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Germination Ecology of *Rhanterium epapposum* Oliv.

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Abstract: Problem statement: *Rhanterium epapposum*, locally called Arfaj is the national flower of Kuwait. The current situation of *Rhanterium epapposum* is quite critical in most areas of Kuwait. In fact it has completely disappeared from most parts of the country due to a combination of overgrazing, collecting for firewood and off road driving. **Approach:** *Rhanterium epapposum* is an excellent species for rangeland restoration and information on its germination ecology is meager. The considerable variations in germination between years lead us to study the variation in the size, mass and number of filled achenes in the capitulum. The variation in capitulum size, mass, number of achenes per capitulum and also the effect of dry heat (1-5 months), Moist chilling (1-5 months) and hydration dehydration cycles (1-5 cycles) on germination of the 6 different year collected *Rhanterium epapposum* (2002-2007) seeds were studied under laboratory condition. **Results:** The seed size, weight, the number of achenes per capitulum of *Rhanterium epapposum* varied considerably from one year to another. Climatic condition significantly decreased seed quality and germination when the precipitation is lower. Heat and moist stratification substantially improved the germination of 2003, 2004 and 2005 seeds. However hydration and dehydration treatment had very little effect on germination. **Conclusion:** It is concluded that results of germination tests differed depending on the presence of filled achenes in the capitulum. There was a strong correlation between the presence of filled achenes in capitulum and the precipitation during the growing season. This suggested that under natural condition precipitation plays key role in the formation of achenes in capitulum of the *Rhanterium epapposum* seeds.

Key words: *Rhanterium epapposum*, Capitulum, achenes, hydration and dehydration, moist stratification, heat stratification

INTRODUCTION

Kuwait is a small arid country situated at the north western corner of Arabian Gulf covering an area of about 18,000 Km (Brown and Al-Mazrooei, 2003). The summer is hot with maximum temperature reaches more than 45°C and the winter is cool with a mean temperature of 12.8°C. Rainfall is erratic with large temporal fluctuations. The vegetation is dominated by short lived annuals, dwarf shrubs and trees are largely absent. *Rhanterium epapposum* is the national flower of Kuwait and Arfaj is the common name for this plant. It is a bushy perennial aromatic shrub approximately 80 cm high with many stems branching out from the base. *Rhanterium epapposum* shed its leaves each summer and shoots out six weeks after the first autumn rain; the small yellow flowers appear in April giving the desert a golden sheen (Dickson, 1955). During the hot, dry summer months, *Rhanterium epapposum* looks completely dry and is leafless. A waxy bloom covers the vital parts and 'A thread of living tissues survives within the fibrous root stock during prolonged dry periods, thus allowing the plant to resume growth when it rains at last fall' (Vesey-Fitzgerald, 1957b). The dry flower heads, seeds of the 'Arfaj' bush are considered good grazing for sheep and in late summer are readily eaten by them (Dickson, 1955). *Rhanterium* is the characteristic and most abundant shrub over wide tracts of north and central Arabia mainly between 25°N (Riyadh) and 30°N (northern Kuwait border) (Vesey-Fitzgerald, 1957b). In Kuwait and adjacent areas, *Rhanterium* is found mainly on deep, sandy soils although it can occur on shallow substrates where it is sometimes accompanied by *Haloxylon salicornicum* (Halwagy *et al*., 1982). In *Rhanterium epapposum* the dispersal unit is the capitulum. In the capitulum the achenes are present only in the peripheral ray florets. The achenes are curved, glabrous without pappus.

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Sheep, goats, camels and hares as being instrumental in long-distance dispersal (Thalen, 1979). The capitula are shed at the end of the spring and remain in the ground throughout the summer. Seeds germinate after rainfall in the following autumn at the earliest and germination occurs whilst the achenes are still located in the capitulum, i.e., there is no dispersal of individual achenes. For germination to take place, the capituli must be positioned with their basal part on the ground, in an upright position. Dormancy over a number of years is a typical feature of the seeds (Thalen, 1979). Often, several seeds germinate at once in a capitulum and it appears that mortality of seedlings due to intra-specific competition may not be the norm. The current situation of *Rhanterium epapposum* is quite critical in most areas of Kuwait. In fact it has completely disappeared from most parts of the country due to a combination of overgrazing, collecting for firewood and off road driving (Brown and Al-Mazrooei, 2003). *Rhanterium epapposum* is an excellent species for rangeland restoration and information on its germination ecology is meager. The considerable variations in germination between years lead us to study the variation in the size, mass and number of filled achenes in the capitulum.

MATERIALS AND METHODS

Capitula of *Rhanterium epapposum* were collected from Sulaibiya, the southern part of Kuwait during the growing season for six consecutive years (2002, 2003, 2004, 2005, 2006 and 2007). The capitulum were dried and stored at room temperature (around 22°C) in Paper bags until use. Each capitulum contains 1-10 achenes. In nature the achenes are not dispersed individually, the germination occurs whilst the achenes are still attached in the capitulum. Hence the whole dispersal unit or capitulum was used for germination experiments.

Germination experiment: Seeds were sown on sand moistened with distilled water in 9 cm diameter disposable petridishes. Four replicates of 25 seeds per petridish were used for each treatment. Seeds were incubated in growth chamber under alternating temperature of 22/10°C with 14 h photoperiod the lowest temperature coinciding with the period of darkness. This temperature regime was chosen to mimic early winter day/night air temperature. Petridishes were distributed randomly within the growth chamber. Germination was recorded every 2 days until no germination was noted on 4 consecutive days. The seeds were considered germinated when the radical protrude to the length of 2 mm.

Table 1: Climatic data for the growing season 2001-2007

Growing season	Rain fall (mm)	Monthly average temperature (°C)
Oct 2001-June 2002	136.4	23.43
Oct 2002-June 2003	158.1	23.57
Oct 2003-June 2004	218.7	23.58
Oct 2004-June 2005	177.3	22.92
Oct 2005-June 2006	143.5	23.81
Oct 2006-June 2007	116.6	22.94

Capitulum weight, size, number of achenes per capitulum and climatic data of the years of collection: The mean mass of 5 groups of 100 capitulum was weighed and the mean length and width were measured to ascertain the average seed weight and size. Hundred randomly chosen capitula of each year were used and the achenes were extracted to determine the number of achenes per capitulum.

The climatic data as monthly values of temperature and precipitation (Oct-June) for the periods 2002-2007 were taken from the meteorological department, Kuwait International Airport. Temperature and precipitation values were taken from October to June, because the initiation of growth was observed during October and the seed collection was initiated in May. The climatic data of the growing season for each sampling year is mentioned in Table 1.

Dry heat: The seeds were placed in an oven at 50°C and the germination experiments were conducted after 1-5 months respectively.

Moist stratification: The seeds were placed on a moist substrate and kept at 5°C for different duration of time (1-6 months). The germination experiments were conducted after 1-5 months respectively.

Hydration dehydration cycles: *Rhanterium epapposum* seeds were exposed to different cycles of 3 days wetting and 3 days drying. This cycle was repeated for 5 times.

Statistical analysis: Statistical analysis was performed with the SPSS 12 statistical package. One way ANOVA was used for the statistical analysis of seed weight, number of achenes per capitulum and seed size. One way ANOVA was used to study the treatment effect and Duncan's multiple range tests to compare means at 5% level of probability. Difference in germination percentage between treatments and years were tested using two ways ANOVA.

RESULTS

The average seed weight and seed length (length F = 16.855, p<0.001) were significantly larger in lots of2005 than in lots of 2002, 2003, 2004, 2006 and 2007 respectively (Table 2). The number of achenes per capitulum (Fig. 1) were significantly different among the 6 seed lots (F = 85.534, p<0.001). Number of achenes per capitulum presents maximum values for the collection of 2004 and minimum value for the collection of 2002 and 2006 year (Fig. 1). Capitulum with filled achenes was abundant in the 2003, 2004 seeds because total precipitation during 2003 and 2004 were higher. The achenes were rare or not present in the capitulum collected during 2002, 2005 and 2006 (Fig. 1). Seed fill was only 3, 8 and 37% for seed harvested during 2002, 2006, 2005 and 2007 respectively. No correlation was observed between climatic data of the collection year and the size and weight of the seeds. Since the seed size and weight present a maximum value for the collection of 2005. Seed quality (the number of seeds per capitulum) varied from year to year.

Table 2: Average capitulum size, number of achenes per capitulum and capitulum weight of 6 lots of *Rhanterium epapposum* collected in different years

	Size/capitulum (mm)			
Year of collection	Length (mm)	Width (mm)	No. of achenes in 100 capitulum	Weight/lot (g)
2002	5.71bc	5.60a	8a	4.95b
2003	5.92c	5.43a	80d	4.88b
2004	6.21d	5.44a	93e	4.94b
2005	6.37d	5.59a	37b	5.83c
2006	5.60b	5.83a	3a	4.25a
2007	5.31a	5.68a	52c	4.72b

Values with same letter are not significantly different at the 5% level according to Duncan's Multiple Range test

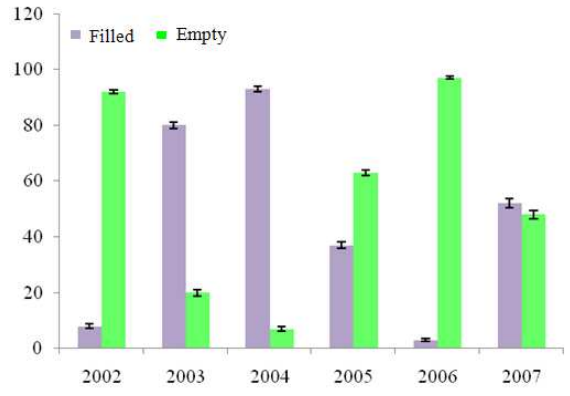

Fig. 1: The percentage of filled and empty achenes in the capitulum of 6 consecutive years collected *Rhanterium epapposum* seeds

An increase in length of dry heat storage period slightly increased germination (Table 3) of 2003, 2004 and 2005 seeds (F = 1.075, p = 0.407; F = 2.823, p<0.05; F = 2.187, p = 0.101). However it did not improved the germination percentage (Fig. 2) of 2002, 2006 and 2007 seeds (F =1.249, p = 0.328; F = 0.486, p = 0.782; F = 0.829, p = 0.546). Germination differed significantly between years and with in duration (F = 153.592, p<0.001; F = 6.698, p<0.001).

Table 3: Effect of dry heat storage on germination of 6 consecutive year collected seeds

	Germination (%)					
Duration of storage at 50°C (months)	2002	2003	2004	2005	2006	2007
0	3a	36a	60ab	5ab	0a	2a
1	4a	33a	42a	12b	1a	2a
2	2a	37a	51ab	8ab	1a	8a
3	7a	48a	61abc	5ab	0a	4a
4	3a	28a	68bc	3a	2a	5a
5	8a	43a	74c	12b	1a	5a
Significance[a]	NS	NS	*	NS	NS	NS

Values with same letter are not significantly different at the 5% level according to Duncan's Multiple Range test; [a]: The data were analyzed by Analysis of Variance (ANOVA); NS: Not Significant; *: Significance at p<0.05

Table 4: Effect of Moist chilling on germination of 6 consecutive year collected seeds

	Germination (%)					
Duration of storage at 5°C (months)	2002	2003	2004	2005	2006	2007
0	3a	38a	51bc	8ab	1a	10ab
1	0a	27a	30a	4a	1a	0a
2	4a	46ab	42ab	18bc	0a	5ab
3	0a	64b	56bc	17bc	0a	10ab
4	4a	46ab	59c	24c	0a	11b
5	3a	43ab	75d	12abc	0a	2ab
Significance[a]	NS	***	***	*	NS	NS

Values with same letter are not significantly different at the 5% level according to Duncan's multiple range test; [a]: The data were analyzed by Analysis of Variance (ANOVA); NS: Not Significant; ***, *: Significance at p<0.001 and p<0.05

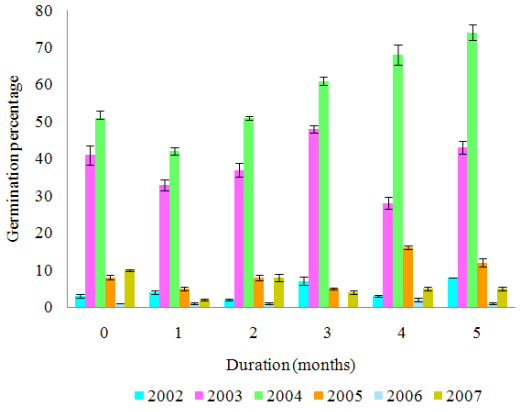

Fig. 2: Effect of heat stratification on germination of 6 consecutive year *Rhanterium epapposum* seeds

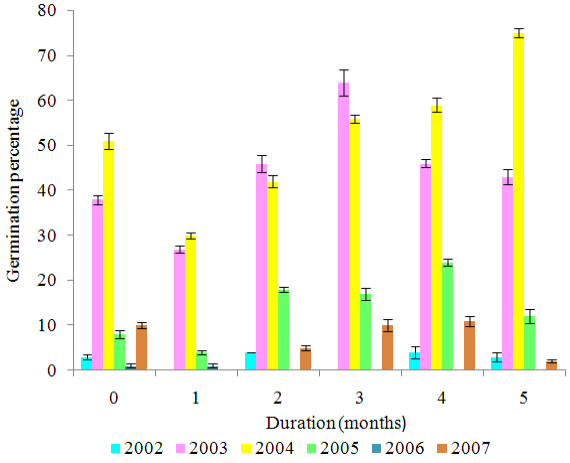

Fig. 3: Germination of 6 consecutive year *Rhanterium epapposum* seeds after varying length of moist stratification

Table 5: Effect of Wetting and drying on germination of 6 consecutive year collected seeds

	Germination (%)					
Cycle	2002	2003	2004	2005	2006	2007
0	3a	20a	51b	8a	1a	5ab
1	1a	37ab	59b	13a	1a	8b
2	3a	29ab	48b	14a	0a	0a
3	3a	43b	49b	7a	0a	7b
4	0a	42b	24a	12a	0a	0a
5	0a	41b	18a	6a	0a	1a
Significance[a]	NS		***	NS	NS	NS

Values with same letter are not significantly different at the 5% level according to Duncan's Multiple Range test; [a]: The data were analyzed by Analysis of Variance (ANOVA); NS: Not Significant;***, *: Significance at p<0.001, p<0.05 and p<0.1

Seeds collected during 2003, 2004 and 2005 showed that moist stratification significantly improved germination (Table 4) from an average of 38 to 64% ($p<0.001$), 51-75% ($p<0.001$) and 8-24% ($p<0.05$) respectively. In contrast, germination decreased in 2006 seeds but remained same in 2002 seeds (Fig. 3). Seed germination differed significantly between years and duration ($F = 179.277$, $p<0.001$; $F = 9.814$, $p<0.001$).

Drying and wetting cycles were ineffective in promoting germination (Fig. 4). Compared to the control the hydration and dehydration treatment slightly increased the germination percentage (Table 5) of 2003 seeds ($F = 2.480$; $p<0.05$). However in 2002, 2004, 2006 and 2007 seeds the germination percentage significantly decreased as the cycle is repeated ($F = 0.785$, $p = 0.574$; $F = 8.387$, $p<0.05$; $F = 0.200$, $p = 0.958$; $F = 4.387$, $p = 0.009$). Germination differed significantly between years and cycles ($F = 144.290$, $p<0.001$; $F = 4.556$, $p<0.001$).

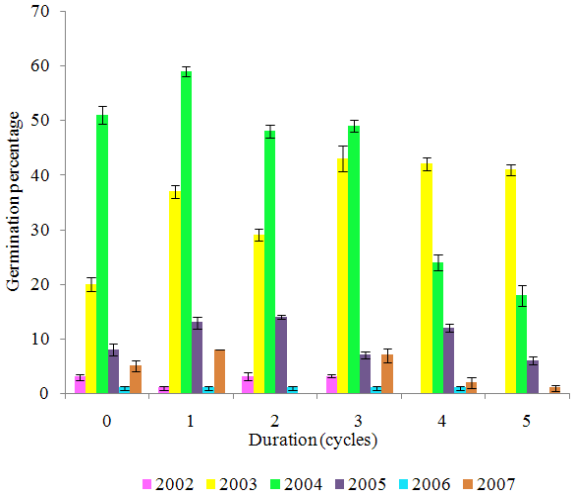

Fig. 4: Effect of hydration and dehydration cycles on *Rhanterium epapposum* seed germination

DISCUSSION

The seed size and weight of *Rhanterium epapposum* vary interannually and the germination is independent of size and weight. Similar results were obtained in *Medicago sativa* (Beveridge and Wilsie, 1959). The number of achenes per capitulum varied considerably from one year to another. The percentage of filled achenes ranges was greater during 2004 year than other years. This can partially be attributed to greater precipitation in 2004 than other years. The presence of fewer filled achenes in 2002, 2006 and 2005 is due to the subsequent drought through the rest of the year. Environment and genotype interactions control the production of achenes in subsequent year. This year to year variation suggested that these traits exhibit phenotypic plasticity. The substantial variation in precipitation during 2002-2007 year study may account for the observed temporal variation. In high rainfall years, *Rhanterium epapposum* plants produce large amount of viable achenes. The production of more achenes in each capitulum in good years than in poor years is that the genotypes best adopted to good years can make a larger contribution to the seed bank than those best adapted to poor years. Thus, the seed bank with its preponderance of seeds produced by the best genotypes in good years minimizes the genetic impact of seeds produced by the best genotypes in poor years and thus prevents the population from responding genetically to the environment each year (Levin and Wilson, 1978; Templeton and Levin, 1979). *Rhanterium epapposum* flower heads stored dry at room temperature were tested for germination after 4

years at room temperature gave 60% of germination, proving the exceptional longevity of the species under dry conditions (Thalen, 1979). Heat stratification had little effect on germination. However the germination percentages were positively related to the presence of filled achenes in capitulum. Germination of seeds collected in 2002, 2006 and 2007 was not affected by heat stratification. However stratification substantially improved germination of 2003, 2004 and 2005 seeds. The heat stratification at 2, 3, 4 and 5 months were significantly higher compared to control. In *Lepidium lasiocarpum* and *Eriophyllum wallacei*, 90 and 48% germination respectively were obtained after 2 weeks at 50°C (Gutterman, 1993).

Moist stratification treatment can either promote or hinder seed germination. Prolonged moist chilling (3, 4 and 5 months) promoted germination of 2003, 2004 and 2005 seeds. In contrast, germination remained same in 2002 and 2007 seeds. Two week cold stratification treatment increased the germination of *Celtis pallida* seeds (Fulbright *et al*., 1986).

Effects of hydration and dehydration cycles of *Rhanterium epapposum* seeds on germination have been varied. For instance, such treatments increased germination of 2003 seeds and slightly increased the germination of 2004, 2005 and 2007 seeds. However, in 2002 and 2006 seeds hydration and dehydration treatment did not have any effect on germination. Low germination after hydration and dehydration cycle suggested that such a treatment may not promote germination of *Rhanterium epapposum* seeds. In contrast, hydration-dehydration effect on germination may well be solved in *Rhanterium epapposum* by the mechanism that at the first rain only one or a few seeds germinate and produce seedlings which die when a dry period follows (Thalen, 1979). After seeds become non dormant in the field, they may be alternately imbibed and dried a number of times before conditions become suitable for germination. During continual showers new seeds may germinate, allowing at least some to achieve successful establishment. There were no detectable differences in dormancy between years although it is recognized that year to year variation in environmental conditions would have a big influence on the production of achenes in capitulum. *Rhanterium* needs regular rainfall over the years in order to thrive and this may account for its increasing rarity further south Arabia, where rainfall is lower and even less predictable than in Kuwait (Vesey-Fitzgerald, 1957a). *Rhanterium epapposum* showed that the seeds had none deep physical and physiological dormancy. The heat and moist cold stratifications are part of the sequence of environmental conditions required to break physiological dormancy. Tsuyuzaki (2006) proposed that the pattern of seed dormancy and survival were determined by soil temperature fluctuation patterns which decreased with increasing soil depth.

CONCLUSION

Germination of seeds collected in 2002, 2006 and 2007 were not affected by Pregermination treatments. However pretreatments substantially improved the germination of 2003, 2004 and 2005 seeds. Apparently, germination of this species differed from year to year. One possible explanation is annual variation in climate during seed formation which causes the production of less filled achenes in the capitulum. This study revealed that conclusion based on a particular year collected seeds of *Rhanterium epapposum* cannot characterize the germination behavior of this species. The knowledge about the germination of *Rhanterium epapposum* will contribute to a more efficient use of this species in degraded rangeland.

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