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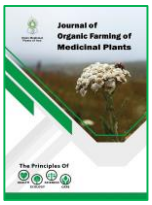

Original Article

Application of bio-priming to enhance osmotic stress tolerance in germinating coneflower (*Echinacea purpurea* (L.) Monch) seeds

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ABSTRACT

Coneflower has antioxidative, antibacterial, antiviral, and antifungal properties, but it is sensitive to environmental stress. Osmotic stress is the main important environmental stress, especially in seed germination, and it is very important to provide environmentally friendly solutions to reduce the risks of this stress. For this purpose, a study was arranged as a factorial based on a completely randomized design with three replications on coneflower seeds. Treatments included four levels of bio-priming [control (no prime), Arbuscular mycorrhizal fungi extract, azotobacter (*Azotobacter chroococcum*), azospirillum (*Azospirillum lipoferum*)] as the first factor and four levels of osmotic stress [0 (control), -0.3 Mpa, -0.6 Mpa and -0.9] as the second factor. The results demonstrated that bio-priming application promoted the germination and growth characteristics of coneflower seedlings compared to control. Osmotic stress reduced germination indicators compared to control, bio-priming application alleviated the negative effect of osmotic stress. In control conditions (without osmotic stress), arbuscular mycorrhizal fungi extract (73.88%), azotobacter (73.74%) and azospirillum (72.31%) raised the germination percentage compared to control of bio-priming in -0.9 MPa of osmotic stress. Interaction of arbuscular mycorrhizal fungi extract and -0.9 MPa of osmotic stress (154.54%) and interaction of azotobacter and -0.9 MPa of osmotic stress (163.63%), increased catalase activity compared to control (without bio-priming and osmotic stress). Therefore, bio-priming is an effective environmentally friendly solution to alleviate the impact of osmotic stress on coneflower seedlings.

1. Introduction

The germination and seedling growth stages are the most important and sensitive stages in plant development, implying plant's tolerance to osmotic stress in the early growth stage (Reddy et al., 2021). Nowadays, various methods have been evaluated in order to improve seed quality with the aim of increasing germination percentage and improving seedling establishment under environmental stress conditions, especially osmotic stress. Among these methods, seed priming can be used. Seed priming is one of the prominent methods that results in quick and consistent germination, seedling establishment and seed quality that leads to boosted plant growth and improves weak seed germination even in severe environmental conditions (Bahcesular et al., 2020; Thejeshwini et al., 2019).

Osmotic stress condition adversely affects plants yield, the extent of which varies significantly depending on the developmental stage and also the duration and intensity of the stress. Osmotic stress in seedlings induces reactive oxygen species (ROS) production, which, in low concentrations, has a signaling role in abiotic stress response pathways. In contrast, higher ROS concentrations cause oxidative damage to cellular biomolecules such as proteins, lipids, and nucleic acids (Vukovic et al., 2022; Muhie et al., 2021). Researchers suggested that Osmotic stress decreased soybean (*Glycine max*) seeds (Langeroodi & Noora, 2017), and also rice (*Oryza sativa*) seeds (Omar et al., 2020).

Biofertilizer is a substance containing micro-organisms, and when it is used on the seed, root surface, or in the soil, it stimulates the growth environment of the root or the plant itself, and by increasing the availability of minerals, it increases plant growth. Some of the effects of nitrogen-fixing bacteria include increasing plant growth, bio-fixation of nitrogen, the ability to produce siderophore, ethylene production in plants, changing root morphology, absorbing nutrients and improving seed germination (Wang et al., 2019). Growth-promoting bacteria improve germination and seedling emergence

and induce plant defense genes through direct and indirect mechanisms and by improving the absorption of water and nutrients by the plant. Seedling emergence enhancing bacteria are a group of plant growth enhancing bacteria that are effective in increasing the speed and amount of seedling emergence and plant establishment in the field (ALKahtani et al., 2020; Kerecki et al., 2022; Wang et al., 2019). Researchers confirmed that azotobacter priming improved sugar beet (*Beta vulgaris*) (Kerecki et al., 2022) and also wheat (*Triticum aestivum* L) seed germination and seedling growth (Rahangdale et al., 2022). Coneflower (*Echinacea purpurea*) is a perennial herbaceous plant belonging to the Asteraceae family from North America. It is one of the most important medicinal plants in the world showing different reactions as affected by osmotic stress. Abiotic stress especially osmotic stress, reduced the germination and growth of coneflower (Sheshbahreh et al., 2019). Coneflower widely used in the pharmaceutical, cosmetic and health industries. The medicinal products derived from the plant root and shoots are used to prevent and treat the colds, coughs, bronchitis, pulmonary infections, and chronic immunodefciency diseases owing to the immune-enhancing properties (Tsai et al., 2012). It is necessary to use environmentally friendly treatments instead of chemical treatments to improve the germination characteristics of plants and deal with osmotic stress. Since coneflower seeds have a low germination capacity and are also sensitive to moisture during germination. Considering the importance of improving the germination and establishment of seedlings in osmotic stress conditions and the matter of the coneflower medicinal plant, this experiment has the purpose of investigating the effect of bio-priming on coneflower seeds under osmotic stress conditions with the aim of alleviating the negative effects of osmotic stress.

2. Materials and Methods

2.1 *Experimental design and treatments*

The experiment was conducted as a factorial based on a completely randomized design with three replications, on coneflower (*Echinacea purpurea* (L.) Monch) seeds in the seed technology laboratory of the Islamic Azad University, Karaj branch, in May 2023. Seeds were purchased from Pakan Bazr Company (Isfahan, Iran). Treatments included four levels of bio priming [control (no prime), Arbuscular mycorrhizal fungi extract, azotobacter (*Azotobacter chroococcum*), azospirillum (*Azospirillum lipoferum*)] as the first factor and four levels of osmotic stress [0 (control), -0.3 Mpa, -0.6 Mpa and -0.9] as the 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, 150 seeds of coneflower 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. To apply osmotic stress, polyethylene glycol (PEG) 6000 was used. For the control treatment, distilled water was used, and other treatments were prepared according to the instructions (Michael & Kaufman, 1976) (Table 1). 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 PEG solution prepared at different levels and added to each experimental unit then were kept in the dark place of the germinator for 14 days at a temperature of 25°C and 45% humidity.

Traits such as seedling length, germination percentage, seedling vigor, allometric coefficient, proline content, ascorbate peroxidase and catalase enzymes activity, and activity of alpha-amylase were measured.

Table 1. Application method of osmotic stress using PEG.

Osmotic Stress (MPa)	PEG 6000 ($g.kg^{-1}$ solution)	Final solution volume (ml)
-0.3	13.08	100
-0.6	18.90	100
-0.9	22.2	100

2.3 *Seedling length and germination percentage and germination rate*

The length of seedlings was measured with a ruler. Germination percentage was calculated by Equation 1 (Scott et al., 1984).

(Eq. 1) $GP=(S/T)\times100$

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

(Eq. 2) GR = Germination rate = number of germinated seeds/ first day counting + number of germinated seeds/ last day of counting (Ellis & Roberts, 1981).

The first day counting was 3 days after germination and the last day of counting was 14 days after germination.

2.4 *Seedling vigor and allometric coefficient*

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

(Eq. 3) Seedling vigor= normal germination percentage × seedling length

(Eq. 4) Allometric coefficient= root length /stem length

2.5 *Proline content and ascorbate peroxidase activity*

The seedlings were dried at a temperature of 25˚C for 72 hours, and after grinding, 0.3 g of dry plant material was poured into a mortar and 5 ml of 3% sulphosalicylic acid

was added to it, then it was homogenized. The samples were centrifuged for 10 minutes at 4°C at 15000 rpm. 2 ml of ninhydrin acid were added to 2 ml of the resulting clarifier, and then they were mixed well, and solutions of 0, 4, 8, 12, 16, and 20 mg l^{-1} proline standards were used. Then 2 ml of ninhydrin acid and 2ml of glacial acetic acid were added to them and mixed well. The samples were placed in a hot water bath (bainmarie) for 90 minutes at a temperature of 100° C, and then placed on ice. 4 ml of toluene were added to the solutions and placed in the shaker for 30 minutes, and the absorbance was read using a spectrophotometer (PG Instruments Itd VIS/UV+T model) at a wavelength of 520 nm and compared with a control sample (Bates et al., 1973). Ascorbate peroxidase enzyme was measured from seedling tissue extract by spectrophotometric method (PG Instruments Itd VIS/UV+T model) at a temperature of 25°C and finally, the enzyme activity was calculated in terms of micromoles of oxidized ascorbate per gram of protein content per minute (Sairam et al., 1998).

2.6 ***Catalase and Alpha amylase enzyme activity***

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 H_2O_2 decomposed per minute in mg of protein. Absorbance changes at 240 nm were recorded for 3 minutes with a spectrophotometer (PG Instruments Itd VIS/UV+T model (Aebi, 1984).

For measuring alpha-amylase enzyme activity, 1 g of germination seed tissue was used. To prepare seed tissue extract, first, 5 ml of a 60 mM 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 (I_2) 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 Itd VIS/UV+T model). It was recorded with a wavelength of 620 nm and compared with the control sample (Xiao et al., 2006).

2.7 ***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 Germination percentage, seedling length and germination rate

The results of the ANOVA for germination percentage and seedling length showed that the main effects of bio-priming, and osmotic stress, as well as the interactions between bio-priming and osmotic stress were significant ($p< 0.01$) (Table 2). The mean values indicated that bio-priming application promoted the germination percentage, seedling length and germination rate compared to the control. Osmotic stress reduced germination percentage, seedlings length and germination rate compared to control, but bio-priming application reduced the negative effect of osmotic stress. In control conditions (without osmotic stress), arbuscular mycorrhizal fungi extract (73.88%), azotobacter (73.74%) and azospirillum (72.31%) raised the germination percentage compared to the control of bio-priming in -0.9 MPa of osmotic stress (lowest treatment). As well as in control of osmotic stress, arbuscular mycorrhizal fungi extract (115.57%), azotobacter (116.10%) and azospirillum (115.75%) promoted seedling length compared to control of bio-priming in -0.9 MPa of osmotic stress (lowest treatment). Also in control of osmotic stress, arbuscular mycorrhizal fungi extract (177.54%), azotobacter (173.83%) and azospirillum (171.15%) increased germination rate compared to control of bio-priming in -0.9 MPa of osmotic stress (lowest treatment) (Table 3).

3.2 Seedling vigor and allometric coefficient

ANOVA results for seedling vigor and allometric coefficient indicated that the main effects of bio-priming, osmotic stress, and the interactions between bio-priming and osmotic stress were significant ($p < 0.01$) (Table 2). Bio-priming treatments decreased the negative effects of osmotic stress, as well as raised the seedling vigor and reduced the allometric coefficient compared to control. Osmotic stress reduced seedling vigor and improved the allometric coefficient compared to the control. In control conditions (without osmotic stress), arbuscular mycorrhizal fungi extract (271.19%), azotobacter (270.73%) and azospirillum (270.37%) increased compared to control of bio-priming in -0.9 MPa of osmotic stress (lowest treatment). Control of bio-priming in -0.9 MPa of osmotic stress (69.90%) increased the allometric coefficient compared to control of osmotic stress and arbuscular mycorrhizal fungi extract (Table 3).

3.3 Proline content and ascorbate peroxidase activity

The results of the ANOVA for proline content and ascorbate peroxidase activity demonstrated that the main

Table 2. Variance analysis of priming and osmotic stress effect on germination traits and enzymes activity of coneflower (*Echinacea purpurea*)

S.O.V	Df	Germination percentage	Seedling length	Germination rate	Seed vigor index	Allometric coefficient	Proline content	Ascorbate Peroxidase Activity	Catalase Activity	Alpha-Amylase Activity
Priming (P)	3	1061.28**	826.48**	759.39**	9856.53**	72.85**	11.68**	21.36**	41.53**	265.79**
Osmotic stress (O)	3	3568.45**	749.35**	921.64**	8236.11**	95.49**	23.72**	16.18**	10.36**	342.21**
P×O	9	4215.73**	916.27**	1023.45**	1011.24**	53.16**	18.64**	13.75**	28.92**	468.37**
Error	32	21.45	12.39	10.81	35.83	3.59	0.97	0.84	1.04	5.13
C.V (%)		13.17	8.76	7.38	10.42	9.35	4.28	7.95	8.32	6.44

** Significant at the 1% probability levels.

Table 3. Means comparison of priming and osmotic stress effect on germination traits and enzymes activity of coneflower (*Echinacea purpurea*)

treatments		Germination percentage	Seedling length	Germination rate	Seed vigor index	Allometric coefficient	Proline content	Ascorbate Peroxidase Activity	Catalase Activity	Alpha-Amylase Activity
Priming	Osmotic stress (MPa)	(cm)	(%)	(%)			(µmol g^{-1}FW)	(µmol H_2O_2 min^{-1} mg^{-1} protein)	(µmol Fw minute^{-1})	(nmol seed^{-1}minute^{-2})
Arbuscular mycorrhizal fungi extract	Control	98.75^{a}	12.18^{a}	32.14^{a}	1203.76^{a}	1.03^{f}	0.39^{e}	0.130^{f}	0.020^{c}	85.79^{a}
	-0.3	95.33^{b}	11.05^{b}	28.45^{b}	1061.35^{b}	1.35^{d}	0.47^{d}	0.145^{e}	0.023^{b}	79.81^{b}
	-0.6	87.45^{c}	9.84^{c}	21.54^{c}	861.59^{c}	1.36^{d}	0.57^{c}	0.163^{d}	0.019^{c}	66.56^{c}
	-0.9	83.35^{d}	7.43^{d}	16.21^{d}	620.41^{d}	1.68^{b}	0.58^{c}	0.178^{c}	0.028^{a}	56.98^{d}
Azotobacter	Control	98.67^{a}	12.21^{a}	31.71^{a}	1202.27^{a}	1.18^{e}	0.39^{e}	0.132^{f}	0.015^{d}	83.93^{a}
	-0.3	95.73^{b}	11.09^{b}	25.52^{b}	1060.94^{b}	1.19^{e}	0.46^{d}	0.163^{d}	0.024^{b}	79.38^{b}
	-0.6	86.89^{c}	9.76^{c}	20.89^{c}	848.59^{c}	1.46^{c}	0.47^{d}	0.164^{d}	0.020^{c}	65.57^{c}
	-0.9	82.66^{d}	9.81^{c}	20.93^{c}	811.25^{c}	1.67^{b}	0.56^{c}	0.178^{c}	0.029^{a}	55.36^{d}
Azospirillum	Control	97.86^{a}	12.19^{a}	31.40^{a}	1201.08^{a}	1.18^{f}	0.57^{c}	0.143^{e}	0.019^{c}	80.79^{b}
	-0.3	94.65^{b}	11.08^{b}	28.31^{b}	1053.21^{b}	1.21^{e}	0.70^{b}	0.162^{d}	0.023^{b}	79.98^{b}
	-0.6	86.45^{c}	9.85^{c}	21.35^{c}	852.38^{c}	1.38^{d}	0.73^{b}	0.179^{c}	0.019^{c}	66.73^{c}
	-0.9	82.33^{d}	9.74^{c}	21.09^{c}	802.47^{c}	1.65^{b}	0.72^{b}	0.199^{b}	0.019^{c}	54.24^{d}
Control	Control	94.34^{b}	9.81^{c}	20.98^{c}	926.16^{bc}	1.49^{c}	0.58^{c}	0.177^{c}	0.011^{e}	46.86^{e}
	-0.3	75.62^{e}	7.39^{d}	16.43^{d}	559.53^{de}	1.63^{b}	0.69^{b}	0.201^{b}	0.014^{d}	48.04^{e}
	-0.6	61.33^{f}	6.55^{f}	16.21^{d}	408.41^{e}	1.64^{b}	0.71^{b}	0.198^{b}	0.015^{d}	36.35^{f}
	-0.9	56.79^{g}	5.65^{g}	11.58^{e}	324.29^{f}	1.75^{a}	0.79^{a}	0.215^{a}	0.024^{b}	31.89^{g}

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

effects of bio-priming, osmotic stress, as well as the interactions between bio-priming and osmotic stress were significant ($p < 0.01$) (Table 2). The mean values showed that the use of bio-priming reduced the proline content and ascorbate peroxidase activity compared to control. Osmotic stress increased proline content and ascorbate peroxidase activity compared to control, bio-priming decreased negative effects of osmotic stress. Control of bio-priming in -0.9 MPa of osmotic stress (79%) increased proline content compared to control of osmotic stress and arbuscular mycorrhizal fungi extract. As well as, control of bio-priming in -0.9 MPa of osmotic stress (48.27%) improved ascorbate peroxidase activity compared to control of osmotic stress and arbuscular mycorrhizal fungi extract and also increased (50.34%) compared to control of osmotic stress and azospirillum (Table 3).

3.4 Catalase and alpha-amylase activity

The results of the ANOVA for catalase and alpha-amylase activity showed that the main effects of bio-priming, osmotic stress, as well as the interactions between bio-priming and osmotic stress were significant ($p < 0.01$) (Table 2). The mean values demonstrated that bio-priming application promoted catalase and alpha-amylase activity compared to control. Osmotic stress reduced catalase and alpha-amylase activity compared to control, but bio-priming application reduced negative effect of osmotic stress. Interaction of arbuscular mycorrhizal fungi extract and -0.9 MPa of osmotic stress (154.54%) and interaction of azotobacter and -0.9 MPa of osmotic stress (163.63%) increased catalase activity compared to control (without bio-priming and osmotic stress). In addition, in control conditions (without osmotic stress), arbuscular mycorrhizal fungi extract (73.88%), azotobacter 73.74% and azospirillum (72.31%) raised alpha-amylase activity compared to control of bio-priming in -0.9 MPa of osmotic stress (lowest treatment). As well as in control of osmotic stress, arbuscular mycorrhizal fungi extract (169.01%), azotobacter (163.18%) promoted alpha-amylase activity compared to the control of bio-priming (lowest treatment) (Table 3).

4. Discussion

The results of this study indicated that with increasing osmotic stress, germination percentage and seedling length decreased. In osmotic stress conditions, due to the decrease in the hydration of the plant cells and the production of hormones that play a role in stimulating

germination, they have problems and the germination of the seeds suffers. The absorption of water by the seed, the movement, and the transfer of seed reserves will also decrease; finally, the lack of excellent materials and protein synthesis in the seed embryo will reduce the germination and growth of seedlings. Primed seeds have a higher metabolism by increasing the rate of water absorption and having enough water, and they can germinate faster. As a result, the germination rate of primed seeds increases compared to the control (Boutasknit et al., 2020; Eshaghi Gorgi et al., 2022). Similarly, other studies confirmed that plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi decreased the negative effects of osmotic stress and increased okra (*Abelmoschus esculentus*) germination and seedling growth (Roslan et al., 2020).

Seed priming with biofertilizers such as azotobacter and azospirillum improved plant growth and performance indicators in a variety of ways, both directly and indirectly (Gouda et al., 2018). In the present study, the use of azotobacter significantly reduced the effect of osmotic stress on the plant. One of the reasons for the increase in germination in the presence of plant growth stimulating bacteria is related to the increase in the production of some hormones, especially gibberellin. This hormone activates some enzymes, such as amylase, which is involved in starch metabolism and affects germination. When plant growth stimulating bacteria adhere to the surface of seeds, they synthesize indole acetic acid (IAA), in response to the secretion of amino acids in seeds. This acid stimulates plant cells and their elongation, and as a result, it can be effective on seed germination. In this study, azotobacter had a significant effect on the seedling enzymes, so it can be said that the use of bacteria from the genus azotobacter through improved seed establishment with increased phytohormones and IAA of seed, and improved nutrients for seeds, especially nitrogen, to the seedling may increase the growth and absorption of some nutrients by the plant. The main cause of raising seed vigor in bio-priming treatments is the boosting germination percentage and seed length. Bio-priming improved germination percentage according to the boosting hormones and organic substances, which improved the total number of germinated seeds and finally promoted the seed vigor (Gouda et al., 2018; Jjagwe et al., 2020).

Mycorrhizal fungi because of having the various hormones and nutrition 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 (Buezo et al., 2019). Phosphorus can be increased in a usable way by using growth-promoting bacteria, (such as azotobacter and azospirillum usage). It increases the cell length and increases the overall growth and multiplication of plant cells. It has also been observed that the inoculation of mycorrhizal fungi causes extensive changes in the morphological indicators of the root, especially the increase of lateral roots. As a result, with the increase in the growth of the root, more water and nutrients are available to the plant, and this improves the growth and performance of the plant (Cheng et al., 2021; Sheteiwy et al., 2021). Similar results were also reported in rice seed; researchers confirmed that osmotic stress reduced rice seed germination and mycorrhizal alleviated osmotic stress effects in rice seed (Kavitha Mary et al., 2018).

Allometric coefficient is an important parameter that is affected by environmental stress. The allometric coefficient is the result of dividing the root length by the stem length, and improving this indicates increased resistance of the plant to environmental stress like osmotic stress. The reason for the increase in allometric coefficient in osmotic 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, which caused a decrease in the length of the shoot (Muhie et al., 2021). Another study reported that arbuscular mycorrhizal fungi improved osmotic tolerance and recovery in two contrasting Carob (*Ceratonia siliqua* L.) ecotypes (Boutasknit et al., 2020).

Ascorbate peroxidase is a key enzyme in the ROS scavenging system that can destroy H_2O_2 produced in chloroplasts. In this study, the activity of ascorbate peroxidase increased with osmotic stress treatment. It seems that the response of the seedling to deal with osmotic stress, and the bio- priming treatment helped the seed to tolerate osmotic stress and reduced the activity of ascorbate peroxidase compared to the control (without prime). The ascorbate peroxidase enzyme detoxifies the H_2O_2 produced in the chloroplast through the ascorbate glutathione cycle. Hydrolytic enzymes like alpha-amylase are responsible for the breakdown of seed reserves and energy production in the early stages of growth; therefore, 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. The hormones and nutrients in bio-primers cause the release of hydrolytic enzymes and the breakdown of starch into oligosaccharides. After that, the oligosaccharides are broken down into glucose. This reduces the water potential of the cell and thus facilitates the entry of water into the cell, and following this process, cell growth is stimulated (Vukovic et al., 2022; Muhie et al., 2021).

Plants have many mechanisms for controlling osmotic stress, one of them is enzymatic defense system. Under osmotic stress, the catalase activity and proline content increased. The average activity of leaf catalase in plants inoculated with azotobacter and mycorrhizal plants showed that inoculation in these plants is able to increase the activity of this enzyme to deal with oxidative damage caused by a lack of water. Therefore, inoculants are able to regulate oxidative reactions and antioxidant defense (Buezo et al., 2019). Other researchers proved that mycorrhizal deal with osmotic stress and increased basil (*Ocimum basilicum*) growth under osmotic stress (Abd-Elghany et al., 2021).

The proline effect depends on the structure of the plant, the duration and the intensity of the stress. Actually, when the content of proline in the plant increases, it indicates the plants ability to react to stress. One of the main causes of improved proline concentration in osmotic stress can be changes in the activities of enzymes involved in proline biosynthesis or degradation. Proline content in stress conditions protects cell membranes, proteins, and cytoplasmic enzymes, inhibits reactive oxygen species, and removes free radicals. Increasing the activity of enzymes involved in proline biosynthesis and decreasing the activity of its decomposing enzymes, such as proline oxidase, causes the accumulation of proline in plants. The increase in proline content was considered to be due to the presence of high-energy compounds from photosynthesis (Vukovic et al., 2022). Usually, seeds treated with mycorrhizal fungi, by using water relations and better nutrition compared to non-mycorrhizal seeds, can easily escape from osmotic stress conditions and suffer less damage, and as a result, the amount of proline increases less compared to non-mycorrhizal plants (Sheteiwy et al., 2021).

5. Conclusion

The results of this study indicated that osmotic stress, especially -0.9 MPa reduced germination indicators and growth of coneflower seedlings compared to control. Application of bio-priming (arbuscular mycorrhizal fungi, azotobacter and azospirillum) improved the germination, growth and enzymes activity of coneflower seedlings compared to control. In addition, bio-priming promotes the resistance of seedlings against osmotic stress. To enhance the osmotic stress, proline content and ascorbate peroxidase enzyme activity increased, which showed an increase in the plant's tolerance to osmotic stress. Accordingly, bio-priming can be suggested as an effective and environmentally friendly technique to alleviate osmotic stress and increased coneflower seedlings growth.

References

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*. 105, 121-126.

Abd-Elghany, S.E., Moustafa, A.A., Gomaa, N.H. & Hamed, B.A. (2021). Mycorrhizal impact on *Ocimum basilicum* grown under drought stress. *Beni-Suef University Journal of Basic and Applied Sciences*. 10: 72-91. Seed biopriming with P- and K-solubilizing Enterobacter hormaechei sp. improves the early vegetative growth and the P and K uptake of okra (*Abelmoschus esculentus*) seedling. Plos One.

ALKahtani, M.D.F., Fouda, A., Attia, K., Al-Otaibi, F., Eid, A.M., Ewais, E., Hijri, M., St-Arnaud, M., Hassan, S. & Khan, N. (2020). Isolation and Characterization of Plant Growth Promoting Endophytic Bacteria from Desert Plants and Their Application as Bioinoculants for Sustainable Agriculture. *Agronomy*. 10, 1325-1343.

Bahcesular, B., Yildirim, E.D., Karaçocuk, M., Kulak, M. & Karaman, S. (2020). Seed priming with melatonin effects on growth, essential oil compounds and antioxidant activity of basil (*Ocimum basilicum* L.) under salinity stress. *Industrial Crops and Products*. 146, 112-125.

Bates, I.S., Waldern, R.P. & Tear, I.D. (1973). Rapid determination of free praline for water stress studies. *Plant and Soil*. 39, 205-207.

Boutasknit, A., Baslam, M., Ait-El-Mokhtar, M., Anli, M., Ben-Laouane, R., Douira, A., El Modafar, C., Mitsui, T., Wahbi, S. & Meddich, A. (2020). Arbuscular mycorrhizal fungi mediate drought tolerance and recovery in two contrasting Carob (*Ceratonia siliqua* L.) ecotypes by regulating stomatal, water relations, and (In) organic adjustments. *Plants Journal*. 9, 80

Buezo, J., Sanz-Saez, A., Jose, M.F., Soba, D., Aranjuelo, I. & Esteban, R. (2019). Drought tolerance response of high-yielding soybean varieties to mild drought: physiological and photochemical adjustments. *Journal of Plant Physiology*. 166(1), 88–104.

Cheng, H.Q., Giri, B., Wu, Q.S., Zou, Y.N. & Kuča, K. (2021). Arbuscular mycorrhizal fungi mitigate drought stress in citrus by modulating root microenvironment. *Archives of Agronomy and Soil Science.* 11, 1–12.

Ellis, R. & Roberts, A. (1981). The qualification of ageing and survival in orthodox seeds. *Seed Science and Technology*. 9, 373-409.

Eshaghi Gorgi, O., Fallah, H., Niknejad, Y. & Barari Tari, D. (2022). Effect of Plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi

inoculations on essential oil in *Melissa officinalis* L. under drought stress. *Biologia*. 77, 11–20.

Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H. S. & Patra, J.K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological research*. 206, 131–140.

ISTA. (1985). International rules for seed testing. *Seed Science and Technology*. 13, 307-520.

Jjagwe, J., Chelimo, K., Karungi, J., Komakech, A. J. & Lederer, J. (2020). Comparative Performance of Organic Fertilizers in Maize (*Zea mays* L.) Growth, Yield, and Economic Results. *Journal of Agronomy*. 10(1), 69-83.

Kerecki, S., Pećinar, L., Karlicic, V., Mirkovic, M., Kljujev, I., Raičevićand, V. & Petrovic, J. (2022). Azotobacter chroococcum F8/2: a multitasking bacterial strain in sugar beet biopriming. *Journal of plant interaction*. 17 (1), 719-730.

Kavitha Mary, J., Marimuthu, P., Kumutha, K. & Sivakumar, U. (2018). Seed priming effect of arbuscular mycorrhizal fungi against induced drought in rice. *Journal of Pharmacognosy and Phytochemistry*. 7 (2), 1742-1756.

Langeroodi, A.R.S. & Noora, R. (2017). Seed priming improves the germination and field performance of soybean under drought stress. *Animal and Plant Science*. 27, 1611–1620.

Michael, B.E. & Kaufman, M.R. (1976). The osmotic potential of polyethylenglycol-6000. *Plant Physiology*. 51, 914-916.

Muhie, S., Memis, N., Ozdamar, C., Gokdas, Z. & Demir, I. (2021). Biostimulant priming for germination and seedling quality of carrot seeds under drought, salt and high temperature stress conditions. *International Agriculture Environment and Food Sciences*. 5(3), 352- 359.

Omar, A., Zayed, B., Abdel Salam, A., Hafez, Y.M. & Abdelaal, K.A.A. (2020). Folic acid as foliar application can improve growth and yield characters of rice plants under irrigation with drainage water. *Fresenius Environmental Bulletin*. 29, 9420–9428.

Rahangdale, P., Kumar, A., Kumar, R., Kumar, A., Kumar, A., Kumar, D., Singh, D., Singh, Sanjiv Kumar, D., Bharti, A.K. & Kumar, S. (2022). Influence of biopriming and organic manures on growth, seed yield and quality of black wheat (*Triticum aestivum* L.). *Journal of Pharmacognosy and Phytochemistry*. 11(1), 132-135.

Reddy, Y.A.N., Reddy, Y.N.P., Ramya, V., Suma, L.S., Reddy, A.B.N. & Krishna, S.S. (2021). Chapter 8—Drought adaptation: Approaches for crop improvement. In Millets and Pseudo Cereals; Singh, M., Sood, S., Eds.; *Woodhead Publishing: Sawston*, UK. 143–158.

Roslan, M.A.M., Zulkifli, N.N., Sobri, Z.M., Zuan, A.T.K., Cheak, S.C. & Abdul Rahman, N.A. (2020). Seed biopriming with P- and K-solubilizing Enterobacter hormaechei sp. improves the early vegetative growth and the P and K uptake of okra (*Abelmoschus esculentus*) seedling. *PLoS ONE*. 15(7), 1-15.

Sairam, R.K., Deshmukh, P.S. & Saxena, D.C. (1998). Role of antioxidant systems in wheat genotypes tolerance to water stress. *International Journal for Experimental Botany*. 41(3), 387-394.

Scott, S.J., Jones, R.A. & Williams, W.A. (1984). Review of data analysis methods for seed germination. *Crop Science*. 24,1192-1199.

Sheshbahreh, M.J., Movahhedi Dehnavi, M., Salehi, A. & Bahreininejad, B. (2019). Physiological and yield responses of purple coneflower (*Echinacea purpurea* (L.) Moench) to nitrogen sources at different levels of irrigation. *Physiology and Molecular Biology of Plants*. 25, 177–187.

Sheteiwy, M.S., Ali, D.F.I., Xiong, YC. Brestic, M., Skalicky, M., Alhaj Hamoud, M.Y., Ulhassan, Z., Shaghaleh, H., AbdElgawad, H., Farooq, M., Sharma, A. & El-Sawah, A.M. (2021). Physiological and biochemical responses of soybean plants inoculated with Arbuscular mycorrhizal fungi and Bradyrhizobium under drought stress. *BMC Plant Biology*. 21, 195-215.

Thejeshwini, B., Manohar Rao, A., Hanuman Nayak, M. & Sultana, R. (2019). Effect of seed priming on plant growth and bulb yield in onion (*Allium cepa* L.). *International Journal of Current Microbiology and Applied Sciences*. 8(01), 1242–1249.

Tsai, Y.L., Chiou, S.Y., Chan, K.C., Sung, J.M., Lin, S.D. (2012). Caffeic acid derivatives, total phenols, antioxidant and antimutagenic activities of *Echinacea purpurea* flower extracts. *Food Science & Technology*. 46, 169–176.

Vukovic, R., Čamagajevac, I.Š., Vuković, A., Šunić, K., Begović, L., Mlinarić, S., Sekulić, R., Sabo, N. & Španić, V. (2022). Physiological, Biochemical and Molecular Response of Different Winter Wheat Varieties under Drought Stress at Germination and Seedling Growth Stage. *Antioxidants journal*. 11 (4), 693-711.

Wang, Z., Chen, Z. & Fu, X. (2019). Integrated effects of co-inoculation with phosphate-solubilizing bacteria and n2-fixing bacteria on microbial population and soil amendment under C deficiency. *International Journal of Environmental Research and Public Health*. 16 (13), 24-42.

Xiao, Z., Storms, R. & Tsang, A. (2006). A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Analytical Biochemistry*. 351, 146-148.