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EFFECT OF SOLID AND LIQUID CHELATED IRON ON GROWTH AND YIELD OF BROAD BEAN

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ABSTRACT. A field experiment was carried out within the randomized complete block design in the agricultural season 2020–2021. The experiment included two factors, the first factor was solid chelated iron (S_0, S_1, and S_2 at concentrations 0, 100, and 200 ppm, respectively), which was added to the soil. The second factor was liquid chelated iron (L_0, L_1, and L_2 at concentrations 0, 2, and 4 ml L^{-1} respectively), which was spraying on the plants. The results showed that the stem diameter, number of pods, and total yield were significantly affected by adding the 100 ppm iron solid chelated treatment (18.36 mm, 25.74 pod plant^{-1}, 5.01 Mg ha^{-1} respectively). While 200 ppm treatment had the highest plant height (30.10 cm, yield (771.35 g plant^{-1}), seeds (6.18 per pod). The treatment of 4 ml L^{-1} liquid chelated iron treatment had the highest plant height (128.55 cm), biggest stem diameter (18.63 mm), highest pods per plant (25.45), yield (755.98 g plant^{-1}), total yield (4.80 Mg ha^{-1}), pod length (24.87 cm), pod weight (28.14 g) and the number of seeds per pod (7.88). The use of the interaction between solid and liquid chelated iron improves the vegetative growth and yield of broad beans.

Introduction

The broad bean *Vicia faba* L. is one of the basic winter crops, and it is one of the annual plants that belong to the legume family and constitutes an important part of the nutrition of people, especially those with limited income (Farhan, 2012). It also has many uses as it is grown as a green crop to consume its pods or to obtain its dry seeds that are used as human food or green feed crop to animals. It is important for improving the fertility of soil through its fixing of nitrogen in the soil. Therefore, it is used within agricultural rotations, and they work on the activity of Rhizobia bacteria. Further, its dry seeds contain carbohydrates 58.41% protein 21.39%, each 100 g dry weight seed 1.6 mg of iron and 90 mg calcium, 3.6 mg vitamin C 100 mg. (Chafiand *et al.*, 2009). It occupies an area of 2.577.201 hectares with a word production of 5.431.503 t (Anonymous, 2021). Foliar nutrition, which is the process of providing plants with the necessary nutrients by spraying nutrients on the vegetative system, which can absorb elements through stomata openings that are found on the upper and lower surfaces of the leaf. The foliar fertilization is one of the most important and easiest ways to deliver nutrients to the leaf stomata. It is a supplement with organic fertilizers in the soil and not a substitute for it (Rajasekar *et al*., 2017; Rachid *et al*., 2020).

Recent experiments have shown that foliar nutrition is efficient and effective compared to soil fertilizing and that it is not an alternative but rather a supplement to it, in addition to the speed of nutrients reaching the tissues of the leaf, taking into account the importance of nutrition from the soil through the roots (Bader *et al.*, 2020). Iron (Fe) deficiency chlorosis is a widespread problem for soybean grown on alkaline, calcareous soils (Hansen *et al*., 2003). Chelated compounds are derived from the word Chelate which is derived from the Greek word claw. In chemistry, 'Chelate' means the cyclic structure resulting from the ion link to two sets or more of electron donors to form a single element molecule. Usually, ferric occupies the first rank in the sequence of elements with the ability to form chelate compounds while ferrous occupies the third rank after the zinc. Chelate compounds can be added to plant either by soil or by spraying since it is easy to absorb, transfer and decompose and does not cause damage if added in appropriate concentrations for the plant (Al-Nuaimi, 1987).

Iron is of great importance in the construction of chlorophyll and the oxidation and reduction processes within the plant tissue, including respiratory enzymes. In addition to its entry into the synthesis of cytochromes and ferredoxin, which are important in the process of carbon metabolism. It is important in the formation of plant proteins and vital processes in the plant by being active for enzymes related to the process of respiration and electron transfer. It is included in the composition of chloroplasts and many enzymes (Barker, Stratton, 2015). It provides a larger surface area for the various metabolic reactions in the plant, which increases the rate of carbonization and thus encourages the demand for mineral elements from the soil, and this leads to the production of more dry matter, as well as keeps the plant from biotic and abiotic stresses (Singh *et al*., 2017). Whereas, Faisal *et al*. (2012) observed that the spraying of chelated iron with iron concentrations 0, 150, and 300 mg Fe L^{-1} on the bean plant led to a significant increase in the average plant height, number of leaves, number of pods, pod length, the weight of 100 seeds, and yield per plant.

The research aimed to study

1. The effect of adding solid chelated iron and determining the best concentration;
2. The effect of adding liquid chelated iron and determining the best concentration.
3. The effect of the interaction between solid and liquid chelated iron and determining the best interaction between them.

Material and Methods

A field factorial experiment was carried out within the randomized complete block design in the agricultural season 2020/2021 at the Haruniya area, kilo 21 – Muqdadiyah – Diyala governorate – Iraq, (latitude 34°53'33" N, longitude 56°04'45" E, altitude 31 m) to study the effect of adding chelated iron, solid and liquid, on the growth and yield of the plant. The field designated for the experiment was prepared by ploughing it horizontally, levelling and ploughing it again vertically, then levelling and smoothing it and it was divided into three blocks, each block had 9 experimental units, 27 experimental units with dimensions of 3×2 m for each experimental unit. The distance was 1 m between one experimental unit and another and 2 m between each block. Irrigation pipes were laid with a 1.3 m distance between one pipe and another inside the experimental unit. The seedlings were planted on one side of the line, with a distance of 40 cm between one plant and another.

The study factors

The experiment included two factors, the first factor was solid chelated iron signed as S_0, S_1, and S_2, at concentrations 0, 100, and 200 ppm, respectively added to the soil. The second factor was liquid chelated iron signed as L_0, L_1, and L_2 with concentrations 0, 2, and 4 ml L^{-1}, respectively which was sequentially sprayed on the plants. Take 6.66 g of chelated iron is weighed with a sensitive scale and dissolved in 1000 ml of distilled water, then the resulting extract was placed in an ultrasonic device for half an hour at a temperature of 40 °C, then the required concentration was prepared to be added by spraying on the foliage. From the dilution equation, the concentration is prepared in a 2000 ml hand sprayer with 2 cm^3 of diffuser T_{20}, to give the concentration of 100 ppm as the first concentration, and in the same way, the second concentration was prepared and sprayed on all sides of the leaf until complete wetness and the time for the addition was in the early morning to avoid evaporation with high temperature.

The solid iron chelated prepare according to the equation:

$$C_1N_1 = C_2N_2$$
$$1000 \times N_1 = 100 \times 2000 \quad (1)$$
$$N_1 = \frac{200\,000}{1000}$$

where
C – concentration,
N – normality.

The process of planting has been done on 01/10/2019 and the harvest on 01/5/2020. Ten samples were taken randomly from the soil with a similar sample of the field to be analyzed before planting, with a depth of 0–30 cm, air-dried, ground with a wooden hammer, sifted with a sieve (2 mm). The sample was preserved in different and known places until analysis. The soil of the study field was classified as a silty loam texture (Table 1). The results were analyzed using the statistical program (SAS). The significant differences between the means were tested according to the (Duncan) polynomial test at the level of probability of 0.05 (Al-Rawi, 2000).

Studied properties

Plant height (cm) was measured from the surface of the soil to the top of the plant using a tape measure. Stem diameter (mm) was measured by (dwarf) the five stems of the plant were collected and the mean was counted. The number of seeds in a pod (seeds per pod) was calculated for: five random pods were selected, then the average was calculated. The number of pods in the plant (resonance per plant): the number of pods in each treatment was calculated, and the average was calculated. Yield per plant (g) was got by weighing all of the pods of treatment then dividing by the number of plants in treatment.

Table 1. The results of the analysis of the chemical and physical characteristics of the study soil before planting

Properties	Value	Unit
EC $_{1:1}$	1.92	dS m^{-1}
pH $_{1:1}$	7.57	
O.M	1.96	%
Nitrogen	45.18	mg kg^{-1}
Phosphorous	12.24	
Potassium	192.92	
Iron	3.47	
Bulk density	1.4	Mega g m^{3-1}
Soil content		
Clay	52.4	%
Silt	8.8	
Sand	38.8	
Soil texture	Silty loam	

Results

Plant height (cm)

The results in Table 2 show that adding solid chelated iron S_2 affected significantly plant height (130.10 cm), compared with control treatment S_0 (119.18 cm). The application of liquid chelated iron L_2 has produced a plant with a height of 128.55 cm, compared with other treatments. The interaction treatment of solid S_2 and liquid chelated iron L_2 was produced the highest plants (136.32 cm), compared with other treatments.

Table 2. The effect of adding solid and liquid chelated iron on plant height (cm) on growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	117.21 ± 7.01 cde	119.88 ± 7.52 cd	122.51 ± 2.50 bc	119.18 ± 7.52 B
S_1	113.21 ± 3.02 e	115.58 ± 8.70 de	126.81 ± 3.01 b	118.53 ± 7.94 B
S_2	119.18 ± 7.52 cd	134.81 ± 10.51 a	136.32 ± 10.06 a	130.10 ± 11.60 A
Average	116.53 ± 5.96 C	123.42 ± 11.70 B	128.55 ± 8.16 A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test. S – solid chelated iron; L – liquid chelated iron

Diameter of the stem (mm)

The results of Table 3 show that there was a significant difference in stem diameter when solid chelated iron S_1 (18.36 mm) was added. The concerning of the adding of liquid chelated iron, the L_2 was revealed highest increase (18.63 mm), compared with other treatments. As for the interaction between the two factors, the treatment S_1L_2 had the highest stem diameter (19.46 mm), compared with the control treatment.

Number of pods per plant (pod plant^{-1})

The results of Table 4 show that there is a significant difference when adding solid chelated iron as treatment S_1, which resulted in 25.74 pod plant^{-1}, compared with the control treatment. As for the addition of liquid chelated iron, the L_2 treatment recorded 25.45 plant pods^{-1}, compared with the control treatment. As for the interaction between the two factors, the highest value was scored at treatment S_1L_2 as it give 27.74 pods plant^{-1}, compared with the control treatment.

Table 3. The effect of adding solid and liquid chelated iron to stem diameter (mm) on growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	17.06 ± 1.41 ab	17.30 ± 4.79 ab	18.91 ± 1.03 A	17.76 ± 2.69 AB
S_1	16.50 ± 1.47 ab	19.12 ± 1.63 a	19.46 ± 3.80 a	18.36 ± 2.60 A
S_2	14.41 ± 2.67 b	16.74 ± 1.49 ab	17.51 ± 2.03 ab	16.22 ± 2.30 B
Average	15.99 ± 2.07 B	17.72 ± 2.85 AB	18.63 ± 2.38 A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test.S – solid chelated iron; L – liquid chelated iron

Table 4. The effect of adding solid and liquid chelated iron on the number of pods per plant (a pod of a plant) on the growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	20.64 ± 3.96 cd	25.70 ± 2.62 a	26.04 ± 6.12 a	24.13 ± 4.68 A
S_1	23.76 ± 8.55 abc	25.71 ± 3.42 a	27.74 ± 1.40 a	25.74 ± 4.96 A
S_2	18.94 ± 6.61 d	20.50 ± 3.49cd	22.56 ± 3.14 bcd	20.67 ± 4.35 B
Average	21.11 ± 6.13 B	23.9 ± 3.807 A	25.45 ± 4.19 A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test.S – solid chelated iron; L – liquid chelated iron

Yield per plant (g)

The results of Table (5) show that there is a significant difference when adding iron solid chelated at the S_2 treatment as it recorded 771.35 g plant^{-1}.

Table 5. The effect of adding solid and liquid chelated iron to the yield of one plant (g) on the growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	343.35 ± 146.82e	545.38 ± 154.58cde	717.05 ± 178.70abc	538.26 ± 213.72B
S_1	436.51 ± 95.34de	467.79 ± 90.79de	653.94 ± 14.26bcd	519.42 ± 121.43B
S_2	632.90 ± 88.47bcd	784.22 ± 349.71ab	896.9 ± 239.634a	771.35 ± 245.05A
Average	470.92 ± 161.26C	602.13 ± 242.21B	755.98 ± 185.24A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test. S – solid chelated iron; L – liquid chelated iron

As for the addition of liquid iron chelate. The L_2 treatment outperformed with result 755.98 g plant^{-1}. As for the interaction between the factors, it was the highest value with treatment S_2L_2 (896.94 g plant^{-1}) but the lowest value was at the treatment of S_0L_0 as it reached 343.35 g plant^{-1}.

Total yield (Mg ha^{-1})

The results in Table 6 show that the addition of iron solid chelated (S_1) significantly affected the total yield (5.01 Mg ha^{-1}), in comparison with the control treatment S0 as it gives 3.53 Mg ha^{-1}. The treatment of (L_2)

iron liquid chelated produced the most total yield 4.80 Mg ha^{-1}. The interaction of the kind of Fe chelate S_1L_2 produced the most total yield (5.45 Mg ha^{-1}), compared with other treatments. Whereas the lowest value was at the interaction S_0L_0 as it gives 2.45 Mg ha^{-1}.

Table 6. The effect of adding solid and liquid chelated iron on the total yield (Mg ha^{-1}) on the growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	2.45 ± 1.05 d	3.95 ± 1.51 c	4.18 ± 1.04 bc	3.53 ± 1.33 B
S_1	4.59 ± 2.35abc	4.98 ± 1.53ab	5.45 ± 1.69A	5.01 ± 1.68 A
S_2	3.73 ± 2.00 c	3.83 ± 1.76 c	4.76 ± 1.34 abc	4.10 ± 1.59 B
Average	3.59 ± 1.87 B	4.25 ± 1.49 A	4.80 ± 1.34 A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test.S – solid chelated iron; L – liquid chelated iron

Pod length (cm)

The results of Table (7) show that there is no significant difference when adding solid chelated iron. As for adding liquid chelated iron, the treatment L_2 outperformed as it give 24.87 cm to the control sample treatment L_0 as it had 22.17 cm. As for the interaction between the two factors, the highest value was in the treatment S_2L_2 as it give 26.39 cm and the lowest value was at the treatment S_0L_0 as it give 21.85 cm.

Table 7. The effect of adding solid and liquid chelated iron to pod length (cm) on growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	21.85 ± 1.88 b	22.81 ± 1.53 b	24.11 ± 5.68 ab	22.89 ± 3.22 A
S_1	22.78 ± 1.54 b	23.32 ± 2.25 ab	24.21 ± 3.95 ab	23.44 ± 3.20 A
S_2	21.88 ± 2.21 b	22.59 ± 2.21 b	26.39 ± 2.69 a	23.62 ± 2.94 A
Average	22.17 ± 1.70 B	22.91 ± 2.70 B	24.87 ± 3.88 A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test. S – solid chelated iron; L – liquid chelated iron

Pod weight (g)

The results of Table (8) show that there is no significant difference when adding solid chelated iron. As for adding liquid chelated iron, the treatment L_2 outperformed as it give 28.14 g to the control sample treatment L_0 as it amounted to 23.41 g. As for the interaction between the two factors, the highest value was in the treatment as it amounted to 29.32 g and the lowest value was at the treatment S_0L_0 as it reached 19.20 g.

The number of seeds in the pod (seeds pod^{-1})

The results of Table 9 show that there is a significant difference when adding solid chelated iron to the treatment S_2 As it gives 6.18 seeds pod^{-1} in comparison to the control treatment as it gives 5.85 seeds pod^{-1}. As for the addition of liquid chelated iron, the treatment L_2 outperformed as it reached 7.88 seeds pod^{-1} to the control sample treatment L_0 as it gives 4.68 seeds pods^{-1} as for the interaction between the two factors, the highest value was at the treatment S_2L_2 as it gives 8.25 seeds pod^{-1} while the lowest value was at the treatment S_0L_0 as it gives 3.91 seeds pods^{-1}.

Table 8. The effect of adding solid and liquid chelated iron to pod weight (g) on growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	19.20 ± 8.98 b	22.67 ± 5.30 ab	25.82 ± 2.02 ab	22.50 ± 6.07 A
S_1	24.23 ± 3.27 ab	28.42 ± 5.81 a	29.32 ± 3.96 a	27.25 ± 4.59 A
S_2	27.27 ± 5.26 a	28.28 ± 4.22 a	29.26 ± 1.21 a	28.25 ± 3.54 A
Average	23.41 ± 6.52 B	26.46 ± 5.29 AB	28.14 ± 2.88 A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test. S – solid chelated iron; L – liquid chelated iron

Table 9. The effect of adding iron chelated steel and liquid in the number of seeds in the pod (pod seed^{-1}) on the growth and yield of broad bean

Factors	L_0	L_1	L_2	Average
S_0	3.91 ± 0.31 d	6.67 ± 0.20 b	7.65 ± 0.64 a	5.85 ± 0.39 A
S_1	5.31 ± 1.07 bc	4.36 ± 0.49 cd	7.73 ± 0.66 a	5.80 ± 0.69 A
S_2	4.82 ± 1.16 bcd	5.47 ± 0.27 bc	8.25 ± 0.34 a	6.18 ± 0.65 A
Average	4.68 ± 0.83 B	5.28 ± 0.32 B	7.88 ± 0.53 A	

Lowercase letters refer to the significant difference between the treatments; capital letters refer to the difference in main factors; the coefficients with similar letters do not differ significantly from each other at the 0.05 probability level according to Duncan's polynomial test.S – solid chelated iron; L – liquid chelated iron

Discussion

The results of the Tables (2, 3, 4, 5, 6, 7, 8, and 9) show that there are significant differences when adding chelated iron, solid and liquid, in some variables of vegetative growth of plants (plant height, leaf area, and stem diameter). The reason for these results is the role of iron. The activation of the meristematic cell division and elongation of the internodes because it is responsible for the formation of cytochrome, ferredoxin, and chlorophyll in the chloroplasts which is important for the photosynthesis process, which is reflected in the height of the plant. This result is consistent with studies by Focus (2003) and Miller *et al*. (1995).

Iron is an essential element in chlorophyll, about 29–35% of the total amount of iron is found in green leaves, and it has an important role in contributing to the building of enzymes and compounds that make up the chlorophyll molecule (Barker, Stratton, 2015). It is believed that the increase in the diameter of the stem is because iron activates several enzymes, including Peptidase, Proteinase, Aconitase and Aminolevulinate dehydrate which works to accumulate photosynthetic products and thus lead to an increase in the diameter of the stem. This is consistent with Gheith *et al*. (1989), Al-Emadi (1991) and Al-Saadi (2021). It is clear from the results of the same tables above that there is a significant difference when adding solid and liquid chelated iron to some of the characteristics of the yield (yield of one plant, total yield, and the number of pods per plant, length, and weight of the pod). The reason is due to the role of iron in increasing the rate of photosynthesis and as a result, encouraging the demand for minerals and producing more dry matter in the plant as well as preserving the plant from various biotic and abiotic stresses. This is consistent with Singh *et al.* (2017). It is also believed that the increase in the yield of the broad bean when adding chelated iron is attributed to the role of chelated iron in activating some enzymes in the plant that have a role in the formation of basic compounds in the plant and the appropriate concentrations of nutrients used and the efficiency of plant for absorption, according to Cheith *et al*. (1989).

It is also believed that adding chelated iron leads to an increase in the qualities of the plant when spraying chelated iron. This may be attributed to its role in influencing the increase of hormones plant which are auxin and gibberellins which leads to an increase in the process of cell division and growth. Iron also has a role in building proteins in addition to its role as a catalyst in the formation of chlorophyll. It is also involved in the synthesis of cytochrome proteins important in the processes of carbon metabolism and respiration, and the formation of an important protein in the process of carbon metabolism, and this agrees with Muhammad and Yunus 1991.

It is also believed that iron is included in the synthesis of oxidation and reduction enzymes, such as cytochrome, cytochrome oxidase, and bio-oxidase, as well as its contribution to building chlorophyll in plants and then increasing the plant's activities in absorbing nutrients and increasing the processes of respiration and photosynthesis. As well as encouraging the growth of meristematic tissues and then cell division and elongation, which increases the height of the plant, this is consistent with the results of Alwan *et al*. (2004).

These results are attributed to the role of iron, which contributes to the vital activities inside the plant body, such as building chlorophyll and stimulating the activity of enzymes, especially those related to photosynthesis and respiration. The reason may be due to the role of iron in increasing the efficiency of important compounds that help in cell division and increase their growth, which was reflected in the characteristics of vegetative growth and total yield of the plant. These results were in agreement with Bozorgi *et al.* (2012) and Sure *et al*. (2012). The increase in leaf area is attributed to the physiological functions of iron in its participation in increasing the activity of respiratory and enzyme cells and photosynthesis processes, as well as its role in the formation of chlorophyll and proteins of cell walls (Abu Dahi, Al-Younis, 1988). These results are in agreement with Al-Mohammadi (2005), Al-Taher (2005) and Al-Khazraji (2011).

The reason for the increase in the yield may be due to the role of iron in increasing the process of cell division and elongation and increasing the process of carbon metabolism, and consequently, the plant height and stem diameter increased and increased The leaf area, which by increasing it, increases the carbonic representation as a result of the availability of energy needed to absorb water and nutrients, and then an increase in the length and weight of the pods, which all contribute to the yield. These results agreed with Boehme *et al*. (2005) and Sarheed (2013).

The chelated iron led to an increase in the vegetative growth characteristics and yield. The reason for this is the effect of iron in many vital processes in the plant. It is an essential element in building chlorophyll and iron-containing proteins which are important in the electron transfer reactions in the photosynthesis process that leads to an increase in the efficiency of photosynthesis, which is reflected in an increase in plant growth rates and this is consistent with Shalash *et al*. (2012) and Ahmed (2016). It is also believed that the role of iron in many of the vital activities of the plant, either through its direct participation as a component of plant materials, and this leads to an increase in the number and size of cells, and this will prompt an increase in growth rates, which was reflected on the increase in stem diameter rates and this is consistent with Abu Khumra, Abbas (2010). It is also believed that iron-works organize the function of plant hormones that encourage the growth of reproductive organs and increase the number of flower facilities and increase their fertilization, which is reflected in the increase in the number of grains, and this is consistent with Eskandri (2011).

Conclusions

1. When adding solid chelated iron, the two treatments S_2 and S_1 had the best results in most studied variables.
2. Adding liquid chelated iron to the treatment L_2 gives the best results in most studied variables.
3. As for the interaction between the two factors, the two treatments S_2L_2 and S_1L_2 had the best results in most of the studied variables.

Recommendations

1. The researcher recommends adding solid chelated iron at a concentration of (200 and 100 ppm) because it gives the best results in most of the studied variables.
2. It is recommended to add liquid chelated iron at a concentration of 4.2 ml L^{-1} because it gives the best results in most of the studied variables.
3. As for the interaction between the two factors, it is recommended to add the same concentrations determined above because they give the best results in most of the studied variables.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Author contributions

MA 25%, BB 25%, MA 25%, HJ 25% – study conception and design;
MA 50%, BB 50% – acquisition of data;
BB 25%, MA 25%, GH 50% – analysis and interpretation of data;
MA 50%, MA 25%, HJ 25% – drafting of the manuscript;
MA 25%, BB 25%, GH 50% – critical revision and approve the final manuscript.

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