

PRODUCTION AND EVALUATION OF A *MORINGA OLEIFERA* LIQUID BIOFERTILIZER TO IMPROVE SEEDLING GROWTH OF THREE CROP PLANTS

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Abstract. The growing demand for food due to the increase in the world's population has led to an increased demand for chemical fertilizers of industrial origin and improper management of crops, causing adverse effects on the environment and soil. In this research, the effect of *Moringa oleifera* as a biofertilizer on morphometric variables (seed germination, root, and stem elongation) was evaluated in lettuce (*Lactuca sativa*), corn (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.), for each species were exposed to 5 dilutions of the aqueous solution of the biofertilizer. The highest percentage of germination was observed at a concentration of 12.5% of the biofertilizer in the three species, however, no significant differences were observed between treatment and species ($p > 0.05$). Likewise, greater growth in stem and root length was observed at a concentration of 12.5% in the 3 species, showing significant differences between species and treatment ($p < 0.05$). On the other hand, it was generally observed that increasing the concentration of the biofertilizer inhibits the growth of the stem and root. The results obtained in this study could contribute as a reference to future chronic studies of exposure to biofertilizers using lettuce, corn, and beans considering other types of bioindicators to be evaluated in the plant, at different concentrations of exposure. This is to contribute to the use of biofertilizers in commercially important sustainable agricultural plantations and thus be able to prevent or mitigate environmental impacts in the short, medium, and long term on soils and crops, as well as the reduction or replacement of the use of inorganic fertilizers of industrial origin and the promotion of the use of biofertilizers.

Keywords: nutrients, corn, *Phaseolus vulgaris*, morphometry, germination

Introduction

In recent decades, the need to meet the growing demand for food due to the increase in population has led to the demand for the consumption of agricultural products, where the objective of producers is to achieve high crop yields per unit area, without

considering the sustainability of production (feasibility, technique, profitability, and environmental damage) (Grageda-Cabrera et al., 2012). In this quest to improve the production rate, elemental nutrients, such as phosphorus, potassium, and nitrogen were supplied to soils by the application of chemical fertilizers (Navarro-García, 2023). Fertilizers increase crop yields, however, it has been observed that the constant use of chemical-based fertilizers affects the quality of soils and poses a health risk to consumers (Calderón et al., 2019). Therefore, producers currently prefer to change production techniques towards green agricultural practices with the use of new biotechnologies, such as the use of biofertilizers. (Deepika and MubarakAli, 2020). Biofertilizers are organic fertilizers that can be composed of living microorganisms, organic debris (plant debris and animal waste), and plant extracts (Armenta Bojórquez et al., 2010; Emongor, 2015). The latter is used as plant growth regulators, as they positively modify plant growth by improving the efficiency of photosynthesis (Pérez-Gómez et al., 2019).

In this sense, one of the plants with the greatest potential for the manufacture of plant extracts is *Moringa oleifera* Lam (syn. *M. pterygosperma* Gaertn). This plant is one of the best-known and widely studied species around the world, due to its versatile usefulness as food, nutraceutical, medicinal uses, and pharmacological properties. (Anwar et al., 2007). By the Phytochemical Analysis of *M. oleifera* researchers from different countries have shown that the leaves are particularly rich in potassium, calcium, phosphorus, iron, vitamin D, essential amino acids, as well as known antioxidants such as carotene, vitamin C, and flavonoids (Al_husnan and Alkahani, 2016). For this reason, various medicinal properties have been attributed to almost all parts of the plant: root, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil (Anwar et al., 2007). Antihypertensive, diuretic, and cholesterol-lowering activity has been reported, due to the combination of diuretic, lipid-lower, and hypotensive components that make the plant very useful against cardiovascular disorders (Patel et al., 2010). Similarly, it has been reported that the roots of *M. oleifera* They antispasmodic, antiulcer and hepatoprotective activity (Anwar et al., 2007; Pérez-Gómez et al., 2019). In addition, ethanol extract and its leaf components have been found to have antispasmodic effects, possibly through blockade of calcium channels. (Cáceres et al., 1992). Finally, it has been shown that *M. oleifera* has an antimicrobial effect on pathogenic bacteria, attributed to antimicrobial peptides and bioactive compounds (Tirado-Torres et al., 2019)

Biotechnology offers alternatives to meet the growing demand for food, through biofertilizers that provide plants with growth regulation to positively modify plant growth and improve yields per hectare. (Emongor, 2015). Therefore, *M. oleifera* appears to be an ideal candidate for the manufacture of extracts for plant breeding of agricultural production due to the active substances it contains. (Olson, 2011). Moringa leaf extract has been reported to contain, zeatin, dihydrozeatin, and isopentyladenine, which are natural cytokinins that stimulate cell division, tissue growth, slow senescence, and aging processes in many plant tissues, promote nutrient partitioning and absorption (Anwar et al., 2007; Cáceres et al., 1992; Emongor, 2015). The use of Moringa extracts as a potential plant growth enhancer has the potential to provide an environmentally safe, easily accessible means of increasing crop yields and meeting the exponentially growing demand for food. The use of biofertilizers, which enhance plant growth, could improve production and yield conditions in agriculture and have significant economic advantages. Therefore, the objective of this study was to evaluate the germination and

growth of agriculturally important seedlings such as beans (*Phaseolus vulgaris*), lettuce (*Lactuca sativa*), and maize (*Zea mays*) through the use of the aqueous extract of *Moringa oleifera* Lam) as a biofertilizer at different concentrations.

Materials and methods

Experimental design with a liquid biofertilizer from Moringa oleifera

The experiment was conducted at the Faculty of Chemical-Biological Sciences of the Autonomous University of Campeche, Mexico. The sowing of the lettuce seeds and the analysis of the samples were carried out in February 2022.

Lettuce (*Lactuca sativa*), corn (*Zea mays* L.), and bean (*Phaseolus vulgaris* L.) seeds were used to perform the bioassay. For this bioassay, 1 liter of stock solution of 3 g L⁻¹ (w/v) of powdered dried leaves of *Moringa oleifera* (Therbal ®) was prepared by infusion with distilled water. The nutritional information of *Moringa oleifera* leaves is shown in Table 1.

Table 1. Nutrition information and average concentration (\pm standard deviation) of nutrients per 100 g dry weight of *Moringa oleifera* leaves

Nutritional content	Concentration/100 g
Energy	304 \pm 47 kcal
Humidity	7.4 \pm 2.0 mg
Protein	22 \pm 5 g
Carbohydrates	28 \pm 9.2 g
Grease	7 \pm 2.5 g
Fibre	9 \pm 7.45 g
Tannins	1.2 \pm 1.4 g
Ca	1890 \pm 748.4 mg
P	298 \pm 149.0 mg
Na	220 \pm 180.0 mg
K	1467 \pm 636.7 mg
Mg	473 \pm 429.4 mg
Fe	32.5 \pm 10.78 mg
Zn	2.0 \pm 1.10 mg
Cu	0.95 \pm 0.40 mg

The analysis of the concentration of nutrients in the leaves of *Moringa oleifera* was carried out according to the Mexican Official Standard (NOM-086-SSA1-1994).

To determine the effect of *Moringa oleifera* as a biofertilizer, 5 treatments were carried out for each species of agricultural importance with concentrations of 12.5%, 25%, 50%, 75%, and 100%, which were obtained from successive dilutions in triplicate and for the negative control of germination and growth, distilled water was used, for a total of 48 experimental units. For each unit and treatment, 15 seeds of each species were placed in a 100 mm diameter polyethylene container (Petri dish), with filter paper (Whatman® No. 3) at the bottom as a support. Subsequently, 15 ml of each of the concentrations of the aqueous solution were applied, 3 times a day. All units were kept at a controlled room temperature of 29°C \pm 1 for 240 h (10 days). At the end of the

exposure period, the number of germinated seeds was counted and the length of the root and stem was measured. The mean and standard deviation of radicle length and hypocotyls were determined as response variables, in the negative control and each treatment (exposure concentrations). In addition, the germination percentage (%G) of the seeds for each concentration concerning negative control was determined according to the equation of Chan Keb et al. (2018) (Eq. 1).

$$\% G = \frac{\text{No. of seeds sprouted at each concentration}}{\text{No. of seeds sprouted in control}} \times 100 \quad (\text{Eq.1})$$

Statistical analysis

To evaluate the effect of *Moringa oleifera* as a biofertilizer on morphometric variables (seed germination, root, and stem elongation) in lettuce (*Lactuca sativa*), corn (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), were compared between the five treatments and the exposed control, to determine the variation or interaction of these two factors, applying a two-way ANOVA. Before this comparison analysis, the normality of the variables was validated with the method of Shapiro and Wilks (1965), with a significance level $\alpha = 0.05$, as it did not meet the assumption of normal distribution, the data were transformed using the Box-Cox method, so that the variables presented the assumptions of normal distribution (Zar, 2010). *Post-hoc* analysis was realized with least significant difference (LSD) Fisher test. All statistical analyses were performed using STATISTICA V.12 (©Copyright StatSoft, Inc., Palo Alto, CA, USA, 1984–2014).

Results

Figure 1 shows the data on the percentage of germination of the seeds of lettuce (*Lactuca sativa*), corn (*Zea mays* L.), and beans (*Phaseolus vulgaris* L.) concerning the negative control of distilled water. The seeds of the germinated species in the negative control were lower than the exposed treatments, however, statistically, no significant differences were observed ($p \geq 0.05$, Table 2). The highest amount of germinated seeds of the three species was observed at concentration of 12.5% and the lowest amount was observed in *Lactuca sativa* at a concentration of 25% (Fig. 1).

Exposure to different concentrations of the liquid biofertilizer made from *Moringa oleifera* leaves showed variation in the development of root length and hypocotyls of lettuce, beans, and corn (Fig. 2A, B). A 2-way ANOVA showed significant differences between species and exposure concentrations in the average root length and hypocotyl development of *Lactuca sativa*, beans, and maize (Tables 2 and 3). From the above, considering all the concentrations of the liquid biofertilizer made from *Moringa oleifera* exposed, greater root growth was observed in the corn seedlings, compared to the root of the bean and lettuce seedlings (Fig. 2A; Table 3); it was also observed that in the treatments to which the 3 species were exposed, the highest root growth was presented at a concentration of 12.5% of the biofertilizer (Fig. 3).

Figure 2B shows the effect of growth on the stem of the 3 species exposed to the different concentrations of the biofertilizer, and it was also observed that the greatest stem growth occurred in the bean seedlings, compared to the stem length of the corn and lettuce seedlings. However, it was also observed in the treatments to which the 3 species were exposed the highest stem growth at a concentration of 12.5% of the biofertilizer (Fig. 3).

Table 2. Two-way analysis of variance concerning germination, root length, and hypocotyls of *Lactuca sativa* corn and beans between concentrations of the biofertilizer (Control, 12.5%, 25%, 50%, 75%, and 100%), with a significance level of $p < 0.05$

Parameter	Factor	df	F	p
Germination (%)	Treatment	5	9.97	0.0001
	Species	2	4.17	< 0.024
	Treatment*Especies	10	1.31	0.260
	Error	36		
Root length (cm)	Treatment	5	19.85	< 0.0001
	Species	2	987.73	< 0.0001
	Treatment*Especies	10	7.08	< 0.0001
	Error	36		
Hypocotyl length (cm)	Treatment	5	34.85	< 0.0001
	Species	2	1086.22	< 0.0001
	Treatment*Especies	10	5.93	< 0.0001
	Error	36		

Table 3. Post-hoc testing by the Fisher method (LSD), of the treatment and species interaction of germination, stem, and root length parameters

Treatment*Species	Germination (%)	Root length (cm)	Hypocotyl length (cm)
Control*Corn	95 ^{de}	13.5652 ^b	4.0783 ^{ef}
12.5*Corn	100 ^a	16.8913 ^a	6.3961 ^d
25*Corn	96.5 ^{cde}	12 ^c	3.7 ^{ef}
50*Corn	96.5 ^{cde}	12.3333 ^{bc}	3.9429 ^{ef}
75*Corn	95 ^{de}	10.3611 ^{de}	3.7222 ^{ef}
100*Corn	96.5 ^{cde}	11.3958 ^{cd}	2.7958 ^{fg}
Control*Beans	95.517 ^{de}	9.8393 ^e	16.2393 ^b
12.5*Beans	99.017 ^{ab}	11.2708 ^{cd}	21.3333 ^a
25*Beans	97 ^{bcd}	10.5185 ^{de}	15.9444 ^b
50*Beans	95.517 ^{de}	9.6364 ^{ef}	15.5455 ^b
75*Beans	98 ^{abc}	8.3478 ^{fg}	12.8043 ^c
100*Beans	98 ^{abc}	7.7273 ^g	12.9773 ^c
Control*Lettuce	95.167 ^{de}	1.3333 ^h	3.85 ^{ef}
12.5*Lettuce	98.936 ^{ab}	1.7083 ^h	5.1542 ^{de}
25*Lettuce	94.252 ^e	1.5714 ^h	4.1821 ^{ef}
50*Lettuce	95.619 ^{de}	1.1706 ^h	3.5588 ^f
75*Lettuce	94.85 ^{de}	1.5368 ^h	3.3421 ^f
100*Lettuce	96.205 ^{cde}	1.445 ^h	1.795 ^g

Means that do not share a letter are significantly different, with a 95% confidence level

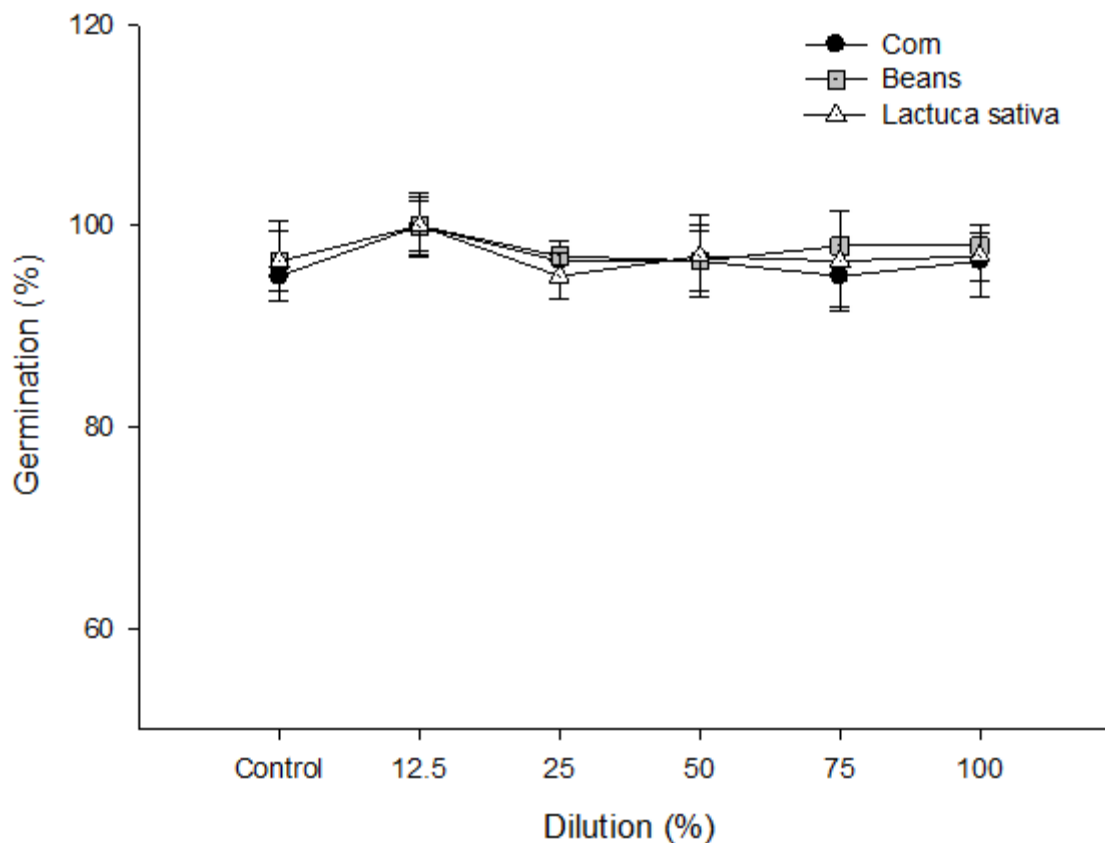


Figure 1. Average germination of seeds of lettuce (*Lactuca sativa*), corn (*Zea mays L.*), and bean (*Phaseolus vulgaris L.*) between the concentrations of the liquid biofertilizer made from *Moringa oleifera* leaves. The error bars represent the standard deviation

Discussion

In general terms, germination, root and stem length of *L. sativa*, *Z. mays*, and *P. vulgaris* showed variation concerning the control, due to the effect of the moringa biofertilizer to which they were exposed at different concentrations, which were absorbed through the roots. The results in the germination of the seeds of *L. sativa*, *Z. mays*, and *P. vulgaris* show that there was no effect of the biofertilizer at different concentrations, the highest percentage of germination was observed at a concentration of 12.5% of the biofertilizer in the three species, similar studies are reported by Deepika et al. (2020), where they studied the production and evaluation of a liquid microalgae fertilizer to improve the growth of four crop plants, the four cultivated species germinated approximately 100% and the highest growth in stem and root were obtained at 20% in the dilution of a biofertilizer made from microalgae.

For this study, the effect of increased root and stem growth of *L. sativa*, *Z. mays*, and *P. vulgaris* was greatest at concentrations of 12.5% of the exposed liquid biofertilizer. In this regard, studies carried out by Pérez-Gómez et al. (2019), determined that the aqueous extract of moringa leaves favored the initial stage of acclimatization of pineapple, this is because *Moringa oleifera* Lam. Leaves have several chemical constituents such as amino acids, mineral ions, ascorbate, phytohormones, and secondary metabolites. This means that its extracts are used to boost the growth of some plants.

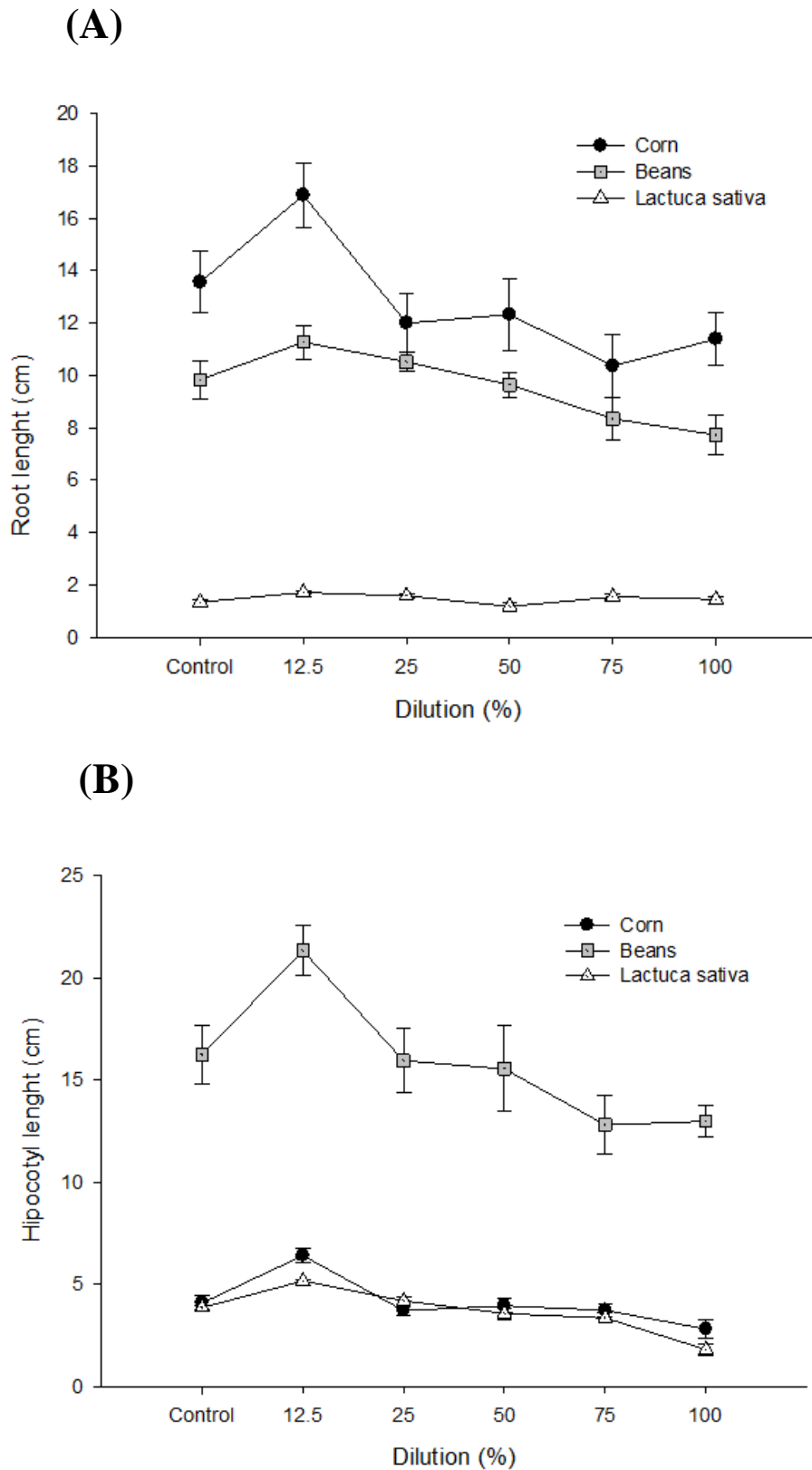


Figure 2. Average growth in (A) root length; (B) length of the hypocotyl of Lettuce (*Lactuca sativa*), corn (*Zea mays* L.), and bean (*Phaseolus vulgaris* L.) between the concentrations of the liquid biofertilizer made from *Moringa oleifera* leaves. The error bars represent the standard deviation

Crops Plants	Exposure concentrations of <i>Moringa oleifera</i> liquid biofertilizer					
	Control (0%)	12.5 %	25 %	50 %	75 %	100 %
(a)						
(b)						
(c)						

Figure 3. Crop plant growth benchmarking: bean (*Phaseolus vulgaris* L.) (a), maize (*Zea mays* L.) (b) and lettuce (*Lactuca sativa*) (c), at different concentrations of the liquid biofertilizer made from *Moringa oleifera* leaves after 10 days

Also, for this study the effects of biofertilizer on root and stem length decrease in *L. sativa*, *Z. mays*, and *P. vulgaris*, was observed at the concentration of 75 to 100%, the effect of decrease was evident in roots and stem length in corn and lettuce. Therefore, we describe that the root was more sensitive to the aqueous solutions of the biofertilizer, due to the direct contact in the absorption of the components of the biofertilizer, generating a physiological stress effect. On the other hand, the absorption of the biofertilizer that is available in aqueous solution, is through the root system, as the main route for transfer and direct contact, where the absorption of nutrients and water is also regulated in the three species of agricultural importance, that is, the morphology, anatomy and biomass production of the roots which is also associated with the effect on the absorption of biofertilizer (Rodríguez-Ruiz et al., 2015), as observed in this study. In this regard, Gómez-Oliván et al. (2014) mention that the presence of high concentrations of isolated inorganic compounds in the soil can induce oxidative stress in plants and influence their antioxidant defenses leading to different responses (phytotoxic effects), this will depend on the type of substance, its concentration and the sensitivity of the plant species (Carvalho et al., 2014; Marsoni et al., 2014; Minden et al., 2017). Authors such as Biruk et al. (2017) found that lettuce seeds were more sensitive to extracts containing inorganic elements than inorganic extracts. On the other hand, Christou et al. (2019) mention that the bioaccumulation of pollutants in plant tissues depends on the type of soil, the

physicochemical properties of the pollutants, and the interactions of these factors, as well as the type of species. Based on the results obtained from this research, more future studies can be carried out on the evaluation of other types of biofertilizer using lettuce, corn, and beans considering other types of biochemical indicators of the plant to be evaluated, different concentrations of the biofertilizer or similar to those used in this study, to contribute to the use of sustainable biofertilizers in agricultural plantations of commercial importance and thus be able to prevent or mitigate short-, medium- and long-term environmental impacts on soils and crops. As well as the reduction or replacement of the use of inorganic fertilizers of industrial origin.

Conclusions

The present study, it showed high percentages of seed germination in all exposed crops that had been treated with liquid biofertilizer made from *Moringa oleifera* leaves at different concentrations (0%, 12.5%, 25%, 50%, 75%, and 100%), and no significant differences were observed between the species and treatment in terms of germination. The growth of root and stem length in the 3 exposed species showed a maximum growth in the concentration of 12.5% of the liquid biofertilizer made from the leaves of *Moringa oleifera*, however, the liquid biofertilizer in a higher concentration inhibits the growth of the stem and root. Based on the results of this research, it shows that the use of biofertilizers is viable for sustainable agricultural practices. Likewise, the application of *Moringa oleifera*-based liquid fertilizer, could be useful to enrich the soil and achieve a high yield in agricultural production. *Moringa oleifera* biofertilizer applied to 12.5% in the cultivation of the 3 species showed better results in growth parameters. This may be due to the high nutrient content contained in the leaves of *Moringa oleifera*, which is also commonly known as the tree of life, due to its high benefits to human health and the high nutrient content it presents. In this way, *Moringa oleifera*-based liquid fertilizer could be applied to various crop plants to enrich the nutrients in the soil and, in turn, increase growth and improve agricultural production.

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