

Original Article

Evaluation of bio-priming technique on germination and some enzymes activity of pot-marigold (*Calendula officinalis* L.) seedlings under salinity stress

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ABSTRACT

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Bio-priming is an innovative seed priming technology that improve seed germination and growth by providing resistance to environmental stresses. For this purpose, this study was conducted as a factorial base on a completely randomized design with three replications, on pot-marigold (*Calendula officinalis* L.). Treatments included five levels of bio-priming [control (no prime), Arbuscular mycorrhizal fungi extract, azotobacter (*Azotobacter chroococcum*), azospirillum (*Azospirillum lipoferum*)] and hydropriming as the first factor and four levels of salinity stress (0 (without salinity), 50, 100 and 150 mM NaCl) as the second factor. The results indicated that salinity stress decreased germination characteristic of pot-marigold. 150 mM of salinity stress treatment reduced seed germination (28.52%) and alpha amylase activity (34.98%) compared to control. The application of biopriming was able to reduce the negative effects of salt stress. Bio-priming effects had a higher percentage of seed germination under salt stress conditions compared to the control (without biopriming). In addition, enzymes activity increased with increasing salinity stress. 150 mM of salinity stress improved superoxide dismutase activity (82.55%) and polyphenol oxidase (76.49%) compared to control. The impact of bio-priming in increasing seedling growth characteristics and control enzymes activity was more than hydropriming. Arbuscular mycorrhizal fungi extract in control of salinity stress (without stress), promoted germination percentage (77.68 %), seed vigor index (375.85%), and alpha-amylase activity (104.7%), compared to the control of bio-priming in 150 mM of salinity stress (lowest treatment). Bio-priming application especially arbuscular mycorrhizal fungi extract, can promote the germination and growth characteristics under salinity conditions by improving germination percentage and control enzymes activity.

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1. Introduction

Seed priming is a pre-germinative enhancement technique that will induce the early emergence of seedlings through the regulation of metabolic processes in the early phases of germination under environmental stresses. Seed priming ensures increased and uniform germination by reducing the imbibition time, increasing the pre-germination enzyme activation and increasing metabolite production (Hussain et al., 2016; Marthandan et al., 2020). Hydro-priming is a promising technique that allows for direct field sowing while also improving seed physiological efficiency and addressing the issue of weak stand establishment (Tania et al., 2020). Bio-priming is a technique that improves seedling establishment in the environmental stresses. It is also environmentally friendly and effective in improving plant yield under extreme eco-physiological conditions

(Kumari et al., 2021). It is also a useful tool for alleviating ecological stress in plants. Seed priming with growth promoting bacteria increase seed germination and set enzymes activity in germination stage (Kerecki et al., 2022). Bio-priming is a seed priming method, which enables bacterial adherence and adaptation to the seeds, improves colonization of rhizosphere and plant tolerance to various biotic and abiotic stresses, such as seed and soil borne pathogens and adverse environmental conditions (Rajendra Prasad et al., 2016). Bio-priming allows microorganisms to adhere to and acclimate to seeds. It ensures germination and enhances seed vigor index and early seedling characteristics (Nawaz et al., 2021). Other study confirmed beneficial effects of seed priming with an *Azotobacter chroococcum* consortium is efficacious to elevate the plant growth in a variety of ways, both directly and

indirectly (Gouda et al., 2018), and that arbuscular mycorrhizal fungi improved germination 25% on average in two contrasting Carob (*Ceratonia siliqua* L.) ecotypes (Boutasknit et al., 2020).

Stress is any environmental factor that can adversely affect plant growth and development and decrease the final yield. All the major abiotic stresses lead to the major declines in the yield of globally important crop plants. Salinity stress produces various detrimental effects on physiological, biochemical, and molecular plants features and also decrease productivity of plants. As a result, plants are not only frequently rather constantly being subjected to various kinds of stresses including both biotic (insect and disease assaults) and abiotic (drought, temperature, salinity, heavy metal) stresses. The occurrence of the abiotic stresses results in a considerable reduction in the yield of plants (Verma et al., 2020; Khan et al., 2021). Researchers reported that salinity stress decreased basil (*Ocimum basilicum*) seeds germination by reducing alpha-amylase activity (Bahcesular et al., 2020). Researchers confirmed that hydro priming (for 20 hours) and bio-priming (*Azotobacter chroococcum*) increased milk thistle (*Silybum marianum*) growth (20%) under salinity conditions and 120 mM NaCl, reduced germination characteristic (Yaghoubian et al., 2022).

Pot marigold (*Calendula officinalis* L.) is one of the medicinal plants belonging to the Asteraceae family. It has a wide range of uses, such as food and pharmaceutical industry, both as an essential oil and due to the fatty oil in the seed. Therefore, both the flower and seed yield are important (Savic Gajic et al., 2022; Pedram Rad et al., 2019). Researchers confirmed that salinity (NaCl) stress (0, 25, 50, 75, and 100 mM of NaCl), by adding NaCl into irrigation water decreased pot-marigold growth and yield with increasing salinity stress (Azizi et al., 2021). Using environmentally friendly priming such as bio-priming is one of the best technique to improve seed germination and control enzymes activity instead of chemical ways. Salinity stress is an important challenge around the world. Seed bio-priming is a novel, beneficial and eco-friendly technique that employ bio-stimulating agents like growth promoting bacteria to improve the physiological functioning of seeds and stress resilience. Therefore, considering the importance of improving the germination and growth of seedlings in salinity stress and the pot-marigold medicinal plant benefits, this experiment aims to investigate the effect of bio-priming to reducing the negative effects of salinity stress in germination stage and seedling growth of pot-marigold.

2. Materials and Methods

2.1 Experimental design and treatments

The factorial experiment was conducted base on a completely randomized design with three replications, on pot-marigold (*Calendula officinalis* L.) seeds in the seed technology laboratory of the Islamic Azad University, Karaj branch, in April 2023. The cultivar name of pot-marigold was Faron and purchased from Royal Bazr Company (Karaj, Iran). Its origin was from Netherlands. Treatments included five levels of bio-priming [control (no prime), Arbuscular mycorrhizal fungi extract, azotobacter (*Azotobacter chroococcum*), azospirillum (*Azospirillum lipoferum*)] and hydropriming as the first factor and four levels of salinity stress (0 (without salinity), 50, 100 and 150 mM NaCl) as second factor.

2.2 Seed priming

Before performing the experiment, the papers were sterilized in an autoclave at 100°C for 20 minutes, and the seeds were sterilized with a 3% sodium hypochlorite solution for two minutes and then washed with distilled water. In each experimental unit, 200 pot-marigold seeds were planted between papers (sandwich method). To prepare mycorrhizal fungi extract, first, 100 g of mycorrhizal fungi was mixed with 400 ml of water and placed on a shaker for 24 hours, then it was passed through a cleaning cloth. The seeds were pre-treated (seed priming) for 10 hours in bio-priming treatments at 20°C temperature and then planted in experimental units. 20 ml of salinity stress solution prepared at different levels and added to each experimental unit then kept in the dark place of germinator for 14 days at a temperature of 25°C and 45% humidity (ISTA, 1985). Traits such as seedling length, germination percentage, germination rate, seedling vigor, allometric coefficient, alpha-amylase activity, superoxide dismutase activity, polyphenol oxidase, and catalase enzymes activity were measured.

2.3 Seedling growth characteristics

The length of seedlings was measured with a ruler. Germination percentage and germination rate were calculated by Equation 1 and 2. (Scott et al., 1984; Ellis & Roberts, 1981).

$$\text{Germination percentage} = \frac{S}{T} \times 100 \quad (1)$$

Where S and T are the number of germinated seeds and total cultivated seeds, respectively.

$$\text{Germination rate} = \frac{\text{number of germinated seeds}}{\text{first day of counting}} + \dots + \frac{\text{number of germinated seeds}}{\text{last day of counting}} \quad (2)$$

Seedling vigor was also calculated according to Equation 3 and allometric coefficient according to Equation 4 (ISTA, 1985).

$$\text{Seedling vigor} = \text{normal germination percentage} \times \text{seedling length} \quad (3)$$

$$\text{Allometric coefficient} = \frac{\text{root length}}{\text{stem length}} \quad (4)$$

2.4. Enzymes activity

For measuring alpha-amylase enzyme activity, 1 g of germinated seed tissue was used. To prepare seed tissue extract, first, 5 ml of a 60 mM pH=6.8 phosphate buffer solution was added to the powdered seeds, and this solution was centrifuged for 15 minutes at 12,000 rpm. 0.5 ml of 2% starch solution was transferred into the test tube and then 0.5 ml of the extract prepared from above was added to it. After 30 minutes of incubation at 37°C, the reaction was stopped by 1 ml of 0.1 normal hydrochloric acid and then 1 ml of iodine reagent (containing 5 mM iodine (I₂) and 5 mM of potassium iodide (KI)) was added to it, after that the volume of the contents of the tube was increased to 10 ml with distilled water and finally the light absorption of the solution concentration was measured by a spectrophotometer (PG Instruments Ltd VIS/UV+T model). It was recorded with a wavelength of 620 nm and compared with the control sample (Xiao et al., 2006).

The activity of superoxide dismutase enzyme from measuring the reduction rate of Nitro Blue Tetrazolium (NBT) was measured at a wavelength of 560 nm. Enzyme activity was expressed in terms of enzyme units in the amount of protein (in milligrams) in the extract. One enzyme unit was considered as the amount of enzyme that prevents the reduction of 50% of NBT under the evaluation conditions (Beers and Sizaer, 1952).

To measure the activity of polyphenol oxidase enzyme, first, 0.5 g of the sample was ground in an ice bath inside a porcelain mortar, then it was homogenized with 1 ml of 0.05 M Tris-HCl buffer with pH 5.7. The resulting solution was centrifuged for 20 minutes at a temperature of 4 degrees Celsius at a speed of 13000 rpm and was used to measure the amount of enzyme in the supernatant solution (Sudhakar et al., 2001).

For measuring Catalase enzyme activity, 1500 µl sodium phosphate buffer containing 2% PVP and 1.3 mM EDTA were added to 350 mg of seedling tissue extract, and after that, the samples were vortexed for 15 minutes at 15,000. They were centrifuged at 1000 rpm and light was used to measure the enzyme extract. The reaction mixture contained 30 mM hydrogen peroxide in 50 mM phosphate buffer (pH=7) and 100 microliters of enzyme extract in a final volume of 1000 microliters. The amount of enzyme activity was calculated in terms of each micromole of H₂O₂ decomposed per minute in mg of protein. Absorbance changes at 240 nm were recorded for 3 minutes with a spectrophotometer (PG Instruments Ltd VIS/UV+T model (Aebi, 1984).

2.5. Statistical analysis

Data analysis was completed using SAS software (Ver.9.4). The mean values were compared using Duncan's multiple range test at a 5% ($p \leq 5$).

3. Results

3.1. Seedling growth characteristics

ANOVA results indicated that the main effects of bio-priming, and salinity stress, as well as the interactions between bio-priming and salinity stress were significant ($p < 0.01$) (Table 1) for germination percentage, seedling length and seed vigor index. Also, according to ANOVA results the main effects of bio-priming, and salinity stress were significant ($p < 0.01$) (Table 1) for germination rate and allometric coefficient. Salinity stress decreased seedling growth characteristics. Bio-priming application (Arbuscular mycorrhizal fungi extract, azotobacter, azospirillum) promoted the seedling growth characteristics compared to the control and reduced the negative effect of salinity stress. Hydropriming increased seedling growth characteristics, but the effect of bioprimer in increasing seedling growth characteristics was greater than hydropriming. Arbuscular mycorrhizal fungi extract, azotobacter and azospirillum enhanced plant tolerance to salinity stress and there were no differences between 50 mM and 100 mM of salinity stress. In control treatments (without bio-priming) there was no differences between control and 50 mM salinity stress. 100 mM of salinity stress (9.28%) and 150 mM of salinity stress (28.52%) reduced seed germination percentage compared to control. The interaction effect of bio-priming and salinity stress on seed germination percentage indicated that arbuscular mycorrhizal fungi extract in control of salinity stress (without stress), improved germination percentage (77.68 %) compared to the control of bio-priming in 150 mM of salinity stress (lowest treatment) (Fig.1.). In seedling length, arbuscular mycorrhizal fungi extract azotobacter, azospirillum and hydropriming reduced negative effect of salinity stress between 50 mM and 100 mM of salinity stress. In control treatments, with increasing salinity content, seedling length decreased and 150 mM of salinity stress (45.06%) decreased seedling length compared to control. In addition, arbuscular mycorrhizal fungi extract in control of salinity stress (167.78%), and azotobacter in control of salinity stress (163.20%), promoted seedling length compared to the control of bio-priming in 150 mM of salinity stress (lowest treatment) (Fig.2.). Also, in seed vigor index, arbuscular mycorrhizal fungi extract and azotobacter reduced negative effect of salinity stress between 50 mM and 100 mM of salinity stress. In control treatments, with increasing salinity content, seed vigor index decreased and 150 mM of salinity stress (60.74%) decreased seed

Table 1. Variance analysis of bio-priming and salinity stress effects on germination traits and enzymes activity of pot-marigold (*Calendula officinalis* L.)

S.O.V	Df	Seedling length	Germination percentage	Seed vigor index	Germination rate	Allometric coefficient	Alpha-amylase activity	Superoxide dismutase activity	Polyphenol oxidase	Catalase activity
Bio-priming (B)	4	83.52**	384**	2459**	456**	21.62**	752**	86.72**	348**	7.61**
Salinity stress (S)	3	69.28**	672**	3745**	673**	18.35**	473**	93.59**	582**	9.43**
B×S	12	92.26**	724**	8659**	7.43 ^{ns}	1.42 ^{ns}	624**	5.28 ^{ns}	9.71 ^{ns}	0.37 ^{ns}
Error	40	5.43	10.38	97.38	12.82	3.44	10.67	8.76	12.62	1.23
C.V (%)		8.95	11.24	12.61	10.37	5.42	9.73	10.45	8.73	4.89

** : Significant at the %1 probability levels, ns: no significance.

Table 2. Means comparison of bio-priming and salinity stress effect on germination rate, allometric coefficient, superoxide dismutase activity, polyphenol oxidase and catalase activity.

	Germination rate (%)	Allometric coefficient	Superoxide dismutase activity (U. mg protein)	Polyphenol oxidase (ΔOD/μg protein min)	Catalase activity (μmol Fw minute ⁻¹)
Bio-priming					
Arbuscular mycorrhizal fungi extract	31.28 ^a	1.01 ^d	3.79 ^d	11.43 ^a	0.005 ^d
Azotobacter	28.12 ^b	1.06 ^e	4.27 ^e	9.86 ^b	0.007 ^c
Azospirillum	27.86 ^b	1.06 ^e	4.35 ^e	9.93 ^b	0.007 ^c
Hydropriming	21.57 ^c	1.09 ^b	5.17 ^b	8.04 ^c	0.010 ^b
Control	18.43 ^d	1.13 ^a	6.01 ^a	6.79 ^d	0.013 ^a
Salinity stress (mM)					
Control	19.25 ^a	1.19 ^d	4.07 ^d	7.02 ^d	0.012 ^d
50	16.73 ^b	1.33 ^c	5.19 ^b	8.73 ^c	0.019 ^c
100	13.15 ^c	1.52 ^b	5.35 ^b	10.27 ^b	0.022 ^b
150	10.98 ^d	1.73 ^a	7.43 ^a	12.39 ^a	0.026 ^a

Means in a column and a treatment followed by the same letter are not significantly different at 1% level.

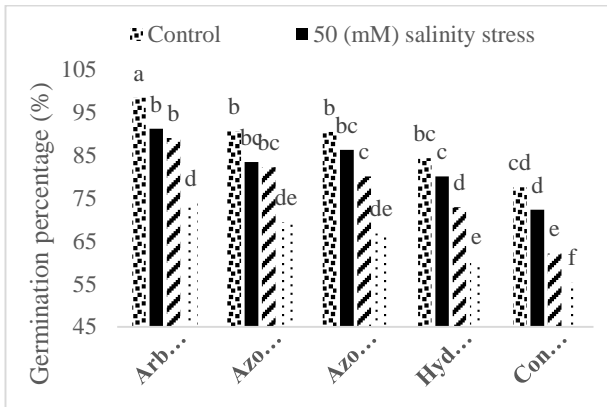


Fig. 1. Interaction effect of bio-priming and salinity stress on germination percentage of pot-marigold (*Calendula officinalis* L.). Dissimilar letters indicate significant differences at the 5% level according to Duncan's test.

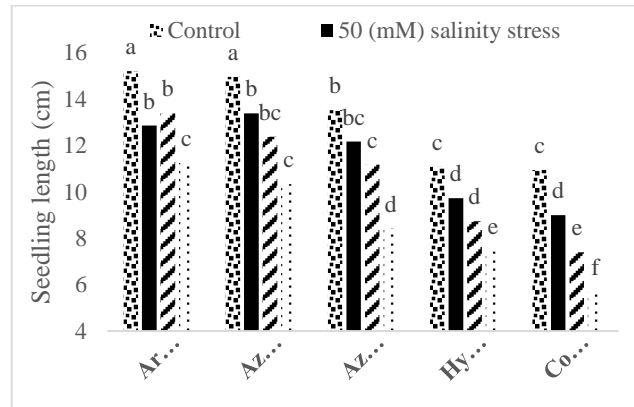


Fig. 2. Interaction effect of bio-priming and salinity stress on seedling length of pot-marigold (*Calendula officinalis* L.). Dissimilar letters indicate significant differences at the 5% level according to Duncan's test.

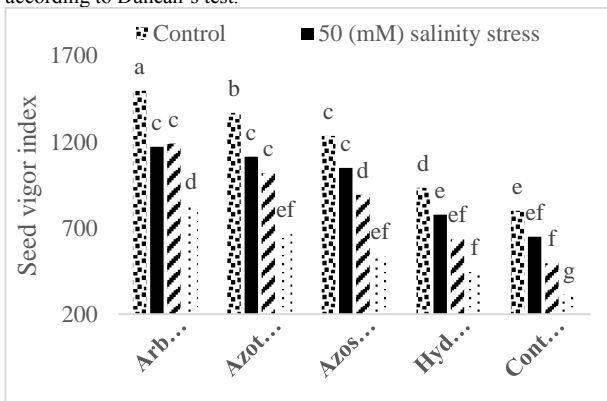


Fig. 3. Interaction effect of bio-priming and salinity stress on seed vigor index of pot-marigold (*Calendula officinalis* L.). Dissimilar letters indicate significant differences at the 5% level according to Duncan's test.

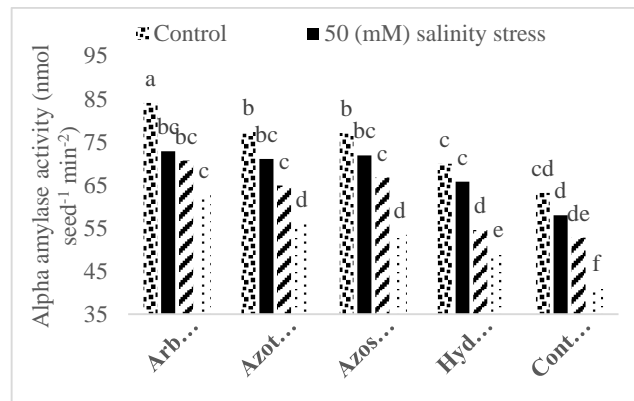


Fig. 4. Interaction effect of bio-priming and salinity stress on alpha amylase activity of pot-marigold (*Calendula officinalis* L.). Dissimilar letters indicate significant differences at the 5% level according to Duncan's test.

vigor index compared to control. Mycorrhizal fungi extract in control of salinity stress (375.82%), increased seed vigor index compared to the control of bio-priming in 150 mM of salinity stress (lowest treatment) (Fig.3.). The results indicated that in bio-priming treatments, arbuscular mycorrhizal fungi extract has the highest germination rate and improved it (69.72%) compared to control (without biopriming). In salinity stress treatments, control (without salinity condition), promoted germination rate (75.31%) compared to 150 mM of salinity stress (Table 2). On the other hand, mycorrhizal fungi extract decreased allometric coefficient (11.88%), compared to control and in salinity stress, 150 mM of salinity stress increased allometric coefficient (45.37%) compared to without salinity condition (Table 2).

3.2. Enzymes activity

The results of the ANOVA showed that the main effects of bio-priming, and salinity stress, as well as the interactions between bio-priming and salinity stress were significant ($p < 0.01$) for alpha-amylase activity (Table 1). As well as, ANOVA results presented that the main effects of bio-priming, and salinity stress were significant ($p < 0.01$) for superoxide dismutase activity, polyphenol oxidase and catalase activity (Table 1). The results showed that salinity stress increased superoxide dismutase, polyphenol oxidase and catalase activity, but decreased alpha amylase activity. Hydropriming decreased superoxide dismutase, polyphenol oxidase and catalase activity, (compared to control), but the effect of biopriming was better than hydropriming. Bio-priming application control the balance of enzymes activity. The interaction effect of bio-priming and salinity stress on alpha amylase activity confirmed that arbuscular mycorrhizal fungi extract, azotobacter and azospirillum promoted plant tolerance to salinity stress and there were no differences between 50 mM and 100 mM of salinity stress. In hydropriming treatments, there was no differences between control and 50 mM of salinity stress. In control treatments, 150 mM of salinity stress (34.98%) decreased alpha amylase activity compared to control. Arbuscular mycorrhizal fungi extract in control of salinity stress (without stress), improved alpha-amylase activity (104.7%), compared to the control of bio-priming in 150 mM of salinity stress (lowest treatment) (Fig.4.). The results showed that in bio-priming treatments, arbuscular mycorrhizal fungi extract decreased superoxide dismutase activity (58.57%), improved polyphenol oxidase (68.33%), and decreased catalase activity (225%) compared to control. In salinity stress treatments, 150 mM of salinity stress increased superoxide dismutase activity (82.55%), polyphenol oxidase (76.49%), and catalase activity

(116.66%) compared to control (without salinity condition) (Table 2).

4. Discussion

The results of this study indicated that the bio-priming especially mycorrhizal fungi extract promoted seedling growth characteristics of pot-marigold. Growth promoting bacteria such as azotobacter and azospirillum make good conditions for seed germination and seedling growth due to the production of phytohormones, nitrogen fixation, and phosphate solubilization in seed (Kumari et al., 2021). Seed bio-priming is an advantageous and environmentally friendly technique that utilizes beneficial microbes to enhance the physiological performance of seeds and increase their ability to withstand stress. Phosphorus is a necessary macronutrient, required for plant growth at all stages, from seedlings to vegetation (Nawaz et al., 2021). The results indicated that mycorrhizal fungi extract and also growth promoting bacteria improved seedling growth, because root morphology and physiology are closely related to IAA (indole-3-acetic) and cytokinins produced by bio-priming that cause cell division and elongation (Sheteiwy et al., 2021). Azotobacter has a high potential for producing auxin and decreasing ethylene levels, which considerably promotes the growth and formation of root structures. PGPBs produce the cytokinin hormone, stimulating cell division and improving root growth and development (Fazeli-Nasab et al., 2019). The beneficial and plant growth enhancing effects of growth promoting bacteria are well reported and explained. Growth promoting bacteria inoculation has increased different plant yields in normal and stress conditions (Kerecki et al., 2022; Rajendra Prasad et al., 2016). Growth hormones such as auxins, gibberellin and cytokinin and also several nutrients in mycorrhizal fungi extract increased amount of nutrients available for seeds, thus improving seedling growth and germination percentage. The combined use of mycorrhizal fungi and growth-promoting bacteria increases antioxidant production, which increases antioxidant activity, reducing reactive oxygen species against stress and protecting cells against oxidative stress (Dief et al., 2021; Boutasknit et al., 2020). Similarly, other researchers confirmed that bio-priming increased finger millet (*Eleusine coracana* L.) germination and reduced negative effect of salinity conditions (Rawat et al., 2022).

Increasing seed vigor index in bio-priming treatments depends on increasing germination percentage. Bio-priming promoted germination percentage due to the increasing growth hormones, which increased the total number of germinated seeds (plants produced) and the

result is the prompted the seed vigor (Dief et al., 2021). Allometric coefficient is one of the factors that affected by environmental stress. This factor may be contributing to resistance to salinity stress. It is the ratio the root length to the stem length, and increasing this indicates the resistance of the plant to environmental stress like salinity. The reason for the increase in allometric coefficient in salinity stress is the lack of access to water for the seeds, because the roots have expanded for water. On the other hand, the transfer of nutrients from the cotyledon to the embryo decreased and that is the causes to decrease in the length of the shoot (Khan et al., 2021). Salinity stress is an important abiotic factor limiting plant productivity. It is an intricate phenomenon involving osmotic stress, specific ion effect, nutrient deficiency, etc. thereby affecting different physiological and biochemical mechanisms related to plant growth and development (Khan et al., 2021). Hydrolytic enzymes like alpha-amylase are responsible for the breakdown of seed reserves and energy production in the early stages of growth; thus, the decrease in growth caused by water stress in the early stages of growth is related to metabolic factors caused by the decrease in water content by salinity conditions (Liu et al., 2018). Researchers confirmed that salinity stress decreased alpha amylase activity (Dief et al., 2021).

Polyphenol oxidase enzyme converts monophenols into diphenols and also catalyzes the oxidation of diphenols into quinones, which play a role in pigment polymerization. The increase in polyphenol oxidase enzyme activity under stress indicates the ability to oxidize and decompose toxic substances such as phenolic compounds, which are mainly accumulated during salt stress (El-Beltagi et al., 2022). Superoxide dismutase enzyme is the first protein that is produced in response to stress and decomposes superoxide ion into oxygen and hydrogen peroxide. The next enzyme is catalase, which reacts with hydrogen peroxide. The creation of stress conditions, including salt stress, causes ionic imbalance and as a result stimulates the production of reactive oxygen species and leads to disruption in the cell membrane and organelles, which changes the metabolic activity of the cell. Molecular signaling changes osmotic regulation and production of secondary metabolites. The plant's defense system is produced through the antioxidant response, which consists of antioxidant components (enzymatic and non-enzymatic), to deal with reactive oxygen species and neutralizes the destructive effects of reactive oxygen species. Therefore, it can be said that with the increase in salinity concentration, the enzyme activity of the plant has increased to deal with the salinity stress (Aleem et al., 2022). Other researchers confirmed that salinity stress increased enzymes activity (catalase, super oxide dismutase and polyphenol oxidase) (El-

Beltagi et al., 2022). This study showed that bio-priming have modulated the destructive effects of salinity stress by increasing the activity of antioxidant enzymes such as polyphenol oxidase and reducing superoxide dismutase and catalase activity. Water absorption by seeds is necessary for the activity of antioxidant enzymes, but simultaneously with the activity of these enzymes in seeds under salinity stress, there are various metabolic pathways that help the production and accumulation of reactive oxygen species (ROS) and have a negative effect on the germination process (Bahcesular et al., 2020). The findings of other researchers supported this study, the authors found that salinity stress changed enzymes activity of mulberry (*Morus alba* L.) (Sudhakar et al., 2001).

5. Conclusion

Bio-priming seed treatments is an ecofriendly technique that can provide a high level of protection against environmental stress such as salinity stress. The results showed that salinity stress reduced seedling growth characteristics and increased enzymes activity (superoxide dismutase, polyphenol oxidase and catalase activity), but decreased alpha amylase activity, and with increasing salinity stress content, seedling growth characteristics decreased. Bio-priming application promoted the seedling growth characteristics compared to the control. Our findings suggest that the constraining impact of bio-priming techniques improved low salinity stress and between bio-priming treatments, mycorrhizal fungi extract was more effective than other priming. Bio-priming and hydropriming reduced the negative effect of salinity stress, but the effect of bio-priming in increasing seedling growth characteristics and control enzymes activity was greater than hydropriming. The results showed that arbuscular mycorrhizal fungi extract was successful in alleviating of salinity stress and it can be suggested as an effective and environmentally friendly technique to alleviate salinity stress, improving seedling tolerance to salinity condition in germination stage, and promoted pot-marigold seedlings growth. Therefore, bio priming with can effectively mitigate stress-responsive criteria and enhance plant tolerance to salinity stress in an eco-friendly way.

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