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Salinity, chitin and GA_3 effects on seed germination of chervil (*Anthriscus cerefolium*)

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Abstract

The seed germination observation of chervil (*Anthriscus cerefolium*) lasted 35 days at a temperature of 20 ^oC. The effect of sodium chloride (NaCl), chitin and gibberillic acid (GA_3) on seed germination of chervil was studied for 35 days at a temperature of 22^oC under controlled growth chamber conditions. For this, various aqueous solutions of NaCl (80, 120, 180, 240 mM NaCl), chitin (1, 2, 3, 4%) and GA_3 (100, 200, 500, 1000 ppm GA_3) were used as germination substrates. The above solutions used solely or combined and added on Petri dishes containing fifty chervil seeds. The seed germination of chervil in the control (H_2O) was 44%. In the 80 mM NaCl concentration there was an increase in the germination (64%) while in higher NaCl concentrations the germination reduced. The seed germination percentage of the chervil in the substrates with 1% and 2% chitin as the 200, 500 and 1000 ppm GA_3 was increased than the corresponding one in H_2O. With increasing rate of chitin and GA3 the seed germination of the chervil was reduced. In the combinations of 80 mM NaCl+ 1% Chitin, 120 NaCl + 1% chitin, 120 NaCl + 100 GA_3, 180 NaCl + 2% chitin and 180 NaCl + 200 GA_3 germination increased and the Timson Index of germination velocity was higher than the corresponding velocity in H_2O.

Keywords: NaCl; germination percentage; germination velocity.
Abbreviations: GA_3, Gibberellic acid; NaCl, sodium chloride.

Introduction

The chervil (*Anthriscus cerefolium* L.) is a fragrant, delicate annual herb belonging to the Apiacae family. Its principal use is as a flavouring agent for culinary purposes, but has been used for medicinal purposes as well. The chemical composition of chervil includes the various flavonoids, such as luteolin (Fejes et al. 2000; Milovanović et al. 2009). Chervil has received relatively little research attention compared to other herbs species. The chervil herb is an interesting plant generally characterized by strong and unique flavour compounds, and in some cases providing important nutrients which can enrich the consumers' diet. The chervil seed needs light and moisture to germinate. However, there is a lack of information about the germination control. Seeds of chervil had a viability of 3 years and germinated after 14 days at a 13°C temperature (Bubel 1988). Using alternating periods of warm (21°C) and cold (4°C) and warm (21°C) in the dark, the seeds of chervil exhibited a 4% germination rate starting at the end of the 2th week after a warm-cold-warm cycle and a 12% germination rate after a warm-cold cycle started at the end of the 1st week and ended at the end of the 3rd week at 21°C and 4 °C in the light (Deno 1993). The optimization of germination conditions are basic to receive high-quality seedling production. The chervil elongates cotyledons and the apical cone are thus 14 mm above the soil surface at the end of the second week (Pütz and Sukkau 2002). Even though the reaction of plants to salinity is one of the most widely studied topics in plant physiology, there is very little information available on the reaction of herb plants to conditions of salt stress. Salinity delimits the germination and reproductive growth of the plants, causing physiological dysfunctions and multiple direct and indirect problems, even in low concentrations (Shannon et al. 1994). The concentrations and composition of salts, the duration of exposal, the plant type, the cultivar, the underlying, the stage of growth and the environmental conditions are some of the factors that play a role in the plants' tolerance (Marschner 1995). Germination under saline conditions is stimulated by applying dormancy-relieving compounds, which counteract the negative change in growth regulator balance in seeds when they are exposed to salt stress. The plant species differ in their sensitivity or tolerance to salt stress (Cony and Trione 1998). At 15 ^oC in the greenhouse a reduction of the germination percentage in three wild green vegetables was observed at concentrations of NaCl (Liopa-Tsakalidi 2010). Chitin [$(C_8H_{13}O_5N)_n$], an aminopolysaccharide is the second most abundant polysaccharide after cellulose in nature and is a natural polymer, a basic structural polysaccharide. The chitin production in the maritime environment is extremely high. In terms of organic farming, chitin have been used as an amendment to control fungal diseases and root-parasitic nematodes (Gooday 1990). Scattered notes appear in literature on the effect of chitin amendment on germination and plant growth. Chitin did not cause any enhancement of germination for corn and soybean (Prithiviraj et al. 2003). D'Addabbo (1995) mentions that when chitin concentration in the soil exceeds 1%, it has a phytotoxic effect. The addition of chitin in the soil at 1% (w/w) eliminated plant-parasitic nematodes in cotton planting, confirming long-term nematode suppressiveness induced by this organic amendment (Hallman et al. 1999). Sarathchandra et al. (1996) mention that the shoot weight of the ryegrass (*Lolium perenne* L.) was greater in chitin amended soil, most probably due to N mineralised from chitin. Ladner et al. (2008) report that the total plant biomass fresh weight and the

Table 1. Timson Index of germination velocity mean (± s.e.) in chervil (*Anthriscus cerefolium*) seedlings at different NaCl, chitin and GA_3 concentrations. Data sharing the same letter are not significantly different (P<0, 05).

H_2O	(mM)	NaCl	(%)	Chitin	(ppm)	GA3
1.31^{e}±0.6	80	1.96^{a}±0.06	1	1.62^{c}±0.06	100	1.42^{e}±0.06
	120	1.53^{d}±0.06	2	1.60^{c}±0.06	200	1.69^{c}±0.06
	180	1.40^{e}±0.06	3	1.47^{d}±0.06	500	1.66^{c}±0.06
	240	0.62^{g}±0.06	4	1.01^{f}±0.06	1000	1.81^{b}±0.06

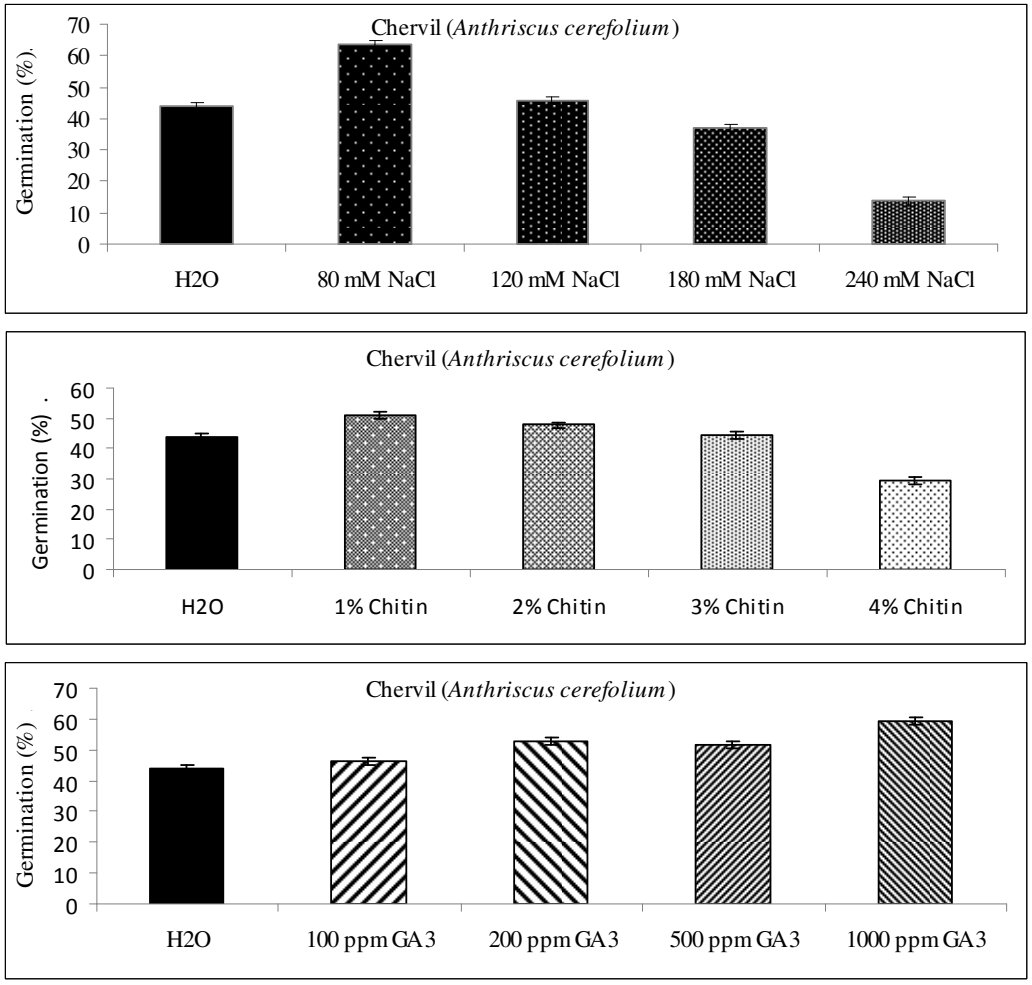

Fig 1. Final germination of chervil (*Anthriscus cerefolium*) seeds at different NaCl, chitin and GA_3 concentrations (±s.e.).

shoot fresh weight at a chitin concentration of 100g in tomato plants was higher when compared to the control. Chitin in the peat substrate did not affect the length and weight of the lemon balm plant. Chitin affected the tarragon leaves resulting in the total chlorophyll content increase (Liopa-Tsakalidi et al. 2010). Giberillic acid (GA_3) is found to play an important part in the germination process (Ritchie and Gilroy 1998) through a multiple regulatory mechanism. However, the stimulating effects of GAs on seed germination are not universal for all plants. (Bell et al. 1995). The exogenous application of GA_3 on germination and seedling growth under salt stress conditions provides an attractive approach to encounter the effects of salinity. Gibberellins have been shown to promote many facets of plant growth and development including germination, and seed development (Sun and Gubler 2004). The focus of the current study was to provide knowledge on the germination of chervil and its reaction to different levels of salinity, chitin and GA_3 in order to have knowledge of its salt tolerance.

Results

Salinity, chitin and GA_3 effects on seed germination

The seed germination observation of chervil lasted 35 days at a temperature of 22 ^{o}C in a controlled plant growth chamber. The radicle emergence from the chervil seeds occurred in day 9 after the seeds were placed in Petri dishes. The seed germination of chervil in the control (H_2O) was 44%. In the 80 mM NaCl concentration there was a significant increase in the germination (64%) of chervil, when compared to the control, in 120 mM NaCl concentration the germination was the same as in the control, while in higher NaCl concentrations the germination reduced (37% at 180 mM NaCl, 14% at 240 mM NaCl) (Fig 1).

Table 2. Timson Index of germination velocity mean (± s.e.) in seedlings of chervil (*Anthriscus cerefolium*) at different NaCl, chitin and GA_3 concentrations. Data sharing the same letter are not significantly different (P<0, 05).

Treatment	80 mM NaCl	120 mM NaCl	180 mM NaCl	240 mM NaCl
1% Chitin +	1.64^{b}±0.06	1.63^{b}±0.06	1.31^{d}±0.06	1.31^{d}±0.06
2% Chitin +	1.15^{f}±0.06	1.32^{d}±0.06	1.82^{a}±0.06	1.57^{b}±0.06
3% Chitin +	1.24e±0.06	1.34^{d}±0.06	1.49^{c}±0.06	1.44^{c}±0.06
4% Chitin +	1.07^{f}±0.06	1.29^{e}±0.06	1.18^{e}±0.06	1.52^{c}±0.06
100 ppm GA_3 +	1.20^{e}±0.06	1.63^{b}±0.06	1.52^{c}±0.06	1.41^{c}±0.06
200 ppm GA_3 +	1.28^{e}±0.06	1.40^{c}±0.06	1.66^{b}±0.06	1.53^{c}±0.06
500 ppm GA_3 +	1.14^{f}±0.06	1.43^{c}±0.06	1.16^{f}±0.06	1.60^{b}±0.06
1000 ppm GA_3 +	1.27^{e}±0.06	1.51^{c}±0.06	1.15^{f}±0.06	1.21^{e}±0.06
H_2O	1.31^{d}±0.06			

The seed germination percentage of the chervil in the substrates with 1% and 2% chitin was significantly higher, with 3% chitin it was the same, and with 4% it was significantly lower that the corresponding one in H_2O (Fig 1). The chervil germination in the substrate with 100 ppm GA_3 was the same as in the control, while in the substrates with higher GA_3 concentrations (200, 500 and 1000 ppm) it was significantly higher than the corresponding one in H_2O (Fig 1). The germination of the chervil in the substrate with 100 ppm GA_3 was the same as in the control, while in the substrate with higher GA_3 concentrations (200, 500 and 1000 ppm) it was significantly higher than the corresponding one in H_2O (Fig 1). Chervil seed germination velocity was reducing the more NaCl and chitin concentrations were increasing and was increasing when the GA_3 concentration was increasing. In the low NaCl (80 mM NaCl) concentration, the Timson Index of germination velocity was statistically higher (1.96) and in the highest NaCl (240 mM NaCl) concentration it was statistically lower (0.62) than the corresponding rate in the control (1.31). The same trend was also obvious with the presence of chitin in the substrate. In the substrate with 1%, 2% and 3% chitin, the germination velocity of the chervil was significantly higher and in the substrate with 4% chitin it was significantly lower than the corresponding velocity in H_2O (Table 1). The Timson Index of the germination velocity was significantly higher in GA_3 substrates compared to the one in H_2O (Table 1).

Chitin or GA_3 addition in the substrates containing NaCl

The addition of chitin or GA_3 in the substrates containing 80 mM NaCl resulted in the reduction of germination. The germination in these combinations was significantly lower than the corresponding one in H_2O, save for the combination that contained 80 mM NaCl, where germination was significantly higher than in H_2O. The addition of chitin or GA_3 in the substrate that contained 120 mM NaCl resulted in increase of germination in the combinations of 120 NaCl + 1% Chitin and 120 NaCl + 100 GA_3, compared to the germination in H_2O. In the substrate that contained 180 mM NaCl the addition of chitin or GA_3 resulted in the increase of the germination rate in the combinations of 180 NaCl + 2% Chitin and 180 NaCl + 200 GA_3 compared to the one in H_2O, while in the rest of the combinations a reduction was observed. High salinity (240 mM NaCl) in the substrates with a combination of chitin or GA_3 resulted in the increase of the germination rate in all combinations, compared to 240 mM NaCl (Fig 2). The Timson Index of the chervil germination velocity was lower or equal in the substrates containing 80 mM NaCl with the addition of chitin or GA_3, compared to the corresponding germination rate by Timson in H_2O.

The only exception was the germination velocity for the chervil seeds that contained 80 mM NaCl. The germination velocity in salinity (120 mM NaCl) in the substrate in combination with 1% chitin (1.63) and in combination with 100 GA_3 (1.63) was statistically higher than the corresponding rate in H_2O. Moreover, the germination velocity in salinity (180 mM NaCl) in the substrate in combination with 2% chitin (1.8) and in combination with 200 GA_3 (1.7) was statistically higher than the corresponding rate in H_2O. In high salinity (240 mM NaCl) in the substrate in combination with 500 ppm GA_3 (1.6) or with 2% Chitin (1.57), it was statistically higher than the corresponding rate in H_2O (Table 2).

Discussion

It is well established that NaCl inhibits seedling germination by delaying seed germination at salinity levels that can cause stress to seeds (Al-Karaki 2001) and salt stress causes a number of changes in plant metabolism (Arafa et al. 2009). In the present study the higher NaCl concentrations of 180 and 240 mM NaCl inhibited the germination process in the chervil, because they probably exceed individual tolerance limits. The study of salt tolerance during germination is important for determining saline limits at each crop developmental stage (Zapata et al. 2004). Some crops that have been classified as salt sensitive can germinate under high concentrations of NaCl (Kurt et al. 1986). However, other tolerant species are more sensitive during germination (Kent and Laüchli 1985). There is a critical lack of research about seed germination control of *Anthriscus cerefolium*. Bubel (1988) referes that seeds of *Anthriscus cerefolium* are germinated after 14 days at a temperature of 13°C. In this paper has been shown that the 80 mM NaCl concentration, compared to H_2O, had an increadibly positive effect on seed germination of chervil. This was reflected in the highest of Timson Index of germination velocity. On the other hand, higher concentrations inhibited seed germination. Other plants react differently under different salt conditions. Zidan and Elewa (1995) mentioned that during germination, anise tolerated salinity up to 160 mM NaCl and coriander up to 200 mm NaCl. It is difficult to quantify the genotypes per each plant species and the individual physiological responses induced by NaCl that are dependent on the stage of development, and on various external factors in a way that could be extrapolated from species to species. The relationships between the the substrates with aqueous solutions, the supplemented chitin percentage and the seed germination features are obviously complex and make it difficult to assess the activities occurring in substrates.

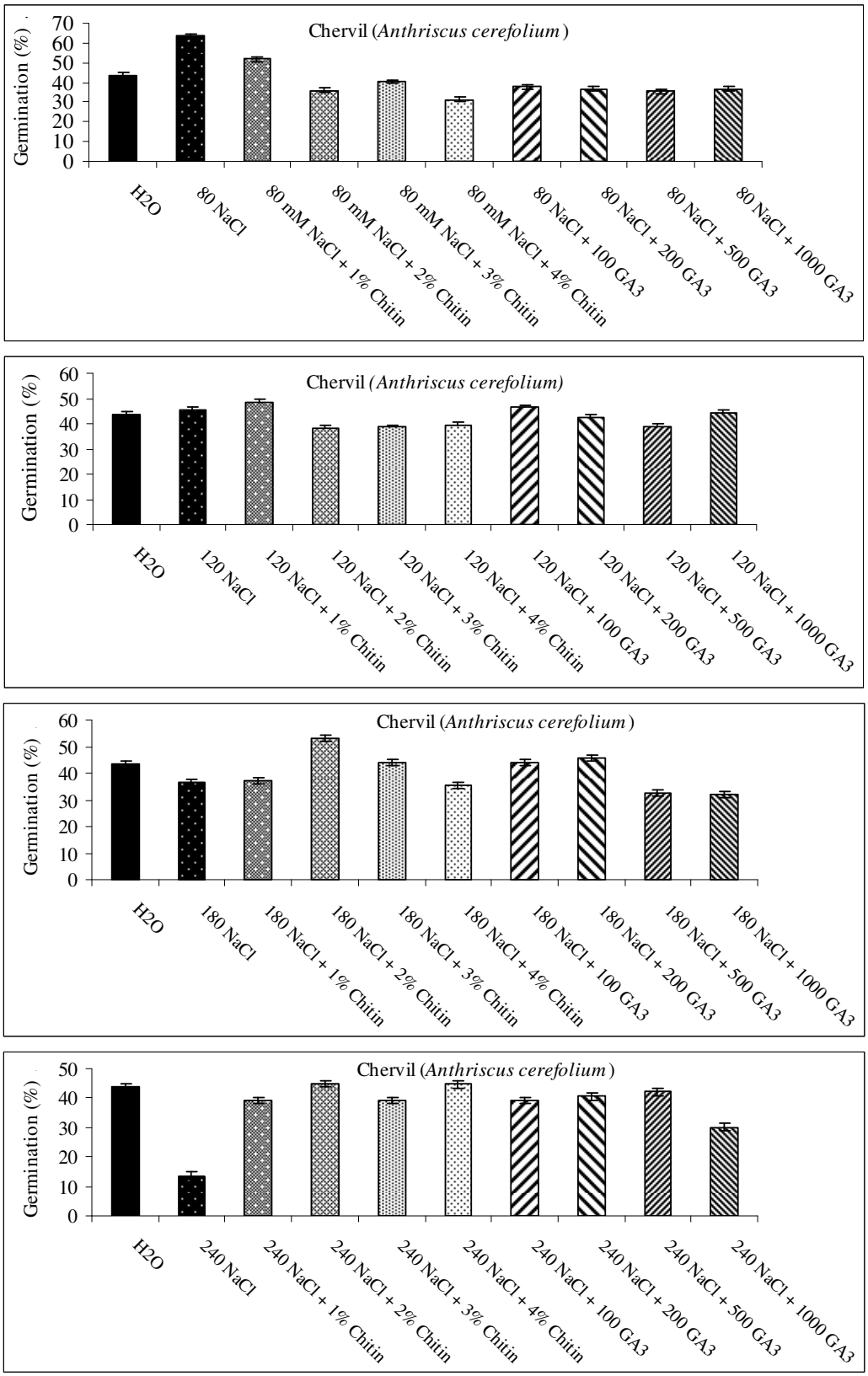

Fig 2. Final germination of chervil (*Anthriscus cerefolium*) seeds at different NaCl, chitin and GA_3 concentrations (±s.e.).

It appears that seed germination features in the supplemented chitin percentage is the result not of one factor but of several different factors. Chitin degradation by microorganisms plays an important role in soil fertility and thus represents a significant source of energy and reflects the development of an adaptive microflora (Iglesias et al. 1994). Chitin has a direct effect on plant functions. The plants have their own ways of benefitting from this situation in the soil-root interaction (Hallmann et al. 1999). Chervil germination in the substrates with 1% and 2% chitin was higher, with 3% chitin it was the same, and with 4% it was significantly lower that the corresponding germination in H_2O. This study shows that the germination responses as a result of chitin amendment in the substrates were higher, mainly in 1% and 2% chitin. Chitin in the two substrates (1% and 2% chitin) had a positive effect on the chervil seed germination. The germination rate of the chervil seed was increasing as the concentration of GA_3 was increasing and in combination with 120 NaCl + 100 GA_3, and 180 NaCl + 200 GA_3 the germination increased and the germination velocity was higher than the corresponding rate in H_2O. The germination velocity was estimated by using a modified Timson index, as a measure of the chervil seed germination. This index is a complete biological indicator, considered as the most sensitive parameter used for evaluating the effect of NaCl. The germination rate illustrates the possible cumulative effects of all the factors connected to the presence of NaCl that could possibly affect the plants. For the farmers who grow herbs, substrates with 1% and 2% chitin could be used for the improvement of *Anthriscus cerefolium* germination in organic farming. Alternatively, the following concentrations of 200, 500 and 1000 ppm GA_3 could be used in the conventional farming system. However further research is needed to confirm the results of the present work.

Materials and methods

Seed germination

Seeds (achenes) of chervil (*Anthriscus cerefolium*) were used in this study fifty seeds were placed on filter paper in 10cm Petri dishes and moistened with 5ml of distilled water (control) or with an equal quantity of the respective test solution. The following treatments were designed: A) H_2O-distilled water (control), B) aqueous solutions supplemented with 80, 120, 180, 240 mM NaCl respectively, C) aqueous solutions supplemented 1, 2, 3, 4% (w/v) chitin (Sigma C7170) respectively, D) aqueous solutions supplemented with 100, 200, 500, 1000 ppm gibberellic acid (GA_3, Sigma-Aldrich) respectively, E) aqueous solutions supplemented with 80 mM NaCl +1%, 2%, 3%, 4% Chitin and 80 mM NaCl +100, 200, 500, 1000 ppm GA_3 F) aqueous solutions supplemented with 120 mM NaCl +1%, 2%, 3%, 4% Chitin and 120 mM NaCl +100, 200, 500, 1000 ppm GA_3 G) aqueous solutions supplemented with 180 mM NaCl +1%, 2%, 3%, 4% Chitin and 180 mM NaCl +100, 200, 500, 1000 ppm GA_3 H) aqueous solutions supplemented with 240 mM NaCl +1%, 2%, 3%, 4% Chitin and 240 mM NaCl +100, 200, 500, 1000 ppm GA_3. Three Petri dishes for each treatment were placed in completely randomized design in a controlled plant growth chamber at a 24h photoperiod, 12 klx light intensity, 22 ± 1^0C temperature regime, and 70 ± 5% relative humidity. Distilled water or test solutions were added to each Petri dish, during the experiment, according to their water requirements. The number of the germinated seeds was recorded every three days, starting from day 9 after the seeds were initially placed in 10 cm diameter Petri dishes. The experiment was conducted three times.

Data analysis

The germination percentage is an estimate of the viability of seeds. The equation to calculate the final germination percentage (GP) is:

$$GP = \frac{\text{number of germinated seeds}}{\text{number of total seeds}} \times 100$$

The germination rate was estimated by using a modified Timsons index of germination velocity $= \sum G/t$, where G is the percentage of seeds which germinated after 7-day intervals and t is the total germination period (Khan and Ungar 1984). The analysis of the seed germination data of chervil in different levels of salinity, chitin and GA_3 was performed according to the completely randomized design with three replicates. The means of the examined traits were ranked according to Duncan's multiple range test and the Post Hoc comparison was used alternatively with the Student-Newman-Keuls, Dunnett and Tukey methods.

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