ISSN: 2476-6909; ECOPERSIA. 2021;9(1):1-10

Effect of Seed Priming with GA and SA on the Improvement of Germination Characteristics of *Hibiscus sabdariffa* Seeds Under Allelopathic Stress of *Eucalyptus camaldulensis*

ARTICLE INFO

Article Type
Original Research

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How to cite this article
Saberi M, Tarnian F, Karimian V. Effect of Seed Priming with GA and SA on the Improvement of Germination Characteristics of *Hibiscus sabdariffa* Seeds Under Allelopathic Stress of *Eucalyptus camaldulensis.* ECOPERSIA. 2021;9(1):1-10.

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Article History
Received: May 23, 2020
Accepted: June 16, 2020
ePublished: October 24, 2020

ABSTRACT

Aims Germination is one of the important stages of plant lifecycles that may be affected by different natural stress such as allelopathic. This study was performed to investigate the effects of priming with gibberellic and salicylic acid on germination improvement and resistance of *Hibiscus sabdariffa* under stress with allelopathic compounds of *Eucalyptus camaldulensis*.

Materials & Methods The experiment was conducted as a factorial experiment in a completely randomized design with four replications in laboratory conditions. The first factor consists of pre-treatment of seeds with gibberellic acid (125, 250, and 500mg/kg) and salicylic acid (100, 200, and 300mg/l), and the second factor was five concentrations (0, 25, 50, 75, and 100mg /l) of allelopathic compounds of eucalyptus. Studied characteristics have consisted of germination percent, germination speed, root length, shoot length, seedling length, and vigor index.

Findings Results showed that Eucalyptus allelopathic extract has an inhibitory effect on seed germination of *H. sabdariffa*. When the extract concentration increased, the germination and seedling growth decreased significantly. In contrast, seed priming with gibberellic and salicylic acid increased the measured characteristics at all levels compared to non-primed seeds. A comparison of means showed that seed priming with gibberellic acid 250mg/kg increased germination 25% compared to an unprimed seed.

Conclusions The response of this species to seed pre-treatment with gibberellic acid has the most effect. The seed pre-treatment technique with the mentioned treatment could increase germination and establish seedling by improving germination percentage and speed under allelopathic stress before planting.

Keywords Allelopathy; Speed Germination; *Hibiscus sabdariffa*; *Eucalyptus camaldulensis*; GA_3

CITATION LINKS

[1] Hibiscus section Furcaria ... [2] Roselle (Hibiscus sabdariffa) seed oil is a rich ... [3] An introduction to botany of tropical ... [4] Hibiscus: Post-production management for improved market ... [5] Uricosuric effect of Roselle (Hibiscus sabdariffa) in ... [6] Germination, postgermination adaptation, and species ... [7] Environmentally induced carry-over effects on seed ... [8] Influence of phenolic acids on ion ... [9] Allelopathic influence of Cannabis sativa on ... [10] Ecophysiological aspects of ... [11] Allelochemicals and ... [12] Allelochemicals and ... [13] Research progress on the use of plant allelopathy in ... [14] Optimization of hydropriming techniques ... [15] Germination and seedling development ... [16] Germination and seedling development ... [17] Manipulation of seed water relations via ... [18] Evaluation of seedling vigor of hydro and matriprimed ... [19] Evaluation of seedling vigor of hydro and matriprimed ... [20] Enhancement of seed vigor following insecticide and ... [21] Effects of stratification and GA3 on seed germination of a sand ... [22] Stimulation of lettuce seed germination ... [23] Changes in salicylic acid and antioxidants ... [24] Chilling tolerance of maize, cucumber and rice ... [25] Salicylic acid alleviates the cadmium toxicity ... [26] Salicylic acid-induced abiotic stress tolerance ... [27] Salicylic acid: An all-rounder in regulating ... [28] Investigation the effects of different levels ... [29] Influence of chemical stimulators to development ... [30] Allelopathic effects of Eucalyptus camaldulensis ... [31] Allelopathic assay of Eucalyptus camaldulensis Labill ... [32] Seed treatments to overcome salt and drought ... [33] Speed of germination aid in ... [34] Polyphenolic allelochemicals from the ... [35] Polyphenolic allelochemicals from the ... [36] Impact of exogenous salicylic acid on the growth ... [37] Evidence for a role of salicylic acid in the oxidative damage ... [38] Effect of salicylic acid on freezing tolerance in winter ... [39] Antioxidant role of glutatnione associated ... [40] The effects of light and some presoaking ... [41] Effects of seed priming on improvement ... [42] Influence of chemical stimulators in ... [43] Enhancement of seedling vigor in rice ...

Introduction

Sour tea or Roselle with the scientific name of *H. sabdariffa* L. is a plant from the Malvaceae family that belonged to the sub-Sahara region in Africa [1]. Sour tea is cultivated for edible and fiber use, as well as wood. Although the seeds of this plant are somewhat bitter, they are widely used as food in Africa because of their high protein content, and likewise, roasted seeds are used as a substitute for coffee. The seeds also contain a significant amount of oil that is similar to cottonseed oil [2]. Cultivation of this plant has been reported throughout India and Africa, part of Asia (China and Thailand are the largest producers), the Americas, and Australia [3, 4]. Consumption of this plant can prevent cancer, reduce blood pressure, and improve the efficiency of the human gastrointestinal tract. The use of sour sepal extract can be an effective treatment for the renal disease [5]. Plotto [4] mentioned that demand has steadily increased for Roselle over the past decades, and it is an ideal crop for developing countries. The propagation of Roselle is through seed and does not naturally propagate through vegetative organs.

Germination is among the earliest events of the plant's life cycle. The transition from seed to germinant is one of the most drastic developmental transitions that plants experience [6, 7]. The germination process begins with the emergence of roots and shoots and their elongation with allocating nutrients to the embryonic axis. Allelopathic compounds interfere growth and development of plants and their important physiological processes with altering cell wall structure, permeability, and dysfunction of the membrane, preventing cell division and activity of some enzymes, disturbing the balance of plant hormones, Nutrient uptake, changes on stomata, photosynthesis, respiration, protein and pigment synthesis and alteration of DNA and RNA structure [8-13]. This process is effective in agronomic ecosystems either directly through interference with plants or indirectly through soil biological and non-biological processes [14].

Seed pre-treatment is a technique by which the seeds are physiologically and biochemically prepared for germination before growing in the environmental conditions. In other words, priming refers to some different methods of seed germination, that all of them are based on water absorption by seeds [15]. This can cause numerous biological and physiological manifestations in priming seeds and the resulting plants so that these cases could also be observed in germination properties, early plant establishment, exploitation of environmental inputs, early maturity, quantitative and qualitative yields [16]. Seed priming is used to improve germination uniformity, reduce germination time and seedling emergence, improve establishment and yield performance, and increase seed strength and reduce damage caused by late planting [17, 18]. Rapid germination is affected by priming induced by RNA, DNA, and protein synthesis [19]. Many researchers have reported that priming increases the germination percentage of weak and damaged seeds [20]. Seed germination begins with water absorption and inflammation and is followed by continuous biochemical processes in seeds [21], including metabolism activation and consuming storage food to transfer to the embryo, cell division, and growth [22]. According to new studies, gibberellic acid plays a key role in these processes. Chemical compounds that penetrate the embryo and stimulate its metabolic activity are often effective in inducing germination. Studies have shown that salicylic acid improves some abiotic stresses such as heat stress in mustard seedlings [23], cold damage in various plants [24], and heavy metal stress in barley seedlings [25]. Several other studies have also addressed the role of salicylic acid in modulating plant responses to abiotic stresses [26, 27]. Saberi *et al.* [28] studied the effect of priming with chemical stimulants on improving germination and early growth of *Festuca arundinace* seedlings under stress with allelopathic compounds. The results showed that gibberellic and salicylic acid are suitable stimuli for germination and seedling growth under stress conditions, and seed priming with the above chemical stimuli had a positive effect on germination improvement neutralizing free radicals or active oxygen. Saberi & Karimian [29], while studying the effect of chemical stimuli on the growth of *Datura stramonium* under allelopathic stress, concluded that pre-treatment with salicylic acid and gibberellic acid had positive effects on the growth of this plant under allelopathic conditions.

In many parts of the world, the cultivation of

Roselle is developing due to its medicinal and industrial applications [4]. Cultivation of medicinal plants, including roselle species, is common in Sistan's Chah-Nime research center. This medicinal plant has important aspects of research and also has economic benefits to native people. The presence of 120-day winds causes severe sandstorms and severe wind erosion so that plants such as eucalyptus trees were planted on the margins of the Rossele fields as *Windbreaks*. The inhibitory effects of Eucalyptus allelopathy on germination and seedling growth of some plant species have been previously reported [30, 31]. In this study, gibberellic and salicylic acid was used as priming to improve the germination properties of Roselle under stress with allelopathic compounds of Eucalyptus. Hence the study is attempted to answer the following questions: 1) what do different concentrations of allelopathic extract affect the germination characteristics of Roselle? Does seed priming with gibberellic and salicylic acid chemical stimulants improve and reinforce germination properties against the inhibitory effects of Eucalyptus allelopathic compounds?

Materials and Methods

This research was conducted to determine the effect of seed priming with chemical stimulants of gibberellic and salicylic acid on the improvement of germination of *H. sabdariffa* under stress with allelopathic compounds of *E. camaldulensis* in the university of Zabol in 2020. To meet this aim, a factorial test (5×7) in a completely randomized design with four replications and two factors were used. Gibberellic acid levels of 125, 250, and 500mg/kg and salicylic acid 100, 200, and 300mg/L were used as pre-treatment, and then allelopathic extractions with 5 levels of 0 (control), 25, 50, 75, and 100 were used. In order to investigate the allelopathic effect of *E. camaldulensis* on *H. sabdariffa,* aerial and underground parts of *E. camaldulensis* were collected from Chah Nime, Zabol, Iran. After air-drying at room temperature, 5g of powder was picked and mixed in 100mL water, placed on a shaker for 24h, and centrifuged at 3000g for 15 min. The obtained mixture was filtered using Whatman filter paper grade 1, and the mentioned concentrations were prepared. Seeds of *H. sabdariffa were* collected from the farm of medical plants located at Chah Nime. The seeds were disinfected by using a 5% solution of sodium hypochlorite before starting of test and were washed by using distilled water several times. Then seeds were pre-treated with salicylic acid 100, 200, and 300mg/L for 10h and gibberellic acid 125, 250, and 500mg/kg for 24h at 25°C and distilled water were used as control treatment simultaneously. All seeds were washed with distilled water after the soaking period. Then dried seeds were placed into Petri dishes with dimensions of 9cm under a filter paper (Watman 1) to test different stress conditions with various concentrations of allelopathic extract related to *E. camaldulensis.* The experiment was done in the germinator with 25°C. Germinated seeds that had length more than 2mm were counted each day over ten days [32], and germination percentage, germination speed, rootlet length, shootlet length, seedling length, and vigor index of seed were measured. Germination percentage and germination speed [33] were measured based on the following equations:

(1) Germination percentage:

$$GP = \frac{\sum G}{N} \times 100$$

GP: Germination percentage, G: Number of germinated seeds, N: Total number of seeds

(2) Germination speed:

$$GR = \sum_{i=1}^{n} \frac{S_i}{D_i}$$

S_i : Number of germinated seed at each counting, D_i: Number of day until n counting, n: Numbers of counting

(3) Seedling length= Rootlet length+Shootlet length

(4) Vigor index:

$$V_i = \frac{\%\,Gr \times MSH}{100}$$

V_i: Vigor index, Gr: Germination percentage, MSH: Mean of seedling length per mm

The obtained data were analyzed using analysis of variance (ANOVA). Means were compared at the 5% level of significance using Duncan's multiple range tests with statistical software MSTAT-C version 2.00.

Findings

Results of variance analysis (Table 1) showed that growth stimulators and various concentrations of *E. camaldulensis* had a significant effect on germination percentage, germination speed, rootlet length, shootlet length, seedling length, and vigor index of *H. sabdariffa* species ($p<0.01$). Gibberellic and salicylic acid have a significant effect on all germination properties of *H. sabdariffa*. Interaction of priming and various concentrations of the extract of *E. camaldulensis* had a significant effect on all studied properties ($p<0.01$, Table 1).

Germination percentage and speed

The mean comparison showed that germination speed and percentage of *H. sabdariffa* decrease by increasing concentrations of the allelopathic extract of *E. camaldulensis,* and differences were significant between control treatment and various allelopathic concentrations. In contrast, priming with gibberellic and salicylic acid caused an increase in germination percentage and speed of *H. sabdariffa's* seeds in all levels in comparison to control. The highest germination percentage and speed were obtained using priming with gibberellic acid 125 and 500mg/kg, respectively (Diagrams 1 and 2). Seed priming with gibberellic acid 125 and 250mg/kg improved germination 25 percent and speed germination 3.2 seeds/day compared to the control treatment.

Rootlet length

The mean comparison showed that increasing the concentrations of *E. camaldulensis* led to a significant decrease of rootlet length. In contrast, all stimulators improved rootlet length in stress conditions with extract of *E. camaldulensis*. The interaction effect of priming and various concentrations of the extract of *E. camaldulensis* had a significant effect on the rootlet length of *H. sabdariffa* so that the highest root length was related to using concentration 500mg/kg of gibberellic acid (Diagram 3).

Shootlet length

Various concentrations of the extract of *E. camaldulensis* caused a decrease in the shootlet length of *H. sabdariffa.* Interaction effects of priming with chemical stimulators and various concentrations of the extract of *E. camaldulensis* were significant on shootlet length. The highest shootlet length of *H. sabdariffa* was related to gibberellic acid 250mg/kg in stress and non-stress conditions, and the lowest shootlet length was related to the control and the highest level of allelopathic extract (100%; Diagram 4).

Seedling length

The interaction effect of chemical stimulators and various concentrations of *E. camaldulensis'* extract was significant on the seedling length of *H. sabdariffa.* The plant length was reduced by increasing the concentration of *E. camaldulensis* in priming and non-priming seeds. The highest seedling length was related to priming with gibberellic acid at 250mg/kg (Diagram 5).

Seed vigor index

Mean comparison showed that the interaction effect of priming and various concentrations *E. camaldulensis* was significant on the vigor index. All priming levels cause an increase in the vigority of seeds against the allelopathic effect of *E. camaldulensis.* The highest increase was related to gibberellic acid atmg/kg ppm and the lowest related to control (Diagram 6).

Table 1) Variance analysis of studied traits of *H. sabdariffa* species

Properties	df	Germination percentage	Germination speed	Rootlet length	Shootlet length	Seedling length	Seed vigor
Priming	6	12/1**	14/9**	23/3**	24/6**	23/6**	36/9**
allelopathy	4	65/1**	60/4**	2701/2**	80/1**	457/1**	617/6**
Priming* allelopathy	24	2/1**	1/8**	6/4**	1/8**	7/8**	6/4**
Error	105	4/01	0/0	0/55	0/29	1/04	1481/9
CV	-	5/02	0/34	2/6	2/4	2/4	4/7

**: significant differences between treatments at 1% level

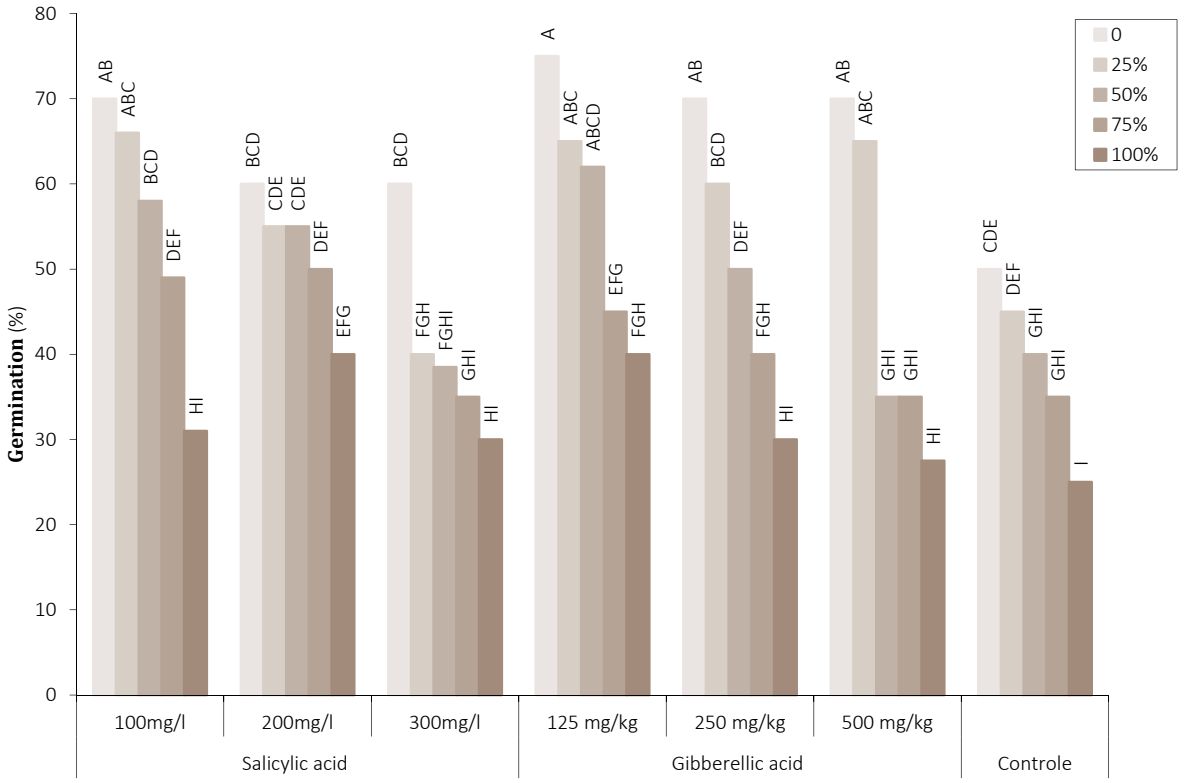

Diagram 1) Comparison of chemical stimulators and various concentrations of the allelopathic extract on germination of *H. sabdariffa*

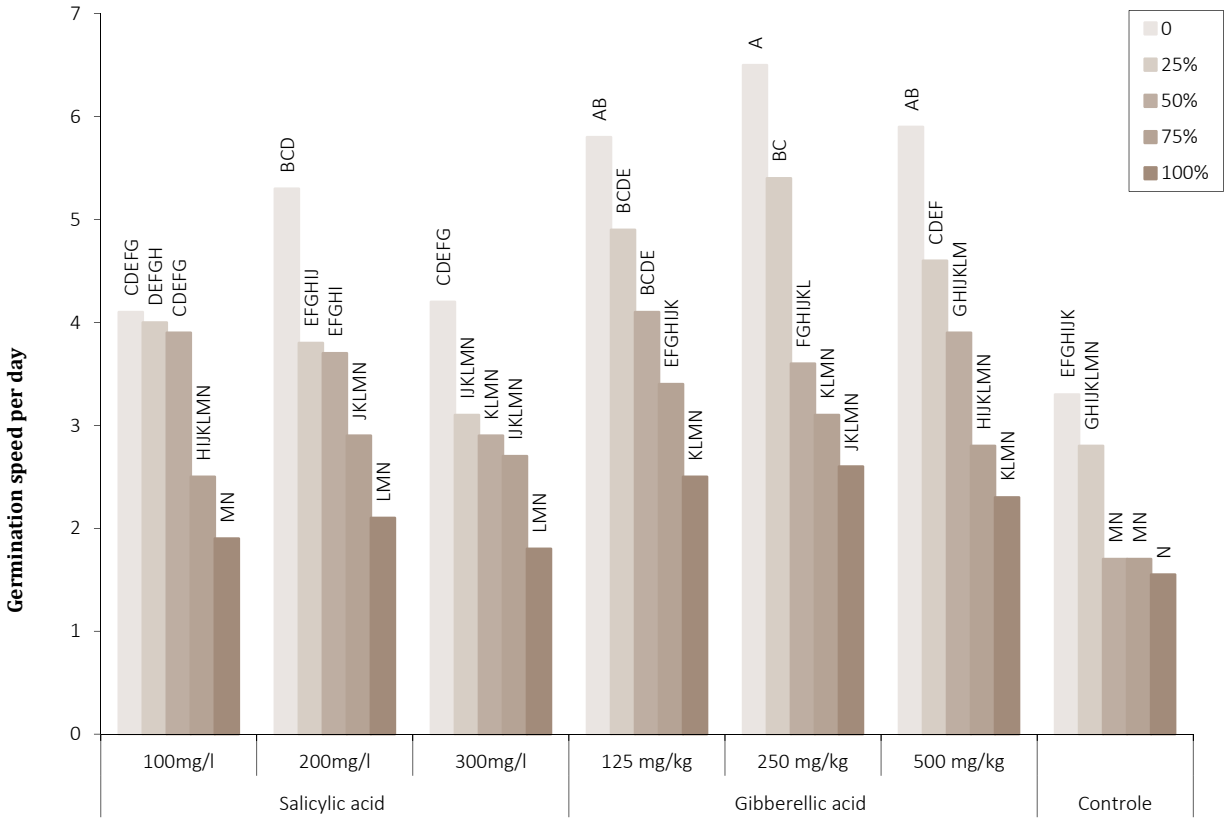

Diagram 2) Interaction effects of growth stimulators and various concentrations of the allelopathic extract on germination speed of *H. sabdariffa*

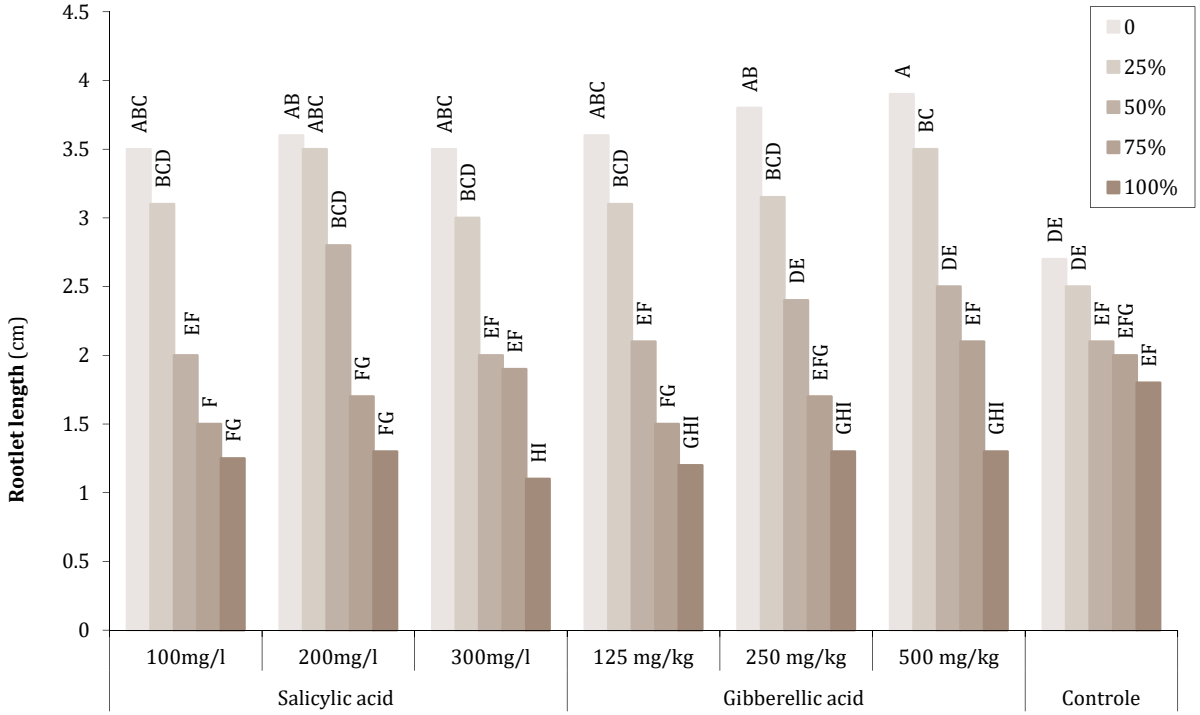

Diagram 3) Effect of priming and various concentrations of the allelopathic extract on rootlet length of *H. sabdariffa*

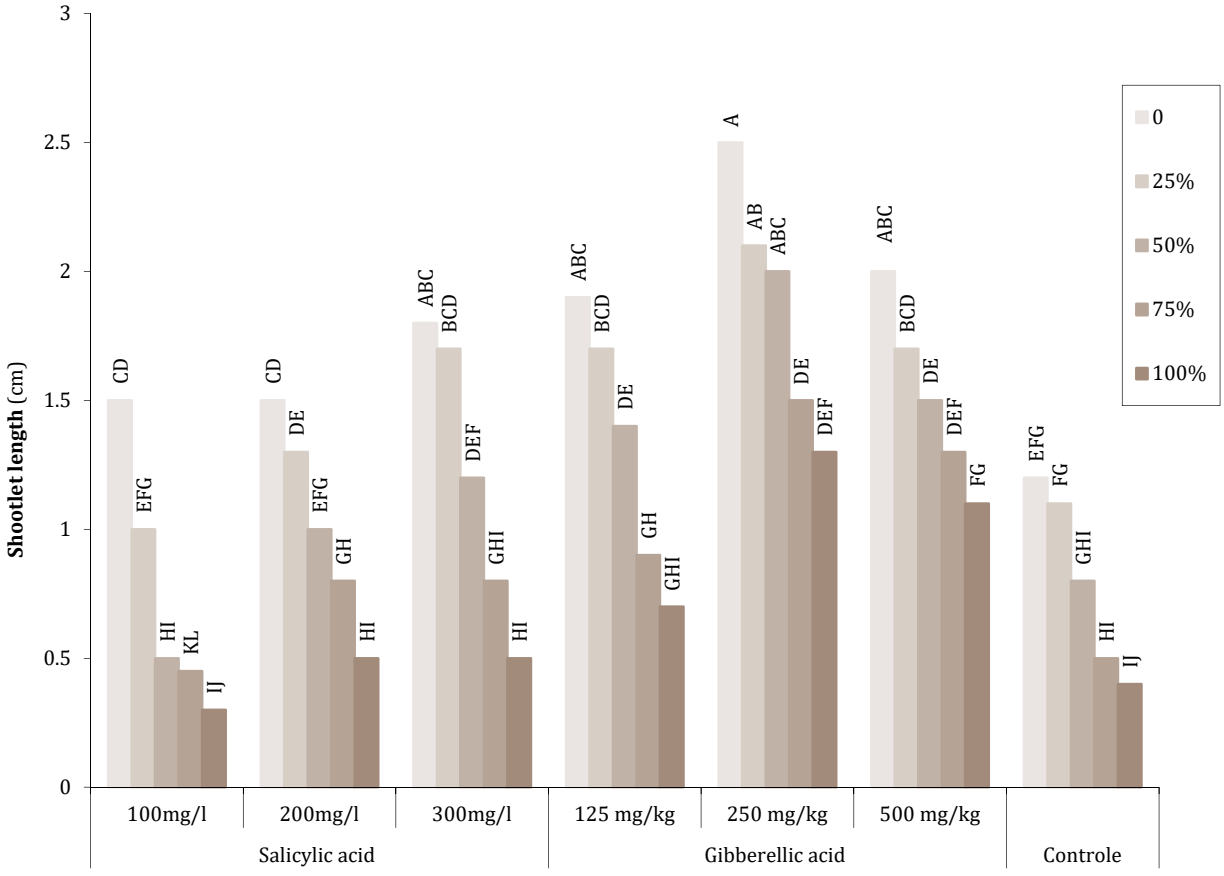

Diagram 4) Interaction effects of growth stimulators and various concentrations of the allelopathic extract on shootlet length of *H. sabdariffa*

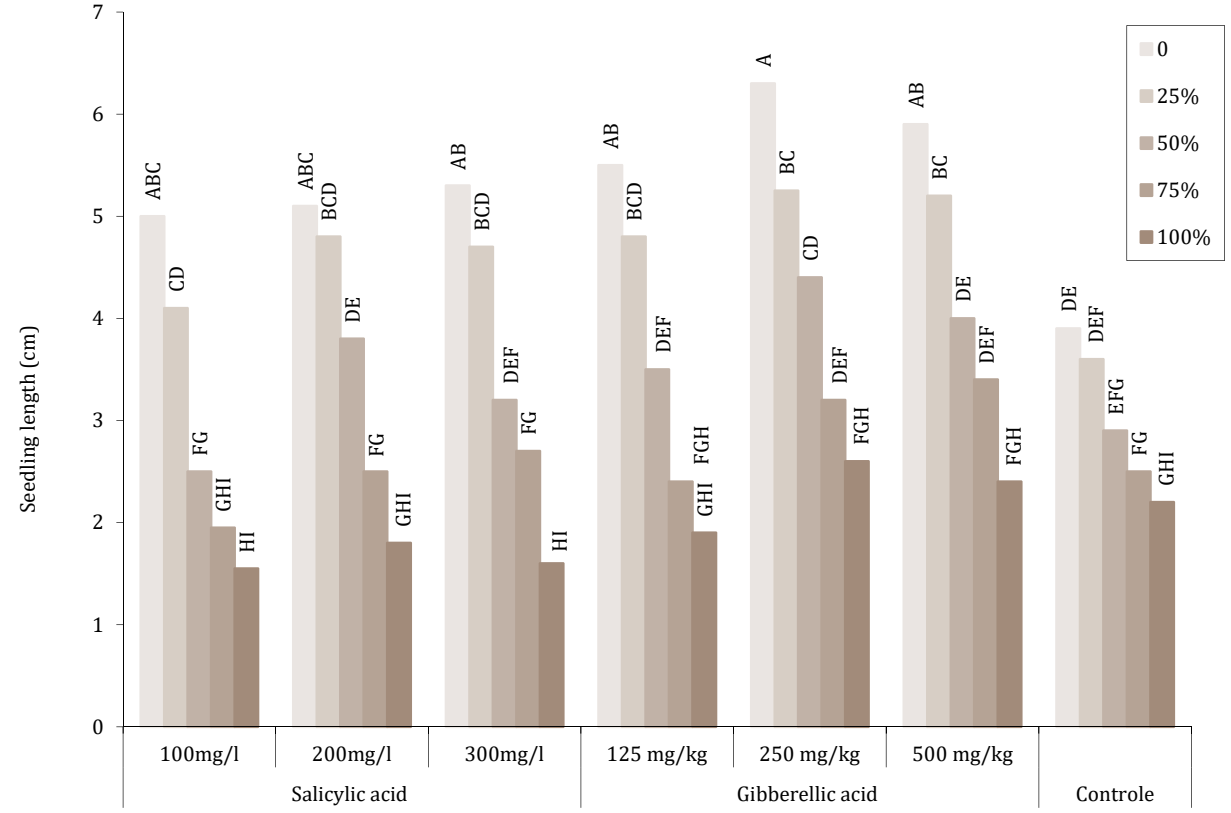

Diagram 5) Interaction effect of growth stimulators and various concentrations of the allelopathic extract on seedling length of *H. sabdariffa*

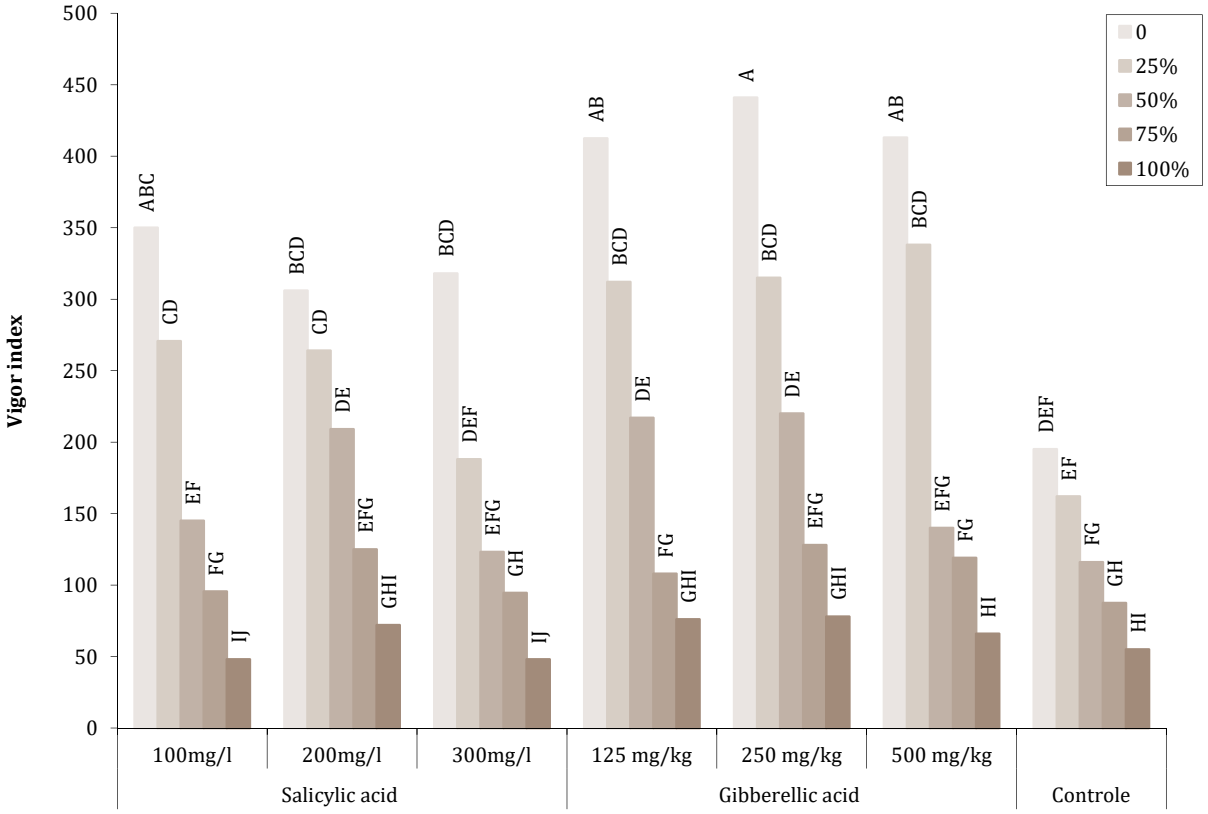

Diagram 6) Effect of growth stimulators and allelopathic extract of *Eucalyptus* on seed vigor of *H. sabdariffa*

Discussion and Conclusion

Eucalyptus contains allelopathic compounds that negatively affect germination characteristics and the growth of species, as results showed for Roselle. Allelochemicals may significantly influence the lifecycle of plants from reducing germination to reduce the growth of plants and the yields of crops [34, 35, 10]. Therefore, pre-treatment of seeds with chemical stimulants was investigated for possible control of the inhibitory effect of Eucalyptus's allelopathic compounds. The effect of different levels of salicylic and gibberellic acid pre-treatments on seed germination improvement and seedling growth was observed under stress conditions with allelopathic compounds, which is in agreement with the results of Saberi and Karimian [29]. Seed pre-treatment with salicylic acid increases antioxidants such as glutathione and ascorbate in the seeds, which decrease lipid peroxidation activity at the germination stage and thereby increase germination percentage [16]. This acid regulates the proline and accumulation of toxic ions in plants [36]. It is also involved in removing oxidative damage during germination [37]. Results from Tasgin *et al*. [38], Kang *et al*. [24], Khan *et al*. [26], and Hasanuzzaman *et al*. [27] suggest that salicylic acid is a suitable stimulus for germination and plant growth under stress conditions. Salicylic acid improves the germination of plant seeds by neutralizing free radicals or reactive oxygen [39]. The results showed that gibberellic acid had the most positive effect on the length of rootlet, shootlet, and seedling and vigor index of Roselle. Herbal hormones, such as gibberellic acid, play an important role in the germination and growth process. One of the important reasons for the positive effect of gibberellic acid on the early growth of roselle seeds could be because of the induced balance between hormonal ratio and reduction in growth inhibitors such as abscisic acid.

Gibberellins increase the synthesis of the hydrolytic enzymes that lie beneath the aleurone layer. The synthesized enzymes are transported to the endosperm, and it decomposes the stored food and provides the energy needed for germination and growth [40]. The positive effect of gibberellic acid on seed germination improvement and early growth of *Datura stramonium* [29], *Vicia villosa* [41], and *Onobrychis sativa* [42] under stress with allelopathic compounds have been reported, which is inconsistent with the results of the present study. Researchers also suggest that the use of gibberellic acid can break seed dormancy and seedling establishment [43]. The results showed that increasing the concentration of Eucalyptus extract reduced germination and growth of roselle seedlings. The decrease in germination may be due to the inhibitory effect of allelochemicals on gibberellin. Stopping germination may also be attributed to changes in enzymes that affect the transfer of storage compounds during germination [8]. Delay or amount of mobility in using storage compounds, a process that usually occurs rapidly during seed germination, could lead to the deficiency of respiratory products and eventually lead to ATP deficiency in seeds exposed to allelochemicals. Irregularity in respiratory rate leads to metabolic energy limitations and ultimately reduces germination and seedling growth [40].

In general, it can be concluded that chemical stimuli could reduce the inhibitory effect of allopathic compounds and improve seedling growth through the organization of antioxidant defense mechanisms, cell division, alteration of hormonal balance, increase in some plant hormones including auxins and cytokines, proline accumulation and nutrient uptake, photosynthesis, respiration, and protein synthesis. Pre-treatment the seeds with gibberellic acid had the best effect on the establishment and early growth of roselle seedlings. Eucalyptus contains allelopathic compounds that have a negative effect on the growth of crop species, including Roselle as a medicinal plant. Results showed that seed priming with gibberellic acidmg/kg ppm increased germination 25% compared to an unprimed seed. Hence it is suggested that seeds were pre-treated using appropriate materials such as gibberellic acid to increase germination percentage and better establishment of seedlings before planting.

Acknowledgments: The authors are grateful to research affairs of University of Zabol for the support.
Ethical Permissions: None declared by the Authors.
Conflict of Interests: There is no conflict of interest.
Authors' Contributions: Saberi M. (First author), Introduction author/Methodologist/Original researcher/Discussion author (50%); Tarnian F. (Second author), Assistant researcher/Statistical analyst/ Discussion author (30%); Karimian V. (Third author), Introduction author/Methodologist (20%)

Funding/Sources: This research was funded by the University of Zabol (Grant number: UOZ-GR-9618-72).

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