African Journal of Biotechnology Vol. 11(41), pp. 9782-9787, 22 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2531
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of seed collecting date and storage duration on seed germination in *Artemisia* spp.

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Accepted 9 November, 2011

***Artemisia* genus (*Asteraceae*) includes approximately 400 and 33 species in the world and Iran, respectively. The species have medicinal properties and is widely distributed in arid and semi-arid regions. Low seed germination due to some phenolic compounds especially in collected fresh seeds of *Artemisia* spp. has been reported. The objective of this study was to determine the effect of seed collecting dates (5 to 6 times from mid-November to early January, 10 days intervals) and seed storage duration (4, 8, and 12 months) at room temperature on seed germination of four *Artemisia* species (*Artemisia sieberi*, *A. diffusa*, *A. kupetdaghensis*, and *A. aucheri*) collected from ten different locations in north east of Iran during two years. The experiment was conducted as a completely randomized design with four replications and combined analysis was done as cross-nested design. The results showed that there were significant differences between seed collecting date for each site and seed storage duration and their interactions (*P* < 0.01). Seed storage duration had higher effect on seed germination than collecting date. Overall, mean seed germination percentages were 37.24, 29.07, 44.69, and 62.84% at 0, 4, 8, and 12 months storage, respectively (*P* < 0.01). The regression model for relationship between final germination percentage (y) with storage duration (x) was fitted ($y = 36.18 - 2.62x + 0.41x^2$, $R^2 = 96.4\%$). The results suggest that delay in seed collecting at late November through early December and seed storage durations of 8 or 12 months improved seed vigor and germination rate of *Artemisia* spp.**

Key words: *Artemisia* spp., seed germination, seed collecting, storage, regression model.

INTRODUCTION

Sagebrush (*Artemisia* spp.) is one of the most important genera dominated and widely distributed in Iran. *Artemisia* genus (*Asteraceae*) includes approximately 400 and 33 species in the world and Iran, respectively. Different species of Artemisia provide forage and habitat for wildlife and also prevent soil erosion (Wijayratne and Pyke, 2009). Sagebrush is a bunch perennial plant from *Asteraceae* family which is used for renovation of rangelands. Their aerial parts, especially fresh or dried leaves were used by traditional healers as antibacterial, anticholesterolemic and antiseptic (Zargari, 1997; Duke, 2001; Danesh et. al. 2010).Baskin and Baskin (2001) reported a physiological inhibiting mechanism of the embryo as the greats cause for seed dormancy in some *Artemisia* species. Sagebrush seeds hold full viability for 2 to 3 years in warehouse storage (6 to 8% relative humidity and less than 10℃) and germination rate documented to be higher than rates immediately after collecting, so are not long-lived (Wijayratne and Pyke, 2009). Seed may also go after ripening in dry storage or come out of dormancy over time. Laboratory germination trails indicated that seed dormancy in some *Artemisia* species may be habitat-specific. For example, *Artemisia tridentate* seed collected from colder sites being more dormant (Wijayratne and Pyke, 2009). Seeds *Artemisia rhodenta* produced in warm years germinated to higher rates and percentage than those produced in cold years (Baskin and Baskin, 2001).

Flowering stage, seed setting and seed ripening of some *Artemisia* species occurred in October, late November and mid-December, respectively. Mean sterile and fertile florets in each capitol were 78 and 22%,

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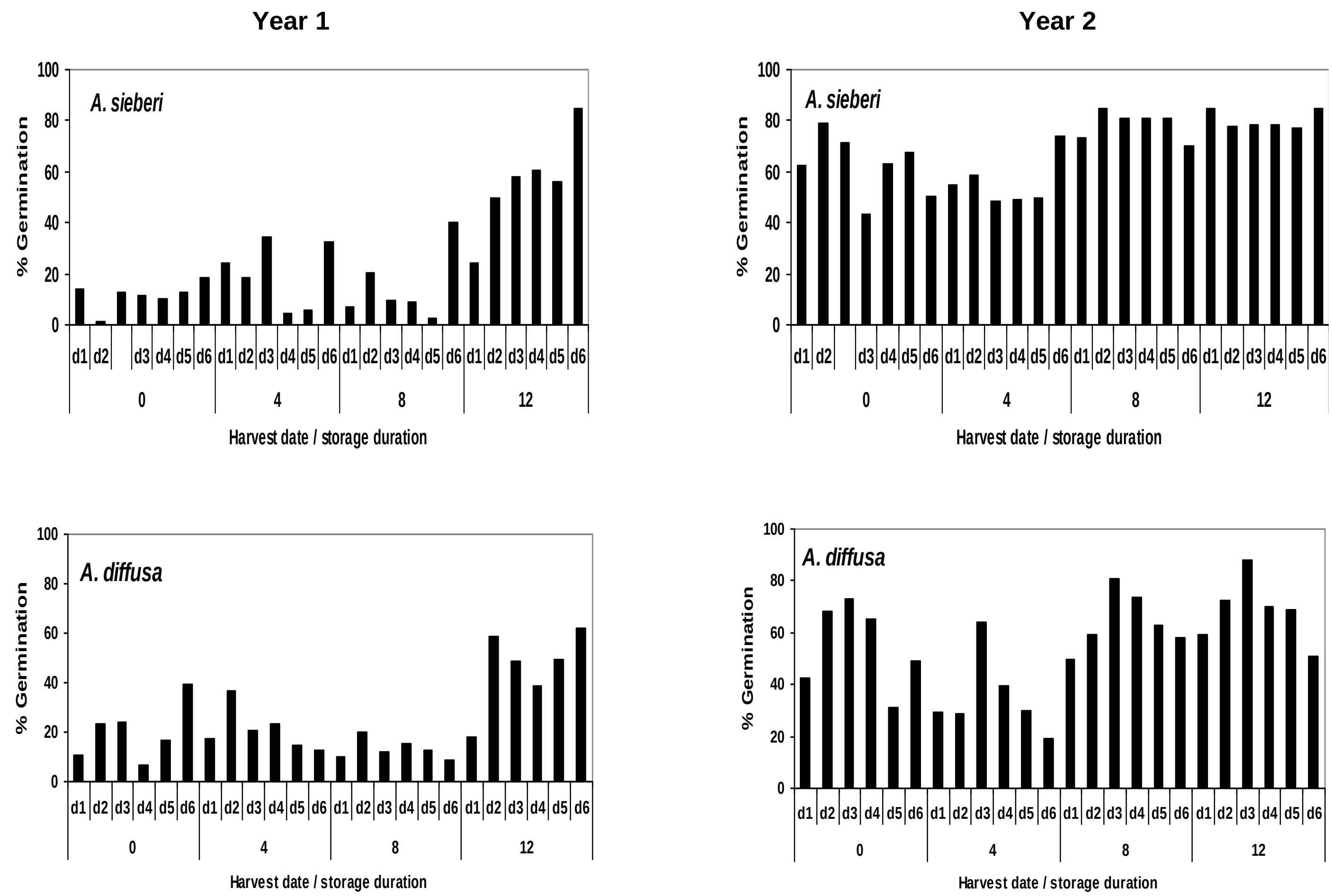

Figure 1. Interaction effects between seed collecting date (from mid-Nov. to early Jan, d1 - d6) and seed storage duration on FGP in *Artemisia* spp. during 1994 and 1995 in east of Iran. The same species such as *A. sieberi* and *A. diffusa* were averaged over different locations. Data not available for d5 and d6 dates in *A. kupetdaghensis* in second year because of seed shattering and also at d1 date in *A. aucheri* for both years due to the seed was not matured.

respectively in east of Iran (Gazanchian et al., 2008). They also reported that seed germination significantly affected different seed collecting locations and date. Balati (1992) in *A. transiliensis* under controlled environment reported that seed germination was affected by temperature, soil water content, light, seed storage and salt concentration.

Al-Charchafchi et al. (1988) also found that decline of seed germination in collected fresh seed of *A. scoparia* is due to dormancy and phenolic components in coverage of flower.

Kelesy et al. (1978) observed that water extracts of the litter of *A. tridentata* containing monoterpene which act as inhibitor for seed germination of sagebrush. Similar results have been reported for other plants (Chen and Leather, 1990; Duke et. al. 1987).

The objective of this study was to evaluate the interaction effects of seed collecting date of *Artemisia* spp. native to east of Iran and their storage durations under sub normal environmental conditions on seed germination and seed dormancy.

MATERIALS AND METHODS

Seeds of four species of *Artemisia* were collected during 1994 to 1995 from nine (first year) and ten (second year) locations of Khorassan, in east of Iran (Figure 1), which represent a wide range of geographical and environmental conditions. Seeds were collected at 10 days intervals from seed setting to seed shattering (6 time from mid November to early January) from all locations except for Sabzevar Norodab in first year (Table 1). Seed germinability was also measured after 4, 8, and 12 month; the seed storage at room temperature. Four replications of 20 seeds were used for each treatment. Seeds were germinated in an incubator at 18 and 12°C in first and second year under dark conditions, respectively. Seeds were considered to be germinated with the emergence of radical (3 to 5 mm). Not germinated seeds were recorded as seed dormant when the seed was as hard seed. In the first year, percentage of germination was measured 3 and 10 days after planting as primary germination percentage (PGP) and final

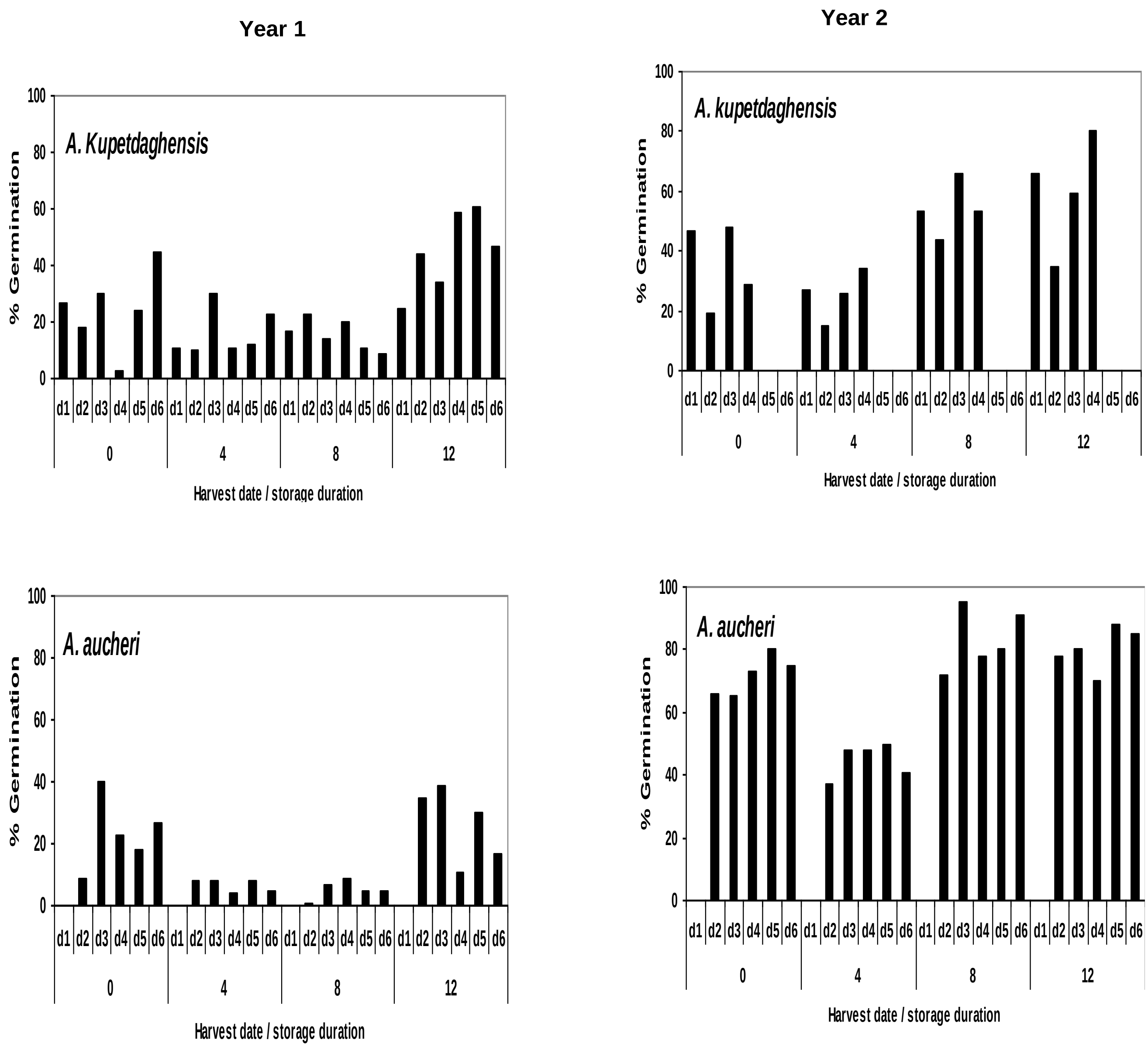

Figure 1. Contd.

germination percentage (FGP), respectively. But in second year, PGP and FGP were 5 and 15 days, respectively.

Data analysis

This experiment was conducted as a completely randomized design with four replications for assessing collecting dates and seed storage duration for each site per year.

Since, there were differences among species, locations and temperature (12 and 18°C); therefore, a combined analysis as nested design was conducted to analyze FGP. Mean of seed storage duration and collecting dates were separated by least significant differences method at 5% level for each location per year. A regression model was fitted to determine the relationship between FGP and seed storage duration by Curve Expert (*ver,* 1.3).

RESULTS AND DISCUSSION

Seed germination responses to collecting dates and storage duration

The results of variance analysis for PGP, FGP and dormant seeds revealed significant differences among collecting dates (from mid-November to early January) and seed storage duration (0, 4, 8, and 12 months) for each location in two years.

Based on mean of square, variation of the seed storage duration was 5 to 10 fold more than collecting dates for each location (data not shown). Seed germination percentages of all species were significantly different for

Table 1. The effect of seed storage duration (0, 4, 8, and 12 months) on seed germination of four *Artemisia* species, collected from ten locations during two years in east of Iran.

Location	Species	Altitude (m)	Seed storage duration (month)											
			0	4	8	12	0	4	8	12	0	4	8	12
			First count				Final count				Dormant seed			
Year 1														
Torbat-Cheshme Gol	*A. Siberi*	1100	3.6 ^{b}	6.2^{b}	3.2^{b}	24.4^{a}	6.6^{b}	8.0^{b}	6.2^{b}	43.6^{a}	93.4^{a}	92.0^{a}	93.8^{a}	56.4^{b}
Gonabad-Bimorgh	*A. Siberi*	950	1.0^{c}	6.4^{b}	11.0^{b}	28.0^{a}	23.8^{b}	13.6^{c}	9.4^{c}	38.2^{a}	76.2^{b}	86.4^{a}	90.6^{a}	61.8^{c}
Gonabad-Bajestan	*A. Siberi*	1100	2.2^{c}	6.6^{b}	13.4^{b}	29.8^{a}	4.4^{c}	14.0^{b}	16.2^{b}	58.4^{a}	95.6^{a}	86.0^{b}	83.8^{b}	41.6^{c}
Gonabad-Ostad	*A. Siberi*	1200	1.2^{c}	6.8^{b}	13.4^{b}	30.8^{a}	4.6^{d}	7.8^{c}	17.0^{b}	55.8^{a}	95.4^{a}	92.2^{b}	83.0^{c}	44.2^{d}
Sabzevar-Ghods	*A. Siberi*	1010	15.7^{b}	8.3^{b}	16.3^{b}	65.3^{a}	22.7^{b}	17.3^{b}	23.0^{b}	77.0^{a}	77.3^{a}	82.7a	77.0^{a}	23.0^{b}
Bojnord-Baghlegh	*A. diffusa*	1300	12.8^{a}	7.0^{b}	0.8^{c}	15.0^{a}	23.8^{b}	13.6^{c}	9.4^{c}	38.2^{a}	76.2^{b}	86.4^{a}	90.6^{a}	61.8^{c}
Bojnord-Ghorkhod	*A. diffusa*	1050	11.0^{ab}	12.7^{a}	8.8^{b}	11.7^{b}	20.7^{c}	30.8^{b}	18.5^{c}	51.3^{a}	79.3^{a}	69.2^{b}	81.5^{a}	48.7^{c}
Bojnord-Bio	*A. Kupetdaghensis*	1750	11.2^{ab}	9.5^{a}	4.2^{b}	9.8^{a}	24.5^{b}	16.2^{c}	15.7^{c}	45.0^{a}	75.5^{b}	83.8^{a}	84.3^{a}	55.0^{c}
Mashhad-Moghan	*A. aucheri*	1050	12.2^{a}	2.2^{bc}	0.8^{c}	6.4^{b}	23.4^{a}	6.6^{b}	5.4^{b}	26.4^{a}	76.6^{b}	93.4^{a}	94.6^{a}	73.6^{b}
Year 2														
Torbat-Cheshme Gol	*A. Siberi*	1100	56.0^{b}	44.8^{c}	74.0^{a}	69.2^{a}	56.0^{b}	44.8^{c}	74.0^{a}	69.2^{a}	44.0^{a}	49.4^{a}	22.8^{c}	30.6^{b}
Gonabad-Bimorgh	*A. Siberi*	950	52.3^{b}	41.8^{c}	75.5^{a}	76.2^{a}	52.3^{b}	41.8^{c}	75.5^{a}	76.2^{a}	47.7^{b}	55.2^{a}	22.7^{c}	23.3^{c}
Gonabad-Bajestan	*A. Siberi*	1100	53.5^{b}	42.7^{c}	67.7^{a}	64.2^{a}	53.5^{b}	42.7^{c}	67.7^{a}	64.2^{a}	46.2^{a}	53.3^{a}	28.7^{b}	30.3^{b}
Gonabad-Ostad	*A. Siberi*	1200	64.7^{b}	48.3^{c}	82.2^{a}	79.8^{a}	64.7^{b}	48.3^{c}	82.2^{a}	79.8^{a}	35.3^{b}	44.0^{a}	16.8^{c}	19.3^{c}
Sabzevar- Nordab	*A. Siberi*	950	74.2^{b}	55.3^{c}	82.2^{a}	82.0^{a}	74.2^{b}	55.3^{c}	82.2^{a}	82.0^{a}	25.8^{a}	33.0^{a}	17.3^{b}	18.0^{b}
Sabzevar-Ghods	*A. Siberi*	1010	85.1^{b}	77.3^{c}	92.3^{a}	92.2^{a}	85.1^{b}	77.3^{c}	92.3^{a}	92.2^{a}	3.8^{b}	5.5^{a}	1.9^{c}	1.9^{c}
Bojnord-Baghlegh	*A. diffusa*	1300	49.4^{b}	24.0^{c}	57.8^{a}	58.6^{a}	49.4^{b}	24.0^{c}	57.8^{a}	58.6^{a}	52.2^{b}	67.0^{a}	38.8^{c}	39.8^{c}
Bojnord-Ghorkhod	*A. diffusa*	1050	64.5^{b}	47.0^{c}	69.5^{ab}	79.5^{a}	64.5^{b}	47.0^{c}	69.5^{ab}	79.5^{a}	32.3^{b}	48.0^{a}	16.8^{c}	18.3^{a}
Bojnord-Bio	*A. Kupetdaghensis*	1750	35.8^{b}	25.5^{c}	54.0^{a}	60.0^{a}	35.8^{b}	25.5^{c}	54.0^{a}	60.0^{a}	64.3^{a}	69.5^{a}	43.8^{b}	39.0^{b}
Mashhad-Moghan	*A. aucheri*	1050	71.8^{b}	44.8^{c}	83.2^{a}	80.2^{a}	71.8^{b}	44.8^{c}	83.2^{a}	80.2^{a}	28.2^{b}	48.2^{a}	16.2^{c}	19.0^{c}

The mean value averaged over six dates for each storage duration. Means with similar letters within each row for each location and trait are not significant at $P < 0.05$.

all collecting dates (from d1 to d6) (Figure 1).

In this experiment, lower germination percentage (below 10%) were measured in the first counting when seeds were collected in November than December which reached up to 30% for both years (data not shown). It seems that low temperature play a key role for increased seed germination of *Artemisia* spp. This would support the result reported earlier by Meyer and Monsen (1992). The effect of collecting date on final germination was also significant. However, no significant differences were found for *A.*

Table 2. Mean square from combined analysis of variance as nested design for FGP as affected by year, location, collecting date and storage duration in *Artemisia* spp.

Source of variation	df	Mean of square
Year	1	338722.26**
Site (year)	17	4340.80**
Harvest date × Site (year)	79	457.12**
Storage duration (D)	3	27870.02**
D × Year	3	11941.87**
D × Site (year)	51	605.414**
D × Year × Date (site)	237	248.146**
Error	1176	52.26**

Table 3. The effect of seed storage duration on FGP for both year in *Artemisia* spp., mean of all treatments.

Seed storage duration (month)	First year	Second year
0	19.30^{d}	53.70^{b}
4	19.31^{d}	43.93^{c}
8	18.98^{d}	61.46^{a}
12	43.25^{c}	60.24^{a}
Mean	18.12 B	66.60 A

Means with similar letters for both years are not significant (P < 0.05.).

sieberi in FGP when tested immediately after collecting among all dates (d1-d6) in first year, but it remarkably increased when stored for 12 months. For example, the mean value in FGP for *A. sieberi* for all collection dates included (d1 - d6, from mid-November to early January) after harvesting; 0, 4, 8, and 12 months storage duration were 10.3, 17.5, 13.6, and 48.1%, respectively (Figure 1). However, in the second year, this species had high FGP in late November (d2) and early December (d3) and it increased for 8 and 12 months storage. Also, in the first year, both species *A. kupetdaghensis* and *A. diffusa* were responsive to collecting date and FGP being significantly higher for the final collecting date (Figure 1). However, seed shatter is also considerable in *A. kupetdaghensis* especially from late December.

These results show that seed germination enhanced about 30% in late seed collection than early time and with increasing seed storage duration, FGP increased for all species. However, a decrease in FGP and increase in dormant seed was measured after 4 month storage especially in second year (Figure 1). This decline could be related to inducing seed dormancy. The effect of seed storage duration (when averaged over six collecting dates) showed that germination percentage of all species were high for first count and FGP after seed storage duration of 8 and 12 months for both years except *A.diffusa* (Bojnord, Ghorkhod) and *A. aucheri* (Mashhad, Moghan) for first count in first year (Table 1). In contrast, dormant seed declined for all species for 8 and 12 months storage (Table 1).

Combined analysis

The results of combined analysis showed significant differences ($P < 0.01$) between years, location within each year and collecting date by location within each year (Table 2).

The effect of year and seed storage duration on FGP was the highest. Overall mean FGP was 18.12 and 66.60 for first and second year, respectively (Table 3). These results indicate that optimum temperature for seed germination was 12°C in the second year. Meyer and Monsen (1992) reported that cool temperature enhanced seed germi-nation, germination rate and vigor in big sagebrush populations.

Overall mean in FGP at 0, 4, 8, and 12 months storage ranged from 19.30 to 43.25% for the first year and from 53.70 to 60.24% for the second year (Table 3). Balati (1992) also reported that the optimum storage period for *A. transiliensis* was one year. A quadratic regression model was fitted for relationship between final germination (y) and seed storage duration (x) ($y = 36.18 - 2.62x + 0.41x^2$, $R^2 = 96.4\%$) (Figure 2). The regression equation showed that the FGP based on linear slope (X coefficient) 2.62% decreased per month up to fourth month of storage and then increased during 8 and 12 months storage (Figure 2).

Generally, the results suggested that delay in seed collection at late November through early December and seed storage duration for 8 or 12 months could be improved final germination percentage and germination rate

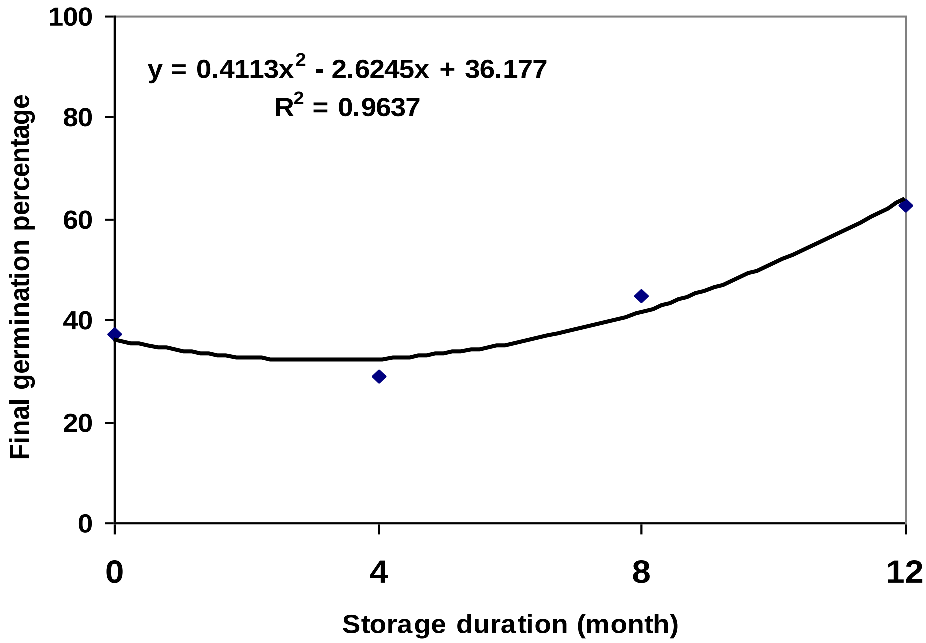

Figure 2. A quadratic regression model for the relationship between final germination (y) and seed storage duration (x) in *Artemisia species*.
Means with similar letters for both years are not significant ($P < 0.05$.).

in *Artemisia* spp.

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