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Original Article

The Influence of Temperatures on Germination and Seedling Growth of Pyrethrum (*Tanacetum Cineraiifolium*) under Drought Stress

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ABSTRACT

Pyrethrum is a perennial daisy grown commercially for the insecticide that is extracted from its flowers. In order to evaluate germination and seedling growth of pyrethrum under different temperature and drought stress treatments, the one year experiment was conducted in seed technology laboratory of Faculty of Agriculture of Islamic Azad University of Isfahan branch in 2018. During this experiment, polyethylene glycol (PEG) at 6 levels (0, -0.2, -0.4, -0.6, -0.8, -1.0 MP) and two temperature treatments (10 °C and 15 °C) were used. Two separate factorial trials, on the basis of absolute randomized design with three replications were used. Drought stress impact on germination percentage, mean time germination, shoot and root length, abnormal germination, germination uniformity, plumule dry and fresh weight, root dry and fresh weight and seed stamina index was significant ($P \leq 0.01$). The influence of temperature on germination percentage, mean time germination, shoot and root length, plumule dry weight, plumule fresh weight, root dry weight and root fresh weight was significant ($P \leq 0.05$).The rate of germination, germination percentage, as well as seedling growth and establishment were significantly lowered with the rise of stress levels using PEG. Control treatment and 15 °C had obtained the highest germination percentage, mean time germination, shoot and root length, germination uniformity, plumule dry and fresh weight, root dry and fresh weight, and seed stamina index. Taking all traits into account, a clear understanding of the germination responses of seeds is useful in screening for tolerance of species to extreme temperatures.

Key words: Drought stress, Germination, Pyrethrum, Seedling growth, Temperature.

Introduction

The seed germination, growth, development and spatial distribution of plants are severely restricted by a variety of environmental stresses (Soleymani and Shahrajabian, 2012; Ogbaji *et al*., 2013; Shahrajabian *et al*., 2018; Safdar *et al*., 2019; Shahrajabian *et al*., 2019a; Shahrajabian *et al*., 2019b; Shahrajabian *et al*., 2019c; Shahrajabian *et al*., 2019d). Drought stress influence the plants in a similar way; reduced water potential is a common consequence of salinity and drought, and both are harmful during early stages of germination and seedling growth (Soleymani and Shahrajabian, 2011; Soleymani *et al*., 2011; Amini *et al*., 2012; Shahrajabian *et al*., 2013; Soleymani and Shahrajabian, 2013; Shahrajabian and Soleymani, 2017; Shahrajabian *et al*., 2017; Soleymani and Shahrajabian, 2017). Pyrethrum which also called chrysanthemum is a perennial with white-yellow flowers that grows to a height of about 60 cm (Zito *et al*., 1983; Kiriamiti *et al*., 2003). It is the most important plant species of the genus *Tanacetum*, the botanical family *Asteraceae* (Delac *et al*., 2018). The common name pyrethrum is used for a number of plant species, including the Dalmatian pyrethrum (*Tanacetumcinerariifolium* (*Trev*.) *Schulz-Bip*. *Syn. Chrysanthemum cinerariifolium* (*Trev*.) *Vis*.) and Persian pyrethrum (*Tanacetumcoccineum syn. Chrysanthemum coccineum Wild*.). It belongs to the Asteraceae family and is cultivated on high lands of tropical and subtropical regions of the world on in lowland regions with temperate climates without frost, but with cool night temperatures which induce blooming (Li *et al*., 2011). Pyrethrum is a native of the Adriatic coastal mountains of Croatia, Bosnia and Herzegovina and has been grown as a crop in Europe, Asia, Africa, New Guinea and S. America (Pethybridge *et al*., 2006; Sladonja *et al*., 2014). It is considered as a 21 st century insecticide (Sladonja *et al*., 2014). Crushed and powdered Chrysanthemum plants were used as an insecticide by the Chinese as early as 1000 BC. The demand for natural insecticides (Erturk *et al*., 2004; Ebadollahi *et al*., 2013) as well as demand for pyrethrum as a botanical insecticide is rising rapidly in the world market (Andreev *et al*., 2008; Li *et al*., 2011; Grdisa *et al*., 2009). A pyrethrum crop is established by direct seeding or by planting in the fields as seedlings. Direct seed sowing is not very successful for a number of reasons including the very small size of the seeds with low and uneven germination and the slow growth of the seedlings. (Sladonja *et al*., 2014) found that seed germination was greatly influenced by soil texture, foremost silt percentage, and soil pH. Medicinally, a pyrethrum root has a pungent efficacy in promoting a free flow of saliva, in relieving toothache, in alleviating chronic catarrh and acne (Rassayana, 2003). The rate of germination usually increases linearly with temperature, within a well-defined range (Ramin, 1997; Phartyal *et al*., 2003). (Phartyal *et al*., 2003) found that this temperature has been defined as having cardinal temperatures, *i.e*., a minimum of base temperature (T_b), maximum temperature (T_M) below and above which the germination rate is zero and optimum temperature (T_o) at which the germination rate is highest. Seed priming has proved to be a successful strategy to reduce the adverse effects of salt and drought stress, and improve the germination percentage and uniformity of emergence in crops and grass (Kaya *et al*., 2006; Li *et al*., 2011). Seed priming is the process of regulating germination by managing the temperature and seed moisture content in order to maximize the seed's potential; it can improve germination rate, reduce time of germination and seedling emergence and improve plant establishment (Heydariyan *et al*., 2014). Water deficit not only affects seed germination, but also increase mean germination time (Willenborg *et al*., 2004). Polyethylene glycol (PEG) is an osmotic chemical which is used to induce water stress, is a non-ionic water polymer, which is not expected to penetrate into plant tissue rapidly (Kawasaki *et al*., 1983; Kaya *et al*., 2006; Li *et al*., 2011). (Mehra *et al*., 2003) showed that the PEG molecules did not enter the seeds, and, hence had no toxicity effect on seeds. Researchers concluded that seed soaking in solutions of PEG was expressed as sowing seeds in an osmotic solution that permits seed to absorb water for germination, but inhibits radical extension via seed coat (Golzardi *et al*., 2010; Soleymani and Shahrajabian, 2018). This research was conducted to study the influence of PEG and temperature on seedling growth traits of pyrethrum.

Materials and methods

This trial accomplished in seed technology laboratory of Faculty of Agriculture of Islamic Azad University of Isfahan (Khorasgan) branch, Isfahan in 2018 (latitude 32°40/N, longitude 51°58/N, and 1570 m elevation). During this experiment, polyethylene glycol (PEG 6000) at 6 levels (0, -0.2, -0.4, -0.6, -0.8, -1.0 MP) and two temperature treatments (10 °C and 15 °C) were used. Two separate factorial trials, on the basis of absolute randomized design with three replications were used. Seeds were germinated at constant temperatures ranging at 10 °C and15 °C in Petri dishes, lined with two layers of moist germination paper which was wetted periodically with distilled water when required. Thermo Gradient System was used in this experiment. In each level, 30 seeds were selected and sterilized in sodium hypochlorite (0.5%) for 30 seconds and then washed in distilled water for three times. During experiments, the seeds were surface sterilized by being soaked in 3 minutes in 1% solution of carbandazime fungicide, and then washed with distilled water and dried. A number of 30 seeds were placed on filter paper and put into Petri dishes. The filter paper was moistened with 5 mL of test solution. To make the moist environment the bottom of Petri dishes were covered with sterile tissue. The dish, then transferred to germinator in appropriate conditions. A seed scored germinated when radical length reached 2 mm. Germinating seeds were counted daily, and terminated when no further germination occurred.

Germination percentage (GP) was calculated using the following formula (Scott *et al*., 1984):

$$GP = \frac{Total seeds germinated\ (When no further germination occured)}{Total number of seeds} \times 100$$

Mean germination time (MGT), which expressed as speed of germination, was calculating using the following modified forumula:

$$MGT = \sum \frac{NiTi}{\sum Ni}$$

Where, Ti is the number of days after sowing, Ni is the number of seeds germinated on the first day (Shoor *et al*., 2014).

Germination rate (GR) was calculating using the following formula:

$$GR = \sum \frac{N}{\sum(n \times g)}$$

Where, n is the number of germinated seed on growth day and g is the number of germination seeds (Ellis and Roberts, 1981).

Germination uniformity was calculated by using following formula:

$$CGU = \frac{\sum n}{\sum[(\bar{t} - t)^2 \times n]}$$

Where, n was the number of geminated seeds in each day, t and $\bar{t}$ were the number of days from beginning of germination and the average of germination time, respectively.

Seed stamina index was calculated by using the following formula:

$$SSI = \frac{G \times (SHL + RL)}{100}$$

Where, G is germination percentage, SHL is average of shoot length, and RL is average of root length (Abdul-Baki and Anderson, 1970). Mean radical and plumule lengths are the end of germination were measured per replication with a millimeter ruler. To obtain radical and plumule, seedlings were placed for 48 h at 70 ^{o}C, in the oven and then the dry was measured with a laboratory balance with an accuracy of 0.0001 g. Analysis of variance (ANOVA) was used to determine the significant differences. Duncan Multiple Range Test was used for the separation of means (5% level probability). All statistics were performed with MSTAT-C program.

Results and discussion

The influence of temperature was meaningful on germination percentage, mean time germination, shoot length, root length, plumule dry weight, root dry weight, plumule fresh weight and root fresh weight. However, the effect of temperature on abnormal germination and seed stamina index (SSI) was not significant. Polyethylene glycol had significant influence on all experimental characteristics, namely germination percentage, mean time germination (MGT), shoot length, root length, abnormal germination, germination uniformity (GU), plumule dry weight, root dry weight, plumule fresh weight, root fresh weight and SSI. (Khodarahmpour, 2011; Soleymani and Shahrajabian, 2018) also mentioned that germination percentage, root and shoot length, plumule dry and fresh weight significantly influences by drought stress induced by polyethylene glycol (PEG). The interaction between temperature and PEG had no significant influence on experimental characteristics (Table 1). (Soleymani and Shahrajabian, 2017) also mentioned that germination and seedling development of cumin cultivars was affected by PEG.

The highest germination percentage (47.33%) and mean time germination (10.05) was related to 15 ^{o}C which had meaningful difference with 10 ^{o}C. Desirable crop yield are achieved by providing seeds with an environment that encourages early germination and emergence (Soleymani and Shahrajabian, 2012; Soleymani *et al.*, 2012). Other scientists also concluded that among several environmental factors, temperature is the single most factor governing the maximum germination percentage and rate of germination (Heydecker, 1977; Phartyal *et al.*, 2003). The higher values of shoot length (0.56 cm), and root length (0.35 cm) were obtained for 15 ^{o}C, followed by another treatment. There were significant differences between these two treatments on root and shoot length. Although, the higher value for abnormal germination (43.82%), and germination unity (0.3) was achieved in 15 °C, no significant difference was found between 10 °C and 15 °C. The higher plumule dry weight (4.05 mg), and plumule fresh weight (123.34 mg) was related to 15 °C, which had significant difference with another treatment. Treatment of 15 °C had obtained the higher value of root dry weight (2.31 mg), root fresh weight (39.89 mg) and SSI (0.56), which had meaningful differences with 10 °C.

The higher values for germination percentage (69.4%) and mean time germination (10.55) were related to control treatment, followed by other treatments. The minimum germination percentage and mean time germination was 13.87% and 6.66, respectively, which was related to -1.0 (MP) application of PEG. There were significant differences between -1.0 (MP) PEG and control treatment on germination percentage and mean time germination. The result of this experiment is consistent with results of (Sayar *et al*., 2010; Soleymani and Shahrajabian, 2018), who affirm that growth medium salinity or drought induced by PEG may affect seed germination. Seed germination and early seedling growth are sensitive to salt and drought stress in many crop species (Ashraf and Foolad, 2005). (Soleymani and Shahrajabian, 2018) also reported that PEG has been used to control water potential in seed germination studies to assess plant drought tolerance at germination and seedling stages. The highest and the lowest shoot length was achieved in control treatment 0.84 cm, and application of -1.0 (MP) PEG (0.14 cm), which had meaningful differences with each others. Like shoot length, the maximum root length also achieved in control treatment (0.84 cm), and it had significant differences with the minimum one which was application of -1.0 (MP) PEG (0.04 cm). (Soleymani and Shahrajabian, 2018) found that drought stress induced by PEG prevented radical extension in cumin. The higher abnormal germination was related to -1.0 (MP) PEW, followed by -0.8 (MP), -0.6 (MP), -0.4 (MP), -0.2 (MP) and control treatment, respectively. Although, the difference between application of -1.0 (MP) and -0.8 (MP) PEG was significant, both of these two treatments had no meaningful differences with other treatments. (Li *et al*., 2011) reported that PEG is an effective tool to improve the quality of pyrethrum seeds. The maximum and the minimum germination uniformity was 0.69 and 0.09, respectively, which had significant difference with each others. All differences between control treatment and other treatments on germination uniformity were significant. The higher values of plumule dry weight (6.07 mg), and plumule fresh weight (180.92 mg) was related to control treatments followed by application of -0.2 (MP), -0.4 (MP), -0.6 (MP), -0.8 (MP) and -1.0 (MP) PEG, respectively. There were significant differences between the maximum plumule dry and fresh weight (control treatment) with the minimum one (-1.0 MP PEG). The maximum root dry weight (3.38 mg) and root fresh weight (59.16 mg), respectively which was obtained for control treatment. Application of -1.0 (MP) PEG had obtained the lowest value for root dry and fresh weight. Besides, all differences between control treatment and application -1.0 (MP) PEG was significant. (Soleymani and Shahrajabian, 2018) also noted that root and shoot length and seedlings fresh and dry weight of maize cultivars were decreased by increasing PEG. The higher value for seed stamina index was achieved in control treatment (1.04), followed by -0.2, -0.4, -0.6, -0.8 and -1.0 (MP) PEG, respectively. The difference between control treatment and the minimum SSI value which was related to application of -1.0 (MP) PEG was meaningful (Table 2). Water shortage, due to drought, is the most significant abiotic factor limiting plant and also crop growth and development. With increasing drought stress, root and shoot length decreased (Bayoumi *et al*., 2008).

Conclusion

Inappropriate and poor germination and seedling establishment are two major problems and these characteristics are considered to be important factors in later plant growth and yield. Temperature treatment had significant effect on germination percentage, mean time germination, shoot and root length, plumule dryand fresh weight, root dry and fresh weight. The higher values of germination percentage, mean time germination, shoot and root length, abnormal germination, germination uniformity, plumule dry and fresh weight, root dry and fresh weight and SSI was related to 15 ^{o}C compare to those for 10 ^{o}C. The prevailing soil temperature determines both the fractions of seeds in a sample, which germinate and the rate at which they germinate, and for completion of phase of development, plants require a particular sum of temperatures that may be different for every development period. A clear understanding of the germination responses of seeds is useful in screening for tolerance of species to extreme temperatures. Drought stress impact on all experimental characteristics was meaningful. But the interaction between temperature and usage of polyethylene glycol had no meaningful influence on experimental characteristics. The rate of germination, germination percentage, as well as seedling growth and establishment were considerable lowered with the rise of stress levels using PEG. Control treatment had obtained the highest value of germination percentage, mean time of germination, shoot and root length, germination uniformity, plumule dry and fresh weight, root dry and fresh weight, and seed stamina index (SSI). Environmental stressed such as drought damage plants during germination which damage to their qualitative and quantitative performance and causing weakness and heterogeny in plant growth. Taking all traits into account, this experiment found that 15 ^{o}C is the best temperature for germination and seed establishment of pyrethrum. Germination and seedling establishment from laboratory does not necessarily mean that germination and seedling emergence from the field soils. To meet the increasing demand, the importance of study of PEG and temperature on germination and seedling growth of pyrethrum is undeniable.

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Table 1. Analysis of variance for experimental characteristics under osmotic potential (ψ) of PEG solutions

S.O.V	d.f.	Germination percentage	Mean time Germination	Shoot Length	Root Length	Abnormal Germination	Germination Uniformity (GU)	Plumule dry weight	Root dry weight	Plumule fresh weight	Root fresh weight	SSI
Replication	2	151.72	6.5	0.02	0.01	196.13	0.001	1.08	0.25	1001.06	77.54	0.11
Temperature (a)	1	1115.67*	58.01*	0.15*	0.05*	10.27ns	0.01ns	7.91*	1.78*	7367.36*	552.09*	0.4ns
PEG (b)	5	3066.88**	15.95*	0.41**	0.27**	11581.4**	0.29**	21.4**	7.93**	19257.39**	2059.29**	0.95**
A*B	5	51.87ns	1.13ns	0.01ns	0.01ns	14.78ns	0.003ns	0.33ns	0.04ns	311.25ns	17.93ns	0.05ns
Error	22	151.47	4.54	0.02	0.01	124.82	0.009	1.06	0.23	975.69	70.43	0.1

nsnon significant, *significant at 0.05 significance in F-tests, **significant at 0.001 significance in F-tests

Table 2. Mean comparison for experimental characteristics under osmotic potential (ψ) of PEG solutions

Treatment	Germination Percentage (%)	Mean Time Germination	Shoot length (cm)	Root length (cm)	Abnormal Germination (%)	Germination Uniformity(GU)	Plumule Dry weight (mg)	Root Dry weight (mg)	Plumule fresh weight (mg)	Root fresh weight (mg)	SSI
Temperature											
10 °C	36.6b	7.51b	0.43b	0.27b	42.75a	0.26a	3.12b	1.87b	94.73b	32.06b	0.35a
15 °C	47.73a	10.05a	0.56a	0.35a	43.82a	0.3a	4.05a	2.31a	123.34a	39.89a	0.56a
ψ (MPa)											
-1.0	13.87d	6.66c	0.14d	0.04d	95.1a	0.09d	0.99d	0.57c	28.82d	10.41d	0.03d
-0.8	17.56d	7.06c	0.27d	0.11d	88.73a	0.13d	1.91d	1.06c	61.16d	18.44d	0.07d
-0.6	41.75c	8.68abc	0.48c	0.29c	62.2b	0.17cd	3.51c	1.93b	106.58c	36.67c	0.34cd
-0.4	49.06bc	9.42ab	0.56bc	0.37bc	9.81c	0.26bc	4.06bc	2.31b	123.73bc	41.59bc	0.48bc
-0.2	61.26ab	10.29a	0.69ab	0.47b	2.45c	0.34b	4.97ab	3.31a	152.99ab	49.6ab	0.78ab
0	69.4a	10.55a	0.84a	0.6a	1.39c	0.69a	6.07a	3.38a	180.92a	59.16a	1.04a

Common letters within each column do not differ significantly