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Research Article

Bacterial bio surfactants stimulate the growth of beans and improve foliar-treated Fe absorption

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Abstract

Due to the eco-systemic hazardous effects of surfactants, biosurfactants are recommended as wetting agents to increase the efficiency of foliar application of nutrients. Moreover, some of them could act as plant growth promoting agents. The aim of the present study was to assess the biosurfactant effect on iron foliar nutrition and its effects on bean growth. Biosurfactant production was investigated in seven *Bacillus* isolates from compost. Among them, *B. nealsonii* 104C, with the ability to produce glycolipid biosurfactant, was selected for the greenhouse study. Three concentrations of Fe, including zero, one, and two percent, and two levels of biosurfactant (zero and 50 mgL^{-1}) were tested in a factorial experiment with a randomized complete block design on the bean plants growing hydroponically. The results showed that foliar application of iron without adding biosurfactant at one and two percent concentrations increased the plant yield by 2.08 and 2.8 times, respectively. Zero, one, and two percent iron plus biosurfactant increased the yield up to 4, 3.3, and 4.2 times compared to the control. The highest bean height, total dry weight, leaf and stem weight, and number of pods were observed in the biosurfactant treatment plus 2% iron. It seems that biosurfactants could be used in the bean plant organic farming as an iron transition facilitator via foliar application or as a plant growth stimulator.

Keywords: *Bacillus*, Bean yield, Glucolipid biosurfactant, Iron sulfate

Introduction

The Food and Agricultural Organization (FAO) predicts that the world population will reach up to nine billion by 2050 and global agricultural production must increase by 70% (Frona *et al*., 2019). Agricultural products aim to meet the growing global demand for foods, mainly with the help of chemical compounds, which have seriously damaged the environment and human health (Sarma *et al*., 2021). Thus, environmentally friendly methods are highly recommended. Beneficial microbes such as plant growth-promoting rhizobacteria and their metabolites have demonstrated their potential and valuable applications in sustainable crop production (Ray *et al*., 2020). With the broad awareness about the various advantages of these organisms, like biosurfactant production, the hopes for their use in sustainable agricultural production systems are increasing.

Biosurfactants were first considered with the dissolution of hydrocarbons in 1990; their use has expanded every year, especially in the oil, food, and pharmaceutical fields (Gayathiri *et al*., 2022). Likewise, modern agriculture also needs enormous quantities of surfactants to either control pests and/or promote plant growth and productivity (Sachdev and Swaranjit, 2013). In agriculture, surfactants are used as emulsifiers and dispersing/wetting agents in stabilizing fertilizers and pesticides (Shah and Bhattarai, 2020). They also act as spreaders, stickers, and penetrants to enhance the accompanying reagents' biological activities (Czarnota and Thomas, 2010).

Biosurfactant biosynthesis is considered an intrinsic growth stimulant trait of bacteria. Recently, studies have shown that they can improve plant growth (Velioglu and Urek, 2015; Marchut-Mikołajczyk *et al*., 2021). Mishra *et al*. 2020, have extracted glycolipid category biosurfactant from *Pseudomonas putida* BSP9 and assayed their effects on *Brassica juncea* L. growth parameters. Their results showed that the germination rate, root length, shoot length, total fresh weight, dry weight, pod number, total oil content, total chlorophyll content, and flavonoid content were increased.

Surfactants are integral components in foliar spraying solutions to stimulate the uptake of the soluble

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active ingredients by plant leaf. Nutrients are received through the leaves in three stages (Fernandez and Eichert, 2009): (i) penetration of nutrients from the cuticle to the cell wall, (ii) surface adsorption on the plasma membrane, and (iii) passing through the plasma membrane and entering the cytoplasm. The hydrophilic molecules are difficult to cross the hydrophobic cuticular membrane covered with insoluble waxy components (Holloway, 1993). Prevention of the leaf surface wetting reduces the penetration rate of foliar nutrients, and forming larger droplets will waste it. Surfactants mixed with the foliar spraying solutions reduce the excess solutes draining off and simultaneously increase the solutes penetration rate, significantly improving nutrient use efficiency (Tagliavini and Toselli, 2005).

Microbial biosurfactants are the most desired "green" surfactants for such use in their biological production process and friendly end-use for both environment and human health. Consequently, their use is attractive in agricultural systems (Sarma *et al*., 2021).

Worldwide, millions of hectares of arable land are deficient in plant-available microelements such as Cu, Fe, Mn, and Zn (Mutwiri *et al*., 2020). Among the most critical risk factors for lowering crop yields in low-income countries are the deficiencies of Zn and Fe, which are ranked fifth and sixth, by WHO, respectively (Singh *et al*., 2018). Legumes are main sources of plant proteins for human and animal nourishment. Beans with 20-25% protein, 55-65% carbohydrates and 1-5% lipid play a major role in nutrition of poor nations which are not able to feed ourselves with animal proteins. Thus, increasing yield and quality of beans is an effective way for reducing protein deficiency in developing countries. One of the factors that reduces yield and quality of beans in calcareous soils of Iran is iron deficiency. High pH and calcium and bicarbonate contents of calcareous soils induce leaf choruses which limits plant growth and yield and reduces the quality of beans (Jozdaemi and Golchin, 2017). Therefore, if the leaf adsorption efficiency of ferrous sulfate as the cheapest Fe fertilizer can be improved by using biosurfactants, it will be a great success in meeting the Fe demands of plants and could be examined and prescribed for the other nutrients in the case of soil-based application challenges. Beans are susceptible to iron deficiency and can be used as a suitable plant in iron nutrition studies. Considering the scarce studies conducted on the biosurfactant-producing bacteria, the principal idea of the current project was to isolate biosurfactants from the bacteria and evaluate (i) their effect on the plant growth potential and (ii) on foliar-treated Fe uptake. Meanwhile, the iron amount in the soils of Iran is enough, but with the large amounts of lime in the soil, and the raised soil pH, it is not available for the plants. Under these conditions, foliar feeding of iron can meet the iron needs of plants.

Material and methods

Microbial strains and culture conditions: This study utilized seven bacteria to extract the surfactants (Table 1). These bacteria were previously isolated from composting tiles by Hemmati *et al*. (2021). The strains were identified by 16S rDNA sequencing and deposited under NCBI GenBank accession numbers (Table 1). The strains were retrieved and sub-cultured in Nutrient-Agar (NA) agar plates (g L^{-1}: 10.0 tryptone, 5.0 yeast extract, and 10.0 sodium chloride with 16.0 agar) and incubated at 37°C for 24 h. The further optimized conditions were applied to culture preparations by the single colony inoculation method using LB broth (pH: 7.0) and incubated in an orbital shaker (150 rpm) for 24 h at 37°C.

Evaluation of the selected isolates for biosurfactant production: Hemolytic activity as a preliminary screening

The blood agar plates containing 5% v/v blood were used to dot culture the isolates and were incubated at 37°C for 48 hours. Then, the plates were examined to form the clear zone around the bacteria colonies, and the size of the clear zone was measured (Dhasayan *et al*., 2015).

Inoculum preparation and biosurfactant production: Biosurfactant production was aerobically carried out in a 500 ml Erlenmeyer flask containing 200 ml of sterile Minimal Salt Medium (MSM) (g.L^{-1}: 30.0 NaCl, 1.8 $MgSO_4$, 0.02 $CaCl_2$, 8.34 KH_2PO_4, 1.62 K_2HPO_4, 0.6 NH_4NO_3, 0.006 $CuSO_4$, 0.006 H_3BO_3, $NaMoO_4$, and 0.5 $FeCl_3$), supplemented with 12 gr sucrose as carbon and energy source. In triplicate flasks, the pre-culture of bacteria was inoculated (1.6×10^4 CFU ml^{-1}) and incubated at 37°C in an orbital shaker at 200 rpm for seven days. At the end of the incubation, the biosurfactant was extracted by refrigerated centrifuge of culture medium at 4°C for 20 min at 3400 × g, and the supernatants were utilized for screening purposes. All the assays were performed in triplicate, and a sterile MSM medium was used as the control.

Oil displacement method: The oil displacement technique was carried out as described previously by Hassanshahian (2014). 10 µl of crude oil was added to 40 ml of distilled water (D.W.) in a petri dish for thin oil layer formation. Then, 10 µl of the supernatant was placed at the center of the oil layer. The diameter of the clear zone on the oil surface can be linked to the biosurfactant concentration. Negative control was maintained with distilled water (without surfactant), in which no oil displacement or clear zone was observed. Triton X-100 was used as the positive control.

Drop collapse test: The drop collapse test followed the procedure described by Patowary *et al*. (2016) with slight modifications. A drop of crude oil was applied to the glass slide. After that, a drop of cell-free culture broth was added to the crude oil drop, and drop collapse activity was noted. Biosurfactant-producing cultures gave flat drops. Deionized water was used as a negative control, and Triton X-100 (a chemical surfactant) solution was a positive control (1 mg/ml).

Emulsification activity: Emulsification activity was

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Table 1. Bacterial strains used to extract the biosurfactants

Name	Strain	NCBI Gen Bank accession numbers
Paenibacillus validus	IVC	MH159206
Paenibacillus koreensis	12C	MH159225
Paenibacillus thailandensis	13C	MH159776
Paenibacillus cellulositrophicus	47YZ	MH160186
Bacillus nealsonii	104C	MH160205
Paenibacillus lautus	151VC	MH159167

measured by adding 2 mL of cell-free culture supernatant to the same volume of oil in a test tube. The mixture was vigorously vortexed for 2 min, and the Emulsification index (E24) was calculated after 24 h based on the following formula (Chandankere *et al*., 2013):

$$E_{24} = \frac{\text{Height of emulsion layer}}{\text{Total height of the mixture}} \times 100$$

Determination of surface tension: For surface tension measurements, 5 ml of broth supernatant were transferred to a glass tube submerged in a water bath at a constant temperature (28˚C). Surface tension was calculated by measuring the height reached by the liquid when it freely ascended through a capillary tube. As a control, the non-inoculated broth was used, and the surface tension was calculated according to the following formula (Viramontes *et al*., 2010):

$$\gamma = \frac{rh\delta g}{2}$$

γ = Surface tension (mN/m), δ = Density (g/mL); g = gravity (980 cm/s^2); r = capillary radius (0.05 cm); h = height of the liquid column (cm).

Extraction of biosurfactants: The selected bacteria (bacteria with high biosurfactant production potentials) were cultured in the production medium for 72 h. Cultures were centrifuged at 10,000 ×g for 20 min. The pH value of cell-free broth was adjusted to 2.0 using 6 M HCl. This was stored at 4˚C overnight to allow precipitation of the biosurfactants. Precipitates were then harvested using centrifugation at 20,000g for 30 min at 4˚C. The precipitates in centrifuge tubes were dried by heating at 37˚C in the oven. Dried materials were dissolved in deionized water and extracted three times with dichloromethane. Equal volumes of dichloromethane and deionized water were briefly used, and the two phases were vigorously mixed. The mixture was centrifuged at 6,000g for 2 minutes to accelerate phase separation. The organic solvent phases, containing the biosurfactants were collected and evaporated at room temperature (Abdel-Mawgoud *et al*., 2011).

Characterization of biosurfactant: Fourier transform infrared (FT-IR) spectroscopy characterized the extracted biosurfactant. The functional groups of the surfactant collected from *Bacillus nealsonii* 104C were qualitatively represented by FT-IR (Perkin-Elmer, Nicolet Nexus–470). The dried biosurfactant was ground with the addition of potassium bromide in the ratio of 1:100, and the pellet was fixed in the sample container and analyzed in the mid-IR region of 400–4000 cm^{-1}.

Greenhouse experiment: This experiment evaluated the impact of biosurfactant application on bean (*Phaseolus vulgaris* L.) cv. Khomein growth and the facilitation of Fe^{++} transfer into the leaves *via* the foliar applications. The experiment was carried out as a factorial based on a randomized complete block design. Factors were included biosurfactant (0 as control and 50 mg L^{-1}, derived from *Bacillus nealsonii* 104C), and Fe^{++} (0, 500 and 1000 mg L^{-1}, from $FeSO_4$ sources). The treatments were sprayed at the V1 (First trifoliolate) and V2 (Second trifoliolate) stages of bean plants. The seeds were sown into 2 kg plastic pots filled with medium-sized perlite. Before planting and to remove any contamination with iron or other trace elements, perlite was washed with 0.01 M HCl followed by three washings with distilled water. Since the seeds have high amounts of elements, especially iron (ref), the diluted Hoagland solution (1/4) was applied at the seedling stage to deplete the elements. To avoid salt accumulation, the pots were washed biweekly with distilled water.

The number of leaves was counted every three days, and SPAD recorded the total chlorophyll index at the same time. The following traits were measured at the R7 stage (Beginning maturity): Total plant fresh weight, plant height, number of pods per plant, number of seeds per each pod, number of seeds per plant, number of flowers, and the oven-dried weight of leaves, stems and, the whole plant. The fully expanded leaves area was measured with a leaf area meter (LI-3000, Li-Cor, Inc.). These equations were used to calculate the tissue water content, single plant yield, and specific leaf area and leaf area index (Pearcy *et al.*, 2012):

$$\text{Tissue water content (\%)} = \frac{\text{Plant fresh weight} - \text{Plant dry weight}}{\text{Plant dry weight}} \times 100$$

$$\text{Plant yield (g pot}^{-1}) = \frac{1000\text{seeds weight} \times \text{number of seeds per plant}}{1000}$$

$$\text{Specific leaf area (cm}^2\ \text{g}^{-1}) = \frac{\text{one sided leaf area surface (cm}^2)}{\text{total leaf dry weight (gr)}}$$

$$\text{Leaf area index} = \frac{\text{Leaf area (m}^2)}{\text{ground cover (m}^2)}$$

Phenology traits, including leaf formation, flowering, and Podding times, were recorded based Pearcy *et al.* (2012).

Determination of leaf Iron content: Leaf samples

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were washed with tap water and then deionized water to clean the dust. The concentrations of Fe in bean samples were determined by a flame atomic absorption spectrometer (FAAS) after digestion by the wet-ashing method. An aliquot of 1 g of the sample was digested in the Kjeldahl flask. The wet-ashing method was applied as a 50 mL mixture of acids HNO_3: $HClO_4$ in the ratio of 8:2 (v/v) was added to the raw plant sample, and the flask was covered with a watch glass and stored at room temperature overnight. The samples were digested at an increasing temperature to 100°C for one hr or more if needed. Then, 4 mL of HNO_3 was added and filtered through glass wool to remove solids. The filtrate was diluted to 10 mL volume with deionized water. The concentration of Fe was determined with Flame AAS (Kalra, 1997).

Statistics: MSTAT-C software was employed to analyse the data obtained from each section. Excel was employed to draw the graphs. The means were compared using Duncan's test at a 5% probability level.

Result and discussion

Screening of the most efficient bacterium for biosurfactant production: Results of the red blood cell lysis, oil spreading, drop collapse, emulsification index, and surface tension are shown in Table 2. Based on the results, *Bacillus nealsonii* 104C was identified as the most efficient biosurfactant producing bacterial species.

According to these physiological roles, biosurfactant-producing microbes can be found in different environments (Walter *et al*., 2010). Some researchers investigated the production of biosurfactants by composter microorganisms. Jahanshah *et al*. (2013) isolated two biosurfactant-producing bacteria, *Bacillus* sp. and *Streptomyces* sp. from compost material and used them to enhance compost quality. Montoneri *et al*. (2008) extracted biosurfactants from urban waste compost and utilized them in textile dyeing and soil remediation.

Various methods were used to screen the biosurfactant production abilities of six composter bacteria. During the screening of biosurfactant production, emulsification activity is one of the most critical methods that determine bio-emulsifier productivity (Bonilla *et al*., 2005). Among the isolates, *Bacillus nealsonii* 104ClB showed an acceptable emulsification index. Drop collapse and oil displacement methods are sensitive and relatively easy to perform, as they require a small quantity of samples and do not require specialized equipment. The drop collapse assay is based on the destabilization of liquid droplets by surfactants. *Bacillus nealsonii* 104ClB was positive for the drop collapse, and oil spread assay. Overall, it was confirmed that *Bacillus nealsonii* 104ClB is a potent biosurfactant-producing bacterium.

Structure analysis of biosurfactants using Fourier Transform Infrared (FT-IR) spectroscopy: Infrared spectroscopy (FT-IR) analyses of the desired surfactant are depicted in Figure 1. The characteristic band in the 3280 cm^{-1} regions indicates the presence of OH bonds in the sample. Absorption at about 2930 cm^{-1} is due to the symmetric stretching of CH bonds of CH_2 and CH_3 groups from aliphatic chains. The absorption band at 1730 cm^{-1} indicates the presence of a carbonyl bond (CO) in COOH. The carbonyl ester group in the 1622 cm^{-1} region is related to the bending vibrations of the CO bond, although other groups also have absorption in this region. Weak bands related to amide bonds of proteins and NH/CO combinations were observed at 1458 cm^{-1} and 1270 cm^{-1}, respectively. Additional bands in 1458 cm^{-1} and 1270 cm^{-1} more possibly belong to the diffusion of polypeptide impurities from cell debris that precipitates during the biosurfactant extraction process. The absorption band in 1138 cm^{-1} is due to the presence of polysaccharide or polysaccharide-like substances in the biosurfactant, and the absorption band in the 617 cm^{-1} region is related to the CH_2 group. Gartshore *et al*. (2000) reported that infrared spectroscopy is suitable for quantifying the concentration of most biosurfactants in a typical growth medium as a quick and straightforward technique.

Biosurfactant and Fe foliar application alter bean growth responses and yield: The interaction effects of ferrous sulfate × biosurfactant were significant on stem height, number of leaves, number of pods, number of flowers, leaf dry weight, stem dry weight, specific leaf area, and total dry weight (Table 3). The sole effects of experimental treatments, i.e., biosurfactant and ferrous sulfate treatment, were significant on stem height and the number of flowers. The main effect of ferrous sulfate treatment was statistically significant on the number of leaves, pods, and specific leaf areas. The individual biosurfactants effects were meaningful on leaf and stem dry weight and plant total dry weight.

A significant increase in bean seed yield was observed in all biosurfactant treatments over the control. Bean seed yield was 2 and 2.8 times higher with 0.01 and 0.02 % concentrations of iron treatment over control, respectively. The simultaneous use of Fe (zero, 0.01 and 0.02 %) and 50 mg L^{-1} biosurfactants increased bean seed yield by about 4, 3.3, and 4.2 times more than the control (Fig. 2).

Biosurfactant application without Fe improved leaf dry weight (82 %), stem dry weight (15 %), total dry weight (56 %), specific leaf area (42 %), plant height (110 %), number of leaves (5.8 %), number of pods (300 %), number of flowers (200 %), and number of seeds per pod (450 %) as compared with control (Fig. 3). Leaf dry weight, total dry weight, and specific leaf area were increased in response to Fe foliar treatment (Fig. 3a, b, and d). Stem dry weight, plant height, the number of leaves, and the number of pods were increased by 0.01 % Fe treatment. The number of leaves decreased by Fe (0.02 %) in compared with the Fe 0 treatment. The number of flowers and seeds per pod was increased by Fe application in respect to non Fe treatment (Fig. 3 h, i).

The simultaneous effects of Fe and biosurfactant on

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Table 2. Six bacterial species which were selected in red blood hemolysis, oil spreading, drop collapse, emulsification index, and surface tension reduction assay

Bacterial species	Hemolysis (mm)	Oil spreading (mm)	Drop collapse	Emulsification index (%)	Surface tension
Control (MMS cell-free medium)	-	-	-	-	27.8
P. validus IVC	0.5	18	+	11	24.7
*P. koreensis*12C	0.3	20	+	19	21.02
*P. thailandensis*13C	0.4	12	+	21	24.7
P. cellulositrophicus 47YZ	0	2	-	12	24.7
*B. nealsonii*104C	0.2	30	+	27	32.01
*P. lautus*151VC	0.4	10	+	20	27.5

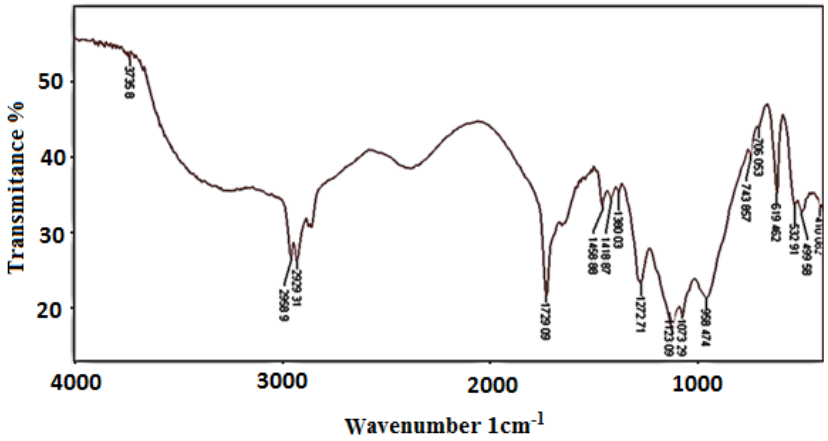


Figure 1. FTIR spectrum of the biosurfactant produced by *Bacillus nealsonii* 104ClB

Table 3. Analysis of variance (ANOVA) for the effects of biosurfactant and Fe floiar use on physiological traits of bean

Source of Variation	Df	Mean of Square								
		Fresh weight	Dry weigh	Stem dry weight	Leaf dry weight	Leaf number	Leaf area	Leaf Specific Surface	Leaf Area Index	Time to Leaf formation
Iron (A)	2	3.35 ^{ns}	0.031^{ns}	0.006 ^{ns}	0.02 ^{ns}	1.08 **	13852^{ns}	170727**	0.086 ^{ns}	0.24 ^{ns}
Biosurfactant (B)	1	2.71 ^{ns}	0.279*	0.020 *	0.14 **	0.00 ^{ns}	4392 ^{ns}	7667 ^{ns}	0.027 ^{ns}	0.50 ^{ns}
(A) × (B)	2	0.88 ^{ns}	0.223*	0.044 **	0.09 **	7.75 **	23284^{ns}	193273**	0.145 ^{ns}	0.17 ^{ns}
Error	12	2.62	0.034	0.004	0.007	0.04	15845	22786	0.09	0.30
CV (%)		14.11	11.8	15.5	10.5	2.5	17.27	17.08	17.27	2.23

Asterisks indicate significant differences according to ANOVA at P < 0.05* and P < 0.01**.

Continued of table 3.

Source of Variation	Df	Mean of Square								
		Stem length	Tissue water content (%)	Flower number	Number of seeds per pod	Seed weight	Number of pods	Plant yield	Time to Flowering	Time to Podding
Iron (A)	2	1098**	7.74^{ns}	1.75**	5.01*	0.01^{ns}	1.08**	0.21**	5.86**	35.93**
Biosurfactant (B)	1	3333**	13.70^{ns}	1.33**	6.18*	0.01^{ns}	0.33^{ns}	0.87**	6.54**	20.04**
(A) × (B)	2	5198**	7.92^{ns}	1.58**	7.90*	0.01^{ns}	1.08**	0.091**	5.53**	33.92**
Error	12	111.2	4.79	0.02	9.85	0.01	0.09	0.01	0.1	0.01
CV (%)		11.2	2.47	6.7	29.11	49.64	14	14.1	0.2	0.9

Asterisks indicate significant differences according to ANOVA at P < 0.05* and P < 0.01**.

the bean growth and yield-related traits were Fe concentration-dependent. Biosurfactant with 0.01 % Fe, decreased leaf dry weight, plant height, and the number of leaves (Fig. 3 a, d, e, and f) and increased the number of seeds per pod. The treatment did not affect the stem dry weight, total dry weight, leaf specific area, number of pods, and number of flowers. Leaf, stem and total dry weight, plant height, and the number of leaves were increased by the simultaneous use of biosurfactant and 0.02 % Fe (Fig. 3 a, b, c, e, and f). The number of seeds per pod and specific leaf area were increased with biosurfactant addition to zero and 0.001 % Fe treatment but decreased at 0.002 % Fe solution at 0.002 % with respect to their controls (Fig. 3 d and i).

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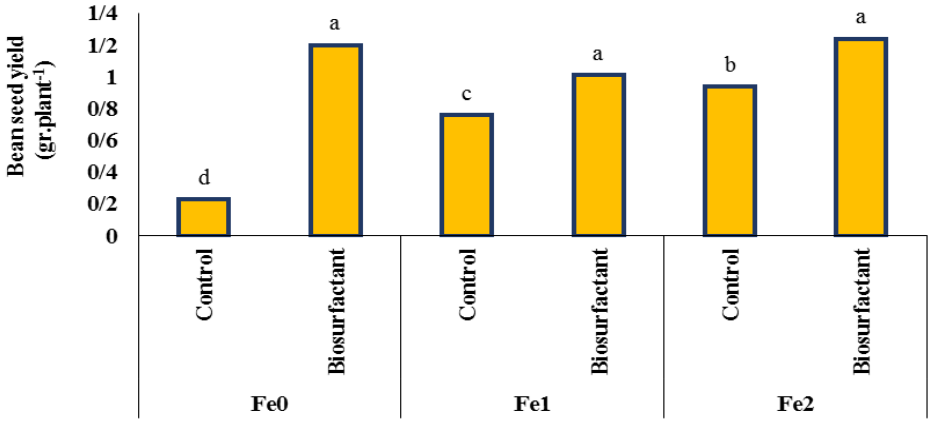


Figure 2. Dependence of bean seed yield on Iron and biosurfactant treatments. Bean seedlings were sprayed with three levels of iron, Fe0 (zero), Fe1 (0.01%), and Fe2 (0.02%) without or with 50 mgL^{-1} biosurfactants. Different letters indicate a difference according to Duncan's test (P < 0.05).

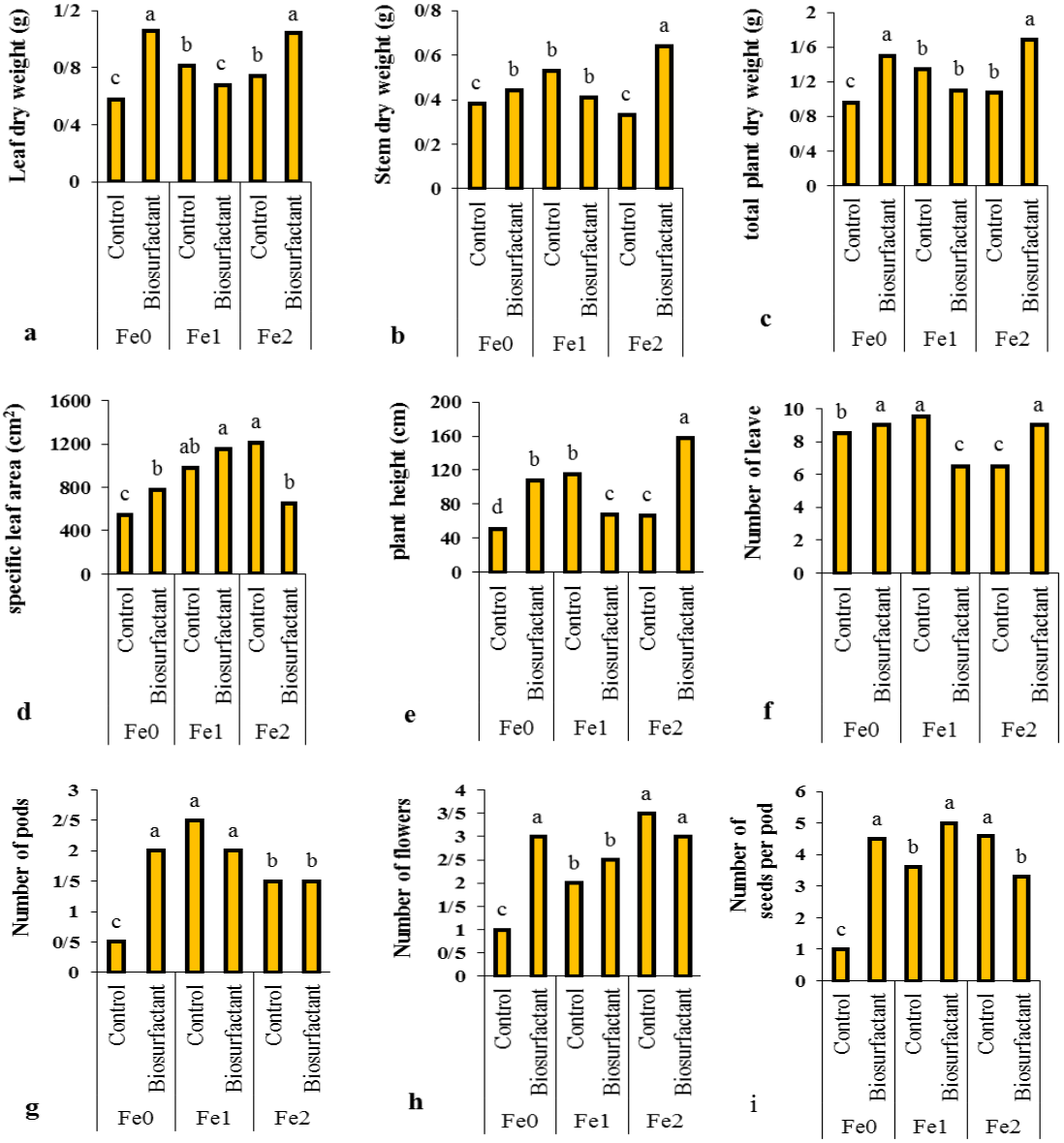


Figure 3. Dependence of some bean traits on Iron and biosurfactant treatments. Bean seedlings were sprayed with three levels of iron, Fe0 (zero), Fe1 (0.01%), and Fe2 (0.02%), without or with 50 mg L^{-1} biosurfactants. Different letters indicate a difference according to Duncan's test (P < 0.05).

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Foliar application of micronutrients, especially iron, can increase crop yield by improving carbon uptake and production and transport of photosynthetic assimilates, stimulating metabolite synthesis, and maintaining water status in plant tissue (Sharma *et al*., 2019). The leaves' dry weight and specific leaf area were increased in all treatments compared to the control (fig3. a, d). Therefore, the photosynthetic potential has been increased, and possibly access to carbon sources for yield improvement has been facilitated. However, the number of leaves did not follow a regular trend, so in some treatments, this trend was increasing, and in others, it followed a decreasing pattern (Table 2). In this study, foliar application of ferrous sulfate with biosurfactant and a 0.02 % Fe treatment increased the number of flowers per bean plant. This can be considered another factor in enhancing the yield potential). Iron deficiency can reduce plant yield by diminishing the number of flowers, pods, or fruits. It has been reported that, on average, iron deficiency can reduce yield by up to 50 % (Sharma *et al*., 2019).

Bean phenology is affected by iron and biosurfactant spraying on leaves: Iron and biosurfactant interactions meaningfully (Table 1) enhanced the bean seedling transition to the flowering and podding phases about four and eight days earlier than the control treatment (Fig. 4).

It is already known that biosurfactants have the potential to enhance metal elements bioavailability in soils compared to synthetic surfactants (Mulligan *et al*., 1989). But there are very few published reports on the effect of foliar application of biosurfactants on agricultural products, and therefore more detailed studies are required to evaluate their potential.

Velioglu and Urek (2015) reported the positive impact of biosurfactant produced by *Bacillus pumilus* 2A on the germination and seedling growth of *Sorghum saccharatum*, *Sinapis alba*, and *Lepidium sativum* that were sown in soils contaminated with hydrocarbons. Marchut-Mikolajczyk *et al*. (2021), using 0.2 % of biosurfactant derived from *Bacillus pumilus* 2A, showed a 4-, 4-, and 2-times higher growth potential for bean, radish, and beetroot, respectively. It was reported that rhamnolipid biosurfactant could act as a plant stimulator by inducing the genes involved in the defense system of plants such as grapevine, cress, cherry tomato, and rapeseed (de Vasconcelos *et al*., 2020). Other research showed that biosurfactants could also induce the biosynthesis of hormones responsible for signaling pathways involved in plant immunity (Chong and Li, 2017).

Liu *et al*. (2016) showed that rhamnolipid biosurfactants increased the penetration of glyphosate herbicide from the cuticular layer of the leaf surface. In addition, the longevity of the herbicide suspension on the leaf surface was significantly increased compared to the control treatment. Therefore, increasing the infiltration of iron in these treatments is one of the possible reasons for enhancing the yield and justifies the differences between the treatments containing biosurfactants and those without. In addition, the biosurfactant itself acted as a factor in increasing bean growth in the current study. There are similar reports of improved performance due to the use of biosurfactants. Marchut-Mikolajczyk *et al*. (2021) showed the addition of purified biosurfactant of *Bacillus pumilus* 2A at a concentration of 0.2 % to the growing medium of the bean, radish, and sugar beet plants increased the weight of 18-day-old seedlings by about four, four, and twice as much, respectively. Studies on the effect of biosurfactants on plant growth are limited to soils contaminated with petroleum hydrocarbons. Therefore, research on the mechanism of action of these compounds on plant growth under normal conditions is minimal. However, in the soil, it is assumed that microbial surfactants may indirectly enhance plant growth by increasing the bioavailability of hydrophobic compounds for rhizosphere-dwelling microorganisms. Higher concentrations may be partially dangerous to the plant root tissue and reduce plant growth (Sachdev and Swaranjit, 2013). Therefore, due to insufficient information, it is necessary to conduct detailed research on the physiology of biosurfactants' effects through foliar applications. Adnan *et al*. (2018) isolated the endophytic fungal strain *Xylaria regalis* from the cones of *Thuja plicata* and investigated its biosurfactants production ability to assess its role as a potential plant growth promotion agent. They reported that the treatment of chili seeds with biosurfactant-producing endophytic fungi, *X. regalis*, significantly improved seedlings' germination and growth. The inoculation of *X. regalis* increased shoot length, root length, dry matter production of shoot and root, chlorophyll, N, and P content of chili seedlings compared to control.

Iron concentration in the bean leaves: Finally, it was found that the Fe concentration in bean leaves is affected by both treatments. (Fig. 5). The leaves Fe content was dependent upon Fe concentration, and the highest content was obtained in a 0.002 % treatment without using the biosurfactant. However, the application of the biosurfactant increased the Fe content at 0.001 % in the treatment compared to the control. The leaves Fe content at 0.001 % treatment + biosurfactant was more than 0.002 % Fe treatment. One of the possible reasons for this event could be the dilution effect. As we showed in figures 2 and 3, this treatment had the most yield-related trait data, so the available Fe in the leaves was consumed in other organs, and the leaves Fe content decreased correspondingly.

As a similar result, Zhiqian (2004) investigated the effect of 0.05 % Lutensol AO5 surfactant and different concentrations of 2,4-D on bean plants as a foliar application. He showed that at 0.05, 0.2, 1, and 2.5 % concentrations of 2,4-D, respectively, 92, 84, 65 and, 56 foliar uptake tacks were placed. With the good emulsification index and oil spreading traits of *Bacillus nealsonii* 104C derived biosurfactant as we reported in table 2, its use in Fe foliar application increased the Fe

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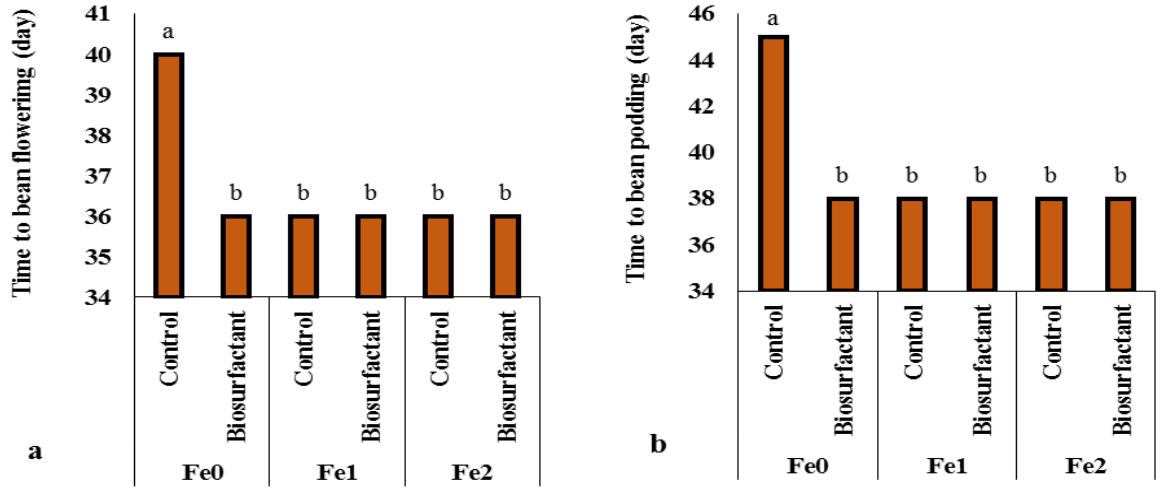


Figure 4. Effects of Iron and biosurfactant treatments on the time of flowering and podding of bean plants. Bean seedlings were sprayed with three levels of iron, Fe0 (zero), Fe1 (0.01%), and Fe2 (0.02%) without or with 50 mg L^{-1} biosurfactant. Different letters indicate a difference according to Duncan's test (P < 0.05).

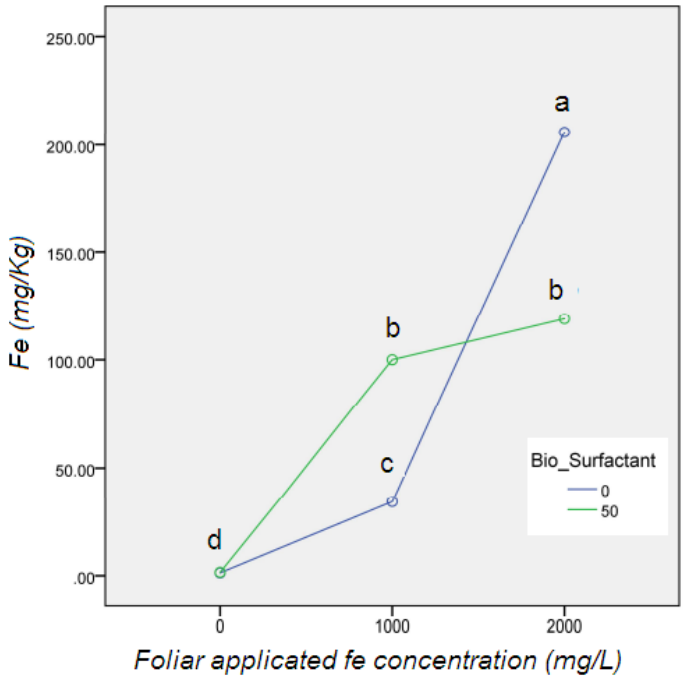


Figure 5. Effects of Iron and biosurfactant treatments on the Fe concentration of leaves in bean plants. Bean seedlings were sprayed with three levels of iron, Fe0 (zero), Fe1 (0.01%) and Fe2 (0.02%) without or with 50 mg L^{-1} biosurfactants. Different letters indicate a difference according to Duncan's test (P < 0.05).

absorption by bean leaves.

Conclusion

All in all, in agriculture, bio-surfactants have often been considered pesticides or leaf penetration facilitators of herbicides via foliar applications. They also act as mediators in increasing the bioavailability of nutrients in contaminated soils with petroleum hydrocarbons and heavy metals. However, their growth-stimulating effects on plant seed germination have been studied. However, there are a few studies on their impact on the growth stages of plants. Here, we showed that biosurfactants alter bean growth characteristics. It would be advisable that the glycolipid biosurfactant-derived bacteria be a promising material for promoting plant growth and improving the microelements acquisition via foliar application. So, we strongly recommend exploring new biosurfactants and their application efficiency on different plants as foliar applications. The action and effectiveness of biosurfactants on plant growth are still unknown. There are some reports on the hormonal-like effects of biosurfactants as plant growth regulations. However, due to the structural diversity of biosurfactants, detailed studies are needed to fully discover their effects on plant growth and productivity.

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Disclosure statement

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