

Influence of Foliar Applications of Micronutrients and Biofertilizers on Plant Growth and Fruit Yield of the Sponge Gourd (*Luffa cylindrica* L.)

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ABSTRACT

This research aimed to explore the effects of three micronutrients (iron, zinc, and manganese) and two biofertilizers (Nitroxin and Phosphate Barvar2) on the growth and yield of sponge gourd (*Luffa cylindrica* L.) at Taleb-Abad experimental station in Anzali Port City, Guilan Province, Iran, between 2019 and 2021. The experiment performed under a factorial design with randomized complete blocks and three replications, resulting in 16 treatment combinations. These combinations included two levels of inoculation and control for both biofertilizers, and foliar sprays of Fe ($\text{FeSO}_4 \cdot x\text{H}_2\text{O}$), Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and Mn ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$) at concentrations of 600, 400, and 500 mg l^{-1} respectively, 30 days after seedling transplanting. Parameters such as fruit length, diameter, weight, yield per plant, yield per hectare, fruit set percentage, flower ratio, and fruits per plant were measured. Results showed that both biofertilizers significantly increased yield, Nitroxin had more pronounced effects than Phosphate Barvar2 and individual micronutrients. The highest sponge gourd yield (18032 kg ha^{-1}), fruit weight per plant (199.23 g), fruit number per plant (12.50) and fruit length (49.50 cm) were achieved from the combination of Nitroxin, Phosphate Barvar2, and iron, compared to 72, 48.17, 58 and 61.61% reduction respectively in control. Micronutrient applications significantly enhanced growth and yield, with iron being the most effective, followed by zinc and manganese. The experiment showed a single Fe, Zn and Mn micronutrients application at concentrations of 600, 400 and 500 mg l^{-1} notably improved fruit yield per plant by 28, 21 and 19% respectively, compared to the control.

Keywords: *Luffa cylindrica*, Micronutrients, Organic fertilizers, Yield

INTRODUCTION

Sponge gourd (*Luffa cylindrica* L.), a climbing annual plant of the Cucurbitaceae family, is widespread in tropical and subtropical regions [1,2]. It thrives in moderate areas with hot summers and frost-free periods, and an optimal pH of 6-6.8 [3-7]. Sponge gourd is known for its medicinal and nutritional properties, serving as a source of carbohydrates, vitamins, minerals, and vegetable protein [8-11]. The leaves, seeds, and fruits of the sponge gourd are utilized for their antimicrobial, anti-inflammatory, antifungal, antiparasitic, analgesic, antidiabetic, antiprotozoan, and antioxidant properties [12-18].

The sponge gourd is a monoecious species which the overall yield is largely influenced by the quantity of female flowers produced [19]. The fundamental principle behind sexual modification in cucurbits involves manipulating the flowering sequence and adjusting the sex ratio, which typically ranges from 25:1 to 15:1 in favor of male flowers [20-22].

The optimal growth and development and the sex ratio of the sponge gourd depend on essential elements, categorized as macronutrients and micronutrients based on their required concentrations [23]. Although micronutrients are needed in smaller amounts, they are as important as macronutrients for plant growth and yield [24]. Deficiencies in any of these elements or imbalances among them can lead to growth suppression or complete cessation of growth [25]. Micronutrients serve multiple vital functions within plants, including acting as cofactors in enzymatic systems and participating in redox reactions [26]. Notably, they are integral to key physiological processes such as photosynthesis and respiration [27]. A lack of these micronutrients can impede these essential functions, ultimately constraining potential yield improvements [28]. Furthermore, the concentration and the method of application can substantially affect the sponge gourd growth, yield of fruit, sex expression and flowering patterns [29]. These factors can lead to either a reduction in the number of male flowers or an increase in the production of female flowers, ultimately influencing the overall yield [30].

Iron is vital for metabolic processes such as photosynthesis, respiration, and DNA synthesis in plants [31]. It also plays key role in biological tasks as a component of important enzymes [32]. Zinc is essential for chloroplast enzyme systems and carbohydrate production, with deficiencies leading to amino acid decay and reduced growth [33-35]. Manganese is involved in photosynthetic proteins and enzymes, affecting various processes including enzyme activity and nutrient utilization [36]. Fertilizers, especially inorganic, are used to boost plant productivity and yield. However, while mineral fertilizers increase production, they can also cause environmental and health issues [37]. Sustainable agriculture practices aim to reduce environmental pollution, making the use of biofertilizers important [38]. Unlike chemical fertilizers, biofertilizers can fix nitrogen, solubilize inorganic phosphorus, and release phytohormones to stimulate plant growth. Studies show biofertilizers

enhance growth, essential oils, and chemical composition in medicinal plants, making them a viable alternative to inorganic fertilizers [39].

This research aimed to assess the yield and components of sponge gourd when treated with foliar applications of micronutrients (Fe, Zn, and Mn) and biofertilizers (Nitroxin and Phosphate Barvar2), either individually or in combination, in northern Iran.

MATERIALS AND METHODS

This study was conducted at Taleb-Abad experimental station in Anzali Port City, Guilan Province, Iran, from 2019 to 2021. The climate of experimental site is very humid, with an average annual temperature of 16.2°C and rainfall of 1819 mm.

Table 1 Experimental soil physico-chemical characteristics in Taleb Abad Experimental Station

Location	Latitude	Longitude	Elevation (m)	O.C. (%)	T.N. (%)	Total N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	EC (ds m ⁻¹)	pH	Soil Texture
Taleb Abad Experimental Station	37°28'N	49°27'E	-22	2.2	22.95	700	12.57	102.45	0.6	6.09	Clay loam,

The experiment was a factorial design with three factors in a randomized complete block with three replicated. Treatments included two levels of Nitroxin and Phosphate Barvar2 biofertilizers (inoculated and non-inoculated) and four levels of micronutrients (control, iron, zinc, manganese). Sponge gourd seeds were prepared from the local agricultural market in Anzali Port City, northern Iran. Given that sponge gourd seeds germinate slowly [40], increasing their vulnerability to weeds [41] and soil pests [42], a nursery bed was prepared in the greenhouse at Shahed University agricultural college. To improve seedling establishment and facilitate planting, particularly for medium vigor seeds like sponge gourd, the seed coats were scraped, soaked in hot water (45-55°C) for 20 minutes, followed by warm water (30°C) for 24 hours before sowing [43]. On February 25th, 2019, seeds were sown in the greenhouse.

After six weeks, seedlings about 15 cm tall with 4-5 true leaves and healthy roots were selected for transplanting into experimental plots. Phosphate Barvar2 and Nitroxin biofertilizers separately and/or together were mixed in water solution and applied to the seedling roots for about 10 minutes in the shade before transplanting on April 25, 2020. Vertical trellising was used to reduce fruit damage and ease management.

Field preparation involved ploughing to a depth of 30 cm in September, 2019, followed by disc, rotavator, and leveling until May. The area of each plot was 15 square meters (5m×5m) with row spacing of 120 cm and 170 cm between rows, at a depth of 5 cm.

As the nutrient demand peak of annuals crops such as sponge gourd is at the phase of vegetative development, micronutrient foliar sprays of Fe (FeSO₄·xH₂O), Zn (ZnSO₄·7H₂O) and Mn (MnSO₄·H₂O) all as sulfate forms were applied 30 days post-transplant at concentrations of 600, 400, and 500 mg l⁻¹ respectively, in the early morning.

General fertilizer (NPK) requirements for sponge gourd suitable growth were 110 kg ha⁻¹ urea (46%N), 90 kg ha⁻¹ diammonium phosphate (46% P₂O₅ and 20% N), and 150 kg ha⁻¹ potassium sulfate (50% K₂O and 17.5% S). A 55 kilograms of urea per hectare, representing half of the total nitrogen requirement, along with the entirety of the phosphate and potash fertilizers, were applied prior to planting. The remaining nitrogen was subsequently applied as a side-dressing at the onset of plant growth. Irrigation was done immediately after sponge gourd transplanting with a 7-day interval, totaling 7-8 irrigations.

Sponge gourd growth being indeterminate, was harvested multiple times when the external coloration shifts from green to shades of brown or yellowish-brown, 120 days after transplanting. Harvesting on August 25, 2020, involved biometric measurements, including the number of fruits per plant, fruit length, fruit diameter, fruit weight, yield per plant, yield per hectare, 100 seed weight, number of seeds per fruit, male to female flower ratio, and fruit set percentage. These observations were collected from five randomly selected plants at the time of harvest in each treatment. The fresh fruit weight was measured for each individual fruit and for each sample plant in separate treatments. This involved weighing the fresh fruit collected from all sampled plants. The number of fruits per sample plant was recorded over a total of six observations by counting the fruits harvested from all sampled plants across these six harvests. Overall Following the completion of the harvest from the entire plot, the total weight of the fruits was recorded to assess yield. Subsequent calculations were conducted to determine the yield for each harvest, standardizing to a plot area of 5 m × 5 m, as well as converting the results to yield per hectare. To assess sexual expression by quantifying the number of male and female flowers per vine and calculating the resultant sex ratio. The differentiation between female and male flowers is characterized by the presence of a swollen ovary at the distal end of the stalk in female flowers. The data were subsequently analyzed statistically using SAS software [44], and mean comparison was performed using the LSD method.

RESULTS

Experimental results showed a 100% survival rate for transplanted sponge gourd seedlings. All measured traits were significantly affected by biofertilizers and micronutrients (Table 2). The analysis of variance revealed that the simple as well as the two- and three-way interactions among the three factors exhibited statistical significance across all traits (Table 2). Notably, the three-way interactions involving Nitroxin, Phosphate Barvar2, and micronutrients were found to be significant through the LSD test. Consequently, this significance led to the exclusion of consideration for the simple and two-way interactions. (Table 2). All the measured traits were uniformly improved by application of biofertilizers and micronutrients (Table 3).

Number of Fruits per Plant

The number of fruits per plant was significantly ($p \leq 0.01$) affected by the application of different biofertilizers and micronutrients (Table 1). The highest and lowest fruits numbers with values 12.5 and 5.25 were obtained in combination of (N, P and Fe), and control, respectively (Table 3). The sponge gourd treated with Fe fertilizer produced more fruits per plant (6.85) than Zn (6.35) and Mn (6.21) treatments. Results showed that Nitroxin biofertilizer produced more fruit per plant (7.15) than Phosphate Barvar2 (7.08) (Table 3).

Fruit Length (cm)

A significant increase was observed in fruit length after the Nitroxin, Phosphate Barvar2 and micronutrients application (Table 2). The sponge gourd size is important to luffs product manufacturers and determines what products can be made from a particular sponge. Fe, Zn and Mn significantly increased fruit length by 29, 24, and 23% respectively (Table 3). The highest sponge gourd fruit length (cm) was obtained in combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment (49 cm) compared with the lowest in control (19 cm) (Table 3).

Fruit Diameter (cm)

Fruit diameter (cm) mean by using Fe, Zn, Mn, Nitroxin and Phosphate Barvar2 fertilizers treatments were 13.4, 11.93, 9.14, 17.98 and 23.64% higher than control respectively (Table 3). The highest fruit diameter was obtained at combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment (13.05 cm) compared to the lowest value 7.75 cm in control (Table 3).

Fruit Weight (g)

Experimental results showed that micronutrients, Nitroxin and Phosphate Barvar2 fertilizers and interaction between them did have a significant influence on the sponge gourd fruit weight (Table 2). The greatest fruit weight (384.45 g) was noted in combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment compared with 199.23 in control (Table 3). There were 14, 9 and 4% increases in the fruit weight (g) by using Fe, Zn and Mn micronutrients compare with control (Table 3).

Fruit Yield per Plant

Results showed that the combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment produced maximum (3680 g) fruit yield per plant as compared to the lowest value of 1046 g in control (Table 3). There were 28, 21 and 19% increases in fruit yield per plant by using Fe, Zn and Mn micronutrients application, respectively. Nitroxin and Phosphate2 at single application had increased the fruit yield per plant by 38 and 32% respectively, compared to the control (Table 3).

Fruit Yield (kg ha⁻¹)

Interaction of Nitroxin, Phosphate Barvar2 and Fe fertilizers produced maximum (18032 kg ha⁻¹) total yield of sponge gourd as compared to control (5125 kg ha⁻¹). Whereas in the case of application of Fe, Zn and Mn micronutrients, the sponge gourd total yield was determined 7080, 6482 and 6301 kg ha⁻¹ respectively. Results showed that both Nitroxin and Phosphate Barvar2 biofertilizers produce more fruit yield than micronutrients fertilizer (Table 3).

Seed Number per Fruit & 100 Seed Weight (g)

The biggest seed per fruit was obtained in both micronutrients and biofertilizer treatments compared with control (Table 3). The highest (474.13) seed number per fruit was obtained at the Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment and the lowest (265) at the control. Similar results were found for 100 seed weight (g) with higher and lower values of 15.5 and 9.3 g in combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment and control respectively (Table 3).

Male and Female Flower Ratio and Fruit set (%)

In the initial phases of flowering, the majority of blossoms produced are typically male. Following this stage, female flowers begin to develop concurrently with male flowers after a few days, allowing for pollination to occur. Female flowers can be distinguished from their male counterparts by the presence of a swollen ovary at the base of the stalk.

The combination of micronutrients and biofertilizers significantly decreased the male and female flower number ratio compared to control (Table 3). The maximum and minimum male and female flower ratio was measured in control (8.25) and the combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment (3.50) respectively (Table 3). On the contrary, the highest value of fruit set (%) was produced at the Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment

(74.5%) compared with the lowest in control (41%). The combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers and control treatments recorded the highest and the lowest average of 45 and 16 female flowers per vine respectively. In contrast, the highest male flower (340) was measured in control, compare to 199 in the combination of Nitroxin, Phosphate Barvar2 and Fe fertilizers treatment (Table 3)

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Table 2 Analysis of variance for morphological traits and yield components of the sponge gourd under different micronutrients (Fe, Zn and Mn) and Nitroxin and Phosphate Barvar2 biofertilizers

SOV	DF	Mean squares (MS)									
		Fruits per plant	Fruit length	Fruit diameter	Fruit weight	Fruit Yield per plant	Fruit Yield	100 Seed weight	Seed per fruit	Male & female flower ratio	Fruit set
Block	2	258.067	0.0001	0.068	0.008	1679.5	3074	0.09	0.001	1568.5	0.07
Nitroxin (N)	1	862081 **	0.0940 **	184.59 **	0.008 **	209686 **	2418 **	0.64 **	0.33 **	21168 **	193.59 **
P Barvar2 (P)	1	1053.6 **	0.0075 **	65.02 **	0.023 *	138336 **	4936 **	1.04 **	0.11 **	14633 **	69.08 **
Micronutrients (M)	3	3407.35 **	0.0248 **	31.67 **	0.115 **	80687 **	7342 **	53.29 **	0.43 **	83487 **	36.76 **
N×P	1	816.6 **	0.0487 **	4.34 **	0.092 **	51568 **	1676 **	2.40 **	0.03 *	55668 **	5.43 *
N×M	3	195.98 **	0.0058 **	13.93 **	0.092 **	3447 **	8270 **	2.38 **	0.041 **	3687 **	16.85 **
P×U	3	66.3 **	0.0011 **	3.26 **	0.021 **	3246 **	359 **	8.15 **	0.02 **	3196 **	5.45 *
N×P×M	3	64.6 **	0.0013 **	4.596 **	0.021 **	2135 **	282 **	1.58 **	0.01 *	1272 **	7.57 **
Error	30	63.4	0.0002	4.596	0.003	124	156	0.06	0.007	119	5.26
CV (%)		2.28	4.9	0.07	1.42	2.36	2.47	1.22	3.67	1.96	0.09

ns, * and **: non-Significant, significant at 5% and 1% probability levels, respectively

Table 3 Mean comparison of yield and yield components of sponge gourd under different micronutrients (Fe, Zn and Mn), Nitroxin and Phosphate Barvar2 biofertilizers

Nitroxin	P-Barvar2	Micronutrients	Fruits No/plant	Fruit Length (cm)	Fruit Diameter (cm)	Fruit Weight (g)	Fruit Yield g/plant	Fruit Yield kg ha ⁻¹	100Seed Weight (g)	Seed No/fruit	Male/female Flower ratio	Fruit Set (%)
N0	P0	Control	5.25 a	19.00 a	7.75 a	199.23 c	1046 a	5125 a	9.30 a	265.00 a	8.25 a	41.0 a
		Fe	6.85 b	26.15 bc	8.95 bc	231.10 b	1445 b	7080 b	10.60 c	298.75 bc	6.11 b	50.5 bc
		Zn	6.35 c	24.90 b	8.80 bc	218.42 c	1323 c	6482 c	10.25 bc	276.23 b	6.45 bc	49.0 bd
		Mn	6.21 c	24.75 b	8.53 b	207.13 d	1286 c	6301 c	10.00 b	280.13 b	6.7 6c	48.5 bd
	P1	Control	7.08 d	30.95 cd	9.45 bc	217.33 b	1539 d	7541d	11.40 d	293.11 bc	5.44 d	51.0 bc
		Fe	8.10 e	34.35 de	10.55 d	249.78 d	1967 f	9638 e	11.75d eg	315.67 c	4.85 e	55.0ce
		Zn	7.95 e	32.70 d	10.50 d	238.16 b	1877 ef	9197 f	11.50 d	310.50 c	5.15 df	54.0 c
		Mn	7.90 e	33.65 d	10.35 d	229.37 e	1812 e	8879 f	11.45 d	308.21 c	5.20 defh	54.0 c
N1	P0	Control	7.15 d	32.25 d	10.15 d	235.17 e	1681 d	8236 g	11.35 e	317.54 c	4.23 gh	53.0 cd
		Fe	8.95 f	37.75 e	11.45 e	263.83 e	2217 g	10863 h	12.90 f	377.23 df	3.99 g	59.0 eg
		Zn	8.75 f	35.32 de	11.30 e	254.67 f	2140 gh	10486 h	11.85 g	359.11 de	4.13 g	57.0 ce
		Mn	8.35 ef	34.18 de	11.25 e	243.42 f	2007 h	9834 e	11.60 de	346.77 de	4.55 h	56.0 ce
	P1	Control	10.21 g	37.05 e	12.15 f	298.23 g	2667 i	13068 i	13.35 h	399.15 f	3.85 g	60.5 eg
		Fe	12.50 h	49.50 f	13.05 g	384.45 h	3680 j	18032 j	15.50 i	474.13 h	3.50 i	74.5 f
		Zn	11.75 fh	44.65 f	12.45 f	345.67 i	3333 k	16331 k	14.65 j	432.19 hi	3.68 i	68.2 i
		Mn	11.50 i	47.59 af	12.33 f	319.21 g	3211 k	15733 l	14.15 j	421.11 i	3.71 i	63.5 g

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using LSD Test.

DISCUSSION

This experiment highlights the significant role of combining micronutrients and biofertilizers in enhancing the growth attributes of the sponge gourd. The findings underscore that biofertilizers, when used alongside micronutrients, serve as an effective alternative to inorganic fertilizers, promoting enhanced growth and increased yield while mitigating environmental damage [45,46]. By promoting soil microbial activity, biofertilizers can improve crop yield and quality, presenting a cost-effective and eco-friendly option compared to traditional inorganic fertilizers [47].

Biofertilizers like Nitroxin and Phosphate Barvar2, which contain nitrogen and phosphate-solubilizing bacteria, are particularly effective in enhancing crop production efficiency [48]. Studies by Azimi *et al.* [39] and Abou-Zeid *et al.* [49] support the significant positive impact of these biofertilizers on crop yield. Additionally, Oboh and Aluyor [10] observed that the application of both nitrogen (N) and phosphorus (P) biofertilizers enhances the absorption of nutrients by roots, resulting in improved yield characteristics. They concluded that under the effect of single and combined application of N and P biofertilizers which induced the uptake ability of the roots to nutrients and positive increase in the yield parameters because of improving the root system.

Research conducted by Jose *et al.* [50] and Din *et al.* [51] supports these findings, suggesting that the application of biofertilizers promotes plant photosynthesis and contributes to increased dry matter accumulation, ultimately leading to enhanced biomass production. This increase in carbohydrate storage post-inoculation with biofertilizers supports enhanced growth, as observed by Kameswari and Narayanamma [46] in sponge gourd.

The combined application of N and P biofertilizers with micronutrients showed superior effects on yield and yield components and the number of female flowers compared to control and single applications [52,53]. This synergistic effect is likely due to positive interactions between microorganisms in biofertilizers and micronutrients, leading to improved yield components [54-56]. As in this study, Kumar and Rao [20], Pandy and Dixit [21] Sharma *et al.* [22] reported that the application of micronutrients had promote the number of female flowers by accumulation of photosynthesis in Cucurbitaceae family.

Among the micronutrients tested, Fe treatment resulted in the highest yield components in sponge gourds, likely due to enhanced photosynthesis and vegetative growth [57]. Similarly, Zn was more effective than Mn, particularly in increasing leaf concentrations during various growth stages [55,58]. This corresponds with findings by Kameswari and Narayanamma [46] who reported improvements in fruit diameter, seed weight, and seed count under biofertilizer and micronutrient treatment. In contrast, the lowest number of total fruits, fruit diameter, 100 seed weight (g) and seed per fruit of sponge gourds were observed in control and Mn treatment application. Many studies agree with this research finding [59,60] which limits of nutrient availability restricted the sponge gourds growth.

Research by Rabbani *et al.* [61] supports the observation that increased vegetative growth under combined biofertilizer and Fe treatment leads to higher fruit yield. This aligns with studies by Oboh and Aluyor [10] who found that chemical and biofertilizers enhance vegetative growth and yield in sponge gourds. Partap *et al.* [11] also reported improved yield components like fruit number and weight under similar treatments.

The results suggest that integrating biofertilizers with micronutrients promotes carbohydrate storage in fruits, enhancing yield [62,63]. Studies by Choudhary [7] and Mukesh *et al.* [59] agree that integrated nutrient management (INM) is vital for sustainable soil fertility and crop productivity. Efficient fertilizer use through INM is crucial for economic and environmental sustainability [64].

The experiment confirms that combining micronutrients and biofertilizers significantly increases sponge gourd yield, as supported by studies by Kumar and Rao [20] and Demir *et al.* [64]. These findings are consistent with research on other Cucurbitaceae family members, where combined organic and inorganic fertilizers enhance yield, quality, and production [65,66].

CONCLUSION

The application of micronutrients, particularly Fe, followed by Zn and Mn, significantly increases the growth and yield of sponge gourds [22,63]. This study confirms the critical role of biofertilizers and in increasing crop yield by using micronutrients such as Fe, Zn and Mn at concentrations at 600, 400, and 500 mg l⁻¹ respectively, 30 days after planting. The findings suggest that using both biofertilizers and micronutrients effectively replaces inorganic fertilizers, enhancing growth and quality while preserving the environment. Further research on the response of sponge gourd and other medicinal plants to different biofertilizers is recommended. The results indicate that combining organic and inorganic fertilizers can halve the consumption of chemical fertilizers, improving plant growth, quality, and environmental sustainability.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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