

## EFFECT OF SOME TREATMENTS ON SEED DORMANCY, GERMINATION AND ANTIOXIDANT ENZYMES OF *KELUSSIA ODORATISSIMA* MOZAFF. SEEDS

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**ABSTRACT.** Seed dormancy provides a mechanism for plants to delay germination until conditions are optimal for survival of the next generation. Dormancy release is regulated by a combination of environmental and endogenous signals with both synergistic and competing effects. In many cases, viable seeds are called dormant, when they are simply not germinating. *Kelussia odoratissima* Mozaff. (wild celery) is a medicinal plant (kind of umbelliferous) of Iran. Seeds of *K. odoratissima* often germinate poorly in the nursery, because of their seeds have a dormancy. Thus shortening the dormancy and increasing germination with laboratory methods can be effective in restoring the plant. The objective of this research was to evaluate the effect different methods of breaking of dormancy on germination of *Kelussia odoratissima*. Experiments used were stratification (0, 3, 6, 9 and 12 weeks), stratification and gibberellin and stratification and nitrate potassium. Results showed that stratification, stratification and gibberellin and stratification and nitrate

potassium increased germination characteristics and catalase and ascorbate peroxidase activity. The highest germination percentage, seedling length, seedling dry weight and catalase and ascorbate peroxidase activity were attained from stratification and gibberellin 500 ppm and stratification and nitrate potassium 1%. In general, results showed that stratification and gibberellin acid (500 ppm) is the best treatment for breaking of *Kelussia odoratissima* Mozaff. seed dormancy and in seeds antioxidant enzymes could trigger germination.

**Key words:** Antioxidant enzymes; Breaking of dormancy; Germination; *Kelussia odoratissima*; Dormancy.

### INTRODUCTION

Seed dormancy could be considered simply as a block to the completion of germination of an intact viable seed under favorable

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conditions, but earlier reviews concluded that it is one of the least understood phenomena in the field of seed biology (Hilhorst, 1997; Bewley, 1997). Seed germination is influenced by internal factors controlling dormancy, including phytohormones (e.g., abscisic acid) inducing dormancy, and by seed coat factors (seed coat-enhanced dormancy) (Bewley, 1997; Hassani *et al.*, 2009). Dry seeds of most temperate trees and shrubs, even though mature, will not germinate and grow until they have imbibed to threshold moisture content under cold conditions (0-5°C) (cold stratification) (Hartmann *et al.*, 1997; Hassani *et al.*, 2009). The dormancy of dormant seeds must be broken to induce germination. Various methods are used for this, depending on the plant species and type of dormancy. Chilling plays an important role in providing the stimulus required to overcome dormancy, increase germination, and produce normal seedlings for *Prunus persica* cv. GF305 (Martinez-Gomez and Dicenta, 2001), strawberry tree (Karam and Al-Salem, 2001) and *Prunus avium* (Jensen and Eriksen, 2001). Exogenous growth regulator treatments – gibberellins (usually gibberellic acid GA3 and GA4+7) and cytokinins (usually kinetin, benzyladenine) – have been shown to break dormancy in many seed species (Dweikat and Lyrene, 1988; Karam and Al-Salem, 2001; Mehanna *et al.*, 1985). Based on this scheme, Baskin and Baskin (2004) have proposed a comprehensive classification system

which includes five classes of seed dormancy.

Dormancy can be broken by GA treatment and also be broken by scarification, after-ripening; moreover cold stratification has been reported to induce an increase in GA3 concentration there for increases seed germination (Nadjafia *et al.*, 2006; Bourgoin and Simpson, 2004).

Reactive oxygen species (ROS) have long been considered as only damaging compounds; however, they have recently emerged as key players in seed physiology (Bailly *et al.*, 2008). Whereas ROS are ubiquitous and present at all stages of the seed life, including embryogenesis through germination, an understanding of the role of ROS as signaling molecules in seed biology is far from complete. It is well known that ROS are produced to a certain level during seed imbibition. It has been proposed that the germination is completed only when the ROS content is within an oxidative window that allows ROS signaling (Bailly *et al.*, 2008). Above or below the "oxidative window for germination", low or high amounts of ROS would not permit progress towards germination. According to this model, seed dormancy, i.e. the inability of seeds to germinate in favorable environmental conditions (Finch-Savage and Leubner-Metzger, 2006), is regulated by ROS signaling.

*Kelussia odoratissima* Mozaff. (Apiaceae family), locally called "karafs-koohi", is a wild rebus, erect, glabrous, perennial aromatic herb, which grows to a height of 120 to 200

## EFFECT OF SOME TREATMENTS FOR BREAKING OF *K. ODORATISSIMA* SEED DORMANCY

cm. The flowers are 1-2 mm in diameter, all hermaphrodites. Petals are yellow and produced in compound umbels. It is native to the central region of the Zagros Mountains, Iran, and has a great biological diversity. Topographic factors have created a very diverse microclimate and edaphic conditions in this region. *K. odoratissima* is dispersed around some parts of the Zagros Mountains at heights of 2500 m, including Isfahan, Chaharmahal, Bakhtiari and the Lorestan provinces. This plant have been dormancy, therefore, the objective of this research was to evaluate the effect different methods of breaking of dormancy on germination of it.

### MATERIALS AND METHODS

Seed collection was collected from the Shahrekord Mountains, Iran, and was stored in cotton bags at room temperature of about 25°C. Germination studies were started in June 2014.

#### Breaking seed dormancy treatments and seed germination

The seeds were surface-sterilized in 1% aqueous sodium hypochlorite (NaOCl) solution for 3 min., and then rinsed with distilled water three times. In the stratification procedure (moist chilling), seeds were placed on sand moistened with distilled water in boxes and stored in a refrigerator at 4 ±1°C, for 0, 3, 6, 9 and 12 weeks. In the gibberellic acid treatments, seeds were imbibed in solutions of GA3 (100, 200, 300, 400, 500 and 1000 mg/l) and solution of nitrate potassium (KNO<sub>3</sub>) for 24 and 48 h. The other pre-germination treatments were combined treatment of GA3 or KNO<sub>3</sub>

concentration × treatment of stratification duration.

In the germination experiments, seeds of all treatments were placed on filter paper moistened in Petri dishes, and incubated at 22 ±1°C, in light conditions and 14 ±1°C, in the dark conditions. Moisture was maintained with distilled water. A seed with at least a 2 mm long radicle was considered to be germinated. After test time expiration, some germination indexes were evaluated such as: germination percentage, seedling dry weight and seedling length.

After the results of breaking treatments effect on investigated traits and determined elite treatment, determination of antioxidant enzyme activity was performed. All extraction procedures were carried out at 4°C. The seed samples, weighting about 0.3 g, were homogenized with 3 ml of tris (pH - 7.8), followed by centrifugation of 20000 g, for 20 min. The supernatants were used for determination of enzyme activity. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometric ally following hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) consumption at 240 nm (Bailly *et al.*, 1996). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Ansari *et al.* (2013). The activities of APX and CAT were expressed per mg protein, and one unit represented 1μmol of substrate undergoing reaction per mg protein, per min.

#### Statistical analysis

The study was conducted in the Seed laboratory of Natural Resources Faculty, University Azad of Shahrekord, Iran. All data were analyzed statistically by analysis of variance, using SAS software. Mean comparisons were performed using an ANOVA protected

least significant difference (Duncan) ( $P < 0.01$ ) test.

## RESULTS AND DISCUSSION

Analyses of variance showed that effect of stratification on germination percentage, seedling dry weight and seedling length were significant (Table 1), effect of stratification, GA3 and their

interaction on germination percentage, seedling dry weight and seedling length were significant (Table 2), furthermore effect of stratification and KNO<sub>3</sub> on germination percentage, seedling dry weight and seedling length were significant (Table 3), but their interaction only on germination percentage were significant (Table 3).

**Table 1 - Variance analysis effect of stratification on germination percentage, seedling dry weight and seedling length in *K. odoratissima* seeds**

S.O.V.	df	Germination percentage	Seedling dry weight	Seedling length
Stratification	4	2503.06**	0.0000003**	8.01**
Error	10	7.46	2.00E-09	0.19
CV%	-	9.67	12/65	19.28

\*\* indicate significant difference at 1% probability level.

**Table 2 - Variance analysis effect of stratification and GA3 on germination percentage, seedling dry weight and seedling length in *K. odoratissima* seeds**

S.O.V.	df	Germination percentage	Seedling dry weight	Seedling length
Stratification (S)	1	21851.5**	0.000003**	67.38**
GA3	6	1330.63**	0.000002**	1.83**
S x GA3	6	415.08**	0.0000001**	0.15**
Error	28	5.24	5.00E-09	0.03
CV%	-	3.89	9.54	5.02

\*\* indicate significant difference at 1% probability level.

**Table 3 - Variance analysis effect of stratification and KNO<sub>3</sub> on germination percentage, seedling dry weight and seedling length in *K. odoratissima* seeds**

S.O.V.	Df	Germination percentage	Seedling dry weight	Seedling length
Stratification (S)	1	19494**	0.000003**	34.8**
KNO <sub>3</sub>	3	49911**	0.0000002**	0.63**
S x KNO <sub>3</sub>	3	85.56**	0.000000008ns	0.03ns
Error	16	5.5	3.00E-09	0.04
CV%	-	4.78	9.13	6.23

\*\* and ns indicate significant difference at 1% probability level, non significantly, respectively.

## EFFECT OF SOME TREATMENTS FOR BREAKING OF *K. ODORATISSIMA* SEED DORMANCY

Results showed that germination percentage (Fig. 1), seedling length (Fig. 2) and seedling dry weight (Fig. 3) increased with increasing duration of stratification, so that, the highest these traits were attained from 12 weeks of stratification.

Results showed that with increasing stratification duration and gibberellic acid concentration, germination percentage, seedling length and seedling dry weight

increased, so that the highest these traits were attained from 12 weeks of stratification treatment and 500 ppm gibberellic acid (Figs. 4-6). With increasing GA3 concentrations higher than 500 ppm gibberellic acid, the measured parameters were decreased (Figs. 4-6). These results indicated that the toxicity may be caused by an increase in the concentrations of gibberellic acid above 500 ppm.

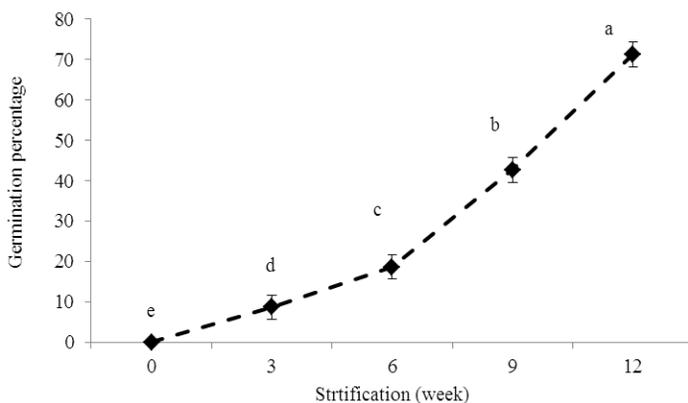


Figure 1 - Effect of stratification on germination percentage of *K. odoratissima* seeds

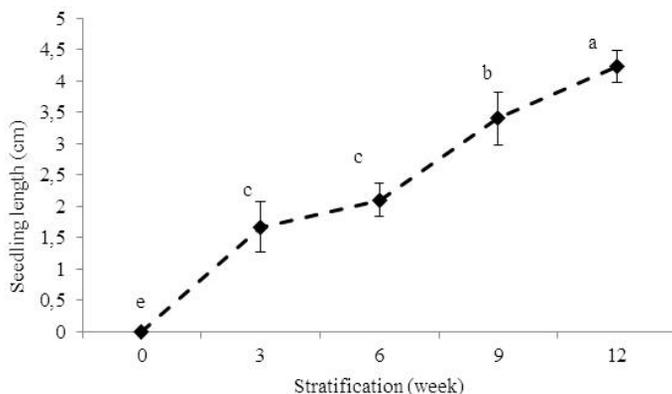


Figure 2 - Effect of stratification on seedling length of *K. odoratissima* seeds

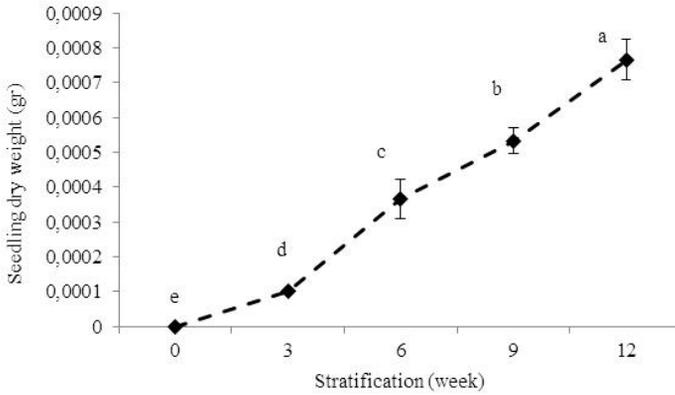


Figure 3 - Effect of stratification on seedling dry weight of *K. odoratissima* seeds

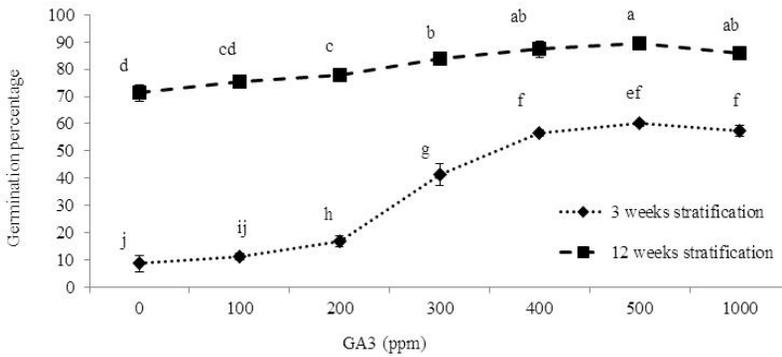


Figure 4 - Effect of stratification and GA3 on germination percentage of *K. odoratissima* seeds

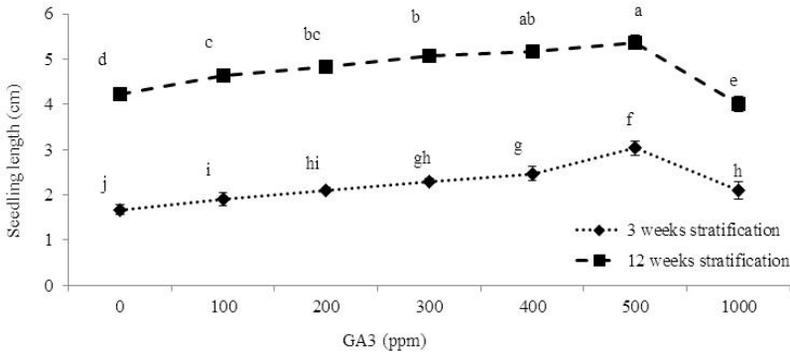


Figure 5 - Effect of stratification and GA3 on seedling length of *K. odoratissima* seeds

EFFECT OF SOME TREATMENTS FOR BREAKING OF *K. ODORATISSIMA* SEED DORMANCY

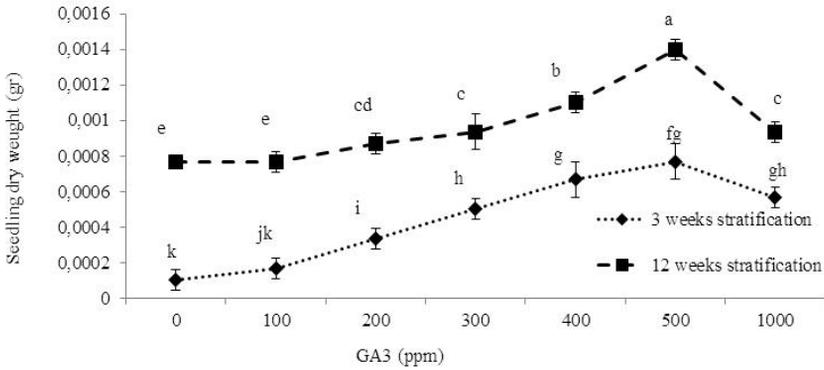


Figure 6 - Effect of stratification and GA3 on seedling dry weight of *K. odoratissima* seeds

Following these results, indicate that with increasing stratification during and nitrate potassium concentration, germination percentage increased, so that the highest these traits were attained from 12 weeks of

stratification treatment and potassium nitrate 1% (Fig. 7). The highest seedling length and seedling dry weight were attained from KNO<sub>3</sub> 1% and 12 weeks of stratification (Figs. 8-11).

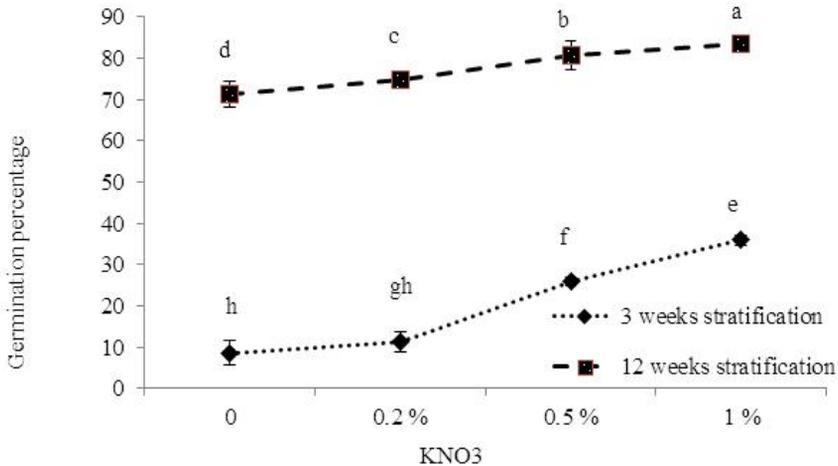


Figure 7 - Effect of stratification and KNO<sub>3</sub> on germination percentage of *K. odoratissima* seeds

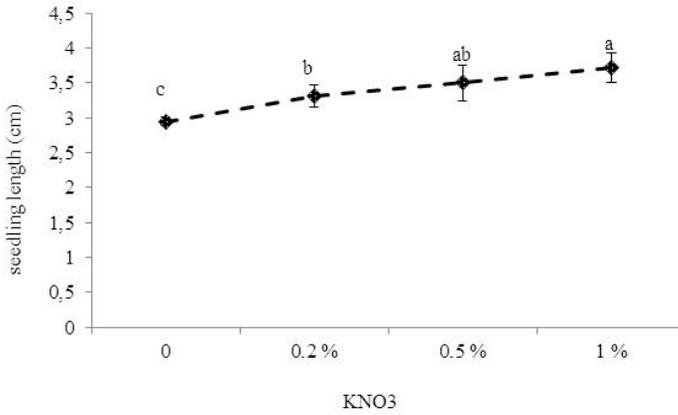


Figure 8 - Effect of KNO<sub>3</sub> on seedling length of *K. odoratissima* seeds

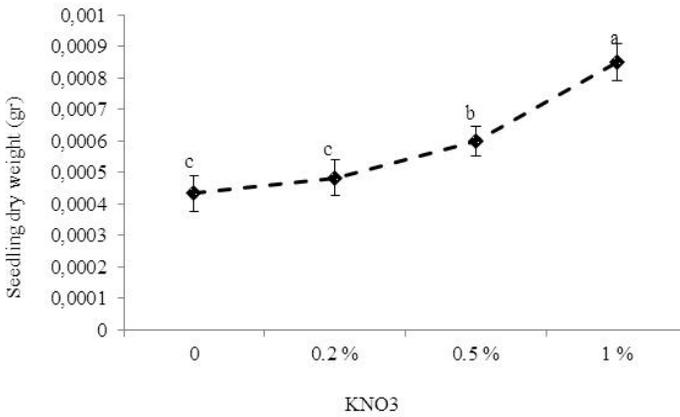


Figure 9 - Effect of KNO<sub>3</sub> on seedling dry weight of *K. odoratissima* seeds

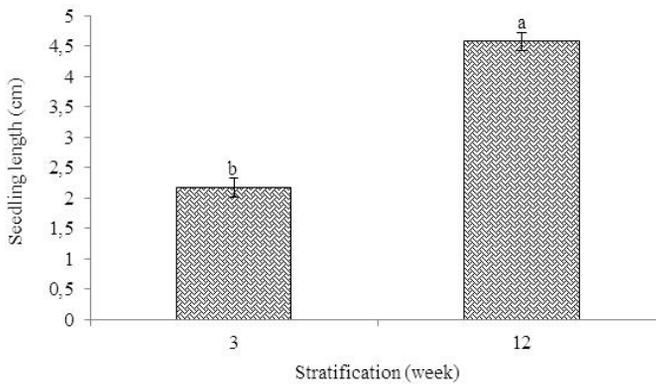
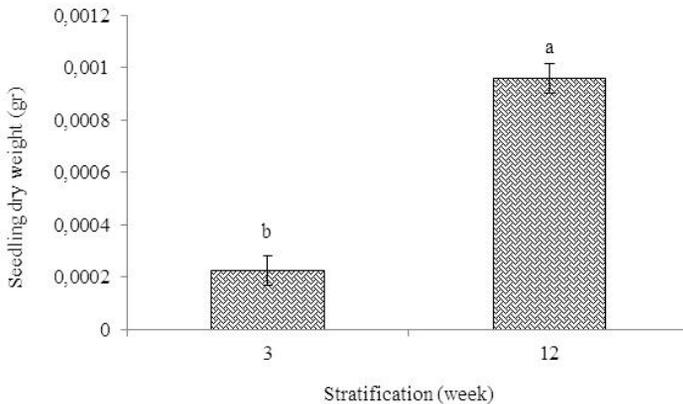


Figure 10 - Effect of stratification on seedling length of *K. odoratissima* seeds

## EFFECT OF SOME TREATMENTS FOR BREAKING OF *K. ODORATISSIMA* SEED DORMANCY



**Figure 11 - Effect of stratification on seedling dry weight of *K. odoratissima* seeds**

The results are in agreement with those Dweikat and Lyrene (1988), Karam and Al-Salem (2001), Mehanna *et al.* (1985), Nadjafia *et al.* (2006), Bourgoin and Simpson (2004).

During the stratification, some biochemicals and phytohormone were changed to be able to germinate dormant seeds. By decreasing abscisic acid (ABA) and increasing GAs, the seeds, with embryo dormancy residing in the embryo, can germinate (Hurtman *et al.*, 1997). Prechiling increases GA biosynthesis, which decreases the accumulation of proteins that control dormancy (Olmez *et al.*, 2004). For an effective ratio of GA:ABA in *Kelussia odoratissima* germination with 45 days of stratification was effective enough, since applying exogenous GA seems ineffectual.

Several workers have reported that  $KNO_3$  improved the seed germination of many plants seed (Cirak *et al.*, 2004) Potassium nitrate was found to be effective in breaking

dormancy of many species. Use of  $KNO_3$  has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its action (Olmez *et al.*, 2004).

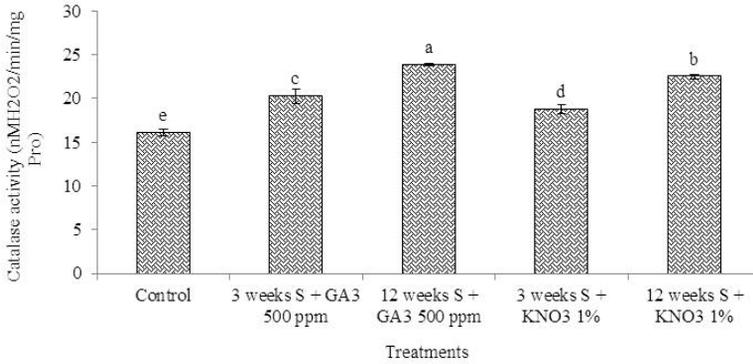
Variance analysis effect of stratification, GA3 and  $KNO_3$  in *K. odoratissima* seeds shows that effect of these treatments on catalase and ascorbate peroxidase was significant (Table 4). The highest value of catalase and ascorbate peroxidase was attained from 12 weeks stratification and 500 ppm GA3 concentration (Figs. 12, 13).

Bailly *et al.* (2008) reported that reactive oxygen species have long been considered as only damaging compounds; however, they have recently emerged as key players in seed physiology. Seed dormancy, i.e. the inability of seeds to germinate in favorable environmental conditions (Finch-Savage and Leubner-Metzger, 2006), is regulated by ROS signaling.

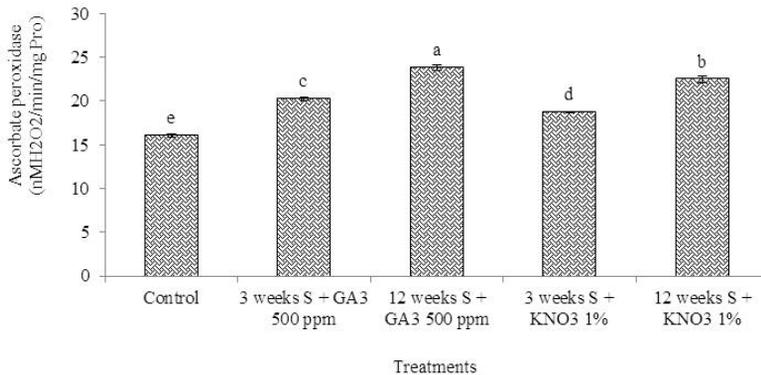
**Table 4 - Variance analysis effect of stratification, GA3 and KNO<sub>3</sub> in *K. odoratissima* seeds**

S.O.V.	df	Catalase	Ascorbate peroxidase
Treatment	4	28.24**	22.55**
Error	10	0.23	0.06
C.V %	-	2.38	1.83

\*\* indicate significant difference at 1% probability level.



**Figure 12 - Effect of stratification, GA3 and KNO<sub>3</sub> on catalase activity of *K. odoratissima* seeds**



**Figure 13 - Effect of stratification, GA3 and KNO<sub>3</sub> on ascorbate peroxidase activity of *K. odoratissima* seeds**

### CONCLUSIONS

Results showed that stratification, stratification and gibberellin and stratification and nitrate potassium increase germination characteristics and

catalase and ascorbate peroxidase activity. The highest germination percentage, seedling length, seedling dry weight and catalase and ascorbate peroxidase activity were attained from stratification and gibberellin 500 ppm and stratification and nitrate

## EFFECT OF SOME TREATMENTS FOR BREAKING OF *K. ODORATISSIMA* SEED DORMANCY

potassium 1%. In general, results showed that stratification and gibberellic acid (500 ppm) is the best treatment for breaking of *K. odoratissima* Mozaff. seed dormancy and in seeds antioxidant enzymes could trigger germination.

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