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Fluctuating temperature and soil storage conditions modulate seed germination and dormancy behavior of *Parthenium hysterophorus* in the changing environment

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Understanding of dormancy and germination behavior is very important for effective weed management of invasive weeds on sustainable basis. This study investigated the germination and dormancy behavior of *P. hysterophorus* seeds under controlled conditions. *P. hysterophorus* seeds were evaluated in standard germination test at different alternate (day/night) temperatures (15/5 ˚C, 20/10 ˚C, 25/15 ˚C, 30/20 ˚C, and 35/25 ˚C). Later, dormant seeds of *P. hysterophorus* were buried in soil filled pots at different moisture conditions (dry, moist and fluctuating moist soil) and kept at different temperature regimes (10, 20 and 30 ˚C) for 150 days. The germination and dormancy behavior of *P. hysterophorus* of buried seed was investigated through standard seed germination test at one-month interval. Alternate temperatures showed significant effects on the germination pattern of *P. hysterophorus* and highest germination at 25/15 ˚C and lowest germination was observed at 15/5 ˚C. Similarly, different soil storage conditions significantly affected germination of *P. hysterophorus* with maximum germination was found under fluctuating soil moist conditions and less germination under dry soil conditions at different intervals of evaluation. However, storage temperature had no effect on germination response of *P. hysterophorus* for different storage intervals. Thus, fluctuating temperature (25/15 ˚C) and fluctuating moist conditions were found favorable for enhancing the germination potential of *P. hysterophorus* through releasing the dormancy behavior. The possible reasons for increase in germination was constant dry/wet fluctuating moisture which helped *P. hysterophorus* seed to release dormancy. The findings of present study may be helpful in development of timely, optimized and effective management strategies for *P. hysterophorus* under field conditions.

Keywords: *P. hysterophorus*, germination, dormancy, alternate temperature, weed management.

INTRODUCTION

Parthenium (*Parthenium hysterophorus* L.) as invasive weed is alarming threat for agriculture worldwide. It belongs to Asteraceae family and popular as aggressive herb in crop world (Tanveer *et al*., 2015). Parthenium has also emerged as rising threats in Asia including Nepal, Bangladesh, Vietnam, Sri Lanka, China, India and Pakistan (Shabbir and Bajwa, 2006; Barman *et al*., 2014). *P. hysterophorus* seed has dormancy behavior to support its survival and invasiveness even under strict environmental conditions. Its seed has maximum germination (100%) when harvested in a cool and dry winter season and low (0 to 7%) when harvested in rainy season because of low viability of seed and high seed dormancy risk in summer harvested weed seed (Javaid *et al*., 2010). About 12% viability in buried seeds of *P. hysterophorus* were observed after two years (Butler, 1984). Recently, it has been reported that *P. hysterophorus* seeds can germinate between 15 and 25 ˚C and emerge from a depth of 0-3 cm (Matzrafi *et al.*, 2021).

Seed dormancy significantly disturb the rate and percentage of germination (Batlla and Benech-Arnold, 2007; Bochenek *et al*., 2007; Batlla and Benech-Arnold, 2010) and environmental factors i.e. temperature, moisture and light affect dormancy, germination and emergence behavior of a seed (Merritt *et al*., 2007). Degree of dormancy of any seed is totally dependent on its strengthen and level at any specific time. In fact, seed of different plant species need rest period

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that have complex mechanism creating uncertain germination problem and rendering their re-use in restoration programs (Merritt *et al.*, 2007; Turner *et al.*, 2013). Moreover, seed dormancy not only affect germination time but also disturb plant life history later (De Casas *et al*., 2012; Huo *et al*., 2016).

Climatic factors have strong evidence to affect mechanism of dormancy in preventing untimely seed germination and likely for seed longevity in soil seed bank (Nguyen *et al*., 2017). The seeds of many weedy species have some type of dormancy, whether unable to germinate directly even when grown under favorable conditions (Baskin and Baskin, 1998). High and low temperature period or some environmental factors including moisture and combination of these factors play important role by changing physical or chemical conditions of the seed to break dormancy to germinate (Baskin and Baskin, 1998). Dormancy level of a specie is changed with time likely on a continuous and gradual basis as they can adjust it with different environmental factors which control the reactions occurring in the environment (Vleeshouwers *et al*., 1995; Baskin and Baskin, 1998). This knowledge about germination and dormancy can be helpful for weeds prevalence in the respective environment and may be supportive for developing and to predict the various effective strategies for their control (Forcella *et al*., 1993; Mortimer, 1997; Benech-Arnold *et al*., 2000). Germination pattern can be well defined and predicted through population-based models under a wide range of conditions (Bello and Bradford, 2016; Bradford, 2018) and are successfully employed to predict germination response in various crops (Bello and Bradford, 2016) and weedy species (Huo *et al*., 2016), consequently helpful for weed management.

Thus, understanding the dormancy behavior of *P. hysterophorus* may be helpful about the invasiveness of this aggressive weed. No doubt, much work has been done on biology of *P. hysterophorus* (Dogra *et al*. 2011; Dogra *et al* 2009; Kohli *et al*., 2009; Kohli *et al*., 2009) but focus of present study is on ecology for its invasion and spread in different agro-ecological conditions through dormancy breaking under changing environmental conditions. Therefore, it is necessary to find ecology based timely weed management of *P. hysterophorus* on sustainable basis. The present study studied the dormancy behavior and germination pattern of *P. hysterophorus* under different alternative temperature and soil moisture to assess the optimum time and favorable environmental conditions. The purpose of this study is to underpin the viability pattern of *P. hysterophorus* seeds under given storage and temperature conditions.

MATERIALS AND METHODS

Fresh achenes (hereafter referred to as "seeds") of *P. hysterophorus* collected from surroundings of Ayub Agricultural Research Institute (AARI), Faisalabad and University of Agriculture, Faisalabad, Pakistan during August, 2019 were sun-dried and cleaned manually. Air dried *P. hysterophorus* seeds having moisture content below than 12 % were kept in plastic box and stored at room temperature (25 ˚C). The required moisture percentage was achieved through repeated drying and then weighed by digital balance (Kern ALJ 220-4). While *P. hysterophorus* seeds collected during rainy season (August) showed risk of dormancy (Javaid *et al*. 2010) as observed with less germination percentage. Therefore, initial quality of seed was determined before conducting the experiments according to International Seed Testing Association (ISTA) protocol. The seed with 30-38% germination and 10.6% seed moisture contents were observed as dormant behavior.

Experiment 1**:** *P. hysterophorus* seeds were taken from storage plastic box and counted on seed counter according to desired quantity (25 seed per petri dish i.e. 9 cm diameter). Ten petri dishes were used per treatment and each petri dish was considered as one replicate. Different alternate temperatures (day/night) viz 15/5 ˚C, 20/10 ˚C, 25/15 ˚C, 30/20 ˚C, and 35/25 ˚C were standardized for germination of *P. hysterophorus*. Experiment was conducted in Plant Physiology Lab at Ayub Agricultural Research Institute, Faisalabad by using programmable growth chamber (GC-600TLH Tsukuba, Japan). Seeds were considered as germinated at the time of emergence of visible radicle having 2 mm length. The counting of germinated seeds was started from 2^{nd} day on daily basis and continued up to 14^{th} day for final germination count (Ardiarini *et al*., 2021).

Experiment 2**:** Dormant seeds (10 g) were taken from storage plastic box and sealed in 5×5-cm nylon mesh bag. Nylon bags with seeds were buried up to 5 cm in soil filled pots (8 cm diameter and 18 cm length) collected from field. After burying, these soil filled pots were kept in a thermo-gradient table at Seed Physiology Lab, University of Agriculture, Faisalabad, Pakistan. This thermo-gradient table was consisted of an aluminum plate having size of 75 × 75 × 3.5 cm with four channels running toward the edges. Different temperatures were maintained under these chambers at a time as cool and hot water is pumped through the channels with the help of two circulating water baths used to create temperature gradient. The temperature level in different chambers was maintained through validation by keeping the thermometer in these chambers.

Seeds were stored at different temperature regimes (10, 20 and 30 ˚C) for the period of 150 days under different soil storage conditions i.e. dry, moist and fluctuating moist (30 days in moist soil and 30 days in dry soil) [moisture level were maintained by gravimetric method]. After this, germination tests were conducted at 30, 60, 90, 120 and 150 days interval to record final germination count and ultimately dormancy behavior of *P. hysterophorus* weed. For germination test, data was recorded starting from 2^{nd} days to final germination count at 14^{th} day of the experiment. Soil filled pots were maintained at 50% field capacity on daily basis and required quantity of water was added when required according to soil storage conditions.

Both experiments were arranged in completely randomized design (CRD) with ten replications. Different soil storage conditions and temperature were considered as different factors affecting the final germination percentage and ultimately dormancy behavior of *P. hysterophorus*. Data of response variables collected from the experiment were analyzed by using data analysis software Statistix v.8.1 and mean values were compared by using Tuckey's test at P ≤ 0.05.

Time to complete 50% germination (days). The time to attain 50% germination (T_{50}) was calculated by using the formula of Coolbear *et al*. (1984).

$$T_{50} = ti + \frac{(N/2 - ni)(tj - ti)}{(nj - ni)}$$

Whereas, N is the final germinated seeds while nj and ni indicate the cumulative number of seeds germinated by adjacent counts at times tj and ti, respectively, where ni <N/2 < nj.

***Mean germination time (days)*:** The data regarding mean germination time (MGT) was calculated according to Ellis and Roberts (1981):

$$MGT = \sum Dn / \sum n$$

where n is the number of seeds that were germinated on day D, and D is day when counted from the start of germination.

Thermal time model / Probit Analysis: For experiment 1, germination percentage was determined through repeated probit analysis using population based threshold models (Bello and Bradford, 2016; Vahabinia *et al.,* 2019). Then cumulative germination percentage was transformed to probit and regressed against time log (Steinmaus *et al*., 2000) and germination time (t_g) was also taken according to Steinmaus *et al*. (2000). Similarly, rate of germination (GR) was calculated through the inverse of germination times for every percentile under each temperature. The estimated parameters values under thermal time were achieved through plotting germination rate against temperature for each percentile. Then repeated probit analysis was used for calculating the exact parameters of thermal time for the whole population.

RESULTS

Germination potential of P. hysterophorus seeds under different alternate (day/night) temperatures (Exp.1): Alternate (day/night) temperature treatments significantly affected the seed germination and germinated index (Fig. 1). Maximum germination (85.60%) was recorded when incubated at alternate temperature (day/night) of 25/15 ˚C and minimum was recorded at 15/5 ˚C and 35/25 ˚C alternate temperatures.

Similarly, maximum germination index (72.6) was recorded when incubated at alternate temperature of 25/15 ˚C and minimum germination count was recorded at 15/5 ˚C and 35/25 ˚C alternate (day/night).

The effect of alternate (day/night) temperature was observed significant for mean germination time (MGT) and time taken to 50% germination (T_{50}) (Fig. 2). Minimum MGT and T_{50} were recorded 6.30 days and 1.90 days respectively at mean temperature of 20 ˚C. For the alternate (day/night) temperature (15/5 ˚C), both MGT and T_{50} values were higher with 7.4 days and 5 days respectively. On average, fewer days were observed for mean germination of *P. hysterophorus* seed when incubated at fluctuating temperature (day/night) of 25/15 ˚C and 30/20 ˚C.

Thermal time modeling for P. hysterophorus weed at mean temperature of (day/night): Model parameters for *P. hysterophorus* weed were calculated based on mean temperature. The parameters θ_T, T_b (50), σ_{Tc} and R^2 had values of 3.09 ˚C h, 7.5 ˚C, 0.75 ˚C and 0.662 respectively (Table 1). These model parameters are helpful in predicting the germination pattern of *P. hysterophorus* under varying alternate (day/night) temperature. Data clearly showed that germination of *P. hysterophorus* weed declined at lower mean temperature as compared to higher mean temperature. The percentage of germinating seeds increased gradually with increase in day and night temperature (Fig. 3a). Thermal time (˚C days) for *P. hysterophorus* seed germination reduced as mean temperature increased indicating higher germination required low thermal time (°C days) at 20-25 °C. Similarly, more time or thermal time was required for germination of *P. hysterophorus* seeds at lower temperature (Fig. 3a).

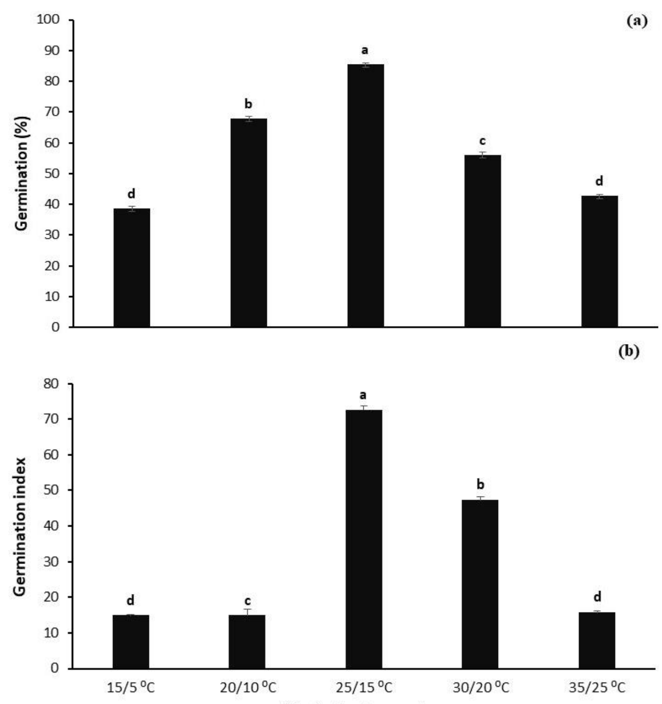

Figure 1. Effect of fluctuating temperature (Day/night) on (a) germination (%), (b) germination index of *Parthenium hysterophorus.* Vertical bars

showing different letters are significantly different. Values are means ± standard error.

Table 1. Model parameters for thermal time model describing germination of parthenium seeds at various mean temperatures of day and night

Temperature (˚C)	θ_T (˚C h)	$T_{b(50)}$ (˚C)	σ_{Tc} (˚C)	R^2
Mean temperature (day/night) (10, 15, 20, 25)	3.09	7.5	0.75	0.662

θ_T = Thermal time constant, $T_{b(50)}$ (˚C) = Base temperature, σ_{Tc} (˚C) = Standard deviation of base temperature, R^2 = Regression value

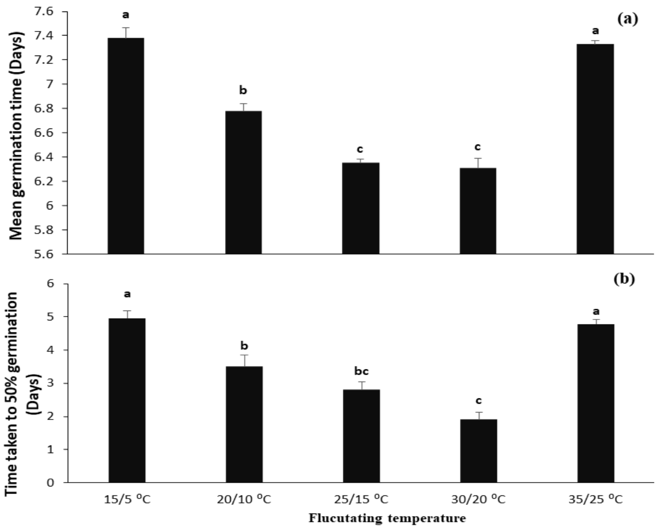

Figure 2. Effect of fluctuating temperature (day/night) on (a) mean germination time, (b) time taken to 50 % germination of *P. hysterophorus*. Vertical bars showing different letters are significantly different. Values are means ± standard error.

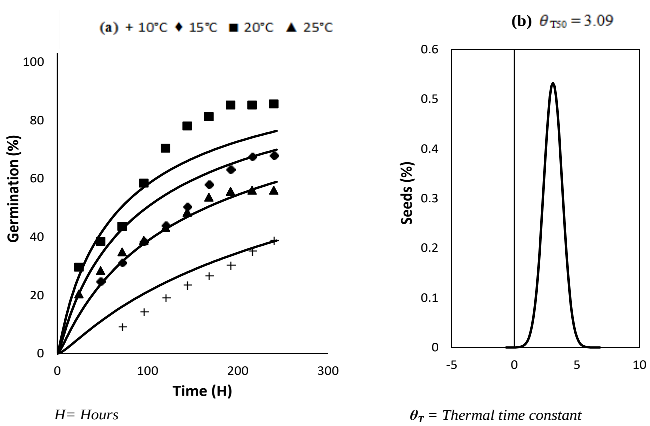

Figure 3. (a) Germination curve for parthenium seeds at mean temperatures (day/night) by using thermal time modelling approach under controlled environment, (b) Model distribution showing the relative frequencies of $T_{b50)}$ values at mean temperature of (day/night) under controlled environment.

Germination (%) of P. hysterophorus seeds after storage period of 30 days (Exp. 2): Storage temperature (10, 20 and 30 ˚C) had no significant effect on the germination% of *P. hysterophorus* (Supplementary Table 1). However, storage conditions (dry, moist and fluctuating moist) have sound effect on germination with highest germination (59%) was found with fluctuating moist condition (Fig 4). Poor germination (47%) was observed in dry soil storage condition. Similarly, interactive effect of different temperatures and soil storage conditions had also significant impact on germination and dormancy behavior of *P. hysterophorus* (Supplementary Table 1).

Germination percentage of P. hysterophorus seeds after storage period of 60 days: The observed data exhibit that minimum germination (43.33%) was recorded from *P. hysterophorus* seeds when stored under dry soil storage condition while maximum germination (72%) was observed when stored at fluctuating moist soil condition at different levels of temperature (10, 20 and 30 ˚C). This was followed by the germination (60%) of *P. hysterophorus* seeds stored under moist soil storage condition at each temperature (Fig.4). Similarly, interactive influence of storage condition and temperature showed that seed buried in fluctuating moist condition had higher germination percentage at each temperature while minimum germination for seed which buried in dry soil condition and followed by germination percentage of *P. hysterophorus* seed buried under moist soil storage.

Germination percentage of P. hysterophorus seeds after storage period of 90 days: The maximum germination (73.33%) *P. hysterophorus* seeds was observed in seeds buried in soil with fluctuated moist conditions and low germination (43.33%) when buried and stored under dry soil at all levels of temperature i.e. 10, 20 and 30 ˚C (Fig. 4).

In interactive effect of temperature and soil storage condition, maximum germination percentage was found when seeds of *P. hysterophorus* buried and stored at fluctuating moist soil condition at all temperature levels. While low germination of *P. hysterophorus* seeds was recorded in dry soil. Likely maximum germination of *P. hysterophorus* seeds was found when storage temperature was 30 ˚C compared to parthenium seeds stored at 10 and 20 ˚C with identical germination.

Germination percentage P. hysterophorus seeds after storage period of 120 days: Maximum germination (73.33%) was recorded *P. hysterophorus* seeds that were buried and stored under fluctuating moist condition at all temperatures regimes while minimum germination (42.66%) in seeds buried and stored under dry soil at different temperatures regimes (Fig. 4). Similarly, due to interactive effects of soil storage and temperature, the germination of *P. hysterophorus* seeds were found more under fluctuating moist condition at each level of temperature and followed by seed stored under moist soil condition at all temperature level. The minimum germination percentage was observed when *P. hysterophorus* seeds were buried and stored in dry soil condition at all temperature level (10, 20 and 30 ˚C).

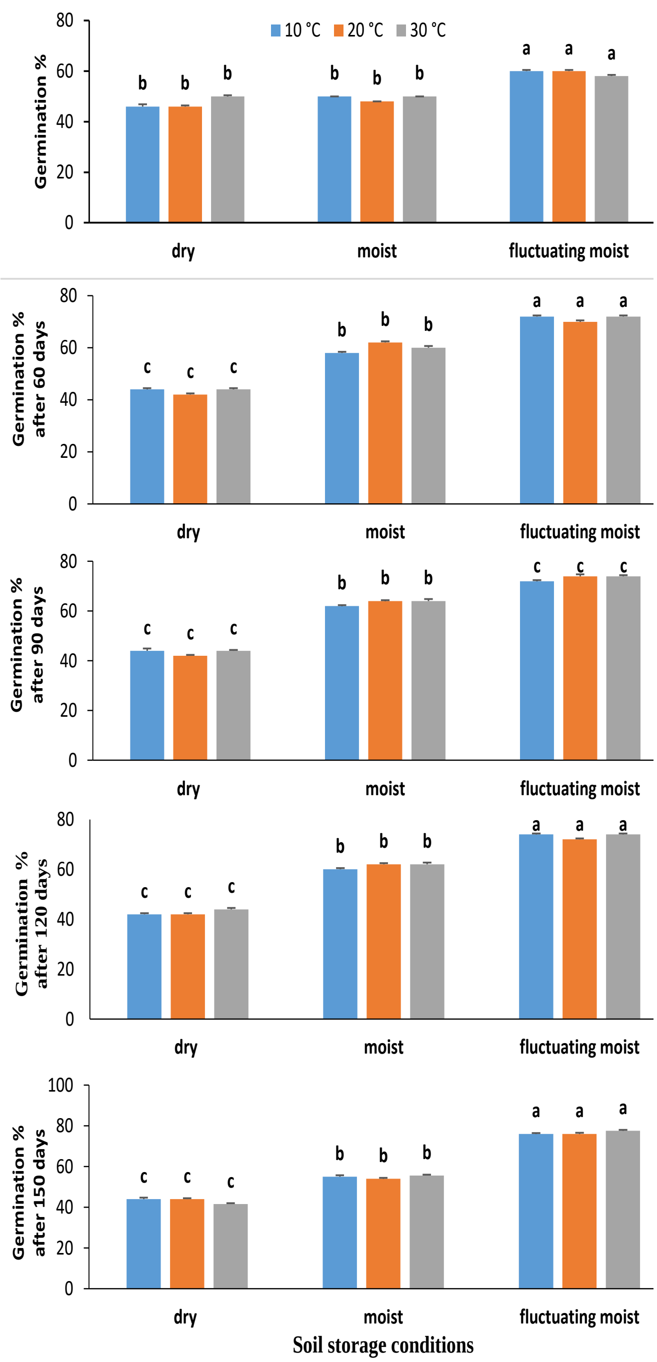

Figure 4. Effect different soil storage conditions and temperature on germination and dormancy pattern of parthenium weed at 30, 60, 90, 120 and 150 days interval of storage. Values are means ± standard error.

Germination (%) of P. hysterophorus seeds after storage interval of 150 days: The highest germination (76.50%) of parthenium seeds was recorded under fluctuating moist condition and followed by seed stored and buried in moist soil condition. While lowest germination (43.16%) were observed in dry soil conditions at different storage temperatures i.e. 10, 20 and 30 ˚C (Fig. 4). Similarly, it was also observed from results of the interactive effect that maximum germination was found under fluctuating moist soil condition while minimum count was observed in dry soil condition at all temperature levels (10, 20 and 30 ˚C).

DISCUSSION

Parthenium *(Parthenium hysterophorus)* weed seed has ability to germinate in the wide range of temperature (Bajwa *et al*., 2016). The present study investigated the germination potential of parthenium seeds at five different alternate (day/night) temperatures. Results showed that parthenium seeds germinated at each temperature regimes providing evidence that weed can germinate under wide range of temperatures (Bajwa *et al.*, 2018). Maximum germination was found at alternate (day/night) of 25/15 °C and 30/20 °C (Fig.1). Earlier, Tanveer *et al*. (2015) observed highest germination rate of parthenium at moderate favorable climatic conditions with optimum temperature of 22-25 ˚C. Other studies also found wide range of temperatures from low to higher level for germination ecology of *P. hysterophorus* as evident in present study (Nguyen *et al.*, 2017; Adkin *et al.*, 2019). This shows potential of *P. hysterophorus* not only germinate during summer as well during cool season with temperature of 20/10 and 15/5 ˚C (Johns *et al*. 2013; Adkins and Shabbir, 2014). The germination behavior of *P. hysterophorus* indicates that temperature is the main climatic factor responsible to enhance biological invasion of this specie (Bajwa *et al*. 2017). The **v**ariations in MGT and T_{50} under fluctuating temperatures regime indicate the adaptation and germinability of parthenium under harsh environment (Bajwa *et al*., 2016; Adkin *et al*., 2019). Nonetheless, this behavior of *P. hysterophorus* may be reflected towards management and control strategies in many regions (Shackleton *et al*. 2019; Shrestha *et al*. 2019).
The base temperature (7.5 ˚C) and thermal constant (3.09 ˚C h) of *P. hysterophorus* proved to be an effective and successful method for predicting germination response of this species (Table 1 and Fig. 3b) and may be supportive to predict the germination behavior of other plant species (Bradford, 2002). Thermal time models can be helpful to predict germination behavior of *P. hysterophorus* as evident in present study which is only possible when base temperature (T_b) is available (Royo-Esnal *et al*. 2010). Furthermore, thermal time constant, optimum temperature (T_o) and ceiling temperature (T_c) calculated by thermal time model for

Supplementary Table 1. Analysis of variance (ANOVA) for germination (%) of parthenium seeds at different soil storage conditions and storage temperature under controlled environment (Lab condition) for storage interval of 30, 60, 90, 120 and 150 days.

SOV[a]	DF[b]	Germination (%) after 30 days	Germination (%) after 60 days	Germination (%) after 90 days	Germination (%) after 120 days	Germination (%) after 150 days
Soil condition (S)	2	496.000***	2487.11***	2800.00***	2865.78*	3433.33***
Temperature (T)	2	5.333NS	1.78NS	0.11NS	7.11NS	0.33NS
S × T	4	3.333*	9.78*	4.78*	4.44*	6.67*

NS= Non-significant, *= Significant at $P \leq 0.05$, **= Significant at ≤ 0.01, *** Significant at ≤ 0.001, a= SOV= Source of variation, b= DF= degree of freedom

different weed plant species including *P. hysterophorus* may be helpful to assess the germination behavior under various constant and mean temperatures (Royo-Esnal *et al*., 2015).

From ecological viewpoint, germination behavior of any plant species is interlinked with dormancy pattern. Moreover, seed dormancy has been shown to affect not only germination time but also disturb plant life history with passage of time (de Casas *et al*., 2012; Huo *et al*., 2016). The present findings clearly showed that *P. hysterophorus* has dormancy behavior like other weedy plant which are produced during rainy season in summer month especially July and August (Javaid *et al*. 2010). This is also evident from present study from germination and dormancy behavior of *P. hysterophorus* as supported by Batlla and Benech-Arnold (2007) that many environmental factors involve in regulating the germination and dormancy pattern of weedy plant species.

Highest germination of *P. hysterophorus* seeds found under fluctuating moist condition may be due to the influence of soil condition as alternate dry wet conditions play important role in breaking dormancy. This is also supported by Batlla and Benech-Arnold (2007) and Xiao *et al*. (2011) in which these studies reported higher germination when seed were stored at fluctuating moist conditions that may be due to release of dormancy in weedy species. Similarly, wet condition also showed increasing trend of germination with increasing storage period of *P. hysterophorus* seeds.

This behavior of dormancy and germination will be further helpful for about the weeds invasion of a specie and further can be used to predict and develope the various effective control strategies for weeds (Forcella *et al*., 1993; Mortimer, 1997; Benech-Arnold *et al*., 2000).

***Conclusions*:** Thermal model curve predicted that germination percentage of *P. hysterophorus* weed was maximum at optimum temperature (20 ˚C) and has maximum capacity to germinate under high/low (day/night) temperature (25/15 ˚C). The possible reasons for highest germination under constant dry/wet fluctuating moisture may be associated with dormancy releasing of *P. hysterophorus* seeds.

***Conflicts of Interest*:** All authors contributed equally and declared no conflict of interest.

***Authors' Contribution Statements*:** M.A. and I.A. were involved in conceptualization, methodology, data analysis and original draft preparation, H.M.A supervised the work and A.T. and G.M. had reviewed and edited this manuscript.

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