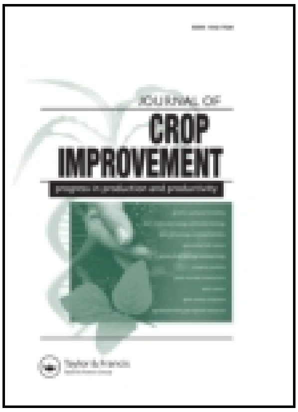

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Constant and Alternating Temperature Effects on Seed Germination Potential in *Artemisia annua* L.

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Artemisinin is a sesquiterpene lactone with a peroxide bridge, and it is a natural anti-malarial drug obtained from Artemisia annua *L. High germination percentages and rates are essential for commercial growers of this species to justify buying premium-priced seed to ensure high performance of their crop. The objective of our research was to determine the seed germination potential of* A. annua *variety 'CIM-Arogya.' An experiment was conducted using three constant (15°C, 20°C, and 25°C) and three alternating (20/15°C, 25/15°C, and 20/25°C) temperature regimes. Seed germination was evaluated in Petri dishes lined with filter paper under daily 16 h light and 8 h dark photoperiod. The highest estimates of mean percentage germination (82.0) and germination energy (20.5) were observed at 15°C, followed by 20/15°C (74.5% germination and 18.6% germination energy). Further, the 3 to 5-day period after seed sowing was ideal for the first germination count, and the 7 to 8-day period after sowing was best for final*

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germination count. Day 7 was best for final germination determination at both constant and alternating temperature regimes. Thus, growers should sow seeds when the mean temperature is 15–20°C for about 7 days.

KEYWORDS Artemisia annua*, Artemisinin, germination, temperature, seed quality*

INTRODUCTION

Quinghao *(Artemisia annua* L.; Family Asteraceae), a plant species endemic to China, is a natural source of the highly potent anti-malarial drug artemisinin (Bhakuni et al. 2001; World Health Organization 2006). *A. annua* is an annual herb/shrub cultivated in many parts of Asia, Africa, Europe, the United States, and Australia (Gupta et al. 2002). In India, commercial cultivation of *A. annua* variety CIM-Arogya has spread across a few of the northern provinces because of the efforts of the Council of Scientific and Industrial Research-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP) and pharmaceutical industries. Because *A. annua* is seed propagated, it is essential to assess seed quality to ensure high crop stand and herb yield.

Assuming that soil moisture is adequate, temperature is the most important abiotic factor controlling seed germination. Temperature effects can vary across varieties of a species and among seeds of the same species originating in different provenances (Bewley and Black 1994; Baskin and Baskin 2001; Yilmaz 2008; Verma et al. 2010; Kumar, Verma, and Singh 2011; Kumar 2012). The temperature that promotes the highest germination percentage and rate is referred to as the optimum temperature. In the natural habitat, seeds germinate under alternating temperature regimes, and various studies on the effects of alternating temperatures on seed germination have found that seeds of some species germinate only at alternating temperature regimes (Harrington 1923; Morinaga 1926; Goedert and Roberts 1986; Ellis and Barret 1994; Kebreab and Murdoch 1999; Baskin and Baskin 2001).

Thus, the purpose of our research was to determine the optimal temperature regime for highest germination so that cultivation could be further diversified in regions where maximum and minimum temperatures are either too narrow or too wide in the context of India. The specific objectives of our research were to determine: 1) the highest germination percentage of variety CIM-Arogya under different temperature regimes, 2) optimum temperature for germination, and 3) the amount of time required for seed to germinate, i.e., the time when full germination potential is achieved.

MATERIALS AND METHODS

Seed

Achenes (hereafter referred to as seeds) of *Artemisia annua* variety CIM-Arogya were collected in December 2011 from plants growing at the Central Institute of Medicinal and Aromatic Plants Resource Centre, Pantnagar, India. Seeds were stored at 27–39°C in paper bags for 5 months until germination experiments were initiated. During this time, any physiological dormancy in the seeds would have been broken via after-ripening.

Germination Test in Petri dish

Experiments were started in June 2012, using constant temperatures of 15, 20, and 25°C and three alternating temperature (10/14 h) regimes of 20/15°C, 25/15°C, and 20/25°C, in Petri dishes lined with filter paper. All seeds received 16 h light (180 lx)/8 h dark each day. The experiment was arranged in a completely randomized design with four replications. Observation on germination percentage, germination energy percent, and germination period were recorded and calculated, as suggested by International Seed Testing Association [ISTA] Rules (2010) and Kumar, Verma, and Singh (2011).

Statistical Analysis

At the end of the experiment, data were subjected to repeated measures analysis using the SAS System (the Mixed Procedure) and the estimates of different test parameters were computed. Temperature and number of days to counting were regarded as fixed factors. For comparing the estimates of different test parameters, least significant difference (LSD) at the 5% level was used.

RESULTS AND DISCUSSION

Only 82% seeds germinated and produced normal seedlings. Of the 18% non-germinated seeds, 10–12% germinated but produced abnormal seedlings, i.e., they either had radicle only or plumule only. Such seedlings died after 4–5 days of seed sowing. Thus, only 6–8% of the seeds were non-viable, i.e., they produced no seedlings at all.

The F-statistics presented in Table 1 reveal that temperature and number of days to counting and their interaction were highly significant. The germination percentage varied from day to day at different temperature

TABLE 1 Analysis of variance (ANOVA) for seed germination percentage (G) and germination energy (GE) of *Artemisia annua* variety CIM-Arogya under different temperature (T) regimes and number of days to counting (D) by REML (Fixed effects SE method: Model based) analysis

		Mean squares	
Effect	DF	G	GE
Temperature	5	3902***	244.13***
Error 1	18	20.95	1.3
Number of days to counting	5	3312***	207.4***
T X D	25	1109***	72.5***
Error 2	89	5.80	0.36

***Significant at probability level ($p \leq 0.001$).

regimes. There was no germination during the first two days at studied constant and alternate temperature regimes. A critical look at estimates reveals that alternate temperature of 20/15°C registered the highest germination percentage as well as germination energy overall; followed by constant temperatures of 20°C and 15°C. Similarly, for number of days to counting, day 8 had highest percentage of germination and germination energy, followed by day 7 and day 6 (Table 2). Further, data in Table 2 reveal that the highest percentage of germination and germination energy were achieved at 15°C on day 8th, though until the 4th day no germination was observed. The germination at other temperatures, though initiated as early as on the 3rd day, continued to progress with passage of time. The alternate temperature 20/15°C was the best for highest levels of germination potential (74.50% for germination and 18.625% for germination energy), but germination progressed slowly up to day 3. This observation provides a basis to hypothesize that, for initiation of the germination process, alternate temperature regimes, i.e., high and low are essential, while for sustaining the process, a minimum temperature of 15°C was invariably required. The above hypothesis is supported by other alternate temperature regimes, i.e., 20/25°C and 25/15°C, where germination on 3rd day was significantly higher than that for alternate regime of 20/15°C.

As far as number of days to counting is concerned, day 7 was the best for both constant and alternating temperature regimes (Table 2). Considering these two factors simultaneously, i.e., number of days to counting and temperatures, constant 15°C was the best temperature, followed by alternating 20/15°C. The germination period (i.e., the period during which the maximum number of seedlings could be obtained) was day 7-8 at constant as well as at alternating temperature regimes. Thus, it seems that temperature is a critical factor in the germination of *A. annua* seeds as in Asteraceae (Forsyth and Van Staden 1983), Poaceae (Verma et al. 2010), Acanthaceae (Kumar, Verma, and Singh 2011), and Lamiaceae (Kumar 2012).

TABLE 2 Estimates of mean germination percentage (G) and germination energy (GE) of *A. annua* variety CIM-Arogya at number of days to counting (D) under six temperature (T) regimes by the SAS System (Fit statistics: Type 3 tests of fixed effects)

	Number of Days to counting (D)													
	Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Mean	
Temperatures (T)	G	GE	G	GE	G	GE	G	GE	G	GE	G	GE	G	GE
15°C	**-16E-15**	**-444E-17**	**-391E-16**	**-444E-17**	**-0.252**	**-444E-17**	**71.50**	**17.8750**	**77.00**	**19.2500**	**82.00**	**20.5000**	38.3747	9.6042
20°C	**38.75**	**9.6875**	**43.25**	**9.6875**	**45.25**	**9.6875**	**46.75**	**11.6875**	**48.50**	**12.1250**	**48.50**	**12.1250**	45.1667	11.2917
25°C	**17.75**	**4.4375**	**22.50**	**4.4375**	**25.25**	**4.4375**	**28.25**	**7.0625**	**29.25**	**7.3125**	**29.25**	**7.3125**	25.3750	6.3437
20/15°C	**22.25**	**5.5625**	**70.00**	**5.5625**	**72.50**	**5.5625**	**74.25**	**18.5625**	**74.50**	**18.6250**	**74.50**	**18.6250**	64.6667	16.1667
25/15°C	**37.25**	**9.3125**	**38.75**	**9.3125**	**39.50**	**9.3125**	**41.75**	**10.4375**	**42.00**	**10.5000**	**42.00**	**10.5000**	40.2083	10.0521
20/25°C	**37.75**	**9.4375**	**39.25**	**9.4375**	**40.75**	**9.4375**	**45.75**	**11.4375**	**48.75**	**12.1875**	**48.75**	**12.1875**	43.5000	10.8750
Mean	25.625	6.406	35.625	8.906	37.166	9.302	51.375	12.844	53.333	13.333	54.167	13.542		
LSD 5% for T						2.78932 (G); 0.69274 (GE)								
LSD 5% for D						1.38821 (G); 0.34372 (GE)								
LSD 5% for T X D						3.9771 (G); 0.98588 (GE)								

The present study revealed that the growers *of A. annua* can germinate seeds at a temperature regime of about 20/15°C. They should be able to determine exact germination percentage of seeds on day 7, at which time they could begin to plan for transplanting on the basis of germinated seedlings.

Conclusions

Temperature affected the amount of time taken to germinate and germination percentage in *A. annua*. The seeds of *A. annua* variety CIM-Arogya showed highest germination at constant 15°C temperature, followed by alternating 20/15°C temperatures, with 82.0% and 74.5% estimates of mean percentage of germination and germination energy, respectively. Further, the 3 to 5-day period after seed sowing was ideal for the first germination count, and the 7 to 8-day period after sowing was best for final germination count. This information should be useful for growers as well as for researchers to determine exact germination potential at final count day and be able to determine the best time for transplanting of seedlings into the field.

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