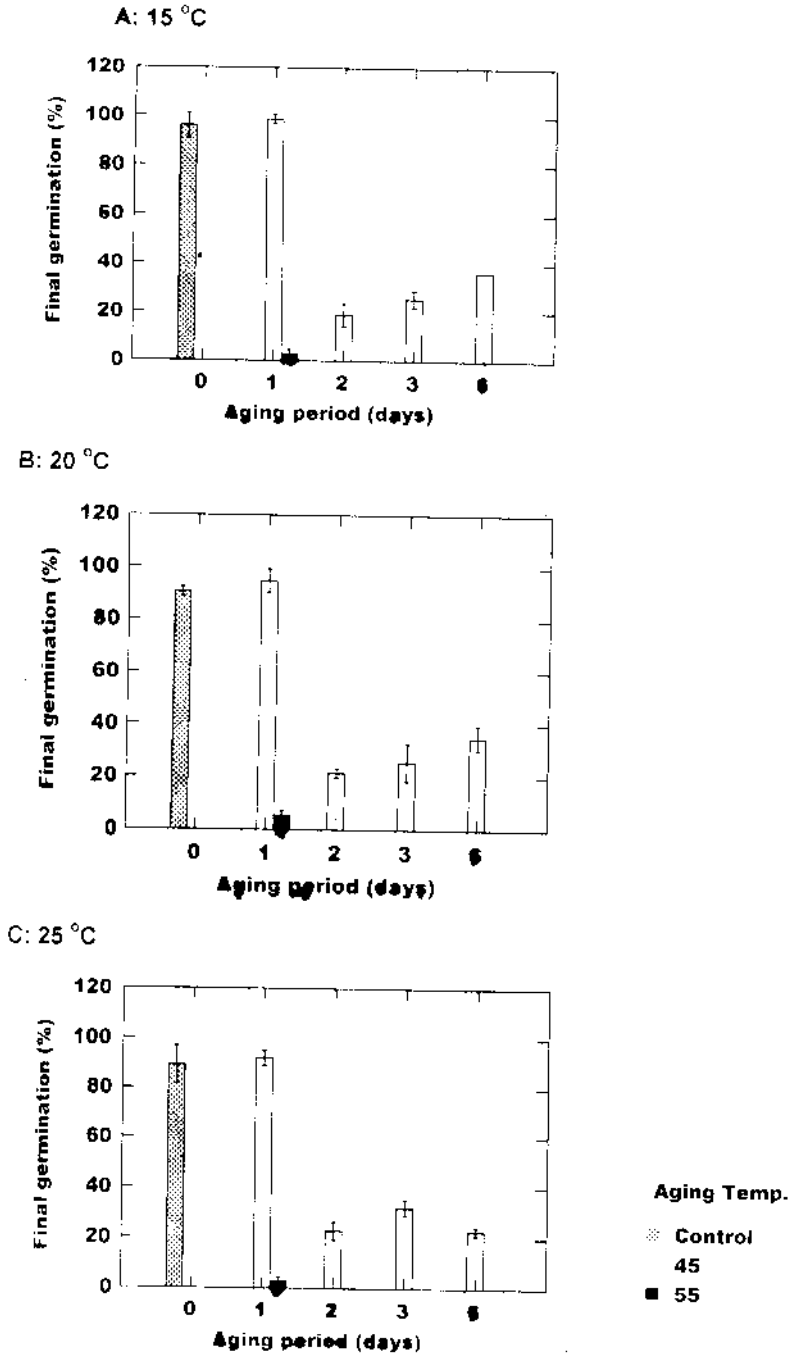


Fig. 2. Effects of period and temperature of accelerated aging and temperature of germination (A = 15. B = 20 and C = 25 °C) on germination rates of *Chenopodium album* seeds.



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