



Phytopriming Technique for Enhancing Seed Germination and Secondary Metabolites Production in Sand Daffodil

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Abstract

The Sea Daffodil (*Pancratium maritimum* L.) is an endangered medicinal plant species that is currently facing several challenges such as overgrazing for pharmaceutical purposes, climate change, and depletion of natural resources. To address these challenges, seed priming techniques utilizing natural bio-simulating substances have emerged as an effective, environmentally friendly, and cost-efficient alternative to chemical methods. These techniques aim to enhance seed quality, promote plant growth, and increase the production of bioactive compounds in *P. maritimum*. Involves the use of distilled water, and phytopriming, which involves the use of *P. maritimum* aqueous extract with concentrations of 10%, 20%, 40%, and 60% derived from *P. maritimum*, as seed priming agents to achieve the goals. The results of the study revealed that both hydropriming and phytopriming techniques had positive impacts on certain germination parameters, although the effects varied for different parameters. Additionally, it was observed that coated seeds exhibited more significant changes in germination parameters compared to uncoated seeds. Furthermore, Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *P. maritimum* treated with various phytopriming methods demonstrated an increase in the total alkaloid content, as well as the levels of pancratistatin, lycorine, and antioxidants, when compared to hydropriming treatment and the control group. The application of *P. maritimum* aqueous extract as a natural bio-simulating agent represents an innovative approach to sustainable agriculture in the field of phytotechnology. This technique can effectively enhance the production of secondary metabolites in *P. maritimum*, thereby furthering the development and utilization of this valuable medicinal plant.

Keywords Alkaloids · Antioxidants · Hydropriming · Medicinal plant · Phytopriming

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Abbreviations

GC-MS	Gas Chromatography-Mass Spectrometry
PMAE	Pancratium maritimum aqueous extract
GP%	Germination Percentage
GVe	Germination Velocity
SG	Speed of Germination
GE	Germination Energy
GI	Germination Index
MGT	Mean Germination Time
SVI	Seed Vigour Index
SL	Seedling Shoot Length
RL	Seedling Root Length
DPPH	2,2-diphenyl-1-picrylhydrazyl
SD	Standard deviation
h	Hours
min	Minutes

1 Introduction

The Mediterranean region is known for its rich diversity of wild medicinal plants, which are highly valued for their bioactive properties and extensively utilized in traditional medicine (Petropoulos et al. 2020). Despite their widespread distribution, these popular medicinal plants face a range of threats from human activities such as tourism, urbanization, coastal degradation, over collection, and other ecological pressures. Consequently, *P. maritimum* has been designated as an endangered species that is inadequately protected (De Castro et al. 2016). In recent years, coastal plants like *P. maritimum* have garnered significant attention for their potential applications in the industrial and pharmaceutical sectors due to their abundance of valuable compounds, including alkaloids, flavonoids, and phenolic compounds (Berkov et al. 2010; Leporini et al. 2018) which are found in their bulbs, leaves, and flowers. However, given its endangered status, it is imperative to consider the bioethics and sustainability of utilizing this species (Juan Vicedo 2018).

Extracts from the bulbs and flowers of *P. maritimum* have been found to possess various effects. These include acaricidal and insecticidal properties (Abbassy et al. 1998), anti-fungal activity (Sür-Altiner et al. 1999), analgesic and anti-cancer effects (Leporini et al. 2018; Taie et al. 2015), as well as purgative, hypotensive, emetic, and anti-inflammatory properties (Tayoub et al. 2018; Nair and van Staden 2017; Leporini et al. 2018). Moreover, the leaves of *P. maritimum*, which are known for their alkaloids, are a rich source of antioxidant compounds (Ibrahim et al. 2014; Pandey et al. 2005; Cedrón et al. 2015; Rokbeni et al. 2016; Sanaa et al. 2013). Many alkaloids have been identified in this species, including pancratistatine and lycorine. Pancratistatine has shown anti-tumor activity (Pettit et al. 1995), while lycorine has demonstrated anti-viral and analgesic effects (Torras-Claveria et al. 2013).

Seed priming is a commonly practiced agricultural technique that entails treating seeds prior to planting in order to optimize germination potential and enhance overall crop performance (Paparella et al. 2015). Hydropriming is a cost-effective and straightforward method in which seeds are soaked in water for a specific duration, dried to a particular moisture level, and then sowed (Singh et al. 2015). It activates early germination events (Lutts et al. 2016), revitalizes seeds, and helps the seeds withstand environmental stress like high heat and drought by improving water uptake efficiency and seed hydration (Waqas et al. 2019; McDonald 2000).

Phytopriming is a technique that involves using aqueous plant extracts as priming agents. Studies have demonstrated that certain crop and tree residues possess anti-fungal properties (Perelló et al. 2013; Rafi et al. 2015) and promote

growth (Farooq et al. 2008; Perelló et al. 2013; Rafi et al. 2015; Jagatap et al. 2014; Imran et al. 2014) due to the presence of essential nutrients such as amino acids, potassium, calcium, iron, and ascorbate, as well as growth-regulating hormones like zeatin (Nouman et al. 2012). Botanicals have been shown that phytopriming may improve seed germinability potential (Imran et al. 2014; Masuthi et al. 2015; Nouman et al. 2012; Rafi et al. 2015) and combat adverse climatic conditions (Masuthi et al. 2015). One of the more modern methods for treating seeds is the use of extracts from medicinal plants rather than artificial chemical seed treatments, as the latter can directly increase environmental pollution and pose health risks. The effectiveness of medicinal plant extracts like Mint (Menthol), Coleus (Forskholin), Sweetflag (Acarin), *Aloe vera* (Oleorin, Barborin) Pongamia leaf extract (Karanzin), Basil (Camphor, Linolinacetate, Eugenol) on seed germination, however, has not been well studied (Nanjappanavar et al. 2017). Soares et al. (2023) recorded that seed priming improved the speed of seed germination and Increased seedling length and vigor of *Moringa oleifera* using *Moringa oleifera* leaf extract. medicinal plants rich in biochemical compounds are known to have a varied range of biological activities as well as bio-stimulant properties (Souri and Bakhtiarizade 2019).

Seed priming is known to trigger various regulatory processes in seed embryos during germination and plant growth, including enzymatic, hormonal, physiological, transcriptomic, metabolomic, and proteomic changes (Jatana et al. 2024). During seed imbibition in the priming process, metabolic regulations can be induced, leading to the de novo synthesis of proteins and nucleic acids, increased production of adenosine triphosphate (ATP), activation of antioxidants (Shetty et al. 2005), and DNA repair mechanisms (Ejaz 2019). Seed priming can enhance the production of bioactive compounds that are used for medicinal purposes. Two eco-friendly and traditional techniques, hydropriming and phytopriming, utilize natural bio-stimulants such as water or plant-derived extracts. These techniques promote growth and help create safe and targeted bioformulations. In this study, we conducted an experiment to investigate how priming affects the germination of vigor seeds, seedling growth, and the production of bioactive compounds. Specifically, we examined two methods of seed priming: hydropriming and phytopriming. Hydropriming focused on the duration of soaking, while phytopriming considered both the duration and concentration of the soaking solution as variable factors. Our main objective was to investigate the effects of hydropriming and phytopriming techniques on enhancing seed germination, promoting sustainable production of *P. maritimum*, and improving the bioactive properties of the plant, particularly the content of alkaloids and antioxidants.

2 Materials and Methods

2.1 Plant Materials

Pancratium maritimum L. (Amaryllidaceae) mature seeds were gathered from closed pods in Matrouh Governorate, located on the Mediterranean coast of Egypt during August 2022. The Faculty of Science at Alexandria University was the site of the experiment. The collected seeds were separated into two groups - coated seeds, which had a layer of auriferous parenchyma, and uncoated seeds, which lacked this coating. The goal was to examine the influence of the seed coat under hydro and phytopriming methods. After being air-dried for 15 days at 25 °C, both groups of seeds were placed in paper bags for storage until use.

2.2 Preparation of Plant Aqueous Extract for Phytopriming

The selected species (*Pancratium maritimum* L.) have been collected in the Spring season (May 2022). After drying the materials in the shade, we ground them to a fine and uniform texture using a Wiley Mill, then stored them in air-sealed glass jars at a controlled temperature of 5 ± 0.5 °C until use. An aqueous extract from the dried plant materials was prepared to investigate the effects of seed priming with natural bio-simulating substances.

To make aqueous extract of *Pancratium maritimum* L. (PMAE), 100 g of dry powders from the selected species were used and extracted with 1000 ml of distilled water. After conducting the extraction in the dark for 48 h at 25 °C, we filtered the solution through the Whatman No. 41 filter paper. The subsequent dilutions of 10%, 20%, 40%, and 60% from the previously stock solution were prepared and the extracts were stored in a refrigerator at 5 °C until it was time for the preliminary test to select the most effective concentration and soaking period of the seeds (Fig. 1).

2.3 Seeds Preparation for Laboratory Experiments

The uniform coated and uncoated seeds of *P. maritimum* were sterilized for twice minutes using a diluted solution of sodium hypochlorite (3%). After that, they were washed three times with distilled water and left to air dry. This process was described by El-Kenany et al. (2017).

2.4 Germination Bioassay

A Petri dish experiment was conducted to study the effects of two seed priming methods (hydropriming and phytopriming) using *P. maritimum* aqueous extract (PMAE) on the germination parameters. The experiment aimed to compare the results between coated and uncoated seeds.

Before sowing, two treatments were carried out on sterilized seeds (coated and uncoated) hydropriming and phytopriming. In hydropriming, the seeds were soaked in water, while in phytopriming, they were soaked in different concentrations of the PMAE (10%, 20%, 40%, and 60%). The soaked seeds were left for different periods (4, 8, and 12 h), washed with distilled water, and then dried back to their original moisture (12 h).

Primed and unprimed seeds (taken as control) were placed in 9 cm Petri dishes on a layer of filter paper soaked with 20 ml distilled water. Each petri dish contained ten seeds arranged in a completely randomized block design. The experiment was carried out under normal laboratory conditions with daytime temperatures between 22 and 27 °C and nighttime temperatures between 14 and 18 °C. Three replicates were included in the experiment design. (Fig. 2).

2.5 Growth Experiment

The experiment involved soaking sterilized seeds in water for hydropriming and in an aqueous extract with different concentrations (10%, 20%, 40%, and 60%) for phytopriming. Each concentration had a different soaking period (4, 8, and 12 h). After soaking, the seeds were washed with

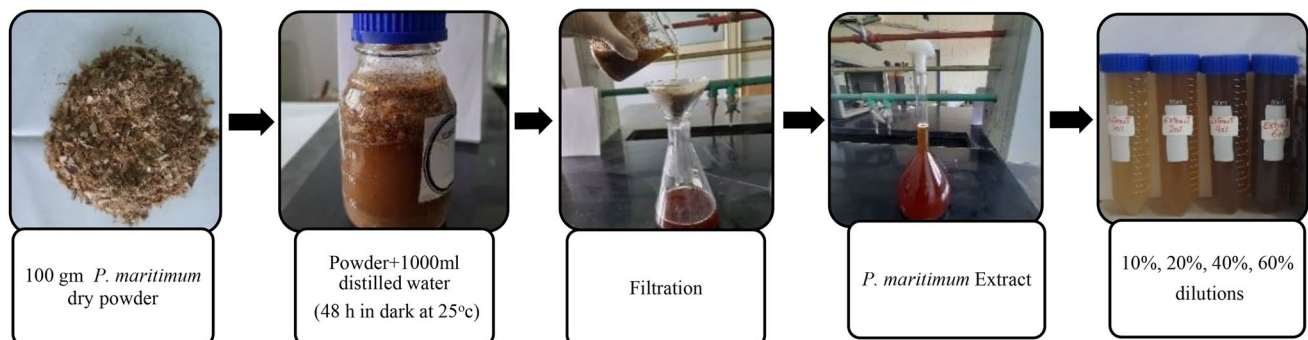


Fig. 1 Preparation of *Pancratium maritimum* L. aqueous extract for phytopriming

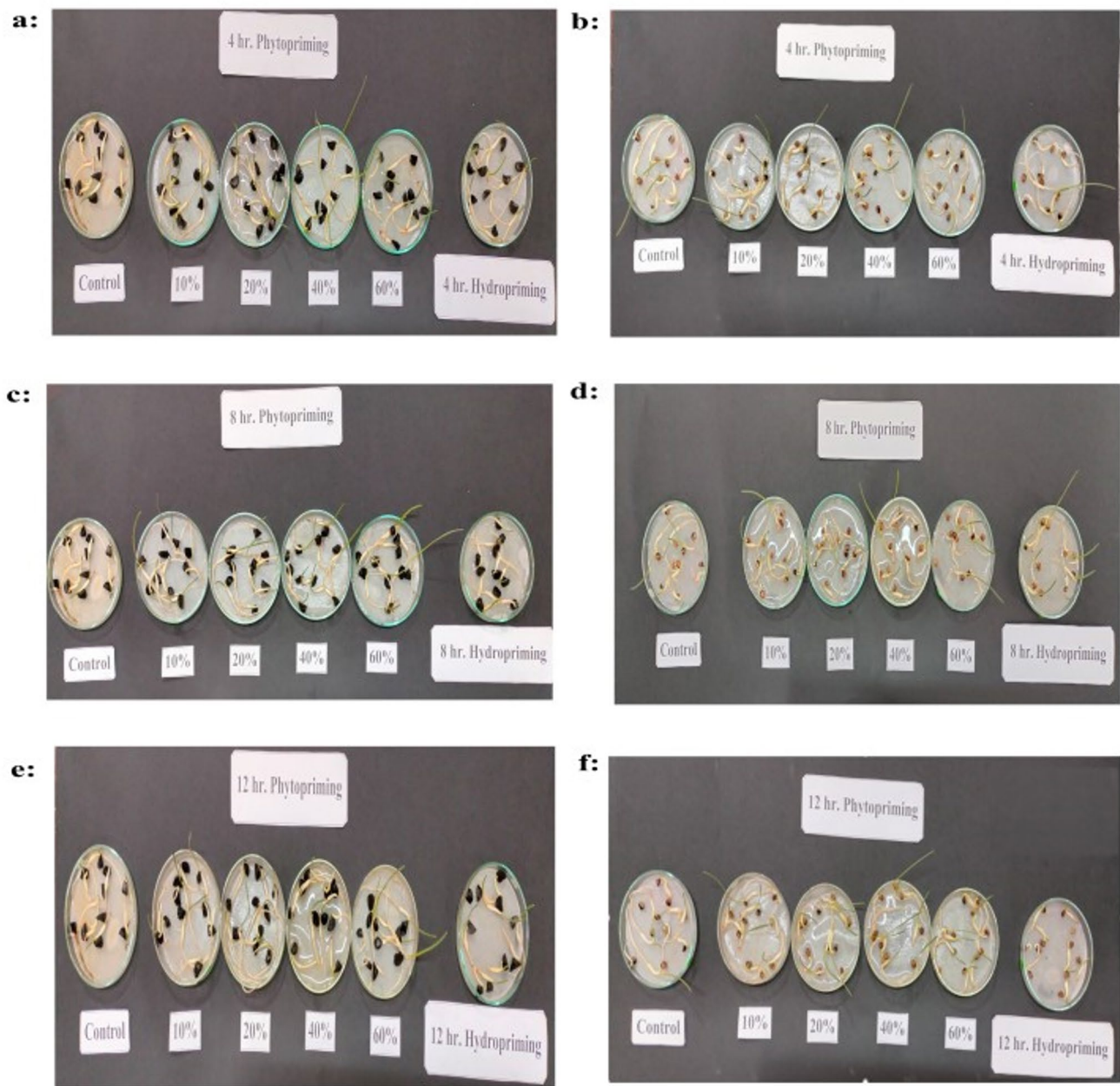


Fig. 2 Effect of different concentration of *Pancratium maritimum* L. aqueous extract (10, 20, 40, and 60%) on germination of coated (a, c & e) and uncoated (b, d and f) seeds of *P. maritimum* L. in petri dish experiment under different soaking durations (4, 8, and 12 h.)

distilled water and left to dry for 12 h between two filter papers. Primed and unprimed seeds were then sown in 15 cm diameter by 20 cm long plastic pots with holes at the bottom, filled with 1 kg of sandy soil. 10 seeds were planted separately in each pot at a depth of 0.5 cm from the soil surface. The pots were arranged in a completely randomized block design with 3 replicates. The amount of water given was determined by the average soil-plant evapotranspiration calculated from weight loss over 24 h intervals; The experiment was conducted under greenhouse conditions at the

Faculty of Science, Alexandria University, with a temperature of $20 \pm 2^\circ\text{C}$, relative humidity of $75 \pm 2\%$, and a 14/10 light/dark photoperiod (Fig. 3).

After 99 days, when the plants were well established, homogeneous seedlings were collected from each treatment. The seedlings were washed with tap water to remove soil particles, and then gently blotted with filter paper dampened with distilled water. The samples were separated into shoots and roots for the determination of growth parameters. Other

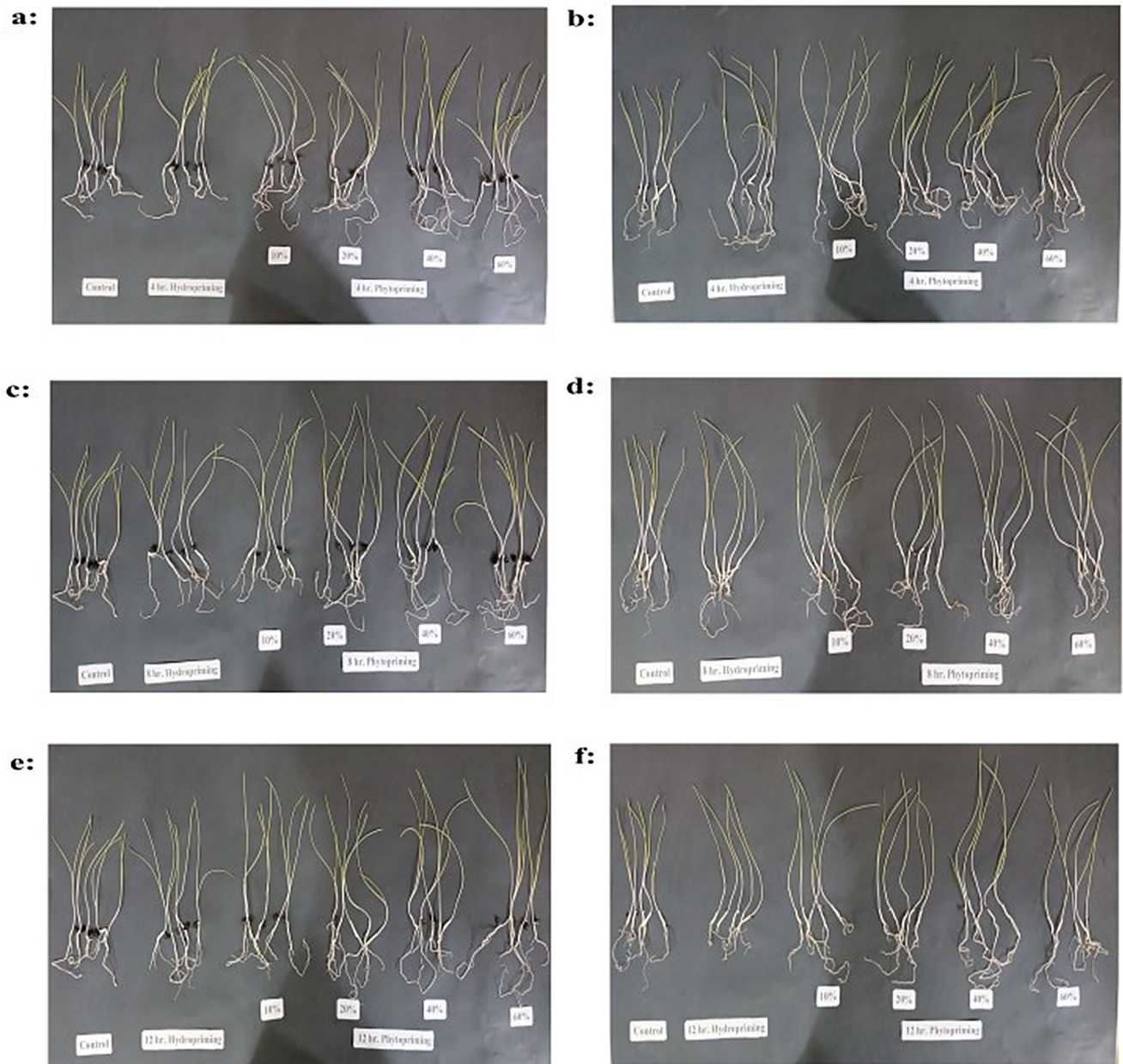


Fig. 3 Effect of different concentration of *Pancratium maritimum* L. aqueous extract (10, 20, 40, and 60%) on germination of coated (a, c & e) and uncoated (b, d and f) seeds of *P. maritimum* L. in pot experiment under different soaking durations (4, 8, and 12 h)

samples were dried at 60°C until a constant weight was achieved, to determine the dry weight.

2.5.1 Growth Parameters

The following parameters were recorded to determine the germination of seeds: **The germination percentage (GP%)** is determined by dividing the total number of seeds by the number of germinated seeds $\times 100$, **speed of germination (SG)** = N_i/D_i number/day, **germination velocity (GVe)** = $\sum$

GP/t , where t is the total germination time and GP is the germination percentage (Khan and Gul 1998) Since D_i represents the number of days after sowing, N_i is the number of seeds that germinated on day i . **The germination energy (GE)** was calculated by dividing the number of germinated seeds at day 4 by the total number of seeds tested $\times 100$. **The germination index (GI)** is equal to $\sum (E_t / D_t)$ where E_t is the number of seeds that emerged in t days; D_t is the number of corresponding germination days, **mean germination time (MGT)** is equal to $\sum f_x / (\sum (f_1 \times x_1) + \dots)$, To

calculate **the biomass** for each treatment, the samples were subsequently dried in an oven at 60 °C until their weight remained constant. For the shoot system, **the percentage of water content** was determined using both fresh and dry samples. **the lengths of the seedling shoot (SL) and seedling root (RL)** were determined for each treatment based on five plant individuals. **Seedling vigour index (SVI)** is equal to seedling total length multiplied by germination percentage %, as stated by Hussain et al. (2006). **The shoot to root ratio (SL/ RL ratio)** was calculated for each treatment. Ten plants were chosen from each treatment, split into shoots and roots, and then weighed independently to calculate **the plant biomass and water content** for each treatment, the samples were subsequently dried in an oven at 60 °C until their weight remained constant. For the shoot system, the percentage of water content was determined using both fresh and dry samples in the following formula: $WC\% = \frac{(\text{Fresh wt.} - \text{Dry wt.}) \times 100}{\text{Fresh wt.}}$

2.5.2 Chemical Analysis

2.5.2.1 Extraction *P. maritimum* was used to create a sample for biological activity assay and GC/MS analysis. To extract the plant material, 500 mg of air-dried and powdered material was mixed with 5 ml of methanol three times, each time for 30 min using an ultrasonic bath at room temperature. The residue was then dissolved in 10 ml of 2% sulfuric acid after the solvent was extracted under vacuum. Diethyl ether (3 × 10 ml) was used to eliminate nonpolar components. The alkaloids (3 × 10 ml) were extracted using chloroform after the acidic aqueous phases were neutralized to a pH of 9 to 10 using 25% ammonia. The extracts were dried over anhydrous sodium sulphate and the organic solvent was vacuum-distilled. (Bozkurt et al. 2017, 2019).

2.5.2.2 Gas chromatography-Mass Spectroscopy Analysis X-calibur 2.2 software (Thermo X-calibur) and a Trace 1300 GC Ultra/Mass Spectrophotometer ISQ QD (Thermo Scientific) equipment were utilized. The carrier gas was helium, flowing at a rate of 1 ml/min with an average velocity of 39 cm/s. 51.14 min was the run time. The GC/MS was worked in electron impact ionization (EI) with an electron energy of 70 electron volts. The temperature program of the oven elaborated heating from 70 °C for 1 min (5°C/min) to 100°C for 1 min, and then heating from 100°C to 220°C for 5 min (7°C/min). The final temperature of 240°C was held for 20 min (20°C/min). The injector temperature was set to 275 °C. A TG-5MS Zebron capillary column (length 30 m × 0.25 mm ID, 0.25 µm film thickness; Thermo) was used. The scanning range was 50–600 m/z at five scans per second. The extracts were dissolved in methanol and the split/

splitless injector was operated in splitless mode. The identification of the compounds was confirmed by comparing and matching their mass spectral fragmentation with standard reference spectra from the NIST (National Institute of Standard and Technology) database.

2.5.2.3 Preparation of Methanolic Plant Extract To assess the antioxidant activity, a fresh plant sample weighing 1 gram was immersed in 10 ml of 80% methanol and left to soak overnight. Subsequently, the extract was filtered through a Whatman No. 4 filter paper, and the resulting filtrate was topped up to 10 ml with 80% methanol. These extracts are now primed for the evaluation of antioxidant activity.

2.5.2.4 Antioxidant Activity Using the DPPH Radical Method The antioxidant properties of the plant extract were assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (Bandonienė et al. 2002). A volume of 50 µl of the plant extract at concentrations of 10–100 µg /ml was mixed with 2 ml of DPPH and left in the dark at room temperature for 30 min. A negative control was created by combining 1 ml of methanol with 2 ml of DPPH, while a positive control was made using only a methanol solution. The level of DPPH radical scavenging activity (%RSA) was determined by measuring the absorbance at 517 nm and then calculating the percentage of %RSA as follows: $\%RSA = \frac{100 \times (\text{Absorbance of the control} - \text{Absorbance of the sample})}{\text{Absorbance of the control}}$, where control is 2 ml of DPPH + 1 ml of methanol.

The percentage inhibition of DPPH was used to express the radical scavenging activities of the tested samples. The calculation of the inhibition was based on the formula proposed by (Molyneux 2004). $A = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$ where A_{sample} is the test sample's ultimate absorbance at 515 nm and A_{control} is the blank sample's absorbance at 515 nm at time t=0 min.

2.6 Statistical Analysis

The standard deviation, or ±SD, was used as a descriptive statistic to compare the variations in mean values. The computer was fed data, and IBM SPSS software package version 20.0 was used for analysis. (IBM Corp, Armonk, NY). The mean and standard deviation were used to characterize the quantitative data. At the 5% level, the results' significance was assessed. The Post Hoc test (Tukey) was utilized for pairwise comparisons and the F-test (ANOVA)

for normally distributed quantitative variables when comparing more than two groups.

3 Results

3.1 Experiment of Petri Dish Bioassay

The experiment in the Petri dish involved utilizing distilled water and PMAE with varying concentrations (10%, 20%, 40%, 60%) as priming agents, along with control (unprimed seeds). There were three distinct soaking times for each treatment (4, 8, and 12 h). The aim was to compare the outcomes of coated and uncoated seeds and assess the germination parameters of *P. maritimum* (Fig. 2). The findings indicated that there were insignificant differences in the germination percentage (GP%) between hydro and phytoprimering treatments in comparison to the coated and uncoated seeds. The GP% of coated seeds was higher than that of uncoated seeds, indicating a significant difference between the two types of seeds. (Fig. 4a.).

Different seed priming techniques were used to assess their impact on germination velocity (GVe) and speed of germination (SG) of coated and uncoated *P. maritimum* seeds. Hydropriming coated seeds for 12 h showed the highest increase in GVe (4.4% compared to the control), while soaking seeds in Phyto 10% for 8 h resulted in a 5.38% increase in GVe in comparison to the control. Hydropriming for 4 h showed the greatest increase in GVe for uncoated seeds (5% in comparison to the control). Phyto 60% showed the highest increase in GVe (11.79% compared to control) when soaked for 12 h. (Fig. 4b.).

Significant differences were noticed in GVe between coated and uncoated seeds with different priming techniques. However, the speed of germination (SG) did not vary significantly for coated and uncoated seeds despite being treated with different priming techniques and soaking durations. In some cases, SG decreased for coated and uncoated seeds with various priming techniques and soaking periods (Fig. 4c.). Coating and priming *P. maritimum* seeds in a 20% PMAE solution for 4 and 8 h before planting increased germination energy (GE) by 19.98% and 79.96%, respectively. Hydroprimed seeds soaked for 4 h showed a 59.99% increase in GE as compared to the control. However, different seed priming techniques caused a decrease in GE when uncoated seeds are soaked for 4, 8, and 12 h. (Fig. 4d.).

The study showed a significant improvement in the germination index (GI) of coated seeds after soaking for different durations. The highest increase was observed in hydro and phyto 10% treated seeds after soaking for 4 h. and in hydro and phyto 40% treated seeds after soaking for 8 h. For

treated uncoated seeds, the highest increase was observed in phyto 20% treated seeds after soaking for 12 h. (Fig. 4e.). The mean germination time (MGT) of hydroprimed and phytoprimered coated seeds was reduced by 9.59% as compared to the control when exposed to hydro for 4 and 8 h, phyto 20% for 8 and 12 h, and phyto 40% for 8 h. The highest MGT reduction percentage (9.59%) was observed in phyto 10% treatment at 12 h for uncoated seeds (Fig. 4f.).

Coated seeds soaked with phyto 40% treatment for 12 h showed a significant increase in the plumule/radicle ratio (Fig. 4g.). The highest increase (73.25% relative to the control) was recorded. Additionally, the plumule/radicle ratio (189.17%) in uncoated seeds significantly when primed with phyto 40% treatment for 8 h compared to the control.

Data showed a significant increase in the seedling vigour index (SVI) of coated seeds after soaking for different durations. The highest increase was observed in phyto 60% treated seeds after soaking for 8 h. For treated uncoated seeds, the highest increase was recorded in phyto 40% treated seeds after soaking for 12 h. (Fig. 4h.). The results also showed an overall increase in biomass of *P. maritimum* that were grown from phytoprimered seeds (Fig. 4i.).

3.2 Growth Experiment

Pots experiment involved utilizing distilled water for hydropriming and PMAE at different concentrations (10%, 20%, 40%, 60%) as priming agents, in conjunction with unprimed seeds as a control. Each treatment was exposed to various soaking periods (4, 8, and 12 h) (Fig. 3.). The aim was to evaluate the germination parameters of *P. maritimum* and compare the performance of coated and uncoated seeds.

Over all, various priming techniques improved GP% of coated *P. maritimum* seeds. Coated seeds had higher GP% compared to uncoated ones. No significant differences in GP% were observed between hydropriming, phytoprimering, and the control in coated seeds. Uncoated seeds soaked in priming agents for 8 and 12 h showed a decreasing trend in GP%. (Fig. 5a.).

Coated seeds revealed varying effects of different treatments on GVe. Soaking seeds in 20% PMAE for different durations, as well as in 40% PMAE for 12 h, increased data by 3.1% as compared to the control. Phyto treatments resulted in GVe increases of 5.08%, 4.925%, and 6.56% after a 4 h soaking period (Fig. 5b.). Coated primed seeds had a substantial increase in SG under different seed priming treatments for three different soaking durations. The results of uncoated primed seeds showed a significant reduction in SG values compared to the control, except for the increase in SG for phyto 10% treatment at a 4 h soaking period and hydropriming treatments at 8 and 12 h soaking periods (Fig. 5c.). It was observed that treating coated

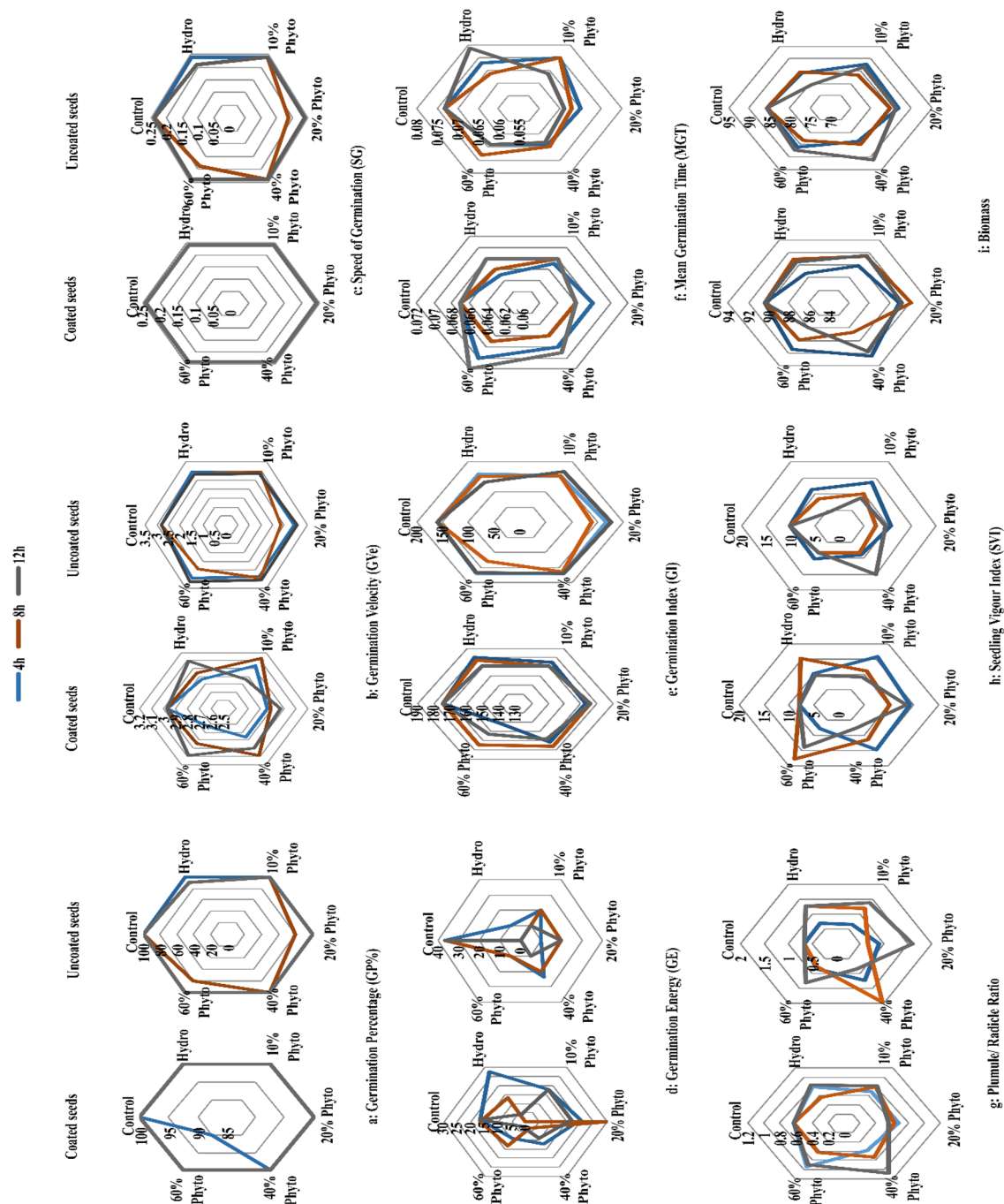


Fig. 4 Variations of **a**: Germination Percentage (GP%), **b**: Germination Velocity (Gv), **c**: Speed of Germination (SG), **d**: Germination energy (GE), **e**: Germination index (GI), **f**: Mean germination time (MGT), **g**: plumule: radicle ratio, **h**: seedling vigour index (SVI) and **i**: Biomass

seeds of *P. maritimum* with phytopriming concentration of 60%, as well as 40% concentration for soaking duration of 4 and 12 h respectively, resulted in the highest increase in GE (41.71% for both). On the other hand, treating uncoated seeds with different phytopriming treatments and soaking them for varying durations resulted in a significant increase

in coated and uncoated seeds of *Pancratium maritimum* L. in petri dish experiment in response to different concentrations of *P. maritimum* L. aqueous extract (10, 20, 40, and 60%) under different soaking durations (4, 8, and 12 h)

in GE ranging from 40.1 to 360.14% as compared to the control. A graphical representation of these results is shown in Figure (5d).

Generally, hydropriming and phytopriming treatments of *P. maritimum* seeds resulted in an increase in germination index (GI) and shoot/root ratio. The highest increases in GI

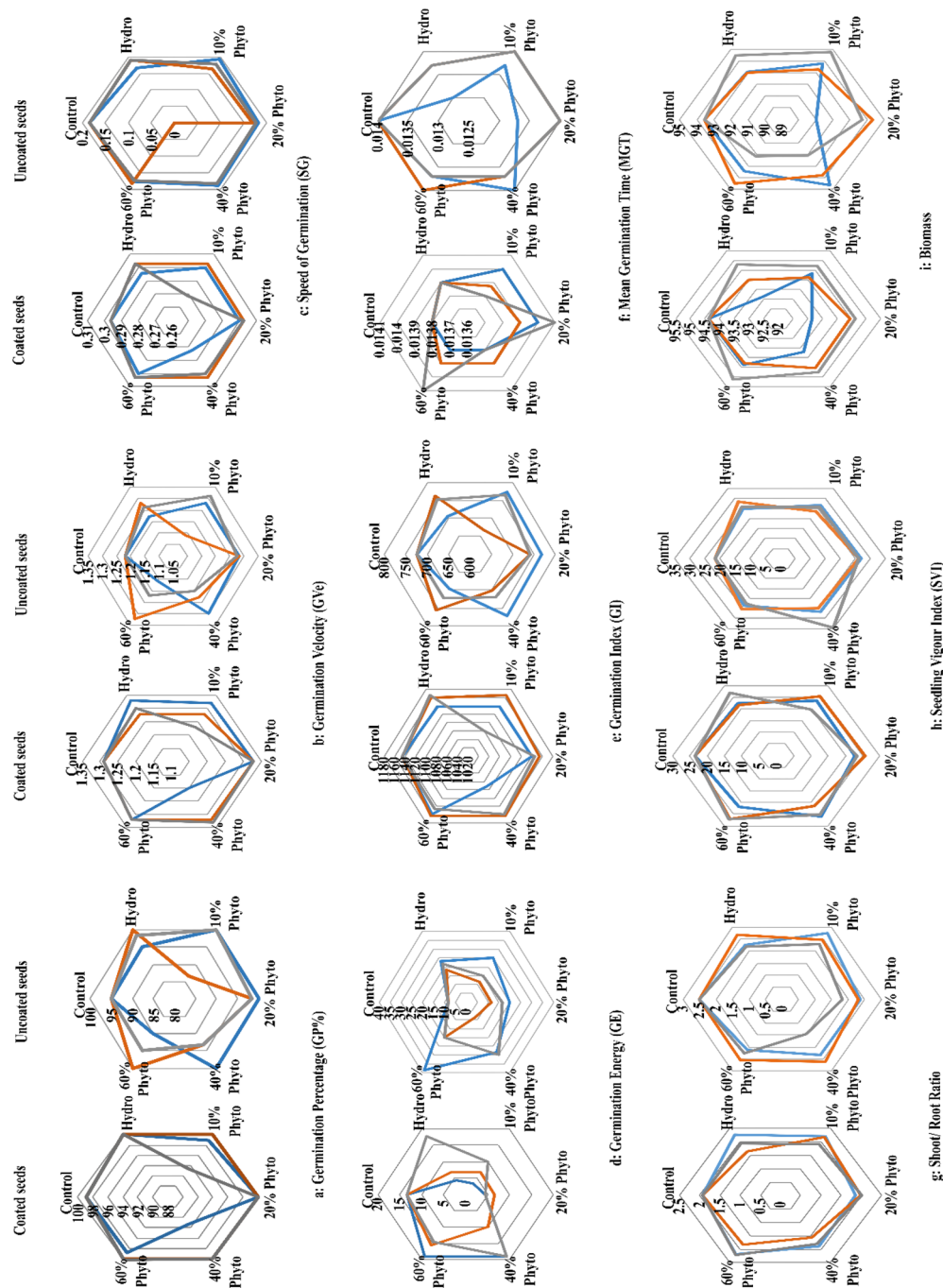


Fig. 5 Variations of **a:** Germination Percentage (GP%), **b:** Germination Velocity (Gv), **c:** Speed of Germination (SG), **d:** Germination energy (GE), **e:** Germination index (GI), **f:** Mean germination time (MGT), **g:** shoot root ratio, **h:** seedling vigour index (SVI) and **i:** Biomass in

coated and uncoated seeds of *Pancratium maritimum* L. in pot experiment in response to different concentrations of *P. maritimum* L. aqueous extract (10, 20, 40, and 60%) under different soaking durations (4, 8, and 12 h)

were observed in the 20% concentration of phytopriming treatment after an 8 h soak and the hydropriming treatment after a 12 h soak (Fig. 5e.). The mean germination time (MGT) increased for hydro and phytoprimed coated seeds, but not for phytopriming treatments with 10% and 40% concentrations, as well as 40% phyto treatment after 4 h of

soaking. The MGT decreased significantly in response to seed soaking for 4 h in hydropriming treatment (Fig. 5f.). The shoot/root ratio increased significantly for coated seeds in response to hydropriming treatment, while the ratio for uncoated seeds of the same plant species showed fluctuations (Fig. 5g.).

Seed priming techniques were also tested on *P. maritimum* seeds to observe their impact on SVI and biomass. Coated seeds soaked for 4, 8, and 12 h showed a significant rise in SVI, with the 60% phyto treatment resulting in the highest increase. Coated seeds soaked in water and a 60% PMAE solution for 8 h also had a boost in SVI. Uncoated seeds showed a trend of increased SVI after soaking for different durations (Fig. 5h.). Soaking coated seeds for 8 h. led to a significant increase in biomass, with the highest increase observed in a 60% phyto treatment. Uncoated seeds showed a significant increase in biomass in response to hydropriming and phytopriming agents. After soaking for 12 h, a significant increase in biomass was observed in response to hydro, Phyto 10%, and 20% treatments (Fig. 5i.).

3.3 Chemical Analysis

Figure 6. shows chromatography-mass spectrometry (GC-MS) analysis of methanolic extract in *Pancratium maritimum* L. and Table 1. Illustrate GC-MS identification for its bioactive constituents. 5-Hydroxymethylfurfural, Palmitic acid and Trans-13-Octadecenoic Acid were the major constituents, with 5-Hydroxymethylfurfural being the most abundant one (35.27%). The effects of different treatments on the alkaloids present in *P. maritimum* were analyzed using GC-MS to gather data on alkaloid metabolism. The results showed a significant increase in alkaloid content ranging from 261.03 to 715.02% in response to

all treatments at various soaking durations. The highest increase was observed when seeds were primed in 60% PME for 12 h. Similarly, there was a noticeable increase in the lycorine content of the *P. maritimum* due to priming treatments after different soaking durations. However, the pancratistatin content in the primed seeds of *P. maritimum* did not differ significantly from unprimed seeds, despite the use of various priming techniques and soaking durations (Fig. 7a, b and c).

Priming treatments were applied to hydroprimed and phytoprimed seeds, and their antioxidant levels were analyzed. Phytoprimed seeds produced the highest quantity of antioxidants compared to hydroprimed seeds and control groups. Soaking *P. maritimum* seeds in 60% PMAE for 4 and 12 h resulted in the highest level of antioxidants observed (Fig. 7d).

4 Discussion

In the present research, the effects of hydropriming and phytopriming as seed priming treatments on *P. maritimum* was explored. with the primary aim of enhancing seed quality, promoting plant growth, and increasing the bioactive compounds. By utilizing natural bio-simulating substances. The research sought to explore the potential of these priming techniques to improve physiological and biochemical attributes of *P. maritimum*, offering insights into their

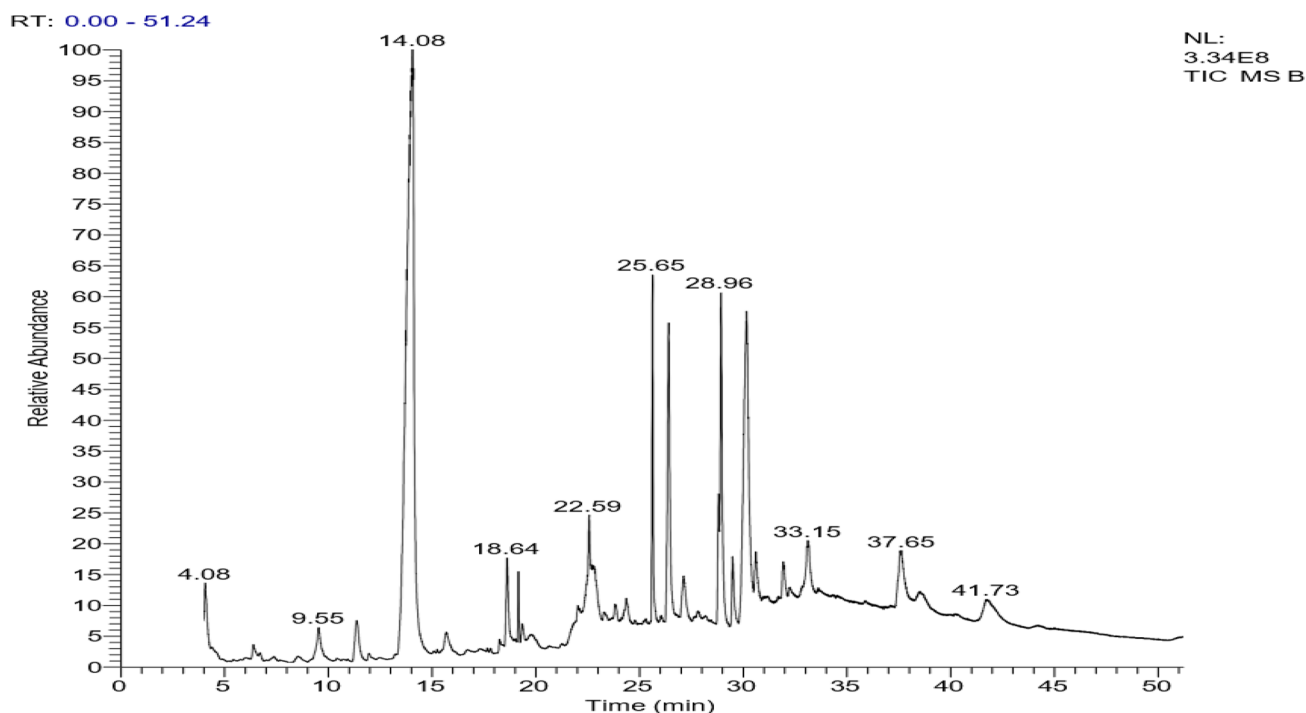


Fig. 6 GC-MS (Gas Chromatography-Mass Spectrometry) analysis of methanolic extract in *Pancratium maritimum* L

Table 1 GC-MS (Gas Chromatography-Mass Spectrometry) identification for the bioactive constituents in *Pancratium maritimum* L. methanolic extract

Compound Name	Molecular formula	Molecular weight	RT	Area%
Furfural	C ₅ H ₄ O ₂	96	4.09	1.00
2,5Furandicarboxaldehyde	C ₆ H ₄ O ₃	124	9.55	1.46
2-Propyl-tetrahydropyran-3-ol	C ₈ H ₁₆ O ₂	144	11.40	1.77
5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126	14.08	35.27
2-Amino-2-cyano-4-methylpentane thioamide	C ₇ H ₁₃ N ₃ S	171	15.71	1.21
N-1-Decenylsuccinicanhydride	C ₁₄ H ₂₂ O ₃	238	18.64	2.44
Methyl laurate	C ₁₃ H ₂₆ O ₂	214	19.19	0.66
Pancratistatin	C ₁₄ H ₁₅ NO ₈	325	23.68	2.24
Tetrahydroactinidiolide	C ₁₁ H ₁₈ O ₂	182	19.38	0.46
Oxiraneundecanoic acid, 3-pentyl, methyl ester, trans	C ₁₉ H ₃₆ O ₃	312	22.59	3.10
1,1-bis(4-methylcyclohexyl)dodecane	C ₂₆ H ₅₀	362	22.79	1.78
Palmitoleic acid	C ₁₆ H ₃₀ O ₂	254	23.86	0.46
2-Methyl-Z-4-tetradecene	C ₁₅ H ₃₀	210	24.40	0.92
Methyl palmitate	C ₁₇ H ₃₄ O ₂	270	25.65	4.28
Palmitic acid	C ₁₆ H ₃₂ O ₂	256	26.42	7.79
Lycorenanone, 9,10dimethoxy-1-methyl-	C ₁₈ H ₂₁ NO ₄	315	27.15	1.71
8,11-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	28.84	2.03
10-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	28.96	7.30
Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl] methyl]cyclopropyl]methyl]-, methyl ester	C ₂₅ H ₄₂ O ₂	374	29.52	1.60
Trans-13-Octadecenoic Acid	C ₁₈ H ₃₄ O ₂	282	30.19	15.03
Stearic acid	C ₁₈ H ₃₆ O ₂	284	30.63	1.09
Ethyl 9,12-hexadecadienoate	C ₁₈ H ₃₂ O ₂	280	31.97	1.20
Cyclohexane, 1,4-didecyl-[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C ₂₆ H ₅₂	364	33.15	2.63
	C ₂₁ H ₃₈ O ₂	322	37.59	1.33
17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	37.64	1.78
1 H-Indene, 5,5'-(1,10 decanediyl)bis[octahydro-	C ₂₈ H ₅₀	386	41.75	1.72

effectiveness as sustainable strategies for enhancing plant development and bioactive metabolite accumulation. Our data revealed that there was no significant variance in the germination percentage (GP%) between hydro and phytopriming treatments when compared to the control for both coated and uncoated seeds. However, a significant difference between coated and non-coated seeds was observed. Generally, the coated seeds exhibited a better germination parameters' values as compared to uncoated ones. Furthermore, phytopriming had a more significant impact on germination parameters than hydropriming. The removal

of the seed coat had an adverse effect on the germination rate (Mohamed et al. 2018), as the seed coat serves as the primary shield against environmental stress and pathogen invasion. It also plays a crucial role in determining seed viability and germination (Mohamed-Yasseen et al. 1994; Souza and Marcos-Filho 2001).

Our findings highlight the significance of seed priming in stimulating seed germination and the resulting seedling's growth and development. Previous studies have investigated plant extracts as plant bio-stimulants. The process of imbibition causes the plant extract to enter the seeds. The increase in seed germination observed in the experiment suggests that priming with medicinal plants affects the seeds' physiological functions (Nanjappanavar et al. 2017). This results in an improved interaction between the seed and plant extract, which leads to a higher germination index, shorter germination and flowering times, increased plant development, and more biomass (Ahmed 2023). The use of plant extracts has the potential to improve seedling vigor during early growth stages by strengthening the antioxidant defense system (Singhal et al. 2023). Therefore, the seed phytopriming method promotes rapid and synchronized germination by enhancing the early stages of germination and seedling establishment. This, in turn, leads to early flowering and maturity, breaks dormancy and stimulates plant growth.

Several researches have revealed that plant extracts are highly effective in boosting plant growth and development, while also promoting the production of secondary metabolites. Bio-stimulants derived from plants have been found to provide a multitude of advantages for plant health and growth. These include improved quality, heightened photosynthesis rates, and enhanced resistance to both biotic and abiotic stressors. Moreover, these bio-stimulants facilitate better resource utilization by optimizing the biochemical, molecular, and physiological processes that govern nutrient, fertilizer, and water uptake (Bulgari et al. 2015; Ertani et al. 2015; Kumar and Alope 2020; Roupael and Colla 2018, 2020; Yakhin et al. 2017; Zulfiqar et al. 2020).

Many studies have showed that *P. maritimum* bulbs contain alkaloids and flavonoids that have potential medicinal uses (Berkov et al. 2004; Bastida Armengol et al. 2011; Taie et al. 2015; Ibrahim et al. 2013). These alkaloids include pancratistatin, which has been found to exhibit anti-cancer effects (Youssef et al. 2022). *P. maritimum* extract possesses various properties, such as analgesic (Çitoğlu et al. 2012), anti-malarial (Cedrón et al. 2010, 2012, 2013), anti-cancer, anti-fungal (Evidente et al. 2004), purgative, acaricidal, insecticidal, anti-migratory, anti-viral, anti-microbial, immunostimulant, and antioxidant. Recent research has revealed that the alkaloids urngimionine, pancratistatin,

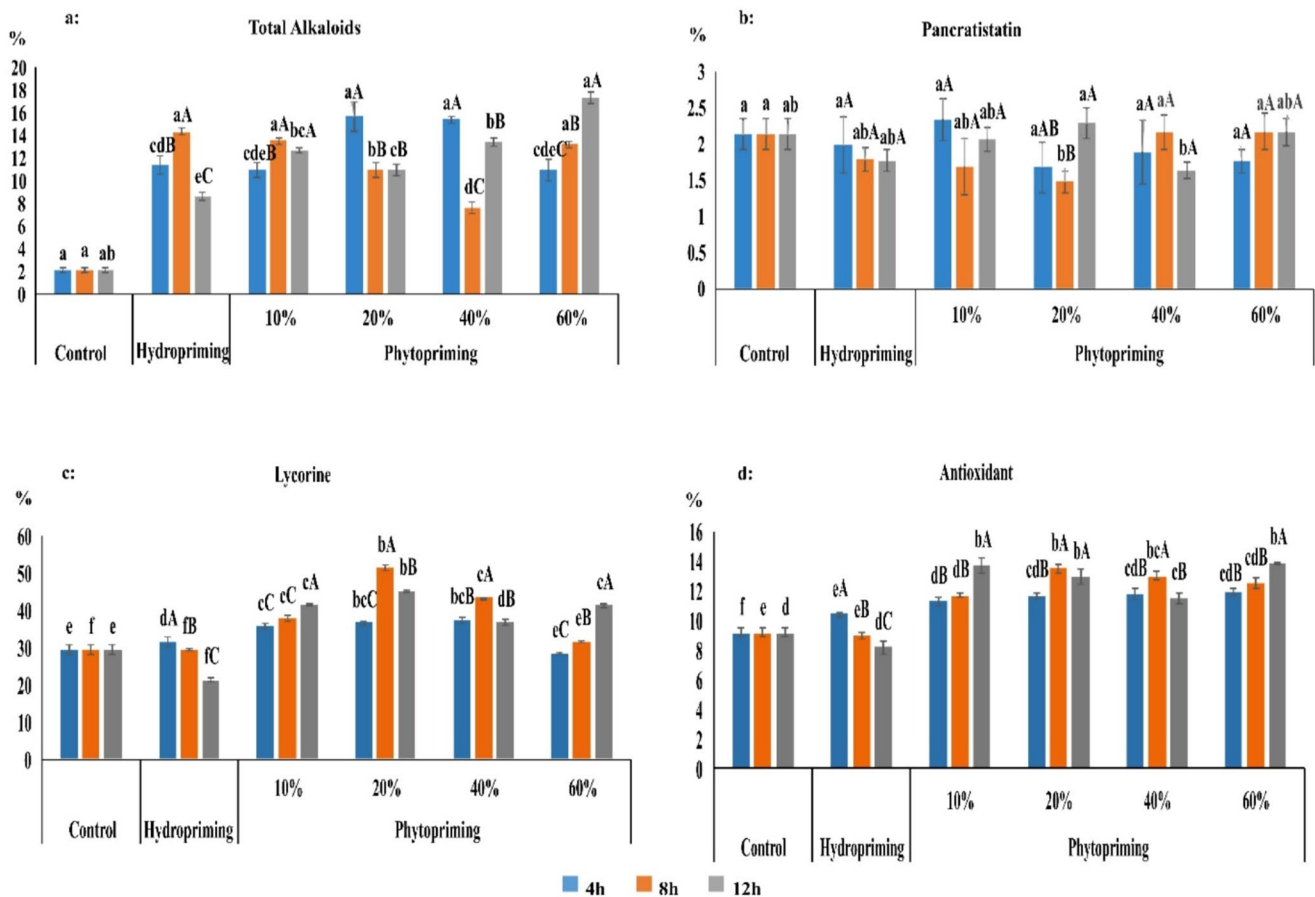


Fig. 7 Mean values of **a:** alkaloids (%), **b:** Pancratistatin (%), **c:** Lycorine (%) and **d:** Antioxidant content (%) (mean $\pm$ S.D., $n=3$) of *Pancreaticum maritimum* L. in response to different concentration of *P. maritimum* L. aqueous extract (10, 20, 40, and 60%) under different soaking durations (4, 8, and 12 h). SD: Standard deviation F: F for

One-way ANOVA test, pairwise comparison bet. each 2 groups were done using a **Post Hoc Test (Tukey)** Means in the same **Column** with **any Small Common letter** are not significant. Means in the same **Row** with **any Capital Common letter**^(A–C) are not significant (**OR** Means with **totally Different letters**^(A–C) are significant)

and lycorine have potent anti-cancer properties (Bastida Armengol et al. 2011; Berkov et al. 2004).

Data of the present study showed that the total alkaloids increased in all hydropriming and phytopriming treatments soaked for 4, 8, and 12 h. as compared to the control. A PMAE application stimulated the production of alkaloids, which have significant bioactive properties that render them very important in the pharmaceutical industries. The data recorded a significant increase in Lycorine content in all treatments compared to control except when the hydroprimed seeds soaked for 12 h. Additionally, there was no significant difference in pancratistatin content across most treatments, except for the 10% PMAE for 4 h, 40% and 60% PMAE for 8 h, and 40% PME for 12 h. For these specific treatments, pancratistatin content increased significantly ($p < 0.05$), with variations ranging from 1.88 to 9.86% compared to the control, potentially due to priming triggering a significant alteration in seed metabolism, leading to an increase in the production of secondary metabolites (Ali

et al. 2019). Allam et al. (2024) indicated that nanopriming of *P. maritimum* seeds increased some compounds such as Pancratistatin and Lycorine.

The activities of antioxidants showed an overall increasing trend across different priming treatments when compared to the control. However, the phytopriming treatments increased the *P. maritimum* antioxidants content in a statistically significant amounts when compared with hydropriming and control. The 60% PMAE for 12 h showed the highest increase by 70.39% and 25.08% as compared to hydropriming treatment and the control, respectively. Our results are consistent with (Noman et al. 2018) who reported that the bio-products based on sugar beet can improve antioxidants' activities in wheat. This may indicate that the relatively high concentration of PMAE enhanced the defense system of seeds by increasing total phenols and antioxidant enzyme activities. It could notably decrease plant oxidative stress and improve crop yield (Balusamy et al. 2022; Ji et al. 2022), and exposure to the selected plant extract increases

both antioxidant and defense enzyme activity (Balusamy et al. 2022). Alkaloids and phenolic compounds present in the leaves of various plants are involved in the production of antioxidant activities and protect the plants against pathogens (Satish et al. 2007).

5 Conclusion

Our results found that using *P. maritimum* aqueous extract as phytopriming improved seed performance, germination, and plant growth. Data demonstrated that phytopriming had a greater impact on the production of bioactive compounds than hydropriming. Seeds treated with *P. maritimum* aqueous extract at a concentration of 60% had significantly higher levels of total alkaloids and antioxidant content. Seeds treated with *P. maritimum* aqueous extract at a concentration of 20% had higher levels of Pancratistatin and Lycorine content. These findings demonstrate the potential of *P. maritimum* aqueous extract as a strategy for improving seed quality and promoting the accumulation of beneficial bioactive chemicals in *P. maritimum*. The underlying mechanisms of *P. maritimum* aqueous extract actions and possible uses in agricultural practices targeted at improving the yield of bioactive compounds in medicinal plants and to better understand the dose-response relationship and find possible harmful or inhibitory effects, we recommended further examinations should be investigated in future research. As well as, Phytopriming-treatment by using the extract of different medicinal plant species itself can be a useful instrument for improving crop quality and nutritional content in the next farming initiatives.

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Author Contributions Salama M. El-Darier and Roufaida M. Elghobashy conceived of the presented idea. Eman S. Allam and Roufaida M. Elghobashy developed the theory and performed the computations. Eman S. Allam verified the analytical methods. Zekry F. Ghattass and Amal M. Fakhry discussed the results and contributed to the final manuscript.

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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