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The Effects of Different Priming Treatments on Germination Rates of Some Winter Weed Seeds

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Abstract Priming is a different application to the seed prior to seeding with controlled water in take to accelerate the metabolic activity that will ensure the germination of the seed. Especially in unfavorable conditions such as low temperature; germination and emergence rate, seedling production which increases the speed of the desired density in a short time provides. Factors which necessary for germination contains temperature, nutrients, light, moisture and gases ratio. In this study was to determine the germination capacity of the seeds of different ages such as *Galium tricornutum* Dandy, *Sinapis arvensis* L., *Melilotus officinalis* L. with different priming and cold stratification processes. Methods used for this purpose; at 30-20℃ (7/17 hours, day/night), halopriming with 2-4 % KNO_3 and cold stratification for 10 days at + 4℃ - 25℃ for 1 month. In this study; seed moisture content (%), normal abnormal germination rate (%) and mean germination time (days) were determined. The moisture content of the seeds used was determined between 8-35%. Germination was determined in *M. officinalis* seeds by halopriming and cold stratification applications. The highest germination rate was observed in *M. officialis* species at age 7 but the highest abnormal germinations rates taken from *G. tricornutum* at age 5 and *S. arvensis* at age 11.

Keywords *Galium tricornutum, Sinapis arvensis, Melilotus officinalis, Priming, Cold stratification*

1. Introduction

Weeds are unwanted factors that competes with cultivated plants in terms of water, light and nutrient and reduces the yield and quality of cultivated plants when their development is not suppressed ([9]; [1]). In Turkey, *Galium tricornutum* Dandy (Rough bedstraw), *Sinapis arvensis* L. (wild mustard) and *Melilotus officinalis* L. (sweet clover) are weeds that become a problem in agricultural lands, especially for winter cultivated plants. The first stage of plant production includes a high rate of germination and seedling emergence. Germination is the start of seed growth, decomposition of nutrients in endosperm by dissolving in water in order to be used for embryo growth and they transfer to embryo. For this process, water intake of seed takes place and softening occurs in testa. However, seed coat dehisces in some species and respiration rate increases, enzymes become active and they are synthesized. Radicle grows as a result of cell division and elongation and emergence from testa. As a result of the changes; germination and seedling emergence of seeds are expected in a short time and homogeneously in plant production. However, due to some environmental (drought, low and high temperature, soil salinity, heavy soil, soil crusting etc.) and seed-based factors, these stages may not be completed. If temperature is not optimum in an environment where water exists, homogeneous emergence does not occur during seed germination. The maximum and minimum germination temperatures apart from optimum temperature value have also importance in seed germination. Low or maximum temperatures may also affect germination negatively, also oxygen is the main essential factor. In addition, germination may not occur under optimum environmental conditions even if it is viabilile due to inhibitors contained in the seed structure. The dormant period varies based on seed type. During this period of seed, storage temperature and relative humidity must be at an optimum level and may not be affecting seed viability negatively. During the seed maturity process, germination rate is low in seeds with immature embryos. In the aging period, various biochemical and physiological changes occur in seeds and this may lead to a continuous decrease in seed quality and finally, viability loss [14]. Germination ability may decrease and heterogenous germination may occur in aging seeds.

Another seed germination inhibitory factor is prevention of water and air get in to testa. As O_2 intake and CO_2 emission slow down in the species because of hard seed coat, there can be occur secondary dormancy. If seeds are

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not kept under suitable storage conditions, testa becomes hard and water/air intake are prevented. Seed dormancy is the most important seed-borne factor in the prevention of germination. The fact that high amounts of inhibitory substances are included in seed causes seed dormancy. Some chemical applications are carried out in order to accelerate and homogenize the germination and seedling emergence of the seeds which are nonuniform and slowly germination. These treatments are provided by plant development regulators encouraging germination and some chemical solutions (inorganic salt, sugar, PEG). In these seed treatments performed using certain chemical solutions and effective substances, the aim is to minimize the seed-borne factors preventing germination. In the seed treatments performed with some other chemical solutions, mainly potassium salts are used. Potassium has germination promoting property, because it does not create osmotic pressure and water intake by the seed can not be controlled rapidly during the priming so radicle can be emergence quickly. Stratification, which is another dormancy breaking treatment, is a method applied for eliminating physiological, morphological and morphophysiological dormancy. There are cold stratification treatment for physiological dormancy and warm dormancy treatment for morphological dormancy [17]. In eliminating physiological dormancy, cold-wet stratification is performed generally in a condition such as sand/peat media and generally at 2-4℃. Stratification has advantages increasing seed vigor, germination speed and obtaining more homogeneous seedlings apart from eliminating dormancy [6]. The presence of weeds in the ecosystem is one of the most important competitive factors for cultivated plants. It occurs variability in environmental factors such as temperature, salt stress, light, pH, seed depth and soil moisture [3]. Temperature and light are considered as the most important environmental factors regulating germination, species distribution and ecological interaction [2]. The aim of the study to determine various treatments performed in some winter weed seeds of different years and their germination properties.

2. Material and Method

The studies were conducted under laboratory conditions in 2017. The main material of the study was the seeds of the species of *Melilotus officinalis* L. Desr., *Sinapis arvensis* L., and *Galium tricornutum* Dandy, of different years, among the broad-leaved weed, that become problem in wheat cultivation areas and cause significant yield loss.

Weeds which used in the test;

***Galium tricornutum* Dandy** is an annual weed that belongs to Rubiaceae family and its seeds have a size of 3-5 mm and they are roundish and their upper parts are stinging. A plant can form approximately 300-400 seeds. ***Sinapis arvensis* L.** is an annual weed that belongs to Brassicaceae family and it can grow up to 30-60 cm. The seeds are in spheroid form and their surfaces are flat and dull. A plant forms approximately 2700 seeds. ***Melilotus officinalis* (L.) Desr.** is an annual weed plant that belongs to Fabaceae family and it has indehiscent fruit, short, protruded, its surface is dressed and it has 1-5 seeds.

Determination of moisture content;

Seed moisture of each species was determined based on [12] rules and the initial weights of the 2 replicate seed samples of 5 g were weighed and then determination of moisture was performed at 130℃ for 1 hour. The initial and final weights were stated in the formula (1) and the initial moisture contents (%) were determined.

$$\text{Moisture content (\%)} = ((FSW-LSW) / FSW) \times 100 \quad (1)$$

FSW: Frist seed weight
LSW: Last seed weight

Germination tests:

Germination tests were performed in weed seeds of the species at alternative variable temperatures and period (day/night). As testa may prevent germination in the seeds of *G. tricornutum* and *M. officinalis* species, so were used scarification for them. As a result of the tests, the moisture content of the seeds (%), the seed amount in 1 gr (number), normal and abnormal seedling rate (%) and mean germination/emergence times (day) were determined [13].

Priming treatments;

Germination by 2-4 % KNO_3 solution; 3 replicate and 25 seeds in each weed species from different years were placed between whatman filter papers which were wet by two different solutions. Seed surface sterilization was carried out with 1% hypo at 1 minute for *G. tricornutum* and *S. arvensis* seeds. The filter papers were initially wetted with 3 ml of 2-4% KNO_3 solution and as required, the solution (depending on application) was added to maintain sufficient moisture. It was kept for 7 hours at 20℃ in the daytime and for 17 hours at 10℃ at night.

Figure 1. Seed surface sterilization and preparation of salt solutions

Cold Stratification;

M. officinalis seeds of different years were kept for 10 days at + 4℃ using stratification method and then kept at 25℃ for 1 month.

Figure 2. Cold stratification treatment

Mean germination time (MGT);

- Mean germination time calculation:

$$MGT = \Sigma\, n.D / \Sigma\, n \qquad (2)$$

In formula;
MGT: Mean germination time
n: number of seeds germinating on a day
D: day since the beginning of germination.

3. Results and Discussion

As a result of the study; seed moisture content varied based on species, years and it were determined to be between 8-35 %. When the number of seeds in 1 g seed size is compared, there is a difference between years and species. Besides that, this variation based on seed collection period, the field conditions and especially pollination time. According to the treatments with salt solutions, the control germination groups had better results. The highest germination rate was obtained from the seeds of *M. officinalis* species for 2010. In addition, *M. officinalis* was the species responding to the salt treatment (4% KNO_3) among the species used. The mean germination time obtained as a result of the daily count results have difference between 3-6 days. The species with the highest germination rate also showed the fastest germination performance (Table 1,2,3).

Table 1. GALTR’s seed germination rate (%) and time (day) in 2- 4% KNO_3 solution medium

Galium tricornutum	**Treatments (KNO_3)**	**2011**	**2012**
Mean germination rate (%)	Control	0	0
	%2	0	0
	%4	12	12
Mean germination time (day)	Control	0	0
	%2	0	0
	%4	6	5
Number of seeds per 1gr (piece)		140	170
Moisture content (%)		20.8	20.4

In a study with *Melilotus* species, it was reported that the relationship between tissue ion concentrations (Na + and Cl−) and salt tolerance was negative (the ion concentrations in the seedlings of the more tolerant species were the lowest) and there were significant differences between the *Melilotus* species. [18]. Similar result was obtained in the *Melilotus* seeds of different years in experiment results (Table 1,2,3,4).

Table 2. SINAR's seed germination rate (%) and time (day) in 2- 4% KNO_3 solution medium

Sinapis arvensis	**Treatments (KNO_3)**	**2011**	**2012**
Mean germination rate (%)	Control	0	0
	%2	0	0
	%4	5	6.6
Mean germination time (day)	Control	0	0
	%2	0	0
	%4	3.6	3
Number of seeds per 1gr (piece)		530	480
Moisture content (%)		14.6	10

Table 3. MELOF's seed germination rate (%) and time (day) in 2- 4 % KNO_3 solution medium

Species		*Melilotus officialis*		
Year		2004	2007	2009
Number of seeds in 1 scale (piece)		201	248	193
Weight of seeds in 1 scale (gr)		0.39	0.34	0.30
Control	Germination rate	37	**48**	**41**
	Mean germination time (day)	4.4	4.7	**4.2**
Cold Stratification	Germination rate	24	15	**58**
	Mean germination time (day)	4.5	11	**2.9**
Moisture content (%)		34.9	8	12.7

Table 4. Cold stratification results of MELOF seeds at +4℃ 10 days +25℃ 1 month

Melilotus officinalis	Treatments (KNO_3)	2004	2007	2009	2010	2012
Mean germination rate (%)	Control	37	48	41	81	45
	%2	1	2.6	0	0	1
	%4	0	0	0	0	4
Mean germination time (day)	Control	4.4	4.7	4.2	2.7	4.7
	%2	4.6	2.6	0	0	1.3
	%4	0	0	0	0	3
Number of seeds per 1gr (piece)		520	730	640	700	700
Moisture content (%)		34.9	8	12.7	13.9	10

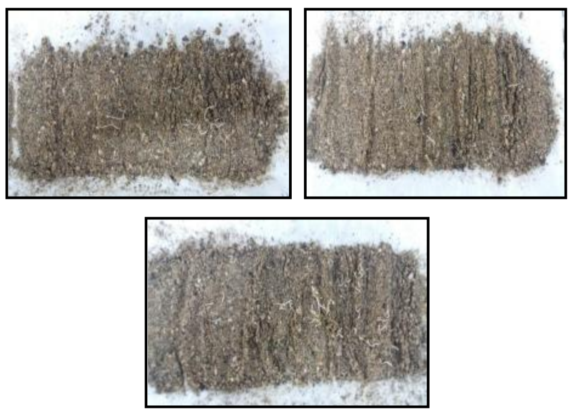
In the study carried out by using Sinapis alba seeds belonging to two different populations (the KKTC/Turkey (Gazimağusa) where germination rates at different temperature (5, 10, 15, 20, 25, 30, 35℃) values were examined, while the germination rate was highest at 5-15℃ (88-74%) in the KKTC population, it was determined as the highest germination rate (79-67%) in the Bornova population at 15-30℃ [20]. In this study, the 6-7-year-old

S. arvensis seeds of viability was determined 5-6 %, with the germination for 7 hours at 20℃ in the daytime and for 17 hours at 10℃ at night (Table 1,2,3). Temperature and light are considered as the most important environmental factors regulating seed germination, species distribution and ecological interaction. Under the conditions in which the other factors (soil moisture, salt salinity and acidity) are not limited, temperature is the main decisive of seed germination [22]. Temperature varies for the species included in the genus [15] and there may be differences between genotypes in the species [10]. However, as a result of the changeable night/daytime temperature and salt solution treatments in the study, it was not found to be effective in the seeds between the ages of 6-14 (Table 1,2,3). The germination rate of the seeds was reduced in nitrogen-rich environments and it was considered that this may be due to the dormancy caused by nitrogen in high concentration in seeds. Testa may affect germination by changing testa permeability as a mechanical barrier for germination [7] and therefore by changing the enzymatic activation in embryo [4] and the appropriate light conditions for embryo [13].

Generally, seed number and weight of the species in a scale varies considerably (Tables 1,2,3,4). This situation may vary depending on harvest maturity, environmental and storage factors as a result of fertilization times. Different results were obtained in seeds of *M. officinalis* based on germination results of the control group as a result of stratification treatment (Figure 3). An increase of about 10% was provided in the germination rate of the seeds of 2009 (Table 4).

In the germination tests performed in different temperatures [11], it was determined that *Amaranthus retroflexus* seeds obtained from Nazilli, germinated at minimum 15℃ and the seeds collected from Menemen germinated at 10℃. The seeds of this plant collected from both locations germinated at maximum 35℃. It was stated in the study on the effects of factors such as temperature, pH, light and salt on the germination of the seeds of *Senecio vulgaris* L. *Taraxacum officinale* L. and *Tragopogon pratensis* L. that the highest germination occurred at 15 for *S. vulgaris*, 20 for *T. officinale* and 10℃ for *T. pratensis*, for 6 months of dormant period, at 16 hours of daylight/8 hours of darkness for *S. vulgaris* and *T. officinale* and in an environment with 24 hours of darkness for *T. pratensis* and the highest germination occurred in the control group based on salt needs [19]. Also in the present study, germination was determined in the seeds in the control group despite it varied based on species (Tables 1,2,3,4). In another study determining the effect of temperature on the dormancy in three different levels, germination was observed during imbibition in 10 mM KNO_3 solution at 2℃ in the dark on the 1st, 2nd and 5th days (dormancy levels 3, 2 and 1) respectively [16]. It was treated by red light and then germination rates of the seeds at different temperatures were determined. Dormancy and temperature affected the total germination percentage in the seeds of *Polygonum persicaria* and it was stated that this rate was higher in the lowest dormancy level in all temperatures. Nitrate is one of the factors stimulating the germination of many (weed) seeds. The stimulating effects of light and nitrate are generally related to germination of seeds. However, this effect was not observed in this study (Table 1,2,3). In the test performed with the seeds buried under soil for three years, the effects of nitrate on the germination of *P. persicaria, Sisymbrium officinale, Chenopodium album* and *Spergula arvensis* seeds were determined [8]. The difference between the unstored (fresh) and stored seeds of *P. persicaria* was determined. The germination of the unstored seeds increased slightly by the nitrate applied and the seeds buried under soil for a longer period did not germinate. This difference is considered to be due to the differences in the endogenous nitrate content of the fresh and buried seeds. As fresh *Polygonum* spp. seeds have very low nitrate content it is possible that the nitrate provided as exogenous reacts. As long as *C. album* and *S. arvensis* seeds are buried, they can use nitrate of soil. It is possible that *P. persicaria* seeds also take nitrate in the first months when the seed is buried and germination is stimulated by the exogenous nitrate. After this first months the nitrate taken by seeds is saturated and the exogenous nitrate cannot stimulate germination any more. Possibly, the other species need higher nitrate requirements for germination. The seeds of this species may have different nitrogen contents. These differences are considered to be due to the different nitrate levels in the soil possibly during seed development.

When this effect is taken into consideration, it is due to the similar structure of the soil conditions in which the seeds used belong to the vegetation process.



MELOF 2004 (24%) MELOF2007 (15%) MELOF 2009 (58%)

Figure 3. MELOF seeds cold stratification at +4℃ 10 days + 25℃ 30 days

4. Conclusions

Depending on weed species, drying the seeds either increases germination [5] or becomes ineffective [21]. It has been stated that the germination of *Chenopodium album*, *Sisymbrium officinale*, *Spergula arvensis* and *Polygonum lapathifolium* seeds are stimulated by drying. Germination of

S. officinale and *S. arvensis* seeds was more stimulated than *C. album* seed, although the drying level was higher and this is proportional to the moisture content of *C. album*, *S. officinale* ve *S. arvensis* seeds.

This difference between the species is associated with nutrient reserve composition, embryo size and the other seed characteristics varying among the species ([5]; [8]). At the beginning, there may be dormancy due to the current membrane in the seed uptake water with low temperature. The dormancy breaking at low temperatures causes phytochrome receptor Xa synthesis. Increasing temperature increases membrane permeability and this allows the receptor to move to the membrane surface. So, it becomes possible that nitrate activates the receptor of co-factors of nitrate (Xa). The moisture levels of the seeds included in the present study were determined between 8-35%. In addition, the temperatures of +4 and 25°C used in stratification process reacted more positively compared to the salt treatments (Table 1,2,3,4). Temperature is the only factor that is known to affect dormancy level directly and light, nitrate, gases environment and moisture content are the important factors affecting seed germination.

As a result of the treatments, a significant advantage cannot be gained for germination in the seeds of the weed species used by the salt and stratification treatments performed as a priming for weed seeds compared to controls. This condition varies depends on seed maturity, harvest period, age, and storage conditions. While *M. officinalis* seeds germinated between 2-4% with salt applications, 81% germination in the control group of seeds having 14% moisture content of 2010 was obtained on mean in 3 days.

Germination was generally observed in seeds of *S. arvensis*, *G. tricornutum* and *M. officinalis*, which are among the winter weeds (Table 1,2,3). The highest germination rate was determined in *M. officinalis* seeds at the age of 7. Also, abnormal seedling emergence was observed in 5-year-old *G. tricornutum* seeds. Results that can be suggestive for further studies in this field were obtained.

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