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# THE EFFECTS OF PRETREATMENT FACTORS ON SEED GERMINATION AND SEEDLING GROWTH OF ANISE (*PIMPINELLA ANISUM L.)*

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**Abstract:***Seed dormancy is one of the major problems in agricultural studies, especially for medicinal plants. Anise (Pimpinella anisum L.)is an important economic medicinal plant with dormant seeds and distributed only in its natural habitats. An experiment was conducted as a Factorial layout within a completely randomized design with four replications to evaluate the effects of some pretreatment factors on primary growth and germination characteristics of anise.Pre-chilling treatments were 0, 15, 30 and 45 days treatments and hormone treatments were $GA_3$ (Gibberellic Acid), BA (benzyladenine), kinetin (Kinetinnetin), $GA_3$+BA, $GA_3$+kinetin BA+kinetin, $GA_3$+BA+kinetin, $KNO_3$, $H_2SO_4$ and distilled water as a control treatment.Prechilling treatment effects on coleoptile and radicle length, seedling length, germination percentage, mean time for germination, germination rate and seed vigor index showed significant differences ($p<0.01$) among them. Similarly,different hormone treatments also had significantly different influence on coleoptile and radicle length, seedling length, germination percentage, mean time germination, germination rate and seed vigor index. The highest germination percentage and germination rate was related to the usage of BA+ kinetin. The highest values for radicle length and uniformity of seed germination were achieved in BA and kinetin, respectively. Moreover, application of $GA_3$+BA+kinetin had given the highest seed vigor index. It seems that application of exogenous $GA_3$+KİNETİN and BA+kinetin concentration, which is provided mostly by chilling treatment, is the most effective factor for breaking the seed dormancy. On the basis of the results, usage of 45 days moist prechilling accompanied with application of $GA_3$+kinetin and BA+kinetin in Esfahan cultivar was appropriate.*

**Keywords**: *Seed dormancy, Seed germination, Seedling growth, Anise*

## 1.Introduction

Anise (*Pimpinella anisum L.*) is a flowering plant in the family Apiaceae native to the eastern Mediterranean region, west and Southwest Asia [1-2-3]. Anise is also famous in traditional Chinese medicine (TCM), Ayurveda and Unani medicine. Anise has been used for different purposes in the traditional medicine system of Iran [4]. Seed priming treatments have been employed to accelerate germination, seedling growth and yield in most seeds under normal and stress conditions [5-6-7-8-9-10-11]. Seed germination can be controlled by many factors like natural germination and growth inhibitors [12-13-14-15]. These are the derivatives of gibberellic acid ($GA_3$), abscisic acid (ABA), cinnamic acid, kinetinnetin (KİNETİN), bonzyladenin (BA), coumarin, jasmonic and etc. The variation in seed dormancy and seedling emergence are controlled by environmental conditions. The origin of research into Gibberellins can be traced to Japanese plant pathologists who were investigating the causes of the bakane (foolish seedling) disease that seriously lowered to the yield of rice crop in Japan, Taiwan, and some other Asian countries [16]. Gibberellic acid is a plant growth hormone that has an important role in seed germination [16]. It has been reported that the stimulating effects of $GA_3$ on seed germination are not similar in all crop species [17]. $GA_3$ has been also reported to promote growth in cotton, rice and in some halophytes under saline conditions [18-19]. Tsygankova et al. [20] confirmed specific auxin-like, cytokinetinnin-like and minor gibberellins-like effect of synthetic heterocyclic compounds on cell division, cell proliferation, cell elongation and cell differentiation that are the basic processes of plant growth and development. Gibberellins is known to eliminate the chilling requirements of peach and apple seed and increased their germination [21]. Primed with gibberelin improve quality of seeds and germination [22]. Liopa-Tsakalidi et al. [23] also suggest that germination and seedling growth of 11 species responded differently to different levels of $GA_3$. Fernandez et al. [24] revealed that cold stratification has a direct influence on the production of gibberellins (Gas) in seeds of *Arabidopsis thaliana*. Exogenously applied GA overcomes seed dormancy in several species [25]. Hormone priming increased antioxidant enzyme activity and decrease the amount of reactive oxygen space.Sharifi and Pouresmael [26] concluded that only cold treatments such as gibberellic acid, cytokinetinnin, potassium nitrate, washing and light treatments are not useful. It has been reported that GA is effective in breaking seed dormancy in snowberry [27]. Nkomo and Kambizi [28] noted that prechilling followed by exposure to a temperature higher than 30$^{o}$C encourages the germination of *C. Olitorious* seeds. Rouhi et al. [29] concluded that applying 500 ppm concentration of $GA_3$ and $KNO_3$ resulted in higher germination in waterlily dormant seeds. Plant hormones are used in breaking seed dormancy [30]. Cytokinins and auxin are the most common plant growth regulators used in in vitro culture of plant tissues [31-32]. Cytokinins constitute a major class of plant growth regulator that is involved in a wide range of physiological processes [33]. Cytokinins have a stimulatory or an inhibitory role in different development processes, such as control of apical dominance in the shoot, root growth and branching, leaf senescence, and chloroplast development [34]. In spite of the fact that Anise is an important and expensive medicinal and spice plant, not enough information is available on the effects of moist pre-chilling and application of hormones on different cultivars of it. So, the aim of this study is to survey the certain effects of different treatments to stimulate seed germination and seedling growth of Anise.

## 2.Materials and methods

The study evaluates the influence of some pretreatments on growth and germination characteristics of anise (*Pimpinella anisum L.*), an experiment was conducted as Factorial layout within completely randomized design with four replications at Research laboratory of Mojgan Agricultural Company, Mahmood Abad, Isfahan, Iran. Pre-chilling treatments were 0, 15, 30 and 45 days treatments and hormone treatments were $GA_3$ (Gibberellic Acid), BA (benzyladenine), kinetin, $GA_3$+BA, $GA_3$+ kinetin, BA+ kinetin, $GA_3$+BA+ kinetin, $KNO_3$, $H_2SO_4$ and distilled water as a control treatment. First, seeds were surface sterilized in 1.5% (w/v) sodium hypochlorite solution for 15 minutes and then rinsed three times with sterile distilled water. For each treatment, 4 Petri dishes were used and 30 seeds were put into each of them, then, each Petri dish was covered with 10 mm of each specific treatment. In the first trial, seeds were chilled for 15, 30 and 45 days, and after that, seeds were soaked and treated with 10 hormone treatments. In the second experiment, the seeds were treated without pre-chilling treatments. In the thirds experiment, seeds treatments were done with polyethylene glycol. Equation number one and number two were used to calculate germination percentage and germination rate, respectively.

Germination percentage= (Number of germinated seed /total number of seed) × 100 (1)

1) GR= $\Sigma\frac{N}{\Sigma(n\times g)}$ (2)

Where N is the number of germinated seed on growth day and g is the number of germination seeds Analysis of variance (ANOVA) was used to determine the significant differences. Uniformity of seed germination and mean time for seed germination (MTG) was evaluated by equation number 3 and 4. Seed vigor index was calculated by equation number 5.

Uniformity of seed germination= $\frac{1}{\frac{\sum(D-\overline{D})^2\times N}{\sum N}}$ (3)

$$MTG=\frac{\Sigma(nd)}{\Sigma n} \quad (4)$$

n: The number of germinated seed in the specific day.
d: The number of days from the beginning of germination.
∑n: The total number of germinated seed.

Seed vigor index: Germination percentage × mean of seedling length (mm)(both coleoptile and radicle) / 100 (5)

The means were separated by Duncan's Multiple Range Test ($p$<0.05). All statistical analysis was performed with the SAS computer statistical software.

**3.Results and discussion**

Prechilling had a significant impact on coleoptile length, radicle length, seedling length, germination percentage, mean time for germination, germination rate and seed vigor index was evaluated. The highest coleoptile length was related to 45 days chilling (2.45 mm) which had significant differences with other treatments. Although, the higher value of radical length was obtained for 45 days (0.8485 mm) chilling, its difference with 30 days chilling was not significant. The minimum coleoptiles (0.7536 mm) and radical length (0.6819 mm) was related to control treatment. The higher values of seedling length (2.98 mm), and germination percentage (70.02%) was obtained for 45 days of chilling followed by chilling for 30 days, 15 days and 0 days (control treatment). There were significant differences in seedling length and germination percentage between 45 days of chilling and other treatments. Control treatment had obtained the highest mean time for germination (11.18) which had significant differences with 30 days and 45 days pre-chilling, although, its difference with control treatment was not significant. The maximum value for uniformity of germination rate (5.87%), and seed vigor index (2.244) was achieved in 45 days pre-chilling which had significant differences with other treatments. Both the germination rate and seed vigor index was increased significantly from control treatment to 45 days pre-chilling. The maximum and the minimum uniformity of seed germination was related to control treatment (0.2165), and 15 days pre-chilling (0.1075), which had no significant differences with each other (Table 1). Gupta et al. [30] reported that prechilling treatment also improved seed germination in Isabgol.

The highest coleoptiles length (1.663 mm), and seedling length (2.574 mm) was related to the application of $GA_3$+ kinetin and the minimum one was observed in $KNO_3$. Patel and Mankad [16] concluded that low concentrations of $GA_3$ influence all developmental and physiological processes in plants. The maximum and the minimum radical length was related to the application of BA (1.7232 mm), and $KNO_3$ (0.5286 mm), respectively, which had significant differences with each other. Benzyladenine (BA) at a high concentration was also shown to be effective in shoot regeneration in *P. vulgaris* [35]. Application of BA + kinetin had obtained the highest value for germination percentage (66.33%) and germination rate (4.126%), which had significant differences with other treatments. Sawan et al. [36] demonstrated that kinetinnetin application improved seed viability and seedling vigor as shown by lengths of the hypocotyls, radical and the entire seedling, as well as seedling fresh weight. Gibberellic acid is also known to play an essential role in seed germination, stem elongation and flower development [37]. The maximum and the minimum mean time for germination was achieved in usage of BA + kinetin (66.33), and $KNO_3$ (25.45), respectively. Narra et al. [38] also found that the seedling under the $GA_3$ influence showed enhanced germination, seedling elongation and dry weight accumulation on *Trachyspermum ammi*. Although the higher value for uniformity of seed germination was related to kinetin (0.1523), followed by other treatments, there were no significant differences between treatments. application of $ga_3$+ba+ kinetin had obtained the highest seed vigor index (1.545), and the minimum one was related to the application of distilled water (0.4076) (Table 1). The efficacy of BA in inducing multiple shoots was also demonstrated in chickpea [39], mungbean [40] and pigeonpea [41]. Gupta et al. [30] concluded that GA has shown promising effect in breaking seed dormancy with accelerated seed germination (speed of germination, vigor index) and seedling growth (seedling dry weight).

**Table 1.** Mean comparison for coleoptile length (mm), radicle length (mm), seedling length (mm), germination percentage (%), meantime for germination, germination rate (%), uniformity of seed germination and seed vigor index.

| Treatment | Coleoptile length | Radicle length | Seedling length | Germination percentage | Meantime for germination | Germination rate | Uniformity of seed germination | seed vigor index |
|---|---|---|---|---|---|---|---|---|
| **Prechilling (day)** | | | | | | | | |
| **0** | 0.7536d | 0.6819bc | 1.463c | 14.22d | 11.18a | 0.3626d | 0.2165a | 0.3287d |
| **15** | 1.000c | 0.5625c | 1.554c | 35.28c | 11.00a | 1.489c | 0.1075a | 0.6328c |
| **30** | 1.340b | 0.7635ab | 2.133b | 53.74b | 7.81b | 3.356b | 0.1336a | 1.239b |
| **45** | 2.145a | 0.8485a | 2.986a | 70.02a | 5.52c | 5.87a | 0.1352a | 2.244a |
| **Hormone** | | | | | | | | |
| **$GA_3$** | 1.352bc | 0.8112abc | 2.185b | 37.91d | 10.44a | 2.533d | 0.1471b | 1.056ab |
| **BA** | 1.428abc | 1.7232bcd | 2.143bc | 61.22b | 9.862a | 3.837b | 0.1066b | 1.365ab |
| **KİNETİN** | 1.536ab | 0.8687ab | 2.385ab | 47.33c | 9.575a | 3.199b | 0.1523b | 1.351ab |
| **$GA_3$+BA** | 1.345bc | 0.6835cd | 2.036bc | 52.26b | 10.61a | 3.007bc | 0.1474b | 1.263bc |
| **$GA_3$+KİNETİN** | 1.663a | 0.9123a | 2.574a | 40.23cd | 10.66a | 2.673cd | 0.1132b | 1.484ab |
| **BA+KİNETİN** | 1.425abc | 0.7348be | 2.176bc | 66.33a | 9.74a | 4.126a | 0.1035b | 1.464ab |
| **$GA_3$+BA+KİNETİN** | 1.563abc | 0.8140abc | 2.356ab | 54.00b | 10.28a | 3.179b | 0.1202b | 1.545a |
| **$KNO_3$** | 0.7537d | 0.5286e | 1.343d | 25.45e | 6.40b | 1.855e | 0.1148b | 0.4661de |
| **$H_2SO_4$** | 1.256c | 0.5573de | 1.915c | 25.69e | 6.26b | 1.632e | 0.1242b | 0.6842d |
| **Distilled water** | 0.8223d | 0.5647de | 1.465d | 25.68e | 5.48b | 1.987e | 0.1247b | 0.4076e |

Common letters within each column do not differ significantly.
$GA_3$= Gibberellic Acid
KİNETİN= Kinetinnetin
BA= Benzyladenine

## 4. Conclusion

Seed germination is a complex physiological process that responds to environmental signals such as light, water, and other factors. Also, Seed germination is very important to know the germination pattern of a plant, especially the medicinal plants. Prechilling treatment effects on coleoptile and radicle length, seedling length, germination percentage, mean time for germination, germination rate and seed vigor index were significant. Different hormone treatments had a significant influence on coleoptile and radicle length, seedling length, germination percentage, mean time germination, germination rate, and seed vigor index. Prechilling treatment for 45 days had obtained the highest coleoptile and radicle length, seedling length, germination percentage, germination rate, and seed vigor index. While control treatment had obtained the maximum mean time for germination and uniformity of seed germination. Application of $GA_3$+kinetin had obtained the highest coleoptile length, seedling length, and mean time for germination. The highest germination percentage and germination rate was related to the usage of BA+kinetin. The higher values for radicle length and uniformity of seed germination were achieved in the application of BA and kinetin, respectively. Moreover, application of $GA_3$+BA+kinetin had resulted in the highest seed vigor index. All in all, in conclusion, it was shown that $GA_3$, kinetin, and BA had greatly enhanced the germination parameters in terms of germination percentage, seedling elongation and other characteristics.

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