# A Study on the effect of moist-chilling and $GA_3$ application on evening primrose (*Oenothera biennis* L.) seed germination

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## Abstract

Evening primrose (*Oenothera biennis* L.) is native to North America, belongs to Onagraceae family, and is a biennial plant. This experiment was performed as factorial based on a completely randomized design in three replications. The main purpose of the study was to evaluate the effect of moist-chilling and $GA_3$ on seed germination and flower induction of evening primrose. The seeds were soaked in distilled water for 30 min, and treated with seven levels of moist-chilling period (0,10, 20, 30, 40, 50, and 60 days) and were kept in 2-4 °C in refrigerator conditions. At the end of day 60 half of the seeds were treated with 500 ppm $GA_3$ solution for 24 hours. After that, both groups were planted in pots with three replications to see the plant reproductive responses. Simultaneously, the same seeds were planted in Petri dishes with three replications for seed germination. Germination was controlled every three days and seed germination test was done for 15 days. Seedling characteristics were measured at the end of germination test. Results showed that moist-chilling and $GA_3$ significantly influenced the seed germination percentage. The highest germination percentage was observed in a combination of 20 days moist-chilling conditions and 500 ppm $GA_3$. Rootlet length increased while moist-chilling was increased from 10 to 30 days. By increasing moist-chilling to 40 - 60 days, rootlet length decreased unexpectedly. Combination of moist-chilling and $GA_3$ did not influence flower stem production and seed yield significantly. On the other hand, the earliest flower stem production was observed in seeds treated with moist chilling for 40 and 60 days. To guaranty flower stem production of spring sowing evening primrose in the areas with warm winter, seeds are strongly recommended to be treated with moist chilling for 40 days at 4 °C.

**Key words:** evening primrose; seed; $GA_3$; moist-chilling; germination

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## Introduction

Evening primrose (*Oenothera biennis* L.) is a biennial plant belonging to Onagraceae family, which can be cultivated as an annual plant (Ghasemnezhad and Honermeier, 2007). This plant which is native to North America, has existed since nearly 70000 years ago in Mexico and Central America and has a long history as a medicinal plant (Hall et al., 1988; Blumenthal et al. 2003). It has been carried from

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America to Europe around 200 years ago (Sekeroglu and Ozguven, 2005). Evening primrose was used to feed livestock nearly 100 years ago during drought and famine in North of China (Deng et al., 2001). It is cultivated as an oil seed crop for production of gamma linolenic acid, a rare fatty acid (Greiner and Kohl, 2014). As a commercial medicinal plant, its seed oil is used in pharmaceutical industries, nutraceutical, cosmetic, and feed sectors (Rodrigues et al., 2015; Ghasemnezhad and Honermeier, 2012).

Gamma linoleic acid is very important in pharmaceutical and nutritional industries (Sohrabi et al., 2017). The common components in all parts of *Oenothera biennis* are fatty acids, phenolic acids, and flavonoids. Also, the seed contains protein, carbohydrates, minerals, and vitamins (Timoszuk et al., 2018). The seed oil content varied from 20-30% depending on different factors including seed age, plant cultivar, and growing conditions (Christie, 1999). The fatty acids profiles of mature seed contains linoleic acid (70-75%) of the oil and gamma linoleic acid (8.0 to 9.9%) (Fieldsend and Morison, 2000).

Seed germination is a very import and vital stage for most plants. The physiological process of seed germination depends on several environmental factors such as temperature, water, light, nutrient, and smoke (Shaban, 2013). Temperature had significant influence on rate and percentage of seed germination (Sarić-Krsmanović et al., 2015). It has been shown that low temperature (4° C) inhibited seed germination in cotton plant (Barpete et al., 2015). In seed production programs the information and evaluation of seed quality after harvesting is important (Vieira et al., 2002). Low germination encounters evening primrose industrial cultivation (Hafez et al., 2013). Non-uniformity in the seed germination of evening primrose is also a big problem in its commercial cultivation (Ghasemnezhad, 2007). In evening primrose, germination characteristics are more important than weight or seed quantity (Mihulka et al., 2003). On the other hand, to have annual culture of evening primrose, cold temperature at the end of winter plays an important role. If the late planted seed do not receive enough cold temperature, flowering stem formation does not occur (Sohrabi, 2017). It has been shown that in persimmon seeds treated with moist-chilling for 4 to 8 weeks at 5° C, germination percentage was increased from 51.2% to 97.2% while non-moist-chilled germination percentage was 22.6% (El-Dengawy and Hussein, 2014). The present investigation was done to see the interaction effect of moist-chilling and gibberellic acid application on evening primrose seed germination and plant flowering homogeneity.

## Material and Methods

This experiment was performed as factorial based on completely randomized design in three replications. Treatments included moist-chilling at seven levels (0,10, 20, 30, 40, 50, and 60 days) and $GA_3$ at two levels (0 and 500 ppm). Seeds were soaked with distilled water for 30 min. The soaked seeds were kept in two layers of wet filter paper in Petri dishes and were stored in a refrigerator (2-4 °C). To prevent drying the seeds, the Petri dishes were carefully closed by parafilm, seed samples were prepared as described and were placed in a refrigerator. At the end of day 60 seed samples were divided into two parts. Half of the seeds were treated with 500 ppm $GA_3$ solution for 24 hours and the other half were planted without extra treatments. After that, both groups were planted in pots in three replications. Simultaneously, the same seeds were planted in Petri dishes with three replications to see the effect of moist-chilling and $GA_3$ on seed germination characteristics. Finally, the influence of moist-chilling and $GA_3$ on seed germination, flowering stem production, and seed yield of evening primrose plants were investigated. Seed germination test was done in a period of 15 days and the germination was controlled each e very days (from 20 April to 5 May, 2018). Each treatment was repeated three times as replication with 100 seeds per Petri dishes (Fig. I). Germination rate and germination speed were calculated as follows (Chebouti-Meziou et al 2014):

$$CG\% = (NGG/NTG^{1}) \times 100$$

[1]CG%= Capacity of germination, NGG= Number of germinated seeds, NTG= Total number of seeds tested

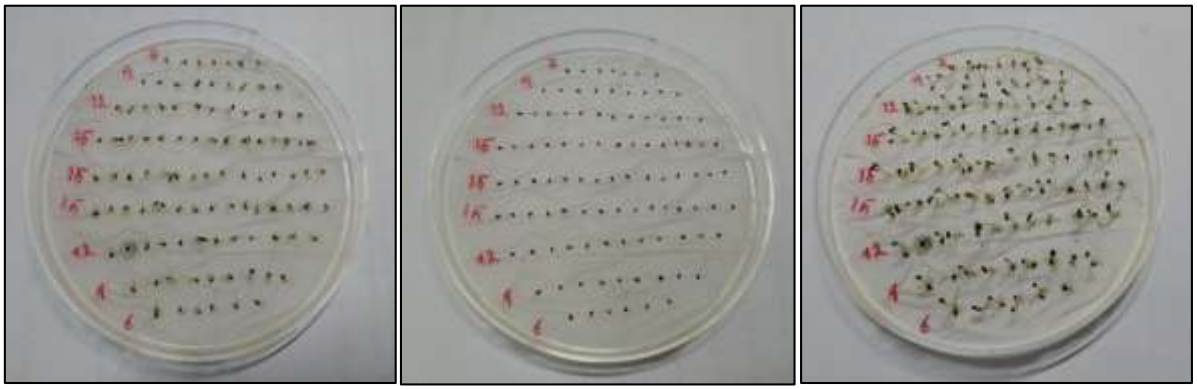

Fig. I. Experimental seeds (a); germination beginning (b); germinated seeds (c)

Table 1
Analysis variance of the effect of moist-chilling and $GA_3$ on seed germination percentage and seedling characteristics of evening primrose

| | Germination | Stemlet L | Rootlet L | Seedling L | Fresh W | Dry W |
|---|---|---|---|---|---|---|
| $GA_3$ | 0.597 | 0.028 | 0.176 | 0.674 | 0.296 | 0.512 |
| Cold | 0.514 | 0.002 | 0.002 | 0.003 | 0.427 | 0.255 |
| Cold×$GA_3$ | 0. 016 | 0.328 | 0.329 | 0.098 | 0.516 | 0.081 |
| LSD $GA_3$ | ns | 0.072* | ns | ns | ns | ns |
| Cold | ns | 0.134* | 0.171* | 0.204* | ns | ns |
| Cold×$GA_3$ | 9.65* | ns | ns | ns | ns | ns |

L: Length; W: weight

$V= NGG/T^2$

At the end of germination period, parameters including stemlet, rootlet, and seedling lengths as well as fresh and dry weights of seedlings were measured for each treatment. For each treatment, six seedlings were measured randomly for fresh and dry weights. Fresh weights of seedlings were recorded using a digital scale with accuracy of 0.0001 g. Then it was dried in an oven at 75° C for 48 h. Afterwards, the dry weights were obtained. Fig. (I) shows an overview of the seed germination test.

## Results

Moist-chilling and $GA_3$ significantly influenced seed germination of evening primrose (Table 1). The highest germination percentage was observed in seed samples which were incubated for 20 days under moist-chilling conditions and treated with 500 ppm $GA_3$ solution. On the other hand, the lowest germination rate was observed in seed samples kept for 10 days under moist chilling conditions and treated with $GA_3$. No significant interaction effect of moist-chilling and $GA_3$ was observed on the length of rootlets, stemlets, and seedling as well as the fresh and dry weights of seedlings. The longest stemlet was recorded in the seedlings treated with 60 days moist-chilling. As moist-chilling treatment increased from 10 to 30 days, so did the rootlet length. Significant decrease in lengths was observed in seedlings treated with moist-chilling between 40 and 60 days.

### Interaction effect of moist-chilling and $GA_3$ on seed germination percentage

Statistical analysis showed that the interaction between moist-chilling and $GA_3$ on seed germination percentage was varied. The seeds treated for 10 days of moist-chilling had the highest percentage of germination. On the other hand, the seeds which were treated with $GA_3$ and were kept for 20 days under moist-chilling conditions showed the highest seed germination percentage (Fig. II).

### Effect of moist-chilling on stemlet length

[2] V = speed of germination, NGG = number of germinated seeds, T = times of germination (days)

According to the analysis of variance (Table 1), moist-chilling affected significantly stemlet length of evening primrose seedlings. In this case, the length of stemlet was better influenced by 60 days moist-chilling than the 50, 40, 30, 20, and 10 days of treatment. Besides, no significant difference was observed between control seeds and and those treated with moist-chilling for 50, 40, 30, 20, and 10 days. In fact, the seeds treated with less moist-chilling preiod had short stemlet length as can be seen in (Fig. III). Although $GA_3$ solution was expected to affect the stemlet length, $GA_3$ at the concentration of 500 ppm did not have any significant effect on the stemlet length as compare with the control in this study.

## Effect of moist-chilling on the rootlet length

Results of variance analysis showed that moist-chilling significantly affected rootlet length of evening primrose. The seeds treated with 30 days moist-chilling showed improved rootlet length. The seeds receiving moist-chilling conditions less than 30 days (10 and 20 days) were not different from the control. Also, the seeds treated with 40, 50, and 60 days moist-chilling were not different from control as shown in (Fig. IV). In this study, $GA_3$ at 500 ppm had no significant effect on rootlet length of evening primrose seedlings. Also, when moist-chilling increased from 10 to 30 days, rootlet lengths increased. Furthermore, by increasing moist-chilling to 40 - 60 days, rootlet lengths decreased.

## Effect of moist-chilling on seedling length

Analysis variance of the obtained data showed that moist-chilling treatment significantly influenced the length of seedlings. The highest seedling heights were observed in seeds which were treated with 30, 40, and 50 days moist-chilling. There was no significant difference between seeds treated with 60, 20, and 10 days moist-chilling and control samples (Fig. V).

## Evaluation of germination rate and germination speed

Moist-chilling and $GA_3$ solution with 500 ppm concentration significantly affected the seed germination percentage. The germination process was evaluated daily. Germination percentage in seeds treated with moist-chilling was 91.2%. Also, the seeds treated with $GA_3$ at 500 ppm concentration showed 90.4% germination. On the other hand, for moist-chilling and $GA_3$ the germination rates were 10.1 and 9.082 per day, respectively.

## Effect of moist-chilling and $GA_3$onflowering stem production and seed yield

While seed yield was not influenced by moist-chilling (Fig. VII), the time needed for flower stem production from seed sowing was significantly affected by the period of moist-chilling. As figures (VI) and (VII) show, flower stem and subsequently, seed production were not observed in control plants.

Germination rate for control (0 days moist-chilling) was 80% after three days, 83.3% after 6 days, 85% after 9 days, and 0% (no germination) 12 days after planting (Fig. VIII). The seeds treated with moist-chilling for 10 days had the highest germination rate (98.3%) after 3 days and 100% after 6 days (Fig. IX). Moist-chilling for 20 days resulted in 86.7% seed germination, 90% after 6 days, and no germination was recorded after day 20 (Fig. X). In the seeds treated with moist-chilling for 30 days, 91.7% germination was recorded 3 days after water imbibition followed by an increase to 93% germination during the next three days with no further increase till the end of experiment (Fig. XI). As seen in Fig. (XII), when the seeds were treated with moist-chilling for 40 days, 85% germination occurred three days after water imbibition and no germination was observed till the end of experiment.

The seeds treated with 50 days moist-chilling showed 93.3% and 95% germination after 3 and 6 days, respectively which was followed by no further germination (Fig. XIII). Similar to the other treatments, in the seeds which were treated with moist chilling for 60 days, the maximum germination (93.3%) was observed during the first 3 days of test and no germination was observed until the end of experiment (Fig. XIV). According to

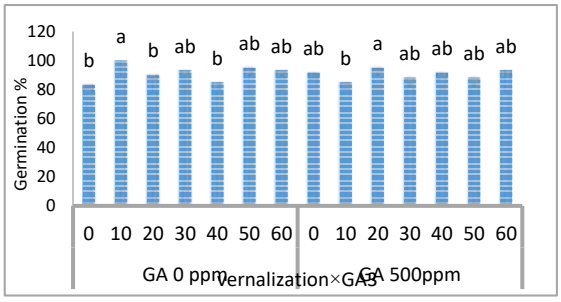


Fig. II. Interaction between moist-chilling and $GA_3$

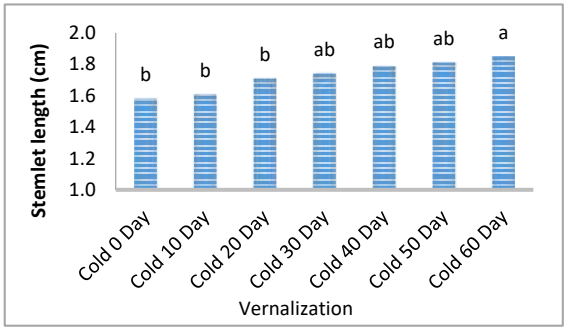


Fig. III. Effect of moist-chilling on stemlet length

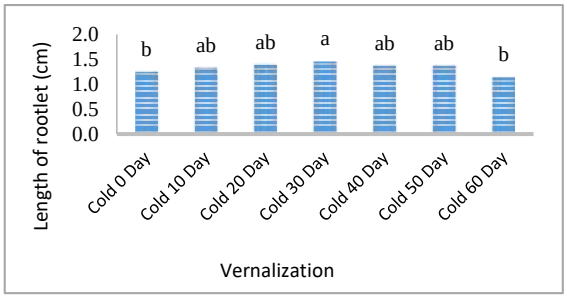


Fig. IV. Effect of moist-chilling on rootlet length

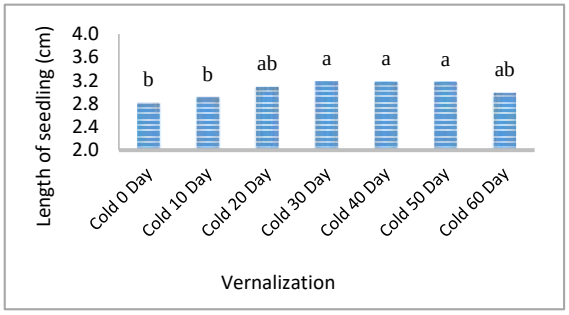


Fig. V. Effect of moist-chilling on seedling length

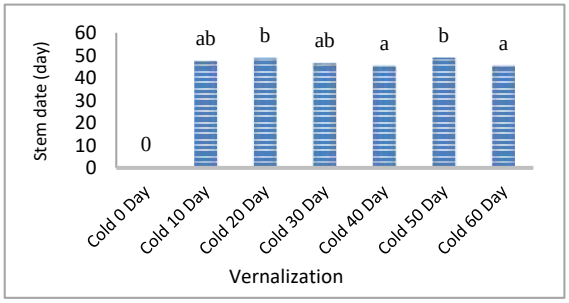


Fig. VI. Effect of moist-chilling on stem date production

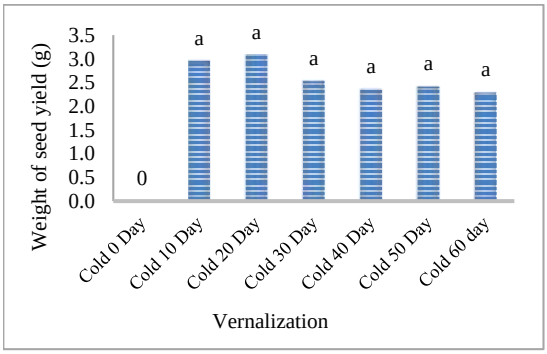


Fig. VII. Effect of moist-chilling on seed yield

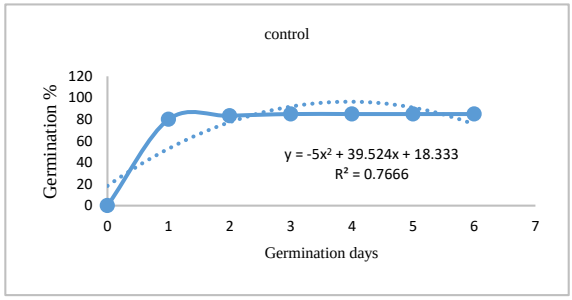


Fig. VIII. Effect of moist-chilling on germination process

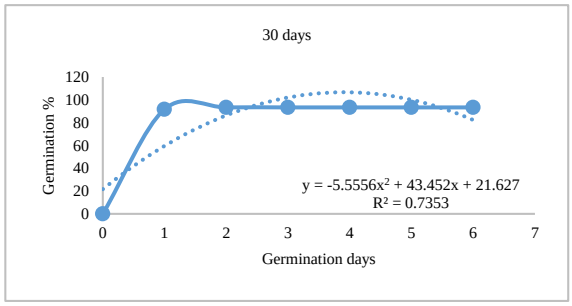


Fig. IX. Effect of moist-chilling on germination process

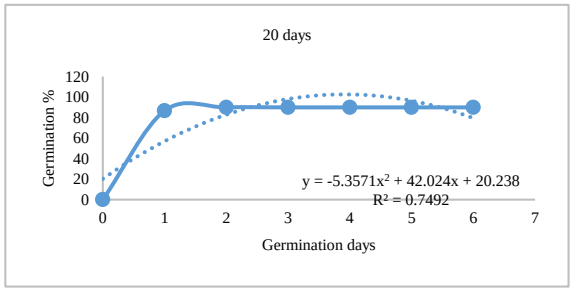


Fig. X. Effect of moist-chilling on germination process

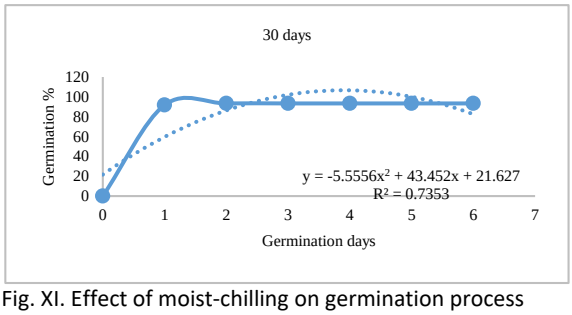


Fig. XI. Effect of moist-chilling on germination process

the statistical results, moist-chilling at 4° C for 10 days had a more pronounced effect on seed germination percentage. The differentiation in germination between different moist-chilling treatments was due to the age of seed maturity and seed quality. It is therefore recommended to use seeds from one capsule to evaluate the effect of moist-chilling duration on seed germination percentage.

As the seeds were planted in Petri dishes, the germination process was evaluated every three days for all samples. For control (500 ppm) gibberellic acid solution applied after 3 days, 86.7% germination was observed and after 6 days, 91.7% seeds were germinated, after which no germination was seen (Fig. XV). The seeds were treated with 10 days moist-chilling and 500 ppm $GA_3$ solution after three days showed 81.7% germination which was then stopped and no germination was observed till the end of the experiment (Fig. XVI). On the other hand, the seeds that received 20 days moist-chilling and were treated with 500 ppm $GA_3$, showed 95% germination (Fig. XVII). Furthermore, around 83.3% of the seed germinated when treated with 30 days moist-chilling and 500 ppm $GA_3$ solution after which no further germination occurred (Fig. XVIII). The germination of seed samples which were treated with a combination of moist-chilling for 40 days and 500 ppm $GA_3$ was 88.3% three days after water imbibition and at the next three days the germination percentage reached to 91.7% and eventually stopped (Fig. XIX). As seen in Fig. XX, when moist chilling reached 50 days, the seeds treated with 500 ppm $GA_3$ showed 88.3% germination during the first three days of experiment and after that, germination did not increase. The seeds treated with 60 days moist-chilling and 500 ppm $GA_3$ showed 91.7% germination and after six days' germination reached 93.3% before it eventually stopped (Fig. XXI). The combination of moist-chilling and $GA_3$ not only reduced the time of germination from seed planting, but also increased the germination percentage and reduced the duration of germination.

## Discussion

Moist-chilling and $GA_3$ had positive influences on seed germination percentage of evening primrose. Similar findings were reported by Keshtkar et al. (2009) on *Ferula assa-foetida* L. seeds where the highest germination percentage was observed (52%) after treatment with pre-chilling and 250 ppm $GA_3$. Also, the highest seed germination (72%) of *Prangos ferullacea* seeds was observed under the treatment of 1000 ppm $GA_3$ and pre-chilling the while control seeds did not germinate. It has been shown that treating seeds of *Arbutus unedo* plant along with one month at 3 to 5°C temperature and 2000 ppm $GA_3$ significantly improved the germination percentage (Pipinis et al., 2017). Rouhi et al. (2005) reported that cold temperature and $GA_3$ positively affected the seed germination of *Amygdalusscparia* plant.

The same results were reported in *Ferula ovina* Boiss (Amoozghaie, 2009). Seed of ferula treated by moist-chilling for six months and soaked in 500 ppm $GA_3$ for 24 hours before planting were demonstrated to have not only improved germination, but also reduced the time of germination compare with control seeds. The best treatment was four and six week moist-chilling followed by soaking in 500 ppm $GA_3$ for 24 hours. These treatments had positive influence on germination percentage and decreased the time to 50% germination compared to the control. Similar study was performed by Parnin et al. (2015) on *Juglansregia* L. who recorded the highest seed germination in samples treated with moist chilling for two months and then treated with 400 ppm $GA_3$. When the seeds of *Thymus satureioids* and *Lavandula dentate* were treated with $GA_3$ their germination percentage significantly increased. The seeds of *Thymus satureioids* treated with 50 ppm $GA_3$ solution showed an increase by 27% compared to the control, and the seeds of *Lavandula dentate* treated with 1000 ppm $GA_3$ solution had maximum germination of 67% compared to the control (Chetouani et al., 2017).

Patel and Mankad (2014) found that $GA_3$ solution had a positive influence on seed germination percentage of *Tithoniarm tundifolia* Blake. In the seeds treated with 500 ppm $GA_3$ maximum effect was observed on germination percentage. Stratified seeds of *Acer pseudoplatanus*

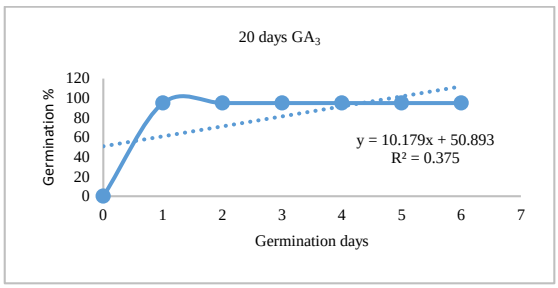


Fig. XVII. Effect of moist-chilling and GA3 on germination process

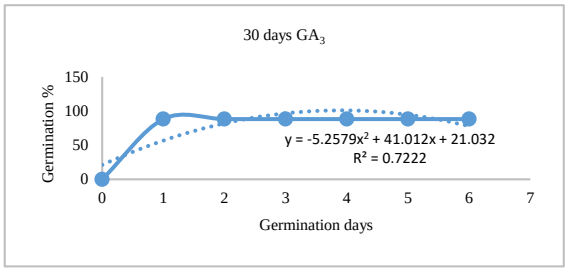


Fig. XVIII. Effect of moist-chilling and GA3 on germination process

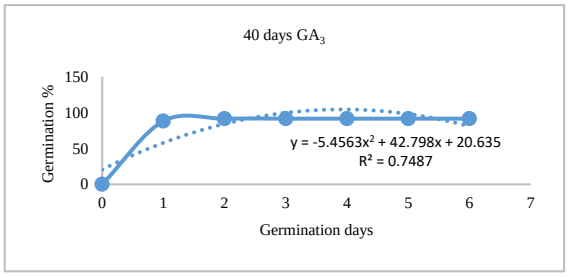


Fig. XIX. Effect of moist-chilling and GA3 on germination process

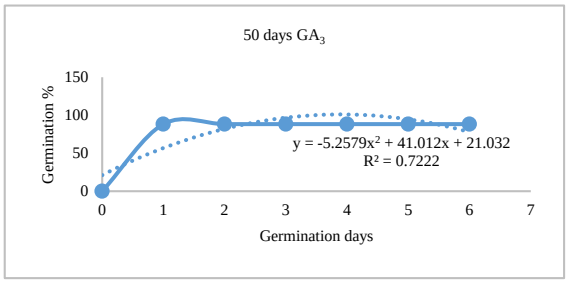


Fig. XX. Effect of moist-chilling and GA3 on germination process

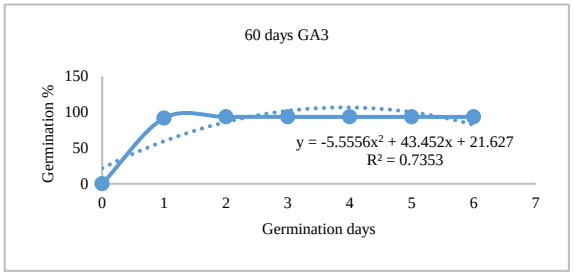


Fig. XXI. Effect of moist-chilling and GA3 on germination process

L. with $GA_3$ were shown to have improved germination rate (Stejskalova et al., 2015). Seeds of *Pinus ponderosa* plant stored in ambient conditions for three and four years at 9° C and then treated with pre-chilling for 21 and 60 days showed 34% and 62% germination, respectively (Pasquini and Defosse, 2012).

In this study statistical analysis showed that the interaction between moist-chilling and $GA_3$ on seed germination percentage was effective. Meziou et al. (2014) reported that mechanical scarification and cold treatment of pistachio seed at 4° C increased the seed germination percentage up to 70% in a period of 30 days. Ghildiyal et al. (2009) found that the germination rate and germination percentage of *Pinus roxburghii* were improved by moist-chilling. Ren et al. (2007) studied the effect of $GA_3$ concentrations (500-1000 mg/L) on three *Pedicularis* species followed by 15-30 days at 4° C resulted in the highest germination percentage. Nevertheless it was found that the effect of moist-chilling treatment alone was similar to the interaction effect of moist-chilling and $GA_3$ treatment.

According to the analysis of variance, moist-chilling effected significantly the stemlet length in evening primrose seedlings. However, $GA_3$ application did not affect seedling length in these plants. On the contrary, Akhtar et el. (2008) showed that application of 10 ppm $GA_3$ significantly affected germination percentage, rootlet length, and number of leaves in *Spinacea oleraceae* L. Fetouh and Hassan (2014) reported that, cold stratification (5° C) for 90 days increased the shoot height in *Magnolia grandiflora* L. plants. Gibberellic acid at the concentration of 500 mg/L had positive effect on stemlet length of *Curcuma alismatifolia* (Khuankaew et al. 2008). Also, Rouhi et al. (2005) reported that a combination of $GA_3$ and low temperature 7° C did not positively influence rootlet length of *Amygdalus scoparia* plant compared with the plants treated with low temperature alone. Borowski and Michalek (2014) reported that low temperature reduced germination percentage, germination velocity, and rootlet length of soy bean seeds. Ramzi et al. (2013) showed that low temperature reduced seed germination percentage rate, vigor index, and shoot and root lengths of *Sorghum bicolor* L. On the other hand, growth index as well as the ratio of root length to shoot length

increased. Low temperature reduced the angel between the primary lateral roots and reduced root activity and volume in *Brassica napus* L. and *Zea mays* L plants (Nagel et al. 2009). Several studies have shown that the moist-chilling decreased the rootlet length of plants.

Analysis of variance of data showed that moist-chilling treatment significantly influenced the length of seedlings. Similar results were reported by Fetouh and Hassan (2014) in *Magnolia grandiflora* L. plant. They showed that cold stratification (5° C) for 90 days increased the seedling length of the plant. Sincik et al. (2004) reported that low temperature reduced maximum growth, root development, yield, and seedling weight of pea plants.

Low temperature is a limiting factor for seed germination and plant growth. For example, 5° C treatment had a negative influence on seed germination and reduced seedling length and fresh and dry weights of *Elymusnutans* Guriseb plant (Fu et al., 2017). $GA_3$ at 250 mg/L concentration significantly improved the seed germination of Loquat, when the concentration of $GA_3$ was increased to 300 mg/L, germination rate decreased, but the length of shoot and root of seedlings increased (Muhamad, 2013). Meziou et al. (2014) reported that, mechanical scarification and cold treatment of pistachio seeds at 4° C increased the seed germination percentage up to 70% in a period of 30 days.

Sohrabi et al. (2017) reported that low temperature and $GA_3$ significantly influenced the flower stem production of evening primrose. Low temperature reduced the flowering time in onion before it produced bulbs (Fukuda et el., 2017). Treatment of garlic bulb with low temperature (4° C) for two months significantly influenced production of flower stem (Kaur and Dhull, 2017).

Nasiri et al (2017) reported that one month moist-chilling treatment at 4° C increased germination percentage to 24.7% while the data for control plants was 6.2% in two genera of chamomile and tansies plants. Amini et al. (2015) found that wet and dry pre-chilling at 4° C for 45 days promoted *Setaria glauca* seed germination. When the seed of *Pedicularis olympica* Boiss. Were treated with moist-chilling at 4° C for 15 days, 75% seed germination was recorded (Kirmizi et al., 2010). Zadeh et al. (2015) reported that 250 mg/l $GA_3$ with 10 days moist-chilling at 5° C significantly increased germination percentage of *Echinacea purpurea* cv. Magnus plant. Sayyad-Amin and Shahsavar (2018) found the same result and the maximum germination percentage was observed at 250 mg/l $GA_3$ concentration with 70 days stratification at 4-7° C in *Diospyros lotus* plants. Stratification at 5° C for 21 days and subsequent treatment with 100 mg/l $GA_3$ solution accelerated the germination percentage of *Alstroemeria ligtu* hybrid seeds (Nasiri et al., 2013). For *Pedicularis* species, 15-30 days stratification at 4° C and treatment with 500-1000 mg/l $GA_3$ solution, the highest germination percentage was observed (Ren and Gaun, 2008). Also, 25 ppm $GA_3$ treatment increased seed germination percentage of *Erica australis* plant (Vera et al., 2010) and in *Pedicularis olympica*, 250 ppm $GA_3$ resulted in seed germination 64% (Kirmizi et al., 2010). Naikawadi et al. (2012) reported that treatment with 5.00 mM $GA_3$ for 36 h increased the germination percentage of *Evolvulus alsinoides* plant. Seven days moist-chilling with 400 ppm $GA_3$ concentration at 15° C in *Scrophularia sp* led to the highest germination percentage (Hosseini et al., 2018). The combination of moist-chilling and $GA_3$ not only reduced the time of germination from seed planting, but also increased the germination percentage and reduced the duration of germination.

## Conclusion

Moist-chilling and $GA_3$ have a main role in removing chilling requirement of the biennial plant, evening primrose. We found that moist-chilling and $GA_3$ solution had positive influences on seed germination percentage of evening primrose. More specifically, only moist-chilling was observed to improve flowering stem production. Suitable time for changing from the rosette to generative situation is 40 days moist-chilling at 4° C for evening primrose plant. Moist-chilling treatment accelerated the germination process in the study and the majority of seeds germinated after three days and the highest the and lowest germination percentages recorded were 98% and 81%, respecively.

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