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Effects of some treatments on seed germination of *Cardopatium corymbosum* (L.) Pers.

Ummahan Öz Arık*

*Medicinal and Aromatic Plants Program, Alaşehir Vocational School, Manisa Celal Bayar University, Manisa, Turkey, orcid.org/0000-0002-0281-1048, *Corresponding author; ummahanoz48@gmail.com*

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Abstract

Cardopatium Juss. has only one species. *Cardopatium corymbosum* (L.) Pers. is a perennial herb belonging to the Asteraceae. In this study germination of *C. corymbosum* have been investigated. The germination experiments were carried out in petri dishes and seedling starter trays and effects of different treatments on germination percentage observed. In germination experiments in petri dishes, best germination rate was obtained in the capitula kept in 10 ppm GA_3 for 24 hours. In the seedling starter trays, highest germination rate was determined in the seeds kept in water for 12 and 24 hours and sown in 1:1 perlite and peat media. The best results were obtained in GA_3 experiments. The rate of germination in the seeds without capitulum is higher than those surrounded by capitulum. The plants which germinate in saline media in petridishes are generally healthier than other applications. It has also been determined that *C. corymbosum* species also have a high germination percentage in saline environments.

Key words: *Cardopatium corymbosum,* GA_3, peat, perlite, seed germination.

1. Introduction

Cardopatium corymbosum (L.) Pers. belongs to the Asteraceae family (Figure 1). Its distribution area includes Italy, Sicily, Macedonia, Greece (Cyclades Islands), Crete, East Aegean Islands, Rhodes, Turkey, Lebanon, Syria, and Cyprus (Hassler, 2017).

Figure 1. Flowering plants of *C. corymbosum.*

C. corymbosum roots are used in cows as mastisis treatment, as an antiseptic in the topical application of wounds and in the treatment of intestinal worms (Tuzlacı and Aymaz, 2001; Pieroni et al., 2006; Bulut et al., 2017).

The aim of this study was to investigate the possibility of using this plant in lanscape beutification because of its showy flowers.

2. Materials and Methods

Germination experiments were carried out with the seeds of *Cardopatium corymbosum* (L.) Pers. collected from the Alaşehir-Manisa in Turkey. Before starting the experiments seeds with similar features were separated and cleaned and then tested for viability. The seeds used in the germination experiments were kept in 1% sodium hypochlorite solution for 10 minutes for sterilization and then washed with sterile water. The experiments were arranged at the temperatures of 26 ± 1 °C under 35-43% humidity in petri dishes and seedling starter trays. The treatments were given using giberellic acid, indole butyric acid, salicylic acid and sodium chloride in petri dishes. Germination was recorded on daily basis.

2.1. Germination experiment in petri dishes

Solutions were prepared for germination pre-treatments. Sterilized seeds were exposed to different pre-treatments. As a control group, the seeds without capitulum and seeds surrounded by the capitulum were kept in water in sterile bottles for 12 hours and 24 hours.

Seeds which are with capitulum or without capitulum were incubated in solutions containing different doses of gibberellic acid (10 ppm, 20 ppm, 50 ppm and 100 ppm), indole butyric acid (2 ppm, 4 ppm, 8 ppm, 16 ppm), salicylic acid (100 ppm, 250 ppm, 500 ppm and 750 ppm) and NaCl (100 ppm, 200 ppm, 400 ppm and 800 ppm) for 12 and 24 hours. 10 seeds exposed to pre-treatment were sown in each petri dishes. The experiment was carried out in four replications. The germination treatments in petri dishes were observed for 21 days and data recorded.

2.2. Germination experiment in seedling starter trays

The seeds with capitulum and without capitulum were used. These were kept in water for 12 and 24 hours. The experiments were carried out in three replications, with 30 seeds per seedling starter tray. The seedings were then transferred to different growth media.

The media used in seed germination experiments in seedling starter trays were: only peat, 50% peat + 50% perlite, only perlite, 1:3:1 pre-prepared mixture, fertilizer (burned sheep manure), perlite mixture, 1:4:1 pre-prepared mixture, fertilizer (worm-fertilizer), perlite mixture, 1: 1: 1 pre-prepared mixture, perlite, peat mixture. The content of the pre-prepared mixture was as follows: 30 % perlite mixed imported peat, pH 5,5-6,5, Peat thickness: 0-6 mm, Organic matter: 95 %, Extra NPK added, Black peat: 50% white peat: 50%. Sphagnum peat experiment was set up as follows: Humus:42-55 g l^{-1}, Weight: 200 kg m^{-3}, EC: Maximum 0,3, pH: 5,5-6,5, Lime: 3 kg m^{-3}, Calcium and magnesium: 2 kg m^{-3}, Surface active agent: 0,1 kg m^{-3}.

The germination treatments in seedling starter trays were observed for 28 days and data recorded.

3. Results

3.1. Seed germination results in petri dishes

Germination was observed on the first day at 20 ppm GA_3. The average germination percentage is 95% at 10 ppm, 90% at 20 ppm, 95% at 50 ppm and 100% at 100 ppm. In this treatment, seeds without capitulum which were kept in solution for 12 hours were used (Figure 2).

Figure 2. Germinating seeds in different doses of GA_3 solution for 12 hours.

Germination was observed on the first day at 10 ppm GA_3. The seeds without capitulum were kept in solution for 24 hours. The average germination percentage was 92,5% at 10 ppm, 90% at 20 ppm, 82,5% at 50 ppm and 80% at 100 ppm (Figure 3).

Figure 3. Germinating seeds kept in different doses of GA_3 solution for 24 hours.

The first germination was observed from the 4th day in the seeds surrounded by the capitulum which were kept in a solution of 50 ppm GA_3 for 12 hours. The average germination percentage is 10% at 10 ppm, 15% at 20 ppm, 7,5% at 50 ppm and 17,5% at 100 ppm (Figure 4).

Figure 4. Germination of seeds with capitulum in different doses of GA_3 solution for 12 hours.

The first germination was observed on the 8th day in the seeds surrounded by the capitulum which were kept in a solution of 10 ppm GA_3 for 24 hours. The average germination percentage was 2,5% at 10 ppm, 2,5% at 20 ppm, 7,5% at 50 ppm and 5% at 100 ppm (Figure 5).

Figure 5. Germination of seeds with capitulum in different doses of GA_3 solution for 24 hours.

The solutions of indole butyric acid and salicylic acid were prepared by dissolving these in 96% ethyl alcohol but seed germination in IBA and salicylic acid were not observed. IBA and salicylic acid tests were carried out by adjusting the alcohol level to 50% and 70% at the end of 21st day. But no germination was observed in petri dishe experiments.

The seeds without capitulum were placed in NaCl solution for 12 hours. On the 2nd day germination was observed in all petri dishes. At 100 ppm the germination percentage was on 6th day in 1st replications. In 3rd replications germination was 100% on 3rd day. At 200 ppm the germination percentage on the 3rd day of the 4th replication reached 100 %. All seeds germinated at 800 ppm concentration on 11th day in 2nd replication and 14th in 4th replication. Average germination percentage at 100 ppm was 95% at 200 ppm: 82,5% at 400 ppm: 77,5% at 800 ppm: 92,5% (Figure 6) .

Figure 6. Germination in seeds kept in different doses of NaCl solution for 12 hours.

When we used seeds without capitulum kept in NaCl solution for 24 hours, germination was observed in all petri dishes on 2nd day The germination percentage reached 100% in all concentrations on 14th day in some replications. The average percentage of germination: at 100 ppm: 87,5%, at 200 ppm: 92,5%, at 400 ppm: 82,5%, at 800 ppm: 92,5% (Figure 7).

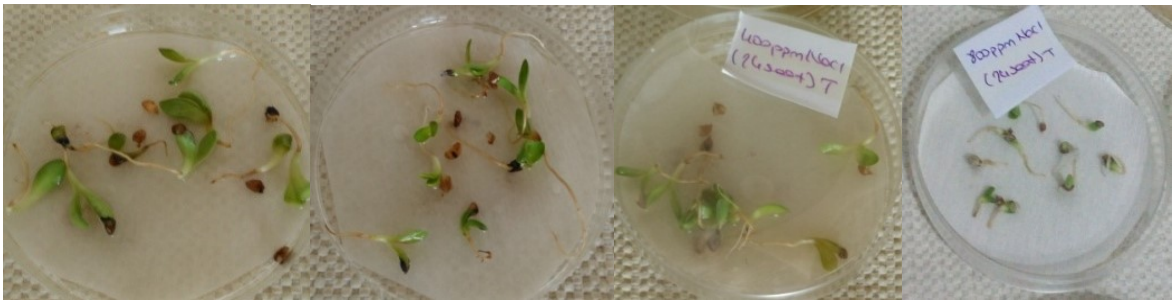

Figure 7. Germination in seeds kept in different doses of NaCl solution for 24 hours.

Germination was not observed in some concentrations in the capitula incubated in NaCl solution for 12 hours. Average percentage of germination: at 100 ppm: 27,5%, at 200 ppm: 10%, at 400 ppm: 22,5%, at 800 ppm: 20% (Figure 8).

Figure 8. Germination in capitulum kept in different doses of NaCl for 12 hours.

No germination occurred in seeds surrounded by the capitulum left in NaCl solution of 100 ppm and 200 ppm for 24 hours. At 400 ppm, germination was observed in the 1st replication and the first germination occurred on the 6th day. At 800 ppm, germination was observed in 3rd and 4th replication and first germination occurred on 10th day. It has been observed that the quality of germinated seeds is better than other treatments. Average germination percentage: at 400 ppm: 7,5%, at 800 ppm: 5% (Figure 9).

Figure 9. Germination in capitula kept in different doses of NaCl for 24 hours.

The best germination rate occurred in seeds kept in 100 ppm NaCl solution for 12 hours. In the seeds without capitulum results are better than those surrounded by capitulum. In applications using capitulum, germination is observed to decrease as the salt concentration increases.

The first germination in control occurred in the 1st replication in the seeds without capitulum kept in water for 12 hours. On the second day of the experiment, germination was observed in other petri dishes. The germination rate reached 100% on the 4th day in some replications. Average germination percentage was 97,5% for 12 hours and 90% for 24 hours. The first germination was observed in the 4th replication and this experiment was conducted with capitulum which were kept in water for 12 hours. Germination was not observed in some replications. Average germination percentage was 27,5% for 12 hours and 5% for 24 hours (Figure 10) .

Figure 10. A and B: Seeds kept in water for 12 and 24 hours (respectively), C and D: Germination in capitulum kept in water for 12 and 24 hours (respectively).

The best germination percentage in the control group was obtained in seeds without capitulum soaked in water for 12 hours. The lowest germination rate was observed in the capitula kept in water for 24 hours. In germination experiments in petri dishes, the best

germination rate was obtained in the capitulums kept in 10 ppm GA_3 for 24 hours. It was observed that seeds without capitula gave better results (Figure 11).

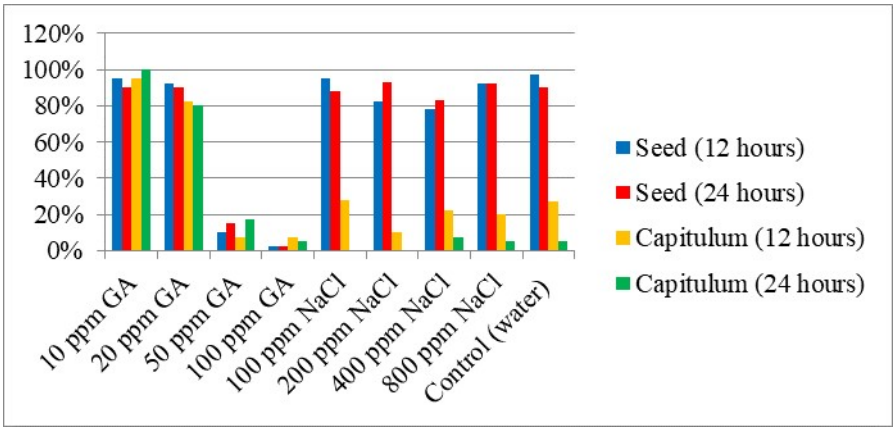

Figure 11. Different applications compared in petri dishes experiments.

3.2. Seed germination results in seedling starter trays

The seeds surrounded by capitulum and without capitulum were used. The materials which were kept in water for 12 and 24 hours were seeding in 6 different media and results were recorded.

The first germination was observed in the seeds without capitulum, kept in water for 12 hours and seedlings in only peat media. The seed germination percentages in only peat media are as follows: seeds which were kept in water for 12 hours (60%), 33,33% for 24 hours (Figure 12); 40% percentage in capitula kept in water for 12 hours, 63,33% for 24 hours (Figure 13).

Figure 12. Germination on only peat media (12 and 24 hours treatment with seed).

Figure 13. Germination on only peat media (12 and 24 hours treatment with capitulum).

The highest germination rate was reached in the capitula kept in water for 24 hours. The lowest rate was obtained in the seeds kept in water for 24 hours. However, some germinated seeds lost their vitality during the experiment. The first germination in seeds was observed on 4th day in 50% peat + 50% perlite media but germination in seeds with capitulum started on 7th day. In addition, it was determined that in these samples two seeds kept in water for 12 hours germinated from a capitulum and when kept for 24 hours, two seeds germinate in two capitula. The seed germination percentages in 50% peat + 50% perlite media are as follows: 86,66% in seeds which were kept in water for 12 hours and 24 hours (Figure 14) ; 74,19 % in capitulum kept in water for 12 hours, 68,75% for 24 hours (Figure 15).

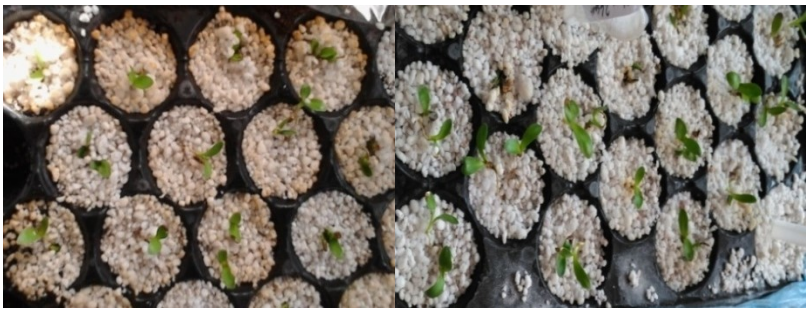

Figure 14. Germination in 50% perlite and 50% peat media (12 and 24 hours treatment with seed).

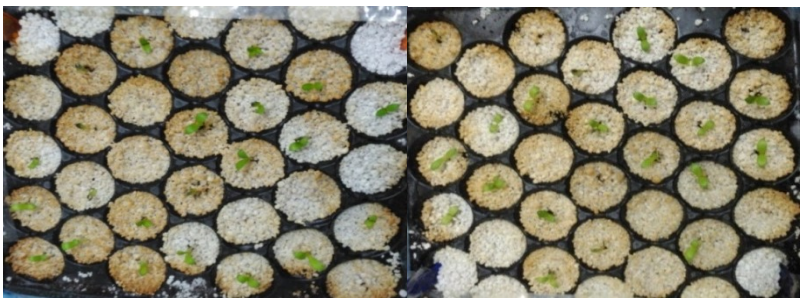

Figure 15. Germination in 50% perlite and 50% peat media (12 and 24 hours treatment) with capitulum.

In perlite application alone, first germination was obtained in seeds without capitulum kept in water for 24 hours and in this application, germination was observed from the 6th day. The latest germination was determined in seeds with capitulum that were kept in water for 12 hours and the first day of germination was 9th day of sowing. The percentage of germination in non-capitulum seeds kept in water for 12 hours is 83.33% and it is 80% for 24 hours (Figure 16). Also percentage in seeds with capitulum is 73,33 % for 12 hours and 71,87 % for 24 hours (Figure 17).

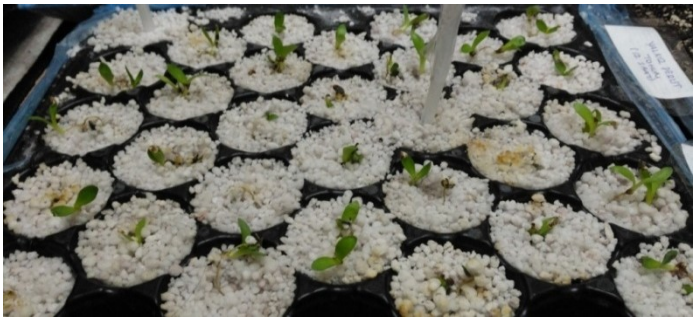

Figure 16. Germination in only perlite media (12 and 24 hours treatment with seed).

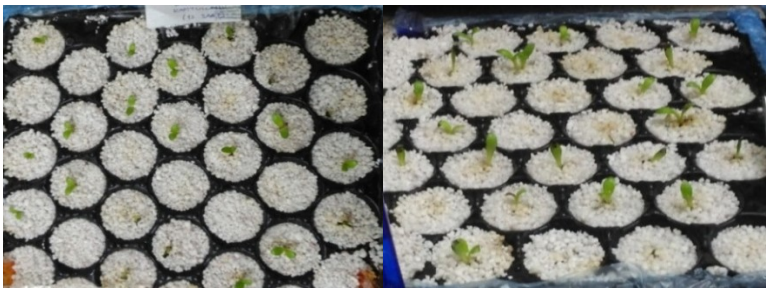

Figure 17. Germination in only perlite media (12 and 24 hours treatment with capitulum).

Germination in seeds without capitulum and kept in water for 12 hours and 24 hours was earlier than in seeds with capitulum in 1:3:1 pre-prepared mixture, fertilizer (burned sheep manure) perlite mixture media. The percentage of germination in the seeds with capitulum removed and kept in water for 12 hours and 24 hours was 66% (Figure 18). However, percentage of germination in the seeds with capitulum and kept in water for 12 hours is 43,33% and for 24 hours is 30% (Figure 19).

Figure 18. Germination in 1:3:1 pre-prepared mixture, fertilizer (burned sheep manure) perlite mixture media (12 and 24 hours treatment with seed).

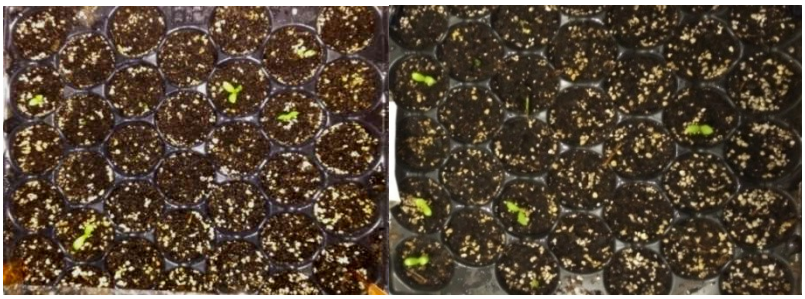

Figure 19. Germination in 1:3:1 pre-prepared mixture, fertilizer (burned sheep manure) perlite mixture media (12 and 24 hours treatment with capitulum).

Germination was observed on the 6th day in the seeds with capitulum removed and kept in water for 12 hours and 24 hours in 1:4:1 pre-prepared mixture, fertilizer (worm-fertilizer), perlite mixture. In this treatment, two seeds germinated from a capitulum. Germination rates of 56,66% and 43,33% were obtained in 12 hours and 24 hours applications with seeds without capitulum, respectively (Figure 20). Germination rates of 51,61% and 61,29% were also determined in the same applications with capitulum respectively (Figure 21).

Figure 20. 1:4:1 pre-prepared mixture, fertilizer (worm-fertilizer), perlite media (12 and 24 hours treatment with seed).

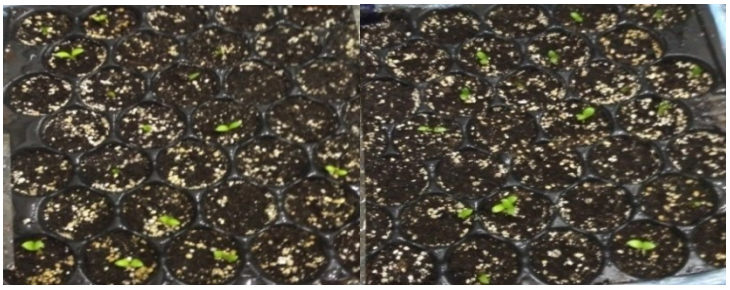

Figure 21. 1:4:1 pre-prepared mixture, fertilizer (worm-fertilizer), perlite media (12 and 24 hours treatment with capitulum).

First germination was observed on the fourth day in seeds without capitulum seeding in 1:1:1 pre-prepared mixture, perlite, peat mixture and on the sixth day in seeds with capitulum. In some applications two germinated seeds emerged from only one capitulum. Germination rates of 56,66% and 36,66 % were obtained in 12 hours and 24 hours applications in the seeds without capitulum, respectively (Figure 22). Germination rates of 66,66% and 45,16 % were also determined in the same applications with capitulum respectively (Figure 23).

Figure 22. 1:1:1 pre-prepared mixture, perlite, peat media (12 and 24 hours treatment with seed).

Figure 23. 1:1:1 pre-prepared mixture, perlite, peat media (12 and 24 hours treatment with capitulum).

The best results were observed in 50% perlite + 50% peat media using seeds without capitulum and the lowest germination was determined in 1:3:1 pre-prepared mixture, fertilizer (burned sheep manure) perlite mixture media with capitulum kept in water for 24 hours (Figure 24).

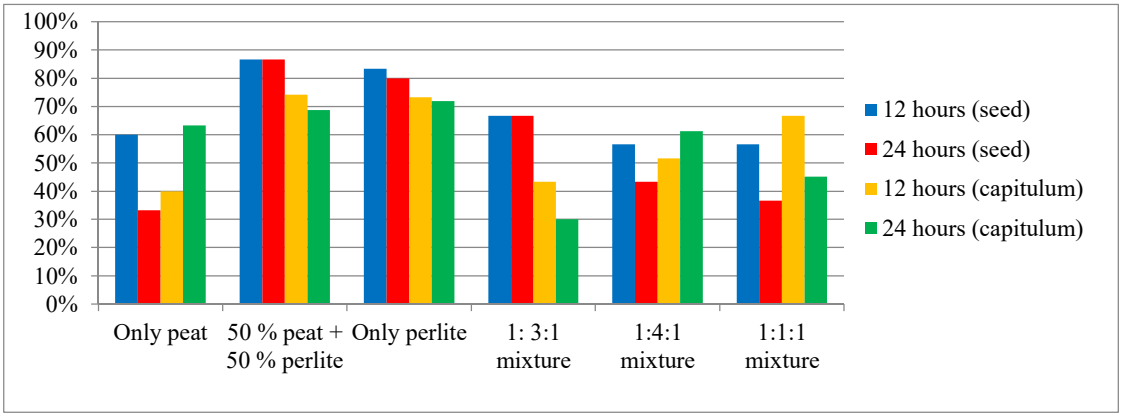

Figure 24. Comparison of different applications in seedling starter tray experiment.

4. Discussion

C. corymbosum is a species with a drug potential. It was used for germination studies in the Kew Royal Botanic Gardens (2018) and report shows that in the seeds with outer layer removed it was 89% after 35 days at 10˚C, 8/16 light period in 1% agar medium; germination percentage was 74% under same conditions at 15 ˚C. It has been reported that germination is 67% at 25˚C after 70 days. It was 93 % at 10 ˚C after 63 days. Our aim here was to undertake more extensive studies in this connection on this species. For this reason the effect of different applications and growth media on germination were followed.

Our studies showed that there are 450-5940 capitulum in a plant and 7-11 flowers in each capitulum. Inspite of this large production there are not so many plants. The plant generally multiplyies by tuber.

When the results of germination experiments carried out in petri and seedling starter trays are examined, gibberellic acid applications have given good results. But these seeds are surrounded by capitulum in nature and the germination rate in the soil is observed to be low. Our research has also shown that germination percentage in seeds without capitulum is higher than in seeds with capitulum.

The highest germination rate (100%) in petri dishe germination experiments was found in seeds without capitulum which were exposed to 100 ppm GA_3 application for 12 hours.

Giberellins weaken the endosperm layer and increase the seed germination in this way by activating the storage material in this layer (Taiz and Zeiger, 2008). According to Okay and Günöz (2009), the shortest germination duration in *Centaurea tchihatcheffii* seeds is obtained at 10 ppm GA_3 application and the highest germination (43,33%) at 100 ppm GA_3.

In a study conducted on *Stevia rebaudiana* (Sugar leaf) to increase germination (Yıldırım, 2017) different concentrations of gibberellic acid were tried and it was reported that most suitable GA_3 application for seeedlings development and germination was at 50 mg/l.

In another study, it was reported that a combination of 3 mg l^{-1} GA_3 and 2 mg l^{-1} kinetin cause 15% germination in *Rhaponticoides mykalea* (Hub.-Mor.) (Emek and Erdağ, 2012).

On the contrary, GA_3 has inhibited germination in *Rhodanthe humboldtiana* seeds (Plummer and Bell, 1995).

Germination in indol butyric acid and salicylic acid solutions was not observed. Different alcohol percentages were tried and no germination occurred. Germination depends on many environmental features such as salinity, temperature, light (Yıldız et al., 2007). Salinity is usually a factor that prevents seed germination. In a research carried out by Khan et al. (1987) seed germination has been suppressed as the salinity rate increases, in the seeds of *Chrysothamnus nauseosus* sp. *viridulus*. But, in our case 77,5-95% germination was observed in seeds without capitulum in petri-germination experiments. In addition, germination of the seeds applied with NaCl solution resulted in healthier plants.

The highest germination rate was obtained in 50% perlite + 50% peat growth media in seedling starter trays.

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