The Effect of Bio-Fertilizers and Phosphorus on Physiological Characteristics, Antioxidant Enzymes Activity and the Essential Oil Content of Green Mint (*Mentha spicata* L.) under Arsenic Stress Condition

Jamal HEKMATI[1]
Yousef HAMIDOGHLI[2]
Behrooz ESMAIELPOUR[3] (✉)
Mahmood GHASEMNEZHAD[4]

Summary

Arsenic is one of the toxic heavy metals for plant growth and development. Therefore, this study was carried out to investigate the effects of phosphorus and bio-phosphate fertilizers to suppress the harmful effects of arsenic on green mint growth and development. Green mint plants were grown under arsenic stress conditions (0, 50, and 100 mg kg^{-1} soil) and after that treated with the bio- phosphate (0 and 50 mg/kg) and phosphate (0, 50, 100, and 150 mg kg^{-1} soil) fertilizer. The results showed that arsenic stress significantly reduced growth characteristics, so with increasing arsenic content the harmful effects on morphological and physiological characteristics increased. The incorporation of bio-phosphate and phosphorus fertilizers suppressed the negative impact of the arsenic stress and improved leaves number, plant height, leaf area, root, shoot fresh and dry weight. The activity of antioxidant enzymes, proline content and essential oil percentage was increased with the application of phosphorous and bio-phosphate fertilizers under arsenic stress. The average activity of catalase and peroxidase enzymes in the treated plants with phosphate and bio-phosphate fertilizers was significantly higher than the control. Furthermore, the application of phosphorous increased the activity of catalase and peroxidase enzymes from about 3 to 3.5 times and about 2.3 to 2.5 times more than the control, respectively, under arsenic stress conditions.

Key words

essential oil percentage, polluting, plant growth, phosphate fertilizer

[1] Department of Horticultural Sciences, University Campus 2, University of Guilan, Rasht, Iran
[2] Department of Horticultural Sciences, Faculty of Agricultural Sciences. University of Guilan, Rasht Iran
[3] Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran
[4] Department of Horticultural Sciences, Faculty of Agricultural Sciences. University of Guilan, Rasht Iran

✉ Corresponding author: behsmaiel@yahoo.com

Received: November 23, 2021 | Accepted: March 21, 2023 | Online first version published: July 20, 2023

Introduction

Mentha (*Mentha spicata* L.) belongs to the family of mints (Lamiaceae). It is the herbaceous, perennial, aromatic plant that is purple due to the presence of anthocyanin and contains essential oil, like other plants of the mint family. This plant is used medicinally and as an edible vegetable. The effective ingredients of mint are used in the pharmaceutical, food, health-cosmetic, confectionery, soft drink, and spice industries (Hekmati et al., 2021). Most Mentha species are perennial, contain essential oils, and are widely cultivated as industrial crops for necessary oil production. Plants from this genus can be found in multiple and diverse environments. (Salehi et al., 2018).

Heavy metals are naturally present in different amounts in the environment. The amount of these metals in soil is affected by various factors such as rock, the presence of contaminating sources, the application of organic and chemical fertilizers in agriculture as well as the use of industrial and urban water runoff in irrigation (Mesa et al., 2015). Extraction and processing of minerals, particularly metal mines, can increase toxicity and reduce plant growth. In the current century, the toxicity of heavy metals is one of the main environmental problems. These elements enter the food chain and are consumed through plant products. Some of the heavy metals, such as copper, zinc, and nickel, in small quantities as micronutrient elements for plant growth and development, are necessary and absorbed by the root from the soil. In contrast, heavy metals such as arsenic, mercury, and cadmium are toxic and harmful to plant and human consumption (Kaplan et al., 2011). At the global level, the contamination of water sources with arsenic is the primary source of pollution. Since these waters are used to irrigate fields and gardens, with the accumulation of arsenic in fruits and vegetables, and other plants, there is a possibility of this contamination entering the food systems of humans and animals. It is estimated that 30 million people in the world are exposed to arsenic poisoning (Mithöfer et al., 2004). Previous studies have shown that in calcareous soils, more than 80% of phosphorus is unusable for plants. On the other hand, the world is faced with deficiency of phosphorous sources. Meanwhile, annually, between 75 and 90% of added phosphorus to soil due to the calcareous nature of most soils, high pH, drought stress and the presence of bicarbonate in irrigation water and deficiency of organic matter in the soil, as well as by combining with calcium, aluminum and iron ions in the soil is precipitated and becomes unavailable for the plants. Therefore, phosphate-solubilizing bacteria are necessary without any risk to human health and the environment (Gulati et al., 2008).

Considering the importance of food security in the world and the growing need of communities for agricultural products, as well as the existence of limiting factors such as heavy metal stress that reduces the quantity and quality of agricultural products (Glick, 2010), it seems the attention to appropriate and helpful management strategies to reduce the destructive effects of stress caused by heavy metals pollution and to achieve maximum yield in the majority of crops is necessary. In addition to common solutions for dealing with heavy metal stress, it is worth considering biological methods, including using the coexistence potential of growth-promoting microorganisms (plant growth-stimulating bacteria and *mycorrhiza* fungi to increase the ability and absorption of pollutants by plants to reduce their harmful effects). These strategies present one of the most effective and reliable methods compared with the other clean-up technologies in terms of environmental compatibility, productivity and cost (Malakouti and Homaei, 2004).

The roots of plants are associated with a large number of different living organisms. Their reaction to each other and soil conditions determines the growth and development of plants. In this case, the reactions between metals, microorganisms and plants are considered due to the biological potential of living organisms for the transfer of metal directly from contaminated soils, the probable transport of accumulated metals in the shoot and the toxicity effects of heavy metals on fuel, as well as the microbial mechanisms and plant growth (Maas and Hoffman, 1977).

Interactions between plants and useful microorganisms in the rhizosphere can increase biomass production and plants' tolerance to heavy metals (Loneragan and Webb, 1993). Among rhizospheric organisms involved in the reaction of the plant and surrounding soil, growth-promoting bacteria such as phosphate and potassium solvents, nitrogen free-living stabilizer bacteria, rhizobium, and arbuscular *mycorrhizal* fungi were considered. The most evident signs of arsenic toxicity in plants are disorders in metabolic processes, the replacement of nutrient uptake by arsenic and cellular transformation (Glick, 2010).

A previous study showed nitrogen biological fixation as susceptible to arsenic toxicity, with the total root nodes reduced (Alonso et al., 2006). Other effects of arsenic at this stage include root necrosis, damage to deadly fibers and loss of root length. In arsenate-treated roots, the amount of glutamine synthetase protein is low and free oxygen radicals are produced in these roots. Also, the contamination with this toxic element causes a decrease in the amount of amino acid reserves and the total soluble proteins (Alonso et al., 2006).

Taking heavy elements as one of the most critical environmental stresses in today's agricultural ecosystems, their presence can lead to reduced plant growth and production, which can endanger human health and cause poisoning. Therefore, discovering methods to reduce and prevent harmful effects arising from heavy metals such as arsenic has been interested to researchers. In this study, the impact of different levels of phosphorus and bio-fertilizers (phosphate) on the physiological and biochemical indicators of green mint under arsenic stress was investigated.

Materials and Methods

Growth Conditions and Treatments

The experiment was conducted under greenhouse conditions at the University of Guilan, Rasht, Iran. The green mint (*Mentha spicata* L.) rhizomes were obtained from a commercial nursery in West Azerbaijan Province, Iran. The soil polluted with arsenic was collected from Zareh Shoran Gold Mining Company in Takab city, located in West Azerbaijan Province (Fig. 1). Then it was air-dried and passed through a 2 mm sieve to homogenize the soil particles. Triple super-phosphate and bio-phosphate as a source of phosphorous were obtained from Biotechnur Sabz Company, and fertilizers were applied as soil fertility at four levels (0, 50, 100, and 150 mg kg^{-1} soil) of triple superphosphate and two levels of phosphate bio-fertilizer (0 and 0.5 g L^{-1}) with six replications.

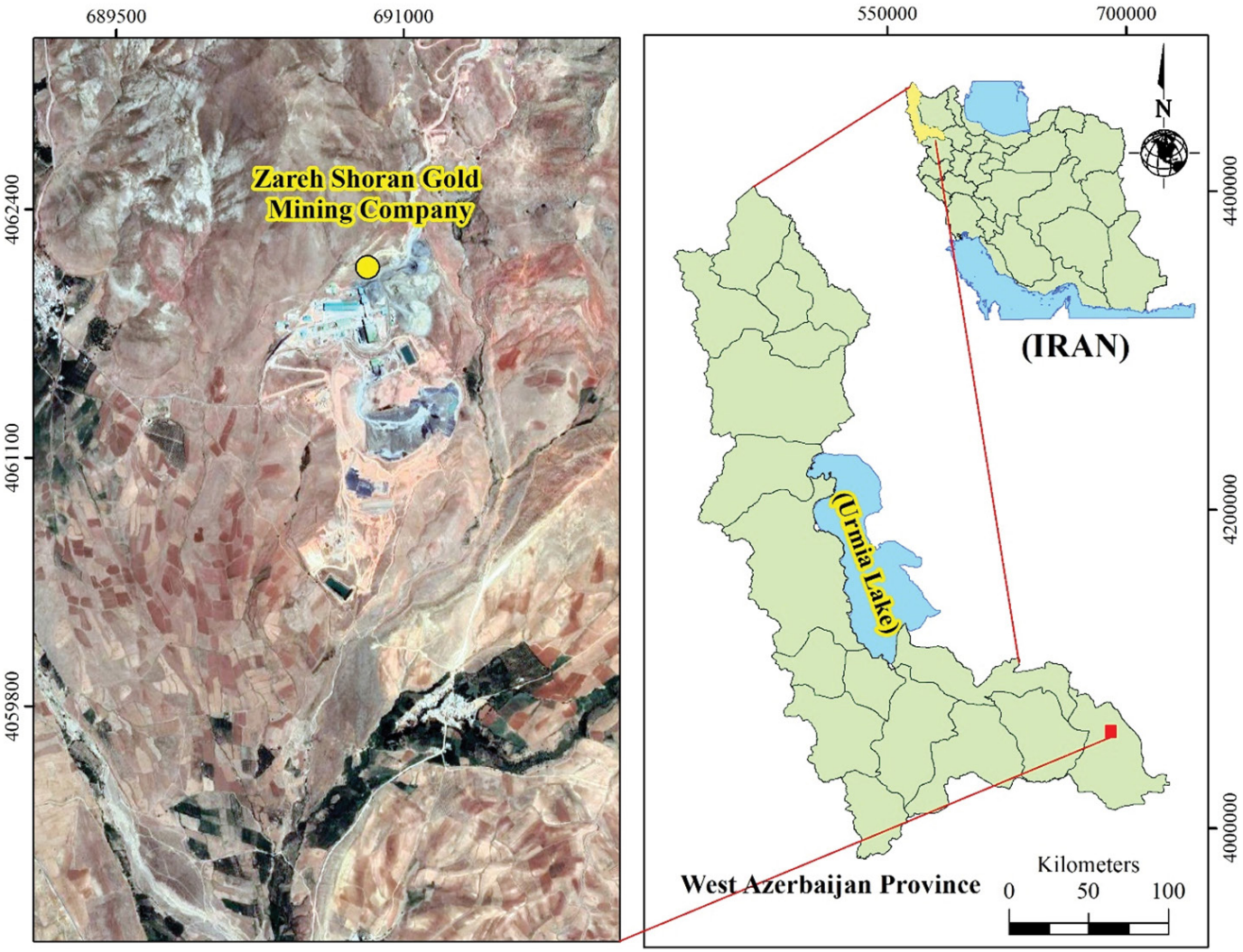

Figure 1. Location of the study area in West Azarbaijan Province, Iran

The plants were grown in pots containing first loamy soil contaminated with three levels: 0, 50, and 100 mg kg^{-1} of the soil from sodium arsenate source ($Na_2HAsO_4 \times 7H_2O$). The green mint plants were grown in the following conditions: 12 h photoperiod, 25 ± 10 °C temperature, 60 ± 10% relative humidity.

Two green mint rhizomes (10 cm length) were planted (6 cm depth) into containers filled with 9 kg of contaminated soil with arsenic in three levels (0, 50, and 100 mg L^{-1}). After planting, pots were irrigated with water as control and 0.50 g L^{-1} as a bio-phosphorus fertilizer in one liter per pot. The physical and chemical properties of the soil mixture are summarized in Table 1.

Evaluation

The green mint plants were grown under greenhouse conditions for two months; after that, the plants were analyzed. The leaf area of some leaves was measured by the leaf area meter ADC model. At the end of the experiment, green mint plants were cut and immediately transferred to the laboratory and weighed by digital balance with 0.001-gram accuracy. After that, they were placed in the oven at 70 °C for 72 hours to dry. Then, the dry weight was determined. Proline extraction and measurement from leaves were carried out using the method of Bates et al. (1973).

Table 1. Physical and chemical properties of soil mixture

Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	$CaSO_4$ (%)	pH	Electrical conductivity (dS m^{-1})	Soil texture
50	36	14	1.45	19.1	7.3	3.8	loam

The super oxide dismutase (SOD) activity was measured spectrophotometrically as described by Beyer and Fridovich (1987). The reaction solution (1 mL) contained 50 mM of phosphate buffer (pH=7), 12 mM of riboflavin, 13 mM of methionine, 0.1mM of EDTA, 7 mM of nitro blue tetrazolium (NBT), and 10 µL of extracted enzyme solution. A solution with no enzyme was used as the control. Test tubes were irradiated under fluorescent lights at 100 mM m^{-2} s^{-1} for 20 min. The absorbance of each solution was measured at 560 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme inhibiting 50% of NBT photoreduction.

The catalase (CAT) activity was assayed as reported by (Brouwer and Brouwer, 1998). The reaction solution (0.5 mL) contained 25 mM of phosphate buffer (pH=7), 10 mM of H_2O_2 and 10 µL of extracted enzyme solution. The reaction was initiated by adding the enzyme solution. Changes in absorbance at 240 nm were read every 10 s for 60 s using a spectrophotometer. One unit of CAT activity was defined as the absorbance change of 0.01 units per minute. The peroxidase (POD) activity was determined using Chance and Maehly (1955) method. POD activity in leaves was assayed by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm. The reaction mixture contained 100 µL of crude enzyme extract, 500 µL of 5 mM H_2O_2, 500 µL of 28 mM guaiacol, and 1,900 µL of 50 mM potassium phosphate buffer (pH=7). POD activity of the extract was expressed as activity U g^{-1} FW min^{-1}.

The essential oil was extracted through water distillation using the clevenger apparatus and the vital oil component was analyzed by the GC/MS. To measure the concentration of arsenic in the plant, one g of dry plant powder was put in a 100 mL balloon, and then 10 mL of 1:1 nitric acid was added and heated for 15 minutes at 95 °C. After cooling, 5 mL of 1:1 nitric acid was added and heated to 95 °C for 30 minutes. Then, 2 mL of distilled water was added to it. After that, 5 mL of concentrated hydrochloric acid was added to it and heated without boiling. After cooling, the extract was filtered with Whatman filter paper and measured by atomic absorption spectrometry (Soon, 1993).

Statistical Analysis

The plants were arranged in a completely randomized design in a factorial layout with three factors: arsenic (0, 50, and 10 mg kg^{-1}), triple super-phosphate concentrations (0, 50,100, and 150 mg kg^{-1}), and two levels of phosphate bio-fertilizer (0 and 0.5 g L^{-1}) before planting, with six replications. All data were analyzed by one-way analysis of variance, and mean comparisons were made by ANOVA with software (SAS, v. 9.0, Cary, NC, USA).

Results and Discussion

Vegetative Growth Characteristics

The results of the analysis of variance (Table 2) on the effects of arsenic stress, phosphorus, and biofertilizers and the interaction impact of stress and fertilizer on the physiological indicators of spearmint showed that the effect of arsenic stress on the number of leaves, plant height, leaf area, fresh and dry weight of roots, weight wet and dry aerial parts was significant at the $P < 0.01$ probability level.

Arsenic stress and phosphorus and bio-phosphorus fertilizers led to changes in the morphological indicators of green mint plants, including the number of leaves, plant height, leaf area, shoot fresh and dry weight and root fresh and dry weight. The most significant decrease in the number of leaves (69), plant height (40 cm), leaf area (126 cm^3), shoot fresh weight (15.83 g), shoot dry weight (1.53 gr), and fresh root weight (0.41 gr) was seen in the treatment of 100 mg kg^{-1} of arsenic without any fertilizer treatment.

However, with the addition of phosphorus alone, this reduction in morphological parameters improved to a great extent; for example, the fresh weight of shoots and roots was 20.7 and 3.48 g, respectively, in the treatment of 150 mg kg^{-1} of phosphorus, but it reached 16.33 and 1.21 grams in the treatment of biological phosphorus under the conditions of severe arsenic stress (100 mg kg^{-1}). The mutual effect of bio-phosphorus and phosphorus had a better effect, and the value of these parameters (fresh weight of shoots and roots) reached 19.62 and 4.5 g, respectively. Therefore, it can be concluded that triple superphosphate has a more significant effect than biological phosphorus in reducing the destructive effects of arsenic stress in the used concentrations. The results of this study indicated that increasing levels of arsenic caused the growth and physiology indices of green mint to decrease. In agreement with this research, the exposure to arsenic significantly affected the natural growth and development of black gram plants. Also, it caused a decrease in fresh and dry weight of shoots and roots as well as plant height, but the phosphorus-treated black gram (*Vigna mungo* L.) plants under stress conditions (especially severe stress; 200 µM As) significantly improved compared to the treatment conditions without phosphorus (Srivastava and Sharma, 2013). Arsenate toxicity can be increased by reducing the phosphate concentration of the growth medium, which can be due to the competition of phosphorus and arsenate in transport. However, it has been reported that phosphate can suppress the inhibitory effects of arsenite and arsenate on fungal growth (National Research Council, 1977). Biswas et al. (2015) reported that arsenic concentration at low concentrations (10 mg kg^{-1}) and high (150 mg kg^{-1}) had harmful effects on sweet basil (*Ocimum basilicum* L.) and arsenic destructive effects were observed. The vegetative growth of basil, such as the length of stems and shoots dry and fresh weight, decreased by 27 and 31.78 % at 150 mg kg^{-1} arsenic concentration (Biswas et al., 2015).

Proline Content

The results obtained from data variance analysis revealed that (Table 3) the effect of simple arsenic was significant at 1% probability level, but the simple effect of fertilizer and interactions of fertilizer × arsenic was not significant. The proline concentration increased significantly and a significant difference was observed between different treatments in which adding phosphorus to the culture medium improved the amount of proline concentration so that the highest amount of proline was in the treatments of phosphorus and biological phosphorus under stressed conditions. Therefore, the highest concentration of proline was observed in 150 mg kg^{-1} phosphorus alongside bio-phosphor under arsenic stress (3.99 µg g^{-1} fresh weight) and the lowest one was observed in control without any stress (0.09 µg g^{-1} fresh weight).

Table 2. Effect of phosphate fertilizer and bio fertilizer effects on vegetative growth characteristics of **green mint** under arsenic stress

Phosphate biofertilizer (gr L^{-1})	Arsenic (mg/kg)	Phosphorus fertilizer (mg/kg)	Leaves Number	Plant height (cm)	Leaf area (cm^2)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
0	0	0	127.42 ^{cde}	97.48 ^{cd}	200.11 ^{def}	17.35 ^{def}	3.45 ^{kl}	1.33 ^{kl}	0.29 ^{m}
		50	128.65 ^{bcd}	98.61 ^{bc}	244 ^{abcd}	18.18 ^{de}	4.68 ^{fg}	3.56 ^{fg}	1.05 ^{i}
		100	135.15 ^{bc}	105.15 ^{bc}	300.12 ^{abc}	20.24 ^{bc}	6.74 ^{cd}	5.62 ^{cd}	2.61 ^{def}
		150	138.55 ^{abc}	108.25 ^{bc}	321.5 ^{bc}	21.35 ^{bc}	7.55 ^{bc}	6.43 ^{bc}	3.41 ^{abc}
	50	0	87.17 ^{fgh}	57.87 ^{fgh}	241 ^{cde}	16.45 ^{ijk}	2.35 ^{mn}	1.23 ^{mn}	0.20 ^{mn}
		50	88 ^{fg}	58 ^{fg}	308.75 ^{cd}	17.52 ^{fg}	3.32 ^{lm}	2.20 ^{lm}	0.99 ^{j}
		100	97 ^{efg}	67 ^{efg}	327.35 ^{bc}	18.64 ^{def}	4.54 ^{ghi}	3.42 ^{ghi}	2.22 ^{efg}
		150	108.65 ^{de}	78.54 ^{de}	350.23 ^{b}	19.72 ^{cde}	5.32 ^{e}	4.20 ^{e}	3.03 ^{cd}
	100	0	69.46 ^{jk}	39.96 ^{jk}	125.75 ^{f}	15.83 ^{l}	1.53 ^{o}	0.41 ^{o}	0.11 ^{o}
		50	74.55 ^{jk}	44.85 ^{ijk}	174.5 ^{ef}	16.92 ^{jk}	2.12 ^{n}	1.00 ^{n}	0.45 ^{l}
		100	78.72 ^{hij}	48.54 ^{hij}	215.75 ^{cde}	17.86 ^{ij}	3.36 ^{lm}	2.24 ^{lm}	2.04 ^{fg}
		150	83.20 ^{ghi}	53.56 ^{ghi}	265 ^{bcde}	20.7 ^{def}	4.6 ^{gh}	3.48 ^{gh}	2.84 ^{de}
0.5	0	0	138.48 ^{bc}	10838 ^{bc}	241 ^{cde}	18.84 ^{cdef}	4.84 ^{f}	3.72 ^{f}	0.99 ^{j}
		50	149.61 ^{ab}	11965 ^{ab}	308.75 ^{abc}	19.26 ^{bcd}	5.66 ^{de}	4.54 ^{de}	1.65 ^{g}
		100	156.15 ^{a}	126.35 ^{a}	347.2 ^{ab}	20.27 ^{b}	7.77 ^{ab}	6.65 ^{b}	3.4 ^{b}
		150	158.25 ^{a}	128.55	374.25 ^{a}	22.22 ^{a}	8.22 ^{a}	7.10 ^{a}	4.01 ^{a}
	50	0	108.87 ^{def}	78.77 ^{def}	209.25 ^{def}	17.26 ^{fgh}	3.26 ^{lmn}	2.14 ^{lmn}	0.59 ^{k}
		50	109 ^{de}	79 ^{de}	271 ^{abcd}	18.28 ^{defg}	4.28 ^{ij}	3.16 ^{ij}	1.45 ^{gh}
		100	119 ^{cd}	89 ^{cde}	326.25 ^{abc}	19.2 ^{cde}	5.2 ^{ef}	4.08 ^{ef}	3.21 ^{bc}
		150	129.54 ^{bcd}	99.54 ^{bcd}	363.5 ^{a}	22.2 ^{a}	8.2 ^{a}	7.08 ^{a}	4.00 ^{ab}
	100	0	90.96 ^{ghi}	60.66 ^{ghi}	265 ^{bcde}	16.33 ^{h}	2.33 ^{mn}	1.21 ^{mn}	0.44 ^{l}
		50	95.85 ^{fgh}	63.55 ^{fgh}	215.75 ^{bcde}	17.72 ^{efgh}	3.72 ^{jk}	2.60 ^{jk}	1.15 ^{h}
		100	99.74 ^{efgh}	63.44 ^{efgh}	174.5 ^{ef}	18.56 ^{efgh}	4.56 ^{ghi}	3.46 ^{ghi}	3.01 ^{bcd}
		150	104.26 ^{ef}	76.46 ^{efg}	125.75 ^{f}	19.62 ^{bcd}	5.62 ^{de}	4.5 ^{de}	3.8 ^{abc}
Analysis of variance									
Arsenic			**	ns	**	ns	ns	**	**
Bio fertilizer and phosphorus			ns	ns	**	**	**	ns	ns
Arsenic stress × bio fertilizer and phosphorus			**	**	**	**	**	ns	ns

Note: Means of the main effects followed by different letters in each column indicate significant difference at $P \leq 0.05$ by least significant range (LSD). ns, * or ** indicate non-significance ($P > 0.05$) or significance at $P \leq 0.05$ or $P \leq 0.01$, by the F-test, respectively

Table 3. Effect of phosphate fertilizer and bio fertilizer effects on physiological characteristics and arsenic concentration of **green mint** under arsenic stress

Phosphate biofertilizer (gr L^{-1})	Arsenic (mg kg^{-1})	Phosphorus fertilizer (mg kg^{-1})	Proline (µg g^{-1})	Membrane stability	Essential oil (%)	Arsenic concentration in Root (mg 100 g^{-1})	Arsenic concentration in Shoot (mg 100 g^{-1})
0	0	0	0.09 ^{o}	0.14 ^{m}	0.500 ^{def}	1.65 ^{bc}	0.6 ^{bc}
		50	0.43 ^{l}	0.9 ^{i}	0.544 ^{abcd}	1.59 ^{cde}	0.54 ^{cde}
		100	2.02 ^{fg}	2.46 ^{def}	0.600 ^{abc}	1.55 ^{ef}	0.5 ^{ef}
		150	2.82 ^{de}	3.26 ^{abc}	0. 621 ^{bc}	1.5 ^{fg}	0.45 ^{fg}
	50	0	0.18 ^{mn}	0.05 ^{mn}	0.541 ^{cde}	1.74 ^{a}	0.69 ^{a}
		50	0.97 ^{j}	0.84 ^{j}	0.608 ^{cd}	1.7 ^{abc}	0.65 ^{abc}
		100	2.20 ^{efg}	2.07 ^{efg}	0.627 ^{bc}	1.55 ^{abcd}	0.5 ^{abcd}
		150	3.01 ^{cd}	2.88 ^{cd}	0.650 ^{b}	1.58 ^{def}	0.53 ^{def}
	100	0	0.27 ^{m}	0.01 ^{o}	0.425 ^{f}	1.75 ^{a}	0.7 ^{a}
		50	1.03 ^{i}	0.30 ^{l}	0.474 ^{ef}	1.72 ^{ab}	0.67 ^{ab}
		100	2.59 ^{def}	1.89 ^{fg}	0.515 ^{cde}	1.68 ^{cd}	0.63 ^{abc}
		150	3.39 ^{abc}	2.6 ^{de}	0.565 ^{bcde}	1.62 ^{cde}	0.57 ^{cde}
0.5	0	0	0.42 ^{l}	0.84 ^{j}	0.541 ^{cde}	1.5 ^{fg}	0.45 ^{fg}
		50	1.13 ^{h}	1.4 ^{g}	0.608 ^{abc}	1.55 ^{ef}	0.5 ^{ef}
		100	2.99 ^{bcd}	3.25 ^{b}	0.647 ^{ab}	1.59 ^{def}	0.54 ^{def}
		150	3.78 ^{abc}	3.85 ^{a}	0.674 ^{a}	1.64 ^{cd}	0.59 ^{cd}
	50	0	0.57 ^{k}	0.44 ^{k}	0.509 ^{def}	1. 7 ^{bc}	0. 65 ^{bc}
		50	1.43 ^{gh}	1.30 ^{gh}	0.571 ^{abcd}	1.68 ^{bc}	0.63 ^{bc}
		100	3.19 ^{bc}	3.06 ^{bc}	0.626 ^{abc}	1.62 ^{cde}	0.57 ^{cde}
		150	3.98 ^{ab}	3.85 ^{ab}	0.663 ^{a}	1.58 ^{def}	0.53 ^{def}
	100	0	0.97 ^{j}	0.29 ^{l}	0.565 ^{bcde}	1.73 ^{b}	0.68 ^{b}
		50	1.63 ^{g}	1.00 ^{h}	0.515 ^{bcde}	1.7 ^{bc}	0.65 ^{bc}
		100	3.38 ^{b}	2.86 ^{bcd}	0.474 ^{ef}	1.68 ^{cd}	0.63 ^{cd}
		150	3.99 ^{a}	3.65 ^{abc}	0.425 ^{f}	1.62 ^{cd}	0.57 ^{cde}
Analysis of variance							
Arsenic			**	**	**	**	**
Bio fertilizer and phosphorus			ns	**	ns	**	**
Arsenic stress × bio fertilizer and phosphorus			ns	**	ns	*	ns

Note: Means of the main effects followed by different letters in each column indicate significant difference at $P \leq 0.05$ by least significant range (LSD). ns, * or ** indicate non-significance ($P > 0.05$) or significance at $P \leq 0.05$ or $P \leq 0.01$, by the F-test, respectively

In the present experiment, with the increase of arsenic levels, proline increased significantly and a significant difference was observed between different treatments. Accumulation of proline is one of the symptoms of environmental stress, with a protective role for the plant. In agreement with this research, Zhang et al. (2010) state that the accumulation of proline in plant tissues is due to a decrease in proline breakdown, an increase in proline biosynthesis and a reduction of protein synthesis and/or protein hydrolysis. Stimulation of proline production from glutamic acid and its increase in plants in soil contaminated by heavy metals has been reported by various researchers (Zhang et al., 2010). Arsenic-treated plants showed a reduction in their growth and pigment content. They significantly enhanced lipid peroxidation, electrolyte leakage and level of proline showing oxidative stress, but the addition of phosphate led to a better development in black gram (*Vigna mungo* L.) (Srivastava and Sharma, 2013).

Enzyme Activity

Arsenic stress and phosphorus and biophosphorus fertilizers led to changes in the measured enzymes of green mint, including phenol oxidase, catalase and superoxide dismutase. The highest amount of polyphenol oxidase (11.4 mg fresh weight), catalase (4.17 mg fresh weight) and SOD (5.49 mg fresh weight), and peroxidase (5.38 mg fresh weight) in the severe stress treatment of arsenic (100 mg kg^{-1}) along with fertilizer treatments of phosphorus (150) and bio-phosphorus (0.5 g) and the lowest amount of polyphenol oxidase (7.5 mg fresh weight), catalase (0.17 mg fresh weight), and superoxide dismutase (1.59 mg fresh weight) and peroxidase (1.48 mg fresh weight) were observed in the control treatment, which shows that the plant increases its enzyme activities by increasing the intensity of stress to reduce free radicals as one of the ways to deal with stress in plants. Also, the use of phosphorus biologically and chemically increases enzyme activities and, as a result, removes more free radicals and reduces the destructive effects of stress (Table 4). In agreement with this research, Srivastava and Sharma (2013) reported that arsenic toxicity was associated with an increase in the activities of anti-oxidative enzymes like SOD, peroxidase and ascorbate peroxidase. But the addition of various concentrations of phosphate fertilizer showed significant alterations in most of the parameters tested under the arsenic stress, which led to better growth in black gram (Srivastava and Sharma, 2013). Changing the activity of antioxidant enzymes such as ascorbate peroxidase and catalase in roots and leaves indicated oxidative stress in soybeans (Pallai et al., 2002). These effects are traditionally attributed to the As-induced accumulation of reactive oxygen species (ROS), consequent lipid peroxidation and damage to cellular membranes (Zhang et al., 2021; Mithöfer et al., 2004). Arsenic causes oxidative stress by increasing the stimulation of oxygen species production reactions and the production of free radicals, especially in the membrane of chloroplasts (Morel, 2008). Increasing production of active oxygen species, followed by increasing activity of antioxidant enzymes such as catalase, under copper stress in buzidan (*Withania somnifera* L.) (Sereda et al., 2008) and nickel stress in nasturtium (*Nasturtium officinale* R.) was observed (Dummen and Ozsturk, 2010).

Essential Oil Content

The obtained results show that arsenic stress causes a decrease in essential oils, but by adding phosphorus to the culture medium improves the amount of essential oils so that the highest amount of essential oil is in the treatments of phosphorus and biological phosphorus, respectively, in non-stressed conditions (0.674), phosphorus treatment with biological phosphorus under moderate stress conditions (0.663) and phosphorus treatment under moderate stress conditions (0.650). The lowest amount of essential oils was observed under extreme stress conditions along with phosphorus and biological phosphorus treatments. These conditions of extreme phosphorus stress along with biophosphorus not only do they not increase the essential oil but also cause it to decrease more than the conditions of extreme stress of arsenic and biological phosphorus without triple superphosphate treatment (Table 4). In agreement with this research, Biswas et al. (2015) stated that Arsenic stress caused a reduction in the growth and biomass of the shoot system at 50 and 150 mg kg^{-1}, respectively. Essential oil yield increased by 3.5 to 4 times at 10 and 50 mg kg^{-1} but decreased significantly by 0.08% at 150 mg kg^{-1} arsenic, respectively (Biswas et al., 2015).

Arsenic Concentration

The variance analysis results of arsenic stress and phosphate and bio-fertilizer effects on arsenic accumulation in the root and aerial portion of the green mint showed that the impact of both factors in both parts of the plant was significant at $P < 0.01$ probability level, the aerial part was significant at 1% level, but that was not meaningful for the root (Table 3).

The results show that the presence of arsenic in the cultivation environment increases the amount of arsenic in the green mint plant. However, soil treatment with different concentrations of phosphorus and biological phosphorus caused competition and reduced its absorption and transfer to the aerial parts of the plant so that the highest amount of arsenic was absorbed under extreme stress conditions (100 mg kg^{-1}) of arsenic and without treatments. Fertilizers were observed and by adding phosphorus both in the form of bio-phosphorus and triple superphosphate, they reduced the amount of arsenic absorption, and bio-phosphorus had less effect than triple superphosphate concentrations in conditions of severe arsenic stress (Table 3). In agreement with these results, other researchers also report that phosphorus reduces arsenic absorption. Zhang et al. (2021) stated that plants growing under arsenic contamination not only reduced plant growth and development but also plants might accumulate significant amounts of arsenic in the edible parts of plants (shoot, root, and grain), which then enter the food chain (Zhang et al., 2021). Arsenic accumulation in barley plants (*Hordeum vulgare* L.) under extreme stress conditions (400 µg L^{-1} arsenic) was 2.4 times higher than the plants treated with 50 µg L^{-1} of arsenic concentrations. In the treatment with a higher phosphorus concentration (300 µg L^{-1}), the plant accumulated 41.4% less than in the treatment with 120 µg L^{-1}. Also, phosphorus prevents the absorption of arsenic in the atmosphere (Saldana-roles et al., 2018). The exact mechanism of producing all these active species is not yet clear, but it seems to be due to the formation of arsenic intermediates (García-Chávez et al., 2003).

Table 4. Mean Analysis of phosphate and bio-fertilizers on enzyme activity of **green mint** under arsenic stress condition

Phosphate biofertilizer (gr L^{-1})	Arsenic levels in (mg kg^{-1})	Phosphorus fertilizer (mg kg^{-1})	Superoxide dismutase (U g FW)	Peroxidase (U g FW min^{-1})	Catalase (U g FW min^{-1})	Polyphenol oxidase (U. g FW (min^{-1})
0	0	0	1.59 ^{o}	1.48 ^{o}	0.17 ^{no}	7.5 ^{o}
		50	1.93 ^{l}	1.82 ^{l}	0.61 ^{l}	7.84 ^{l}
		100	3.52 ^{fg}	3.41 ^{fg}	2.20 ^{fg}	9.43 ^{fg}
		150	3.32 ^{de}	3.21 ^{de}	2.00 ^{de}	9.23 ^{de}
	50	0	1.68 ^{mn}	1.57 ^{mn}	0.36 ^{mn}	7.60 ^{mn}
		50	2.47 ^{j}	2.36 ^{j}	1.15 ^{j}	8.38 ^{j}
		100	3.70 ^{efg}	3.59 ^{efg}	2.38 ^{efg}	9.61 ^{efg}
		150	4.51 ^{cd}	4.40 ^{cd}	3.19 ^{cd}	10.42 ^{cd}
	100	0	1.77 ^{m}	1.66 ^{m}	0.45 ^{m}	7.68 ^{m}
		50	2.53 ^{i}	2.42 ^{i}	1.21 ^{i}	8.44 ^{i}
		100	4.09 ^{def}	3.98 ^{def}	2.77 ^{de}	10.00 ^{def}
		150	4.89 ^{abc}	4.78 ^{abc}	3.57 ^{abc}	10.8 ^{abc}
0.5	0	0	1.92 ^{l}	1.81 ^{l}	0.6 ^{l}	7.83 ^{l}
		50	2.63 ^{h}	2.52 ^{h}	1.31 ^{h}	8.55 ^{h}
		100	4.49 ^{bcd}	4.38 ^{bcd}	3.27 ^{bcd}	10.4 ^{bcd}
		150	5.28 ^{abc}	5.17 ^{ab}	3.96 ^{abc}	11.19 ^{ab}
	50	0	2.07 ^{k}	1.96 ^{k}	0.75 ^{k}	7.98 ^{k}
		50	2.93 ^{gh}	2.82 ^{gh}	1.61 ^{gh}	8.84 ^{gh}
		100	4.69 ^{bc}	4.58 ^{bc}	3.37 ^{bc}	10.6 ^{bc}
		150	5.48 ^{ab}	5.37 ^{ab}	4.16 ^{ab}	11.39 ^{ab}
	100	0	2.47 ^{j}	2.36 ^{j}	1.15 ^{j}	8.38 ^{j}
		50	3.13 ^{g}	3.02 ^{g}	1.81 ^{g}	9.04 ^{g}
		100	4.88 ^{b}	4.77 ^{b}	3.56 ^{b}	10.79 ^{b}
		150	5.49 ^{a}	5.38 ^{a}	4.17 ^{a}	11.4 ^{a}
Analysis of variance						
Arsenic			**	**	**	**
Bio fertilizer and phosphorus			**	ns	ns	**
Arsenic stress × bio fertilizer and phosphorus			**	ns	ns	*

Note: Means of the main effects followed by different letters in each column indicate significant difference at $P \leq 0.05$ by least significant range (LSD). ns, * or ** indicate non-significance ($P > 0.05$) or significance at $P \leq 0.05$ or $P \leq 0.01$, by the F-test, respectively

Conclusion

Overall results of this study showed that adding different concentrations of arsenic to green mint reduced the growth of the very plant, but adding bio-phosphorus decreased the stress caused by adding arsenic as a result of adding different amounts of phosphorus and fertilizer. Biosynthesis of arsenic-contaminated soils will reduce arsenic-induced stress in these soils.

References

Alonso-Alvarez C., Bertrand S., Faivre B., Chastel O., Sorci G. (2006). Testosterone and Oxidative Stress: The Oxidation Handicap Hypothesis. Proc Biol Sci 274 (1611): 819-25. doi: 10.1098/rspb.2006.3764

Bates L , Waldren R , Teare I. D. (1973). Rapid Determination of Free Proline for Water-Stress Studies. Plant and Soil 39 (1), 205-207. doi: 10.1007/BF00018060

Biswas S., Koul M., Bhatnagar A. K. (2015). Effect of Arsenic on Trichome Ultrastructure, Essential Oil Yield and Quality of *Ocimum basilicum* L. Med. Plant Res 5 (6): 1-9. doi: 10.5376/mpr.2015.05Garc.0006

García-Chávez E., Santamaría A., Díaz-Barriga F., Mandeville P., Juárez B. I., Jiménez-Capdeville M. E. (2003). Arsenite-induced formation of hydroxyl radical in the striatum of awake rats. Brain Res 20 976 (1): 82-89. doi: 10.1016/s0006-8993(03)02697-0

Glick B. R. (2010). Using Soil Bacteria to Facilitate Phytoremediation. Biotechnol Adv 28 (3): 367-374. doi: 10.1016/j.biotechadv.2010.02.001

Gulati A., Rahi P., Vyas P. (2008). Characterization of phosphate-solubilizing fluorescent pseudomonads from the rhizosphere of seabuckthorn growing in the cold deserts of Himalayas. Curr Microbiol 56 (1):73-79. doi: 10.1007/s00284-007-9042-3

Hekmati J., Hamidoghli Y., Esmaielpour B., Ghasemnezhad M. (2021) Effect of Arsenic and Phosphate Biofertilizer on Physiological and Biochemical Properties of Green Mint (*Mentha spicata* L.). J Plant Prod Res 28 (1):127-39

Kaplan, Olcay, Yildirim, Nuran C, Yildirim, Numan, & Cimen, Murat. (2011). Toxic Elements in Animal Products and Environmental Health. Asian J Anim Vet Adv 6 (3): 228-232. doi: 10.3923/ajava.2011.228.232

National Research Council. (1977). Arsenic: Medical and Biologic Effects of Environmental Pollutants, 332 pp.

Loneragan, Jack F, Webb M. J. (1993). Interactions between Zinc and Other Nutrients Affecting the Growth of Plants. In: Zinc in Soils and Plants, Springer, pp. 119-134

Maas E. V, Hoffman G. J. (1977). Crop Salt Tolerance–Current Assessment. J. Irrig Drain Div 103 (2), 115-134

Malakouti M. J., Homaei M. (2004). Soil Fertility of Arid and Semi-Arid Regions (Difficulties and Solutions): Tarbiat Modares University Press, Iran

Mesa J., Mateos-Naranjo E., Caviedes M. A., Redondo-Gómez S., Pajuelo E., Rodríguez-Llorente I. D. (2015). Scouting Contaminated Estuaries: Heavy Metal Resistant and Plant Growth Promoting Rhizobacteria in the Native Metal Rhizoaccumulator Spartina Maritima. Mar Pollut Bull 90 (1-2): 150-159. doi: 10.1016/j.marpolbul.2014.11.002

Mithöfer A. S. B., Boland W (2004). Biotic and Heavy Metal Stress Response in Plants: Evidence for Common Signals. FEBS Lett 566 (1-3): 1-5. doi: 10.1016/j.febslet.2004.04.011

Morel F. M. M. (2008). The Co-Evolution of Phytoplankton and Trace Element Cycles in the Oceans. Geobiology 6 (3): 318-324. doi: 10.1111/j.1472-4669.2008.00144.x

Pallai R. (2002). Effect of Plant Growth Promoting Rhizobacteria on Canola (*Brassica napus* L.) and Lentil (*Lens culinaris*. Medik) Plants. MSc Thesis. University of Saskatchewan Saskatoon, 140 pp

Sereda O., Ribas J., Stoeckli-Evans H. (2008). New 3D and Chiral 1D CuIICrIII Coordination Polymers Exhibiting Ferromagnetism. Inorg Chem 47 (12): 5107-5113. doi: 10.1021/ic702234y

Saldana-Robles A., Saldana-Robles N., Saldaña-Robles A., Ana-Zanor G., Ruiz-Aguilar G. M., Gutierrez-Vaca C. (2018). Phosphorus Effect on Arsenic Accumulation of Barley (*Hordeum vulgare* L.) through Irrigation with Contaminated Water. Agrociencia 52 (3):407-18.

Salehi B, Stojanović-Radić Z, Matejić J, Sharopov F, Antolak H, Kręgiel D, Sen S, Sharifi-Rad M, Acharya K, Sharifi-Rad R, Martorell M. (2018) Plants of *Genus Mentha*: From Farm to Food Factory. Plants (Basel) 7 (3):70. doi: 10.3390/plants7030070

Srivastava S., Sharma Y. K.. (2013). Impact of Arsenic Toxicity on Black Gram and Its Amelioration Using Phosphate. ISRN Toxicol 2013: 340925. doi: 10.1155/2013/340925

Soon Y. K. (1993). Lead and Nickel. In: Soil Sampling and Methods of Analysis, pp.101.

Zhang J., Hamza A., Xie Z., Hussain S., Brestic M., Tahir M. A., Ulhassan Z., Yu M., Allakhverdiev S. I., Shabala S. (2021). Arsenic Transport and Interaction with Plant Metabolism: Clues for Improving Agricultural Productivity and Food Safety. Environ Pollut 290: 117987. doi: 10.1016/j.envpol.2021.117987

Zhang J., Huang, X.-Y., Ye M.-L., Luo C.-X., Wu H.-Y., Hu Y., Zhou Q.-G., Wu D.-L., Zhu L.-J., Zhu, D.-Y. (2010). Neuronal Nitric Oxide Synthase Alteration Accounts for the Role of 5-HT1A Receptor in Modulating Anxiety-Related Behaviors. J Neurosci 30 (7):2433-2341. doi: 10.1523/JNEUROSCI.5880-09.2010

aCS88_35