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Original Article

Effect of Different Treatments on Dormancy Breaking for Seed Germination Enhancement and Metabolite Analysis of *Capparis spinosa* L.

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ABSTRACT

Caper (*Capparis spinosa* L.) is a Mediterranean multifunctional crop recognized for its drought resistance as well as its capacity to preserve soil moisture. Caper is important in the world due to the important medicinal and nutritional compounds in its roots and leaves and has been considered by the pharmaceutical and food industries. Seed germination is poor owing to physical and physiological dormancy, which makes large-scale cultivation difficult. This research was done on February 2020 in the department of Agriculture, IROST. The effects of gibberellic acid (GA_3) at three concentrations of 200, 400, and 600 mg/L, as well as pretreatment with concentrated sulfuric acid (H_2SO_4 98%) at four-time levels of 15, 30, 45, and 60 minutes, on dormancy breaking in capers, were investigated. Also, total flavonoids, total polyphenols, DPPH assay, and rutin content were analyzed. The findings revealed that 45 minutes of H_2SO_4 followed by 400 mg/L GA_3 was the most efficient method for breaking the dormancy of dried caper seeds, with 81.5% germination, a 3.22 germination rate, and a 10.48 germination index. The highest concentration of metabolic properties in caper leaves was related to total polyphenols (2012.7 mg/g DW). On the other hand, the lowest value was related to rutin content (0.41 mg/g DW). The total flavonoid content was 64.28 mg/g DW, and the DPPH content was 69.06%. So, the results of this study can help improve and grow the output of capers because of the importance of growing them in dry conditions because of their deep root system.

INTRODUCTION

Capparis spinosa grows in most parts of Iran at altitudes above 2500 meters. Among the areas of caper distribution in Iran can be the slopes of Alborz, West Azerbaijan, Ardabil (Moghan plain), Marand, Khoy, Tafresh, Nahavand, Arak, Golpayegan, Kermanshah, Isfahan, Baluchistan, Shiraz, Yazd (around Taft and Mehriz), and Lamerd (Fars) pointed out [1]. Due to its many biological actions, such as antioxidant, anti-cancerous, antibacterial, and anti-mutagenic, it has been recognized to have health-improving advantages [2]. Alkaloids, glucosinolates, flavonoids, and phenolic acids are abundant in this plant species. Flavonoids, the biggest category of secondary metabolites found in plants, are engaged in a variety of biological processes. The multifunctional caper plant demands a semi-arid or arid environment [3]. This plant is utilized as a multifunctional and adaptable plant that gives a wonderful chance to provide greenery to locations with difficult climatic conditions. It has a deep root system and can withstand temperatures well beyond 40 ℃ [4]. Caper propagation via seed is the most frequent and easiest way. Because seed propagation results in segmentation, there is a risk of obtaining new types that outnumber existing ones. If a better variety can be acquired at the same time, or if a well-known variety is fitted to a specific place, it may proliferate by vegetative propagation. Nevertheless, many varieties have poor rhizogenesis in vegetative propagation [5]. Caper plants grown from fresh seeds do not always develop completely, and only around 30% of new seeds germinate [6]. Barbera and Di Lorenzo [7] discovered that only 5% of caper seeds germinate within the first two to three months of planting. Bond (1990) found that just 10% of fresh caper seeds put in pots grew after 10 days [8]. Foschi *et al* [9] reported that the seed

moisture content, which was obtained from caper seeds, increased germination percentages up to 90%, and all viable seeds sprouted. As a result, caper seeds do not have a water-impermeable covering and do not demonstrate physical dormancy when wet. External treatments are required to break the predominant dormancy in this crop, which is caused by the stiff seed coat.

Seed priming is a method of managing germination by altering the temperature and moisture content of the seed in order to maximize the seed's potential. Seed priming has been shown to improve germination, seedling emergence time, and plant establishment. Chemical cues have been described in the application of chemical cues to speed up growth and germination [10]. Auxin, abscisic acid, polyamines, ethylene, salicylic acid, and ascorbic acid are some of the common growth hormones utilized for seed priming [11]. Several seed treatments (mechanical or acid scarification, and treatment with KNO_3 or gibberellic acid (GA_3)) were used to increase germination percentage while decreasing germination times [12]. It was discovered that caper seed germination may be increased by using concentrated sulfuric acid (H_2SO_4 98%), GA_3, and KNO_3 [6]. The use of H_2SO_4 as a pre-treatment for 15–30 minutes was proven to be an efficient way to boost germination. High germination percentages were achieved with concentrated H_2SO_4 and a 90-minute soak in a 0.01% GA_3 solution [6]. It's also been reported that a warm water treatment paired with a 65–70-day chilling stratification improves germination. Caglar *et al* [13] discovered that using indole-3-butyric acid (IBA) boosts caper cutting rhizogenesis. Scraped and broken coat seeds in the batch are one reason for the poor viability and germination of sold caper seeds. The degradation caused by the extraction, cleaning, drying, and storage operations reduced the viability and vigor of the seeds, and hence their germination capability [9]. Previous research has concentrated on characteristics such as fruit ripeness, harvesting time, storage period, and processing techniques, among others. There is little and inadequate research on caper seed germination [4]. In light of these facts, we applied GA_3 and H_2SO_4 at different concentrations and time courses, respectively, to enhance caper seed germination. Due to the morphological characteristics of this plant, especially the deep root system, and metabolic properties such as total poly phenol, total flavonoid, anti-oxidant and rutin content, the purpose of this study is to seed germination enhancement by priming and other treatments of braking dormancy.

MATERIALS AND METHODS

Plant Materials

Caper seeds were collected from Tafresh region of Markazi province with cold and dry climatic conditions and geographical characteristics (longitude 50.01 and latitude 33.28), altitude 1890, minimum recorded temperature -20 and maximum 39 °C. The average humidity was 47% and the average annual rainfall was 316 mm, with a weight of one thousand seeds of 5.5-6 grams and was approved with the herbarium code 478Cs020MPISB obtained from the Research Institute of Medicinal Plants. This research was done on February 2020 in the department of Agriculture, Iranian Research Organization for Science and Technology (IROST). For viability test, a tetrazolium was used to evaluate the potency of caper seeds (ISTA, 2011). In this method, first a certain number of healthy seeds, selected and cleaned of soil and contamination, washed with distilled water and then immersed in 0.1% solution of triphenyltetrazolium chloride. This experiment was performed in four replications with 50 seeds was placed on 9 cm petri dishes on filter paper. In order to create a dark environment, the containers containing seeds were covered with aluminum foil and kept at 25 °C for 24 hours. The seeds were then washed and the live seeds and embryos and their viability were calculated under a microscope. Root emergence with a length of about 2 mm was considered as the germination criterion [14].

Seed Priming

Seed priming is a controlled watering before sowing and seed germination that is used to promote germination speed and uniformity under stress and non-stress circumstances. Pretreatments are now utilized to speed up seed germination, force seed germination, and allow seed germination in adverse environments. Pre-sowing treatments help grain seed germination. These techniques can be employed to break mechanical or physiological dormancy. Researchers used mechanical scraping, cooling, immersion in concentrated sulfuric acid (H2SO4), gibberellins, cytokinins, light, and

temperature changes [15]. According to the studies performed [6] on caper seeds, different levels of caper seed priming, including seed pretreatment with GA_3 at three levels of 200, 400, and 600 mg/L (ppm) and pretreatment with concentrated H_2SO_4 98%, were selected at four-time levels of 15, 30, 45, and 60 minutes.

Concentrated H_2SO_4 (98%) and GA_3 Treatment

In order to apply the acid treatment, H_2SO_4 (98%), purchased from the German Merck company, was used, and the time factor of the treatment was considered. About 5 g of cleaned and threshed seeds were mixed in the ratio of one part of seed and two parts of acid (volume ratio) and passed through 15, 30, 45, and 60 minutes of acid washing. To remove the effects of acid, the acid-washed seeds were thoroughly washed with sterile distilled water at the end of the specified time. The seeds were then placed in GA_3 solution in three replications for each of the concentrations of 200, 400, and 600 mg/L. In order for the hormone to penetrate better into the seed tissue, the containers were placed on a shaker for 90 minutes. After immersion of the seed in GA3, the seed samples were dried in room temperature for complete of priming process. Finally, after dividing the seeds into equal proportions on a filter paper inside a petri dish (each container containing about 50 seeds) and checking for germination was performed at a laboratory temperature of 25 ±2 °C, with 16 hours of light, and eight hours of darkness (lighting conditions 3000 lux). In order to prevent possible contamination, the seeds were disinfected with benomyl fungicide solution at a concentration of two per thousand. Leached seeds with distilled water were used as control. The seeds were irrigated with distilled water during the experiment. The treatments studied in this experiment are described in Table 1. The letters "A" for H_2SO_4 (98%) and "G" for GA_3 were used to facilitate treatment.

Seed Germination Percentage

In order to evaluate the germination percentage index (GP) in this test, germinated seeds were counted daily in each petri dish. Seeds with a root length of more than two millimeters were considered as germinated seeds. During the test period, 22 counts were performed, and after summing the number of germinated seeds in each treatment, the germination percentage was calculated as the average. The germination percentage was calculated using the following equation (ISTA, 2006).

$\%GP = n/N \times 100$

Where n represents the number of germinated seeds and N represents the total number of seeds sown.

Seed Germination Index

The seed germination index (GI) was obtained from the ratio of the total number of germinated seeds to the number of days after sowing and was calculated using the following equation:

$GI = \Sigma^{(ni \times ti)}/S$

ni = the number of germinated seeds at a given time (ti).

ti = the number of days after the start of germination.

S = total number of seeds

Seed Germination Rate

Germination rate (GR) was studied as another important indicator of drought tolerance in plants. In this test, the germination rate was calculated using the equation [16] as follows.

$GR = \Sigma^{n/t}$

GR = germination rate

n = the number of seeds that germinated at the time (t).

t = number of days after germination

Planting of Seedlings

After germination of seeds in the laboratory, these buds were first planted in small pots containing cocopeat and perlite in a ratio of 2: 1.

Table 1 Combined treatments of H_2SO_4 98% and GA_3 with control. Acid washing of seeds with concentrated H_2SO_4 at 98% for 15, 30, 45, and 60 minutes and immersion of seeds in GA_3 for 90 minutes at concentrations of 200, 400, and 600 mg/L (A = 98% H_2SO_4 and G = GA_3).

Control	A15G200	A30G200	A45G200	A60G200
-	A15G400	A30G400	A45G400	A60G400
-	A15G600	A30G600	A45G600	A60G600

The samples were kept in growth chamber 24 ± 2 °C, relative humidity of 40%, and 16 hours of light and eight hours of darkness until full growth and the formation of three to four leaf seedlings. Necessary care and watering of pots was done daily until the seedlings grew properly and took root (Fig. 1). Seedlings with three to five leaves are planted in plastic pots with an opening diameter of 22 and a height of 25 cm with sand, clay, and arable soil and transferred to the greenhouse after planting. The pots were kept for twenty days in greenhouse conditions with a minimum of 18 °C and a maximum of 25 °C, a relative humidity of 40-50%, and natural light conditions of the greenhouse.

Fig. 1 Caper Seedlings in Growth Chamber

Metabolic Properties

The total flavonoids and total polyphenols were measured by [17] and [18] methods, respectively. In order to measure the antioxidant properties of the extracts, the DPPH method of Miliauskas *et al*. [19] was used.

Extraction

Leaf samples were dried in the shade at a constant temperature and then pulverized. One gram was soaked in 20 cc of solvent consisting of 85% methanol, 12% distilled water, and 2% acetic acid. The glass doors were completely closed and placed on a magnetic stirrer for 24 hours at room temperature with dark condition. The solvent and plant mixture were then separated using filter paper (Whatman), and the extracts were stored in sterile dark glass at 4 °C until the experiments were carried out.

Rutin Content

These studies were conducted in the laboratory of the Medicinal Plants Research Institute, Shahid Beheshti University, using HPLC, the PDA detector, and a method developed by [20] with a flow rate of 0.5%. The column was 150 mm long, 4.6% in diameter, and 3.5 microns in size. In the mobile phase of water, methanol was used at half a milliliter per minute at 200-600 nm. The extracts were purified with paper filters (Labconco, USA) and then dried in a RapidVap dryer. To use the dried extract in the column, it was dissolved in 30% methanol and calibrated twice with methanol and distilled water before the experiment. 2 ml of extract, 1 ml of distilled water, 2 ml of 80% methanol This was followed by injecting the diluted extract into an HPLC syringe with a 0.45m filter. The oven was set to 20 °C. The rutin standards were developed by Sigma. Existing standards and peak curve surfaces were used to figure out how much of each compound there was [21].

Statistical analysis of seed germination data

Germinated seeds were counted daily. All the data obtained was recorded in Excell software version 2019 and analyzed using Minitab software version 18, and the means were compared with the Tukey test.

RESULTS AND DISCUSSION

Germination Percentage

The results showed a significant difference between the treatments ($p \leq 0.05$). The highest germination rate, with an average of 81.5%, was obtained in the combined treatment of GA_3 with a concentration of 400 mg/L and concentrated H_2SO_4 (98%) for 45 minutes. Untreated seeds (control) showed the lowest average (1.6%) germination. GA_3 treatments with concentrations of 200 and 600 mg/L did not show significant differences, with 63.8% and 62.2%, respectively (Fig. 2 and Fig. 3).

Results showed that eliminating physical seed dormancy enabled caper seeds to germinate and had an enhancing role in the germination of this species. Ramezani-Gasak *et al*. [22] also found that caper seeds grew best when they were treated with concentrated H2SO4 for 30 minutes and GA3 200 mg/L for 60.2% of the time. This study, on the other hand, found that the results were better than theirs.

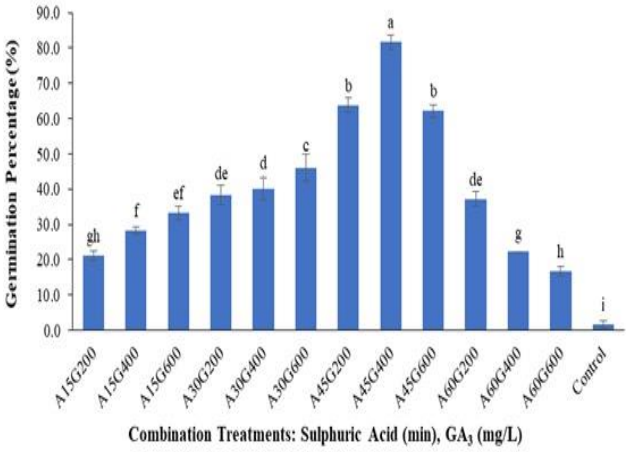

Fig. 2 Germination percentage of caper seeds under combined treatments of H_2SO_4 and GA_3. A15G200 (concentrated H_2SO_4 for 15 minutes and GA_3 at a concentration of 200 mg/L) is an example.

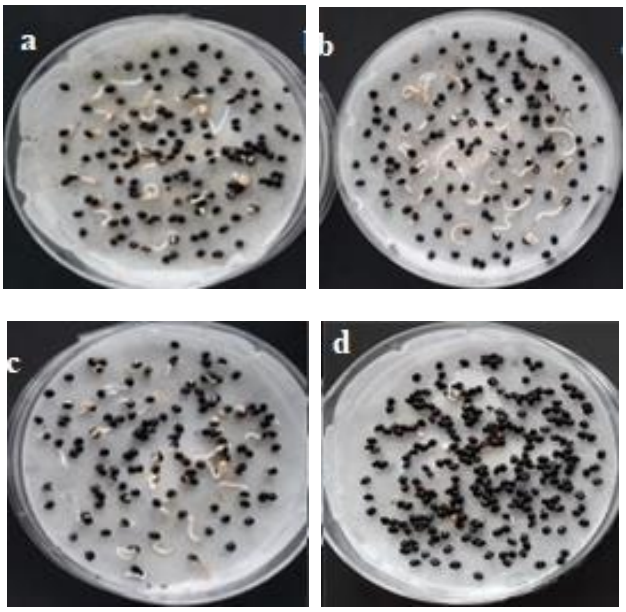

Fig. 3 Comparison of seed germination percentage under combined treatments of H_2SO_4 and GA_3. a) $A_{45}G_{200}$, b) $A_{45}G_{400}$, c) $A_{45}G_{600}$ and d) Control.

Germination Rate

Germination rate is one of the oldest concepts of seedling vigor and is commonly used as an indicator in crop and forage breeding programs. This index is usually determined at the same time as the germination percentage, but in some cases, it is a more valuable criterion. The variance analysis indicated a significant difference between the treatments ($p \leq 0.05$). The results showed that the combined treatment of concentrated H_2SO_4 for 45 minutes with the application of GA_3 at a concentration of 400 mg/L was able to increase the germination rate (number of germinated seeds per day) to 3.22 which is somewhat consistent with the results of [22]. The application of two concentrations of 200 and 600 mg/L GA_3 with 45 minutes of H_2SO_4 did not show a significant difference (Fig. 4 and Fig. 5). The germination rate was equal to 0.05 in control seeds. Research showed that the seed germination rates of two species, *Poa pratensis* and *Poa palustris*, were different from each other, while the germination percentage was almost the same [22].

Slowing down the germination rate and increasing the time required for germination is usually attributed to the ability of the seed coat to propagate at very negative water potentials [23]. Cultivars with a higher germination rate under stress conditions have a higher chance of emergence [24].

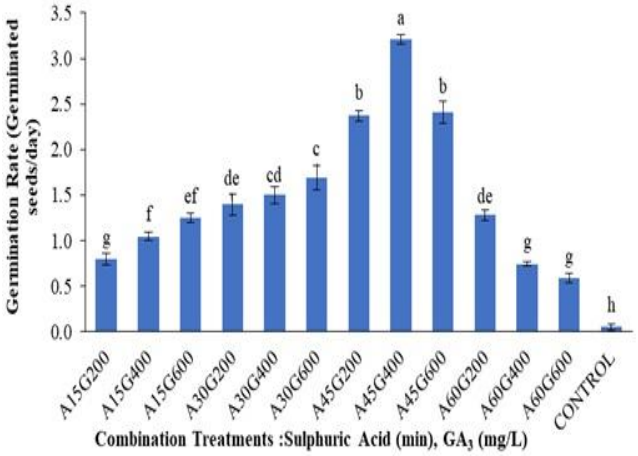

Fig. 4 Germination speed of caper seeds under combined treatments of H_2SO_4 and GA_3.

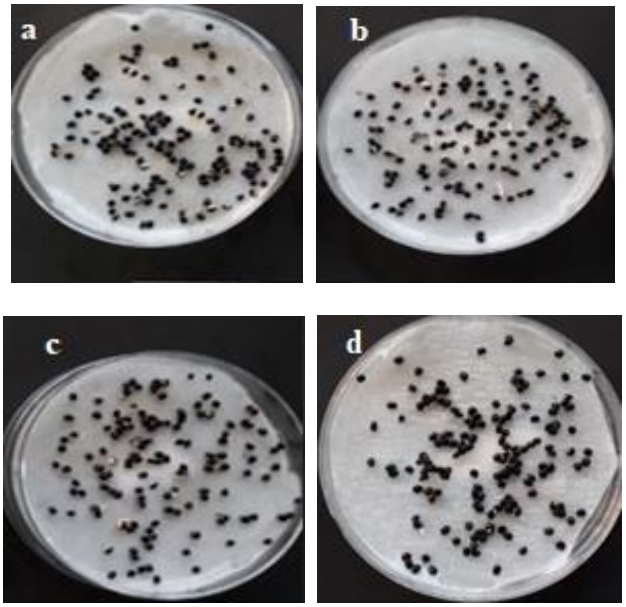

Fig. 5 Comparison of germination rates in combination treatments of caper seed dormancy breaking. a) $A_{45}G_{200}$, b) $A_{45}G_{400}$, c) $A_{45}G_{600}$ and d) Control.

Germination Index

The combined treatments of H_2SO_4 and GA_3 used in this experiment had a significant effect on the germination index of caper seeds. The results indicated that the control samples with 0.51 had the lowest index and the application of GA_3 with a concentration of 400 mg/L with concentrated H_2SO_4 for 45 minutes had the highest germination index (with a value of 10.48). No significant difference was observed between GA_3 treatments of 200 and 600 mg/L with values of 8.49 and 8.21 (Fig. 6 and Fig. 7). Ramezani-Gasak *et al* [22] observed the highest germination index of caper seeds by combining treatments of H_2SO_4 for 30 minutes and GA_3 with a concentration of 400 mg/L.

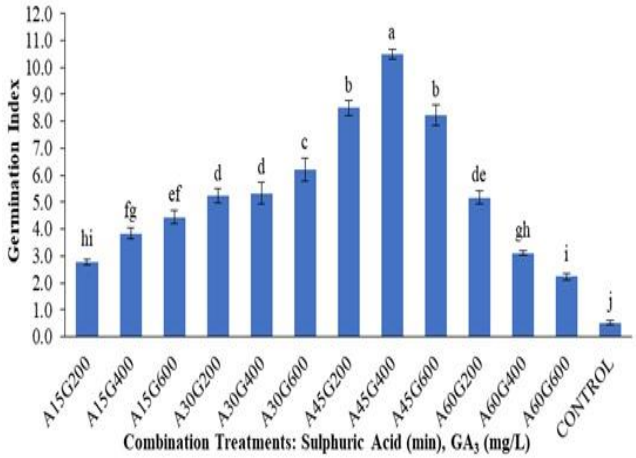

Fig. 6 Germination index of caper seeds under combined treatments of H_2SO_4 and GA_3.

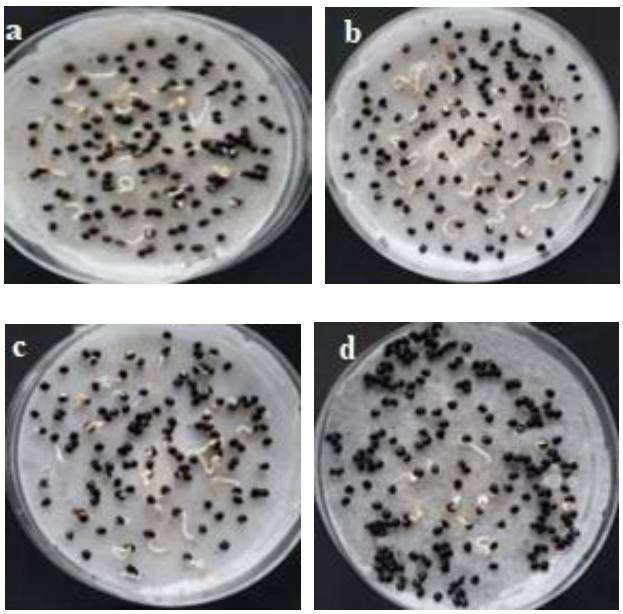

Fig. 7 Comparison of germination index in combined treatments of caper seed dormancy breaking. a) $A_{45}G_{200}$, b) $A_{45}G_{400}$, c) $A_{45}G_{600}$ and d) Control.

To successfully domesticate and cultivate therapeutic plants, an understanding of seed germination is required. Most medicinal plants gathered from natural regions take longer to germinate than crops or modified plants [25]. This might be owing to poor germination rates or species-specific ecological requirements. Drought and water constraints have increased demand for stress-tolerant plants. To domesticate and develop the caper plant, one must first understand seed dormancy and germination. The findings of this study suggest that using H2SO4 and GA3 together can considerably accelerate the germination of caper seeds, which is in line with prior studies on this species and others. Caper seed dormancy has been linked to both mechanical and embryonic dormancy [26].

Following the administration of growth regulators such as gibberellic acid, the ratio of this chemical to abscisic acid in the seed rose, followed by free and active alpha amylase enzyme. Activating enzymes break down carbohydrates and dissolve seed store starch into embryonic components, which helps seeds grow [27,28]. Gibberellins, the principal germination stimuli, influence the transcriptional or translational phases of certain genes, affecting protein synthesis. It is thought that they help produce energy and necessary chemicals for fetal growth and development [29]. Gibberellic acid is produced in the fetus and diffuses through the endosperm to the aleurone layer. It then releases alpha-amylase hydrolyzing enzymes, breaking starch down into oligosaccharides [30]. According to Olmez *et al*. [31], treating celery seeds with gibberellic acid decreased the inhibitory impact of seed coat chemicals and increased germination time and seedling growth. In the study by Rostamipour *et al*. [32], 400 mg/L of gibberellic acid had a bigger effect than 500 mg/L on Iranian eagle seedlings, which matches the results of this investigation on the germination rate of celery seeds. Nowruzian *et al*. [30] [33] found that 10 mg/L of gibberellic acid caused the maximum germination rate compared to the control, which increased the hormone concentration to 15 mg/L and higher. Germination was decreased, highlighting the study's findings.

Gibberellic acid concentrations above the threshold have been shown to inhibit germination and other seed characteristics in *Ferula assa foetida* L. plants, which is consistent with the results of this study.

The seed coat and the mucilage formed on the seed coat are the principal barriers to caper seed germination. Mucilage blocks the caper embryo's oxygen intake. By softening the shell and lowering the mucilage around the shell, concentrated H2SO4 enhances the capacity of cotyledons to break the seed coat and reduces the inhibitory effect of these shells on the germination process. Orphanos *et al*. [34] studied the effect of H2SO4 on Spinoza caper seeds and found that a 15-30 minute H2SO4 treatment improved seed germination by up to 40%. In this investigation, 45 minutes of intense H2SO4 enhanced germination by 66%. Labbafi *et al*. [35] discovered that treating H2SO4 for 15 minutes with 2000 mg/L gibberellic acid at two temperatures of 20 and 30 °C increased germination by 75.92%, which is consistent with the findings of this study. Regardless of the temperature, we employed a lower concentration of gibberellic acid in our experiment. Soaking Carex hetrostachya seeds in concentrated H2SO4 for 20–30 minutes boosted germination by 88%, similar to this study [36]. Other research [37, 38] has shown that breaking caper seed dormancy requires many treatments because of its physiological and mechanical characteristics, confirming the efficiency of the H2SO4 and gibberellic acid treatment combination used in this work. H2SO4 and gibberellic acid were employed to break seed dormancy and caper germination by Ramezani-Gasak *et al* [22]. This research found that treating seeds with H2SO4 for more than 30 min damages seed and lowers germination %, which contradicts the conclusions of this study.

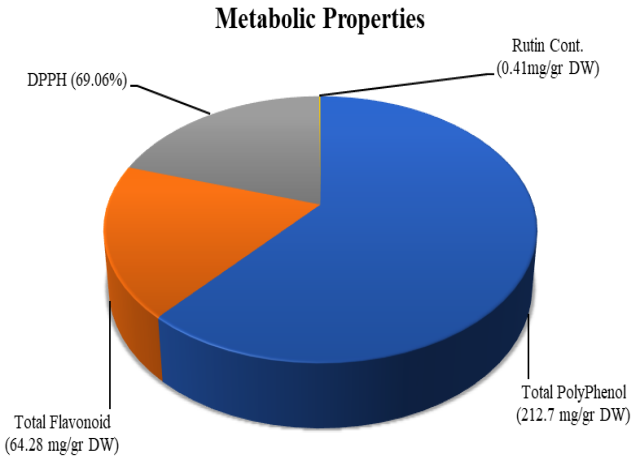

Fig. 8 Metabolite properties of *C. spinosa* L**.** planted in the greenhouse condition.

More than 40 to 45 minutes of H2SO4 treatment reduced seed germination. Caper seeds become sensitive to gibberellic acid after being treated with H2SO4. After the shell was ruptured, gibberellic acid created an enzyme to replicate the chromosome and activated enzymes involved in material handling. Several research on seed dormancy breaking and germination of plants have demonstrated that germination ecology and effective treatments for seed dormancy breaking can vary greatly amongst plants, species, and even ecotypes [39]. The mechanisms of seed dormancy in plants vary, as do the treatments and methods for breaking dormancy.

The Metabolic Properties of Caper Seedlings

The content of total phenolic and flavonoid compounds, the percentage of inhibitory and antioxidant power of these compounds, as well as rutin content, were studied to investigate the metabolic properties of caper seedlings under greenhouse conditions. The result indicated that the highest amount of metabolic properties in caper was related to total polyphenols (2012.7 mg/g DW). On the other hand, the lowest value was related to rutin content (0.41 mg/g DW). Total flavonoid was 64.28 mg/g DW and DPPH was 69.06% (Fig. 8).

Several research has been carried out to determine the presence of various beneficial chemicals and metabolites in various regions of the caper. Polyphenols, flavonoids, alkaloids, steroids, terpenes, and tocopherols are among the bioactive substances found in this plant [40, 41]. A range of flavonoids and different alkaloids, such as rutin [42], may be found in aerial organs such as leaves and stems. In the study by [43] on the phytochemical, antioxidant, and antibacterial activities of the caper plant, the presence of flavonoid, phenolic, alkaloid, tannin, and glycoside compounds in the methanolic extract of the leaves of this plant was proved, and rutin was introduced as the most important and most flavonoid compound in caper leaves. Tlili *et al*. [44] confirmed celery's phytochemical properties and therapeutic benefits in diabetes, sclerosis, inflammation, and antimicrobial and antiviral activities. In addition to its many beneficial and medicinal properties, the caper plant has anti-cancer effects due to its rutin and quercetin [45]. Aliyazicioglu *et al*. [46] were able to identify sixteen phenolic compounds in the caper plant using

high-performance reverse phase liquid chromatography. Also, the amount of total phenolic compounds in this study according to the gallic acid standard is about 37.0 mg equivalent of gallic acid per 100 g dry weight of the sample. The study by [47] on the phenolic content and antioxidant activity of ether extract of caper also showed high levels of phenolic, flavonoid, and anthocyanin compounds in this extract. They reported a concentration of 427.27 mg/g dry weight for the total phenolic content of the core extract and 57.93 mg/g dry weight for the total flavonoid content. Numerous studies have been performed on the content of polyphenolic and flavonoid compounds in different organs of the caper. The amount of these materials varies because of a number of factors, including where they are, how they are grown, when they are harvested, and how they are extracted.

CONCLUSION

The caper plant has a wide ecological area in the field of nature and is able to survive in different ecological conditions due to drought tolerance. The presence of high amounts of phenols and flavonoids in various organs shows the importance and necessity of paying more attention to this valuable plant for commercial, industrial, and medical purposes. The results of this study showed that pretreatment of caper seeds with combined treatments of 400 mg/L GA_3 and 45 minutes of concentrated H_2SO_4 can significantly increase germination indices with 81.5% germination and thus the optimal establishment of seedlings (A45G400). This treatment was considered as the best one. In view of these considerations, research was carried out to investigate the efficiency of different seed dormancy breaking procedures on the germination and metabolic properties of caper seed. We used GA_3 and H_2SO_4 at various concentrations and time courses to see how they would react. Because of the physical properties of this plant, particularly the deep root system, and also medicinal characteristics such as total flavonoids, total polyphenols, DPPH assay, and rutin content, this research may represent to optimize and improve crop production, with particular emphasis on the necessity of cultivation under drought situations.

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Conflict of Interest

The authors have not declared any conflict of interests.

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