ORIGINAL ARTICLE

EFFECT OF PRIMING TREATMENTS AND ACTIVE CARBON ON SEED GERMINATION OF TWO CALENDULA SPECIES

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ABSTRACT

Background: Priming teratments are methods used to increase seed germination and seedling development. **Objective**: In this study, it was aimed to increase the germination percentage for C. officinalis and Calendula arvensis seedling formation. **Methods**: This study was conducted to evaluate the effect of various priming applications on the embryo germination including different combination of cold stratification at +4°C for 7 days, hydropriming for 24h, 2g/l KNO_3 for 2h, GA_3 (20 mg/l) for 24h, 20% H_2SO_4 for 2 min to breaking of *Calendula officinalis* seed dormancy and promote its germination and seedling growth. And then the embryo of *C. officinalis* were incubated to MS0 in petri dishes. In addition to this, the embryo of *C. officinalis* were incubated to MS0 with the addition of 1 g active carbon in petri dishes. **Results**: The germination percentage increased in all of treatments than control. I revealed that the highest germination percent were gotten in cold stratification and GA_3 applications. The mean germination time was also increased in all application group than control. **Conclusion**: The lowest mean germination percent was obtained in acid application.

Keywords: *priming, Calendula species, germination.*

1.INTRODUCTION

Calendula officinalis (pot marigold) L. is a medicinal plant which is belonging to Asteraceae (Compositae) family. The species has 20 varieties. Its flower appears yellow [1]. Its chemical constituents include triterpene glycosides, triterpene alcohols, flavonol glycosides, essential oil, polysaccharides and fatty oil [2]. Many studies have reported that the plant have pharmacological effects such as anti-microbial [3, 4], anti-leishmanial [5], anti-HIV [6], antioxidants [7], cytotoxic, anti-tumor, anti-inflamatuar [8], hypoglycemic [9], lymphocyte activator effect [10] and for biligenic function [11].

Seeds are important in the reproduction of plants. Seed quality refers to plant productivity in agricultural ecosystems. In order to increase the seed quality, applying the methods to optimize the conditions for seed germination can increase the productivity of the product. Seed germination and seedling formation are important in plant life cycle [12]. If the environmental stress effect during seed germination can be reduced, a good crop can be obtained by providing economic crop production [13].

The development of strategies to ensure the growth and development of plants from seed has been researched for many years. Seed priming is a pre-planting strategy that regulates metabolic activity before germination [14; 15]. During seed priming, seeds can be soaked in water. In this way, while pre-germination metabolic activities continue, root emergence is prevented. The seeds are then dried to their original moisture level [16]. The priming applications have been employed to increase the seed germination speed. The most used priming techniques include hydropriming, osmopriming, halopriming etc [14]. It can be said that such simple, low-cost pre-treatment also had positive effects on the wider agricultural system and livelihoods. This method is also popular among farmers [17].

The objective of this study was to determine different priming treatments which are able to stimulate germination percentage of two Calendula species that is important pharmacologically.

2. MATERIAL AND METHODS

2.1.In vitro Seed Germination

In this research, I have used that the *C. officinalis* and *C. arvensis* seeds were purchased from Ceylan Agricultural Company in Turkey.

C. officinalis and *C. arvensis* seeds were pretreated with pre-chilling (+4°C), sterile pure water, KNO_3 (2 g/l), GA_3 (20 mg/l), acid (20% H_2SO_4).

Hydropriming: Seeds were soaked in sterile pure water for a week.
Pre-chilling: Seeds were placed between filtre papers inside petri dishes and stored in the dark at +4°C during a week. Darkness is maintained by sealed with two layers of aluminum foil around petri dishes.
GA_3 treatments: Seeds were soaked in 20 mg/l (ppm) gibberellic acid for 2 hours.
KNO_3 treatments: Seeds were soaked in 2 g/l KNO_3 for 2 hours.
Acid treatment: Seeds were treated with H_2SO_4 (20%) for 2 min.

The different priming treatments for *C. officinalis* and *C. arvensis* seeds were practiced before surface sterilization. At the beginning of all primings, the seeds were treated at +4°C for one week and then with pure sterile water for 24 hours. In addition, the four different primers were applied to the seeds and the non-pre-treated seeds were evaluated as controls (unsoaked seeds) (Table 1).

Table 1: The presents the primings practiced to *C. officinalis* and *C. arvensis* seeds.

	Priming				
Groups	**Pre_chilling**	**Hydropriming**	**KNO_3**	**GA_3**	**H_2SO_4**
Control	-	-	-	-	-
1	1 w	24 h	-	-	-
2	1 w	24 h	2 g/l, 2 h	-	-
3	1 w	24 h	2 g/l, 2 h	20 mg/l, 2h	-
4	1 w	24 h	-	-	20%, 2 min

Otherwise, 1 g of activated carbon was added to the MS0 medium for another application. Thus, this control group, the effect of four different groups of combined priming techniques and the effect of activated carbon were evaluated.

2.2. In vitro Culture Conditions

After the primings, the seed surface sterilization was carried out that the seeds was treated with 98 % EtOH during 1 min., 50% NaOCl during 30 min. and then rinsed with sterile pure water. Subsequently, the seed coats were removed with forceps and lancet in the sterile cabine. The germination medium was comprised of MS0 medium containing with ¼ MS [18]. 15 g/l sucrose and 8 g/l agar. And second germination medium was prepared with addition of 1 g active carbon. Both of germination medium was adjusted to ph 5,8. Then sterilized at 125°C for 30 min under pressure using an autoclave and was distributed in petri dishes. I carried out *in vitro* transferring of *C. officinalis* and *C. arvensis* embryo to the petri dishes containing the MS0 medium under complete sterile conditions. It was surrounded by the petri dishes with parafilm. This petri dishes were wrapped around alumirinium foil. And then petri dishes were placed in a growth chamber at 25 ± 2°C and stored in the total darkness. All germination experiments were conducted twenty five seeds per each application. Seeds and embryos of two Calendula species were given in Figure 1.

Figure 1: The figure presents: **a**) Seeds of *C. officinalis* and *C. Arvensis* and **b**) embryos of *C. officinalis* and *C. arvensis* that is seed coat has been removed.

3. RESULTS

3.1. The Results of Seed Germination

The germination percent (%) was determined according to the primings made to the seeds (Table 2).

Table 2: The table presents the primings made to the seeds of *C. officinalis* and *C. arvensis* and the germination percent (%).

	Priming					Germination percent (%)	
Group	**+4°C Degree**	**Sterile pure water**	**KNO_3**	**GA_3**	**H_2SO_4**	***C. officinalis***	***C. arvensis***
Control		-	-	-	-	26,666	33,333
1	1w	24 h	-	-	-	53,333	40,000
2	1 w	24 h	2 g/l, 2h	-	-	86,67	93,333
3	1 w	24 h	2 g/l, 2h	20mg/l, 2h	-	53,333	40,000
4	1 w	24 h	-	-	20%, 2min.	-	-

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The highest percentage of germination were obtained from the seeds chilled at +4°C for 7 days, treated with hydropriming and KNO_3 as compared with control group. Whereas the most favorable priming combination was established to be group 2 for both *C. officinalis* (86,67 %) and *C. arvensis* (93,333 %) species, the less favorable priming combination was determined to be control group for both *C. officinalis* (26,666 %) and *C. arvensis* (33,333 %) species in this study. I revealed out that group 1 and group 2 priming techniques had same effect for germination of *C. officinalis* and *C. arvensis* species. It was fixed that acid treatment was worse so as to germination of the embriyos for two Calendula species. I revealed that the combined effect of pre-chilling, hydropriming and KNO_3 treatment significantly more effective in improving seed germination and seedling establishment for two Calendula species compared other treatments. It has been detected that more root formation occurs in the seedling growing in the nutrient medium to which activated carbon is added compared to the other application groups.

Figure 2: The figure presents *C. officinalis* seeds grown on MS0 nutrient medium added with ¼ MS.

Figure 3: The figure presents *C. officinalis* seeds grown on MS0 nutrient medium added with KNO_3, GA_3, ¼ MS.

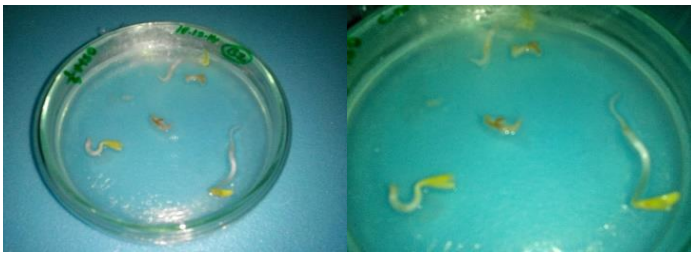

Figure 4: The figure presents *C. officinalis* seeds grown on MS0 nutrient medium added with KNO_3, GA_3, ¼ MS.

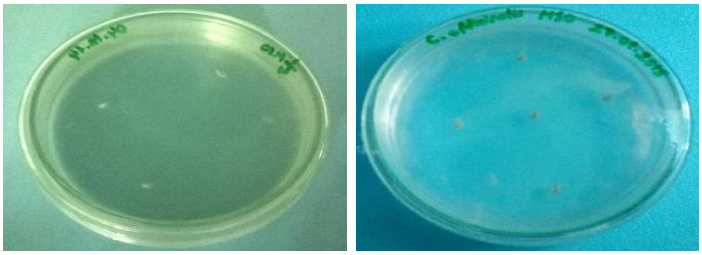

Figure 4: The figure presents the Acid treatment.

Figure 5: The figure presents *C. officinalis* seeds grown on MS nutrient medium with addition of 1 g active carbon.

4. DISCUSSION

Our research results are consistent with previous researches [19, 20, 21]. which reported about priming techniques. We found that the germination percentages of chilled achenes of *A. halodendron* and *A. scoparia*, were higher than that of fresh achenes [19].

Seeds of *Echinacea purpurea* family were treated to different priming treatments such as GA_3, KNO_3 and cold stratification. The germination percentage increased significantly in all priming treatment. We have found that the detected that the highest germination (about 98%) were showed in cold stratification application. Also, It was that the mean germination time was more in all of application than control. Compared with other priming treatments, it indicated that lowest mean germination time was in cold stratification application [20].

The highest radicle length observed in GA_3 (600 ppm) which had no significant difference by control, H_2SO_4 for 2 min and 2% KNO_3, while the lowest was observed in chilling for 12 weeks and 3% H2SO4. We revealed that the most effective reduction among all treatments was observed at chilling for 12 weeks and 3% H2SO4 treatment [21]. In other reserach was indicate that germination percentages of chilled achenes of A. halodendron and A. scoparia belonging to Asteraceae family, were higher than that of fresh achenes [19].

The combined GA_3 and cold scarification treatment was significantly more effective in improving seed germination of *E. purpurea* compared other treatments. Results of this study that is exogenous GA3 useful, when participant with chilling because combined GA_3 and scarification treatment increased endogenous seed GA_3 and help dormant seed to germination. In conclusion, the marked improvement in *E. purpurea* germination following combine GA_3 and scarification indicated that these were suitable treatments to remove dormancy compared use of GA_3 or scarification alone. After these treatments, use of scarification for 4 and 7 weeks was better for germination compared other treatment like GA_3 soaking [22].

Baskin et al., (1992) found cold scarification increased seed germination of some Echinacea angustifolia. Prolonged time of cold stratification has been found to increase seed germination of several plants from Asteraceae family [24]. Accordingly, our results showed that seed germination and seedling fresh weight was higher in seeds of *C. officinalis* that treated with cold scarification alone and combine with GA_3 or KNO_3. Results of this study was asserted that exogenous application of GA_3 is useful for germination of *C. officinalis* seeds, when applied GA_3 alone and combined with chilling [22]. The GA_3, which is applied exogenously, improved the germination of many plant species, for example *Fagus sylvatica* [25] and *Echinacea spp* [24].

5. CONCLUSION

Priming techniques was effective for germination of seeds. However, we found that acid seed treatment gave negative results in terms of germination.

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