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FERTILIZING EFFECT OF BLOOD FLOUR ON LETTUCE CROP

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Abstract
The investigation evaluated the effect of blood meal on the soil of a lettuce crop, and a completely randomized design was used with four treatments and three repetitions, with doses of 0%, 5%, 10 % and 15%. These values were used in the soil before transplanting the crespa-type lettuce seeds. For the soil analysis, each treatment sample was taken as a reference before and after the investigation. To collect data on the variables height, the number of leaves and coloration, they were measured 25 and 50 days after seed transplant. The results, through the statistical analysis of variance and Tukey 5% test, determine significant differences in height at 25 days; for T1, regarding the number of leaves, T0 presented better values for T1, T2 and T3. For the soil, T3 shows an increase in electric conductivity, Nitrogen and iron, and the pH shows a reduction in its values of 4.97 and 4.83 (acid). When applying the blood meal in the soil as a nutritional component in a 5% application, favorable results are achieved during the first 25 days, maintaining small differences in their values until the 50th day corresponding to the harvest.

Keywords: blood meal, fertilizer, transplant, lettuce, soil.

Resumen

La investigación se la realizó con el objeto de evaluar el efecto de la harina de sangre en el suelo de un cultivo de lechuga, se empleó un Diseño Completamente al azar con cuatro tratamientos y tres repeticiones, con dosis de 0%, 5%, 10% y 15%. Estos valores se emplearon en el suelo previo a trasplantar las semillas de lechuga tipo crespa. Para el análisis del suelo se tomó como referencia una muestra de cada tratamiento antes y después de culminar la investigación. Para recolectar los datos de las variables altura, número de hojas y coloración se midieron a los 25 y 50 días del trasplante de la semilla. Los resultados mediante el análisis estadístico de varianza y prueba de Tukey 5%, determinan que existen diferencias significativas en la altura a los 25 días, para el T1, en cuanto al número de hojas el T0 presento mejores valores con respecto al T1, T2 y T3. Para el suelo, el T3 muestra un aumento en la conductividad eléctrica, nitrógeno y hierro, el pH muestra reducción en sus valores 4,97 y 4,83 (ácido). Concluyendo así que al aplicar la harina de sangre en el suelo como componente nutritivo en un 5% de aplicación se logra resultados favorables durante los primeros 25 días, manteniendo pequeñas diferencias en sus valores hasta el día 50 correspondiente a la cosecha.

***Palabras Clave*:** Harina de sangre, fertilizante, trasplante, lechuga, suelo.

I. INTRODUCTION

Biological fertilizers have been used for their high content of nutrients that help develop plants (1). According to (2), products are formulated with one or more microorganisms and nutrients in their composition that enhance crop growth and yield by increasing access to soil nutrients by plants. This represents a fundamental key to improving soil fertility with more balanced agroecosystems and nutritious products (3).

During the last 100 years, the dosage of artificial nitrogen compounds in water, soil and air has doubled due to the increase primarily driven by the widespread use of synthetic fertilizers (3). However, it should be noted that plants can absorb between 30% and 50% of chemical fertilizers, causing the remainder to be lost in the soil (4). As plants and soil convert fertilizer into nutrients, carbon dioxide (CO2), nitrous oxide (N2O) and methane (CH4), known as greenhouse gases, are generated (5).

This type of agrochemicals and their misuse brings environmental problems because they make pests more resistant, making crops more prone to destruction (6). These environmental problems have generated awareness among agricultural producers due to the evidence of the damage caused by agrochemicals, thus generating the use of biofertilizers as an alternative for more sustainable soil management. (7)

Using chemical fertilizers to improve production and agro-economic yields constitutes a severe problem of environmental contamination and damage to the health of people exposed to these chemicals. (8). according to the INEC (9), 1’699,135.54 hectares use chemical fertilizers corresponding to Nitrogen, NPK and others, compared to organic fertilizers that only use 396,619.68 hectares corresponding to manure, guano, compost, humus and liquids. Furthermore, it is important to mention that this product establishes an alternative to reduce soil degradation by promoting the growth and quality of the final product in the crops (10).

New alternatives have emerged to avoid using chemical fertilizers, such as bovine blood meal. This meal is known to be a sustainable fertilizer since it has a nitrogen concentration similar to that of certain fertilizers with chemical components and higher than that of other organic residues of animal origin (11). This is how the use of this component in the quantities determined to use it as organic fertilizer or raw material in formulations for swine and poultry species (12)

II. MATERIALS AND METHODS

This research was carried out in the General Proaño Parish, Morona canton, Morona Santiago Province, Amazon region of Ecuador, at an altitude of 1099 m above sea level, as shown in Figure 1.

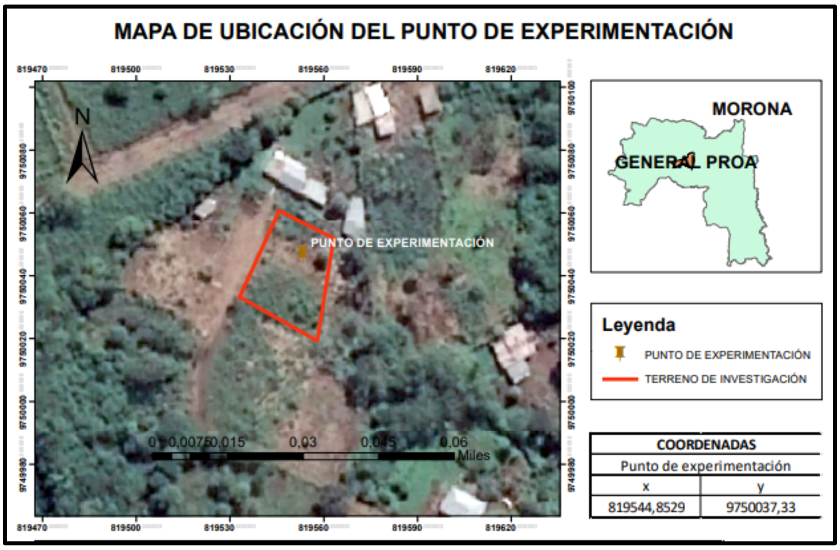

Figure 1. Base map of the experimental area.

Once the experimental site has been defined, Table 1 describes the climatic characteristics of the site:

Table 1. Climatic characteristics of the experimental area

Climatic characteristics	
Maximum temperature	28°C
Minimum temperature	22°C
Maximum precipitation	3000 mm
Minimum precipitation	2500 mm

Plots were used to transplant head lettuce seedlings, following the scheme shown in Table 2, where the characteristics of the experimental area can be observed, graphically represented in Figure 2 to simulate the area used.

Table 2. Description of the experimental area

Total area	Experimental units	Area of the experimental unit	Distance between plants	Distance between grooves	Plants per experimental unit
9 m2 (3m x 3 m)		1.8 m2 (3m x 0.60 m)	15 cm	60 cm	9

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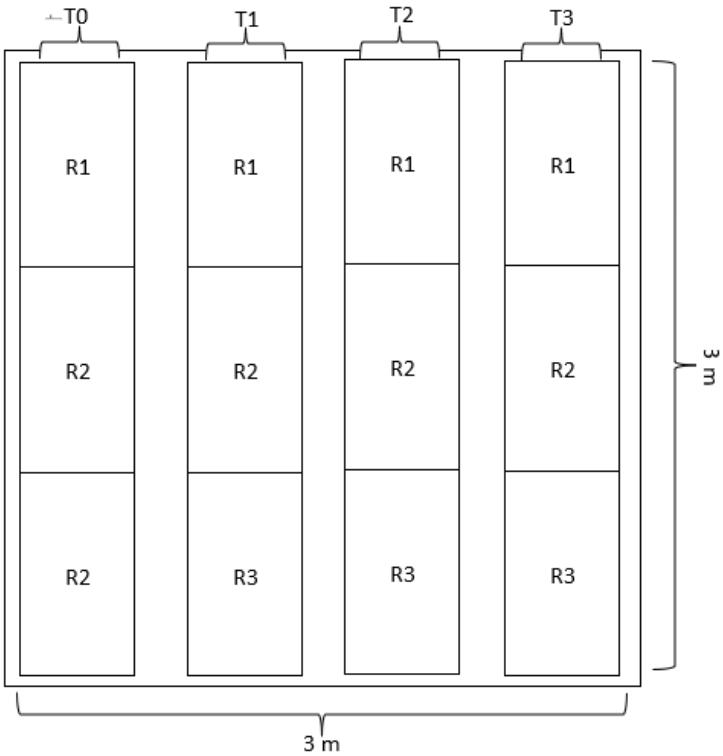

Figure 2. Field distribution scheme of the experimental plot.

As a starting point for the collection of soil samples, we proceeded to remove the weeds from the surface, then a 30 cm hole was dug, and the shovel was placed 3 cm from the edge; the sample was placed in a plastic container to proceed to remove the remains of degradable matter, weeds, among others; finally, the percentages to be used per treatment were calculated using the following formula:

$$TA = \frac{Tr * 100}{\% N}$$

Where:

TA: Application cup
Tr: Cup required
%N: Percentage of the nutrient contained in the fertilizer.
Direct fertilization was used in the soil on which it was applied and homogenized several times, then seeds with 22 days of germination were placed in the furrows of the different treatments with 15 cm between seeds. For this process, the methodology mentioned by (14) was taken into account, which indicates the following "transplants should be done in the morning hours in moist soils and ensuring that the root system has good humidity." Finally, the harvest was carried out 50 days after transplanting the seeds; for this purpose, samples were selected at random according to the following treatments

A. Soil analysis.

Before and after the application of the blood meal, the soil was analyzed by simple random sampling at a depth of 20 cm, collecting only the central part of the available space and analyzed in the laboratory of the National Institute of Agricultural Research (INIAP) where physical and chemical parameters such as N, P, pH, Fe, among others, were determined.

B. Lettuce plant measurements

Height (cm): With the help of a pleximeter, the plant was measured from the basal surface to the final height of the highest leaf; for this purpose, plants were chosen randomly from the furrows and this value was taken every 25 days after transplanting the seed.

Number of leaves**:** To evaluate the number of leaves, plants were randomly selected from the intermediate furrows per treatment and counted.

Coloration: To observe the coloration of the plants, plants were randomly selected, and their coloration was observed.

II RESULTS AND DISCUSSION

The values presented in Table 3 correspond to the percentage in grams used for each treatment except for T0, which corresponds to our control treatment; these values were placed in the soil of each treatment by direct fertilization.

Table 3 *Application rate per treatment*

Treatment	Formula	Application cup (g)
T1	$TA = \frac{5\% * 100}{14.29\ \%}$	34.99
T2	$TA = \frac{10\% * 100}{14.29\%}$	69.98
T3	$TA = \frac{15\% * 100}{14.29\ N}$	104.97

Source: Own elaboration, 2022

A. **Plant measurements**

Initial height

For the initial height variable, the measurement from the beginning of the stem to the highest leaf was taken as a reference; Table 4 shows the following results.

Table 4 *Analysis of variance for the variable height at 25 days.*

Sources	SC	GL	CM	F	P-value
Model	15,56	3	5,19	5,79	0,021
Treatments	15,56	3	5,19	5,79	0,021
Error	7,17	8	0,9		
Total	22,73	11			

Note: SC = Sum of squares; GL = Degrees of freedom; CM = Mean square; F = F-ratio.

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The results of the statistical analysis for the height variable corresponding to data taken 25 days after transplanting and considered as initial height show that there are significant differences between treatments ($F = 5.79$ and $p = 0.021$) in Figure 4, which refers to the average value of each treatment, the first place is occupied by T1 (5%), second place by T2 (10%) and T0 (0%), leaving T3 (15%) in third place. As for the comparison with the control treatment, it can be seen that it had more variation with T1, in which a 5% blood meal percentage was used.

Table 5 *Tukey's test for initial height.*

Treatments	Stockings	n	E. E		
T1	8.33		0,55	A	
T2	6,33		0,55	A	B
T0	6,33		0,55	A	B
T3	5.17		0,55		B

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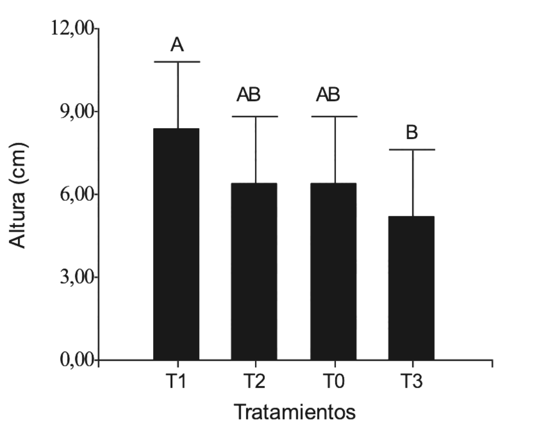

Figure 4. Height of the plant at 25 days.
Source: Peñaranda, Jennifer, 2022

<u>Final height</u>

For the data collection corresponding to final height, the measurement was taken into account from where the root begins to the highest leaf after harvesting.

Table 6 *Analysis of variance for the variable height at 50 days.*

Sources	SC	GL	CM	F	P-value
Model	46,92	3	15,64	5,21	0,0275
Treatments	46,92	3	15,64	5,21	0,0275
Error	24,00	8	3,00		
Total	70,92	11			

Note: SC = Sum of squares; GL = Degrees of freedom; CM = Mean square; F = F-ratio.

Source: Peñaranda, Jennifer, 2022

The statistical analysis results for the height variable corresponding to 50 days after transplanting and considered as initial height show significant differences between treatments (F = 5.21 and p = 0.0275), considering the coefficient of variation of 24.45 %. In Table 7**,** we can observe the differences between treatments obtained by Tukey's test (5%), taking into account the ratio of fertilizer and the control treatment T0; differences are found 50 days after the treatment.

Table 7 Tukey's test for final height in the different treatments.

Treatments	Stockings	n	E. E		
T0	9,67	3	1,00	A	
T1	8,33	3	1,00	A	B
T2	5,33	3	1,00	A	B
T3	5,00	3	1,00		B

Performed by: Peñaranda, Jennifer, 2022

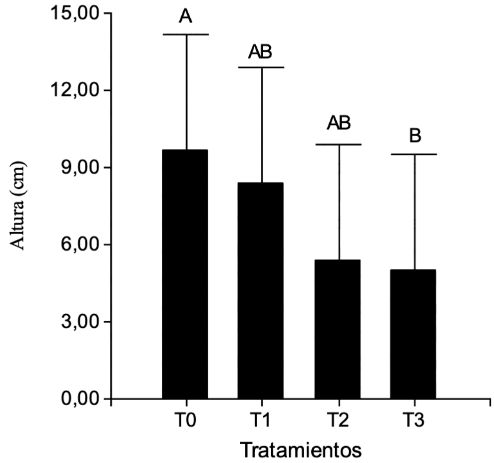

Figure 3. Height of the plant at 50 days after transplanting.

Comparison of initial and final height

The comparison between heights was made with data from days 25 and 50.

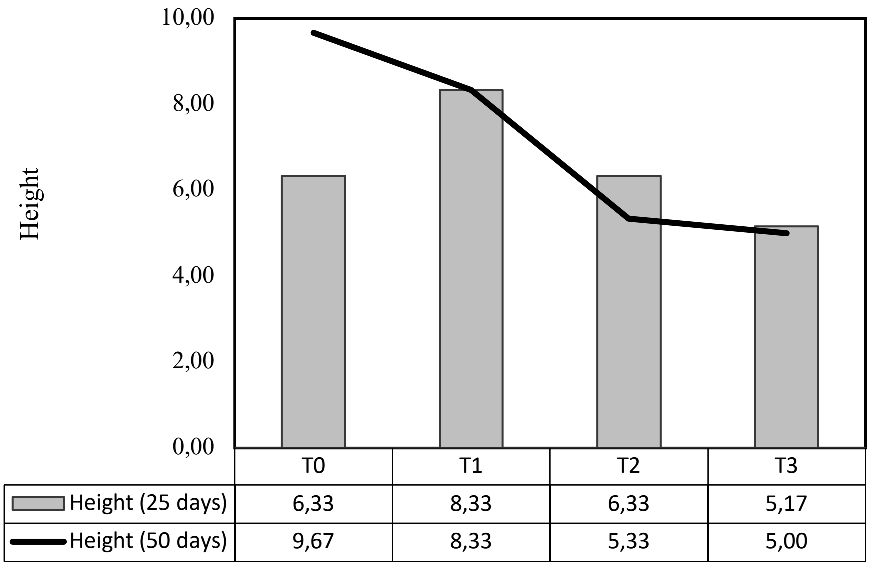

Figure 4: Comparison of heights at 25 and 50 days.
Performed by: Peñaranda, Jennifer, 2022

The variation in height at days 25 and 50 had an increase of 3.44 cm for T0, surpassing T1, which maintained its measurement; T2 presented a reduction of 1 cm and T3 with a reduction of 0.17

cm. In the research carried out by (4); (10), when evaluating different concentrations of blood meal at 100% and with combinations of chemical fertilizers in broccoli crops, results were obtained 15 days after transplanting with 100% blood meal, with an average height of 10.79 cm and 79.23 cm at 50 days, these data indicate that blood meal contributes to growth during the first 25 days.

On the other hand, (15) when using four different fertilizers in a jicama crop in which blood meal was included for its evaluation, obtained negative results on crop development when using 100% blood meal and positive effects when combining it with another chemical fertilizer (16) point out that when increasing the nitrogen concentration, better results in plant height will be obtained.

Initial number of sheets

Data on the number of leaves are presented in Table 8.

Table 8. Analysis of variance for the variable number of leaves at 25 days.

Sources	SC	GL	CM	F	P-value
Model	3,58	3	1,19	0,90	0,4842
Treatments	3,58	3	1,19	0,90	0,4842
Error	10,67	8	1,19		
Total	14,25	11			

Note: SC = Sum of squares; GL = Degrees of freedom; CM = Mean square; F = F-ratio.

Authors, 2022

The statistical analysis results for the variable number of leaves corresponding to data taken 25 days after transplanting and considered as initial number of leaves indicate differences between treatments (F = 0.90 and p = 0.4842), considering the coefficient of variation of 20.8 %. Table 9 shows the differences between treatments according to Tukey (5%); in Figure 5, the average value of each treatment.

Table 9. Tukey's test for initial number of leaves.

Treatments	Stockings	n	E. E	
T0	6,67	3	0,67	A
T1	5.67	3	0,67	A
T2	5,33	3	0,67	A
T3	5,33	3	0,67	A

Performed by: Peñaranda, Jennifer, 2022

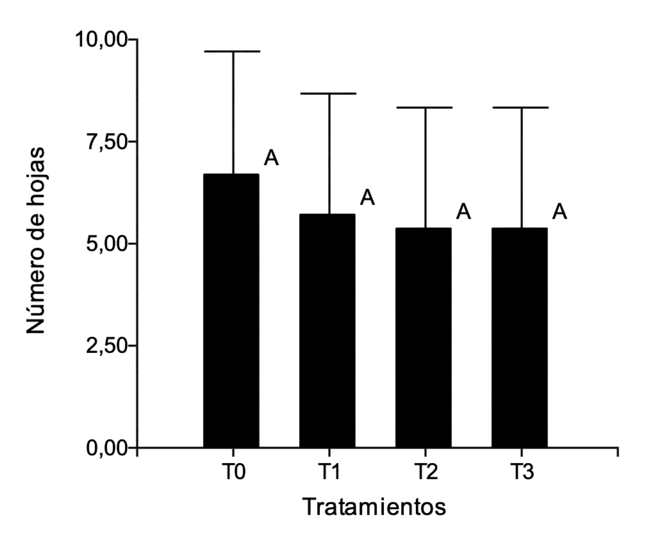

Figure 5. Number of leaves 25 days after transplanting.
Source: Own elaboration, 2022

1.1.1 Final number of sheets

Data on the number of leaves at 50 days are presented in Table 10.

Table 10. Analysis of variance for the variable number of leaves at 50 days.

Sources	SC	GL	CM	F	P-value
Model	128,25	3	42,75	6,26	0,0171
Treatments	128,25	3	42,75	6,26	0,0171
Error	54,67	8	6,83		
Total	182,92	11			

Note: SC = Sum of squares; GL = Degrees of freedom; CM = Mean square; F = F-ratio.

Authors, 2022

The statistical analysis results for the variable number of leaves corresponding to data taken 50 days after transplanting and considered as the initial number of leaves indicate differences between treatments (F = 6.26 and p = 0.0171), considering the coefficient of variation of 35.25%. Table 11 shows the differences between treatments obtained by Tukey's test (5%), which was considered to have a minimum significant difference (MSD) of 6.83502. In graph 5, we refer to the average value of each treatment, and the first place is occupied by T0 (0%), second by T1 (5%), third by T2 (10%), leaving T3 (15%) in fourth place.

Table 11. Tukey's test for the number of leaves 50 days

Treatments	Stockings	N	E. E		
T0	12,00	3	1,51	A	
T1	9,00	3	1,51	A	B
T2	4,67	3	1,51		B
T3	4,00	3	1,51		B

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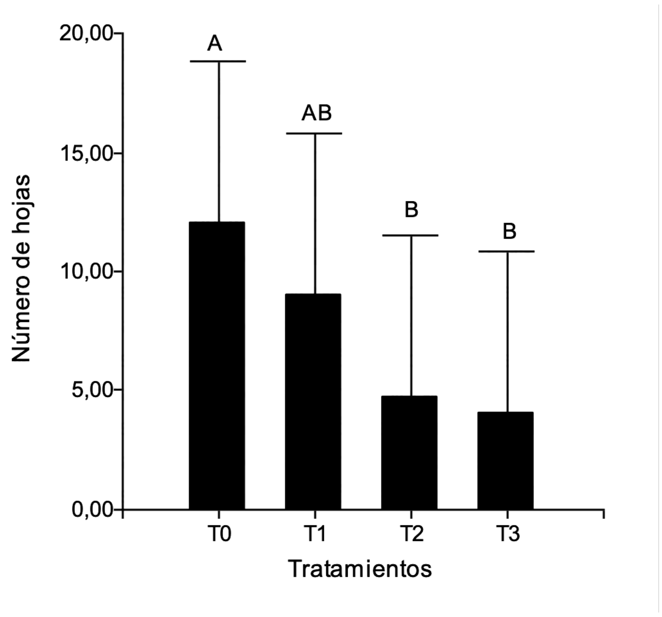
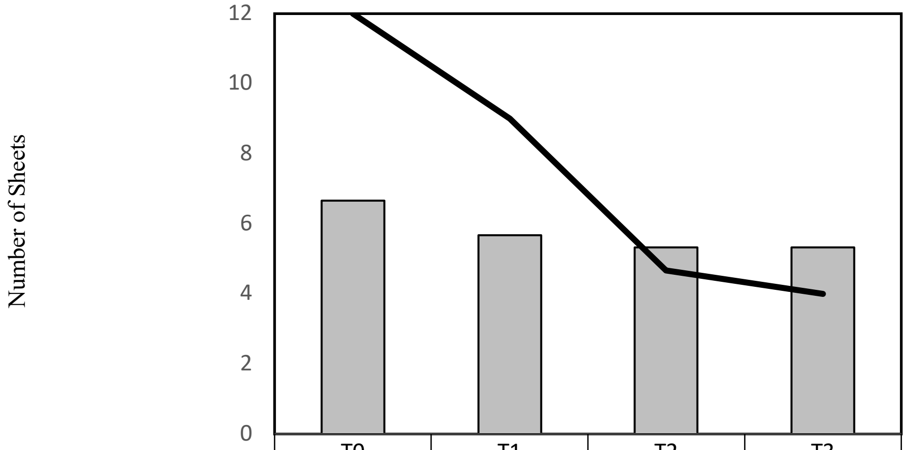


Figure 6: Number of leaves at 50 days after harvest.

1.1.2 Comparison of initial and final number of leaves

The number of leaves was compared with the data obtained on the 25th and 50th day, obtaining the following differences between their values.



	T0	T1	T2	T3
N. Leaves (25 days)	6,67	5,67	5,33	5,33
N.sheets (50 days)	12,00	9,00	4,67	4,00

Figure 7. Comparison of the number of leaves at 25 and 50 days .

Figure 7 shows the variations in the number of leaves for the different periods, the control (T0) obtained better results, considering that this treatment did not receive the application of biofertilizer. Regarding the analysis of variance at 25 days after transplanting, the null hypothesis is taken into account, which states that the application of blood meal will not cause differences between treatments; this hypothesis was accepted because the p-value was 0.4842, showing that all treatments had the same effect and this, in turn, was verified by a Tukey test at 5%.

Comparing the effect of the plants produced by the soil characteristics, the low yield of leaf development in T2 and T3 could have been affected by the high micronutrient indices of the soil and the degree of acidity present throughout the experiment.

The value of blood meal in T3 (104.97 g), adverse effects were evidenced in the plant three days after transplanting, causing the weakening of the crop and generating as an adverse reaction the separation of its leaves from the stem.

Coloring

The following data were obtained for the variable crop coloration at 25 and 50 days after transplanting, as shown in Table 12.

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Table 12. Coloration of plant leaves at 25 and 50 days.

Treatment	Coloring 25 days		Coloring 50 days	
T0	Green		Green	
T1	Green		Green	
T2	Green		Green	
T3	Brown/y ellowish		Brown	

Authors, 2022

The coloration of T3 did not vary greatly because it did not show any improvement in coloration during the entire cycle. Although the other treatments showed good results in coloration, in crops, there is a disease called bacterial spot that causes the presence of brown coloration on the leaves of the plants and is caused by high humidity. According to (17), the area's climatic conditions affected the crop because there was high rainfall during the research development, which could have caused this effect.

The brown coloration on the leaves of the plants corresponding to T2 and T3 may be linked to the high concentration of iron in the soil (Table 18). A low pH causes iron toxicity in the soil of the crop, or when high concentrations of this element are applied, it causes a brown tone on the edges of the leaves (18), thus showing that the concentration of iron and pH influenced the growth of T3.

1.2 Physical-chemical characteristics of the soil

The physicochemical characteristics of the soil samples taken before and after the implementation of the experiment are analyzed below.

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pH

The data corresponding to pH is presented in Table 13 for each treatment, corresponding to values before the application of the biofertilizer and at the end of the experiment.

Table 13. Differences in the hydrogen potential variable of the soil samples.

Hydrogen potential (pH)				
Treatment		Initial	Final	Diff
T0	0% HS	5,1	4,70	-0,40
T1	5% HS	5,02	4,62	-0,40
T2	10% HS	5,29	4,70	-0,59
T3	15% HS	4,97	4,83	-0,14

Note: HS = blood meal; "-" = decrease; "+" = increase; Diff = difference.

Authors, 2022

The soil analysis data presented at the beginning and end of this study indicate a decrease in potential hydrogen content in all treatments, considering that no biofertilizer was applied to T0, which could have had a variation due to the lettuce crop. For soil to be of good quality for vegetable crops, it must have a pH between 6 and 8. According to (13) cited by (19), the values presented for all our treatments before and after cultivation indicate soil acidity. Making a comparison with our T0 (0% HS) that had a decrease in hydrogen potential as the other treatments, T2 (10% HS), before the experimentation presented the best pH value of 5.29, which at the end had a reduction of 0.59 that made the soil more acid with a value of 4.70, which was caused by applying a concentration of 69.98g of blood meal in the soil, thus affecting the quality of the soil by making it highly acidic.

1.2.1 Electrical conductivity

The electrical conductivity results are shown in Table 15.

Table 14. Differences in the electrical conductivity variable.

Electrical Conductivity (dS/m)				
Treatment		Initial	Final	Diff
T0	0% HS	0,10	0,02	-0,08
T1	5% HS	0,07	0,03	-0,04
T2	10% HS	0,08	0,10	+0,02
T3	15% HS	0,05	0,20	+0,.15

Note: HS = blood meal; "-" = decrease; "+" = increase; Diff = difference.

Authors, 2022

Regarding the electrical conductivity, a decrease in these was presented in T0 (0% HS) and T1 (5% HS); on the contrary, T2 (10% HS) and T3 (15% HS) presented an increase of 0.02 ds/m and 0.15 ds/m. The differences presented do not generate significance since soil with a low salt content should remain between a range of 1-2 ds/m; however, to be free of these, their values should be in a range <1 ds/m.(20). Considering the above, the values of T0, T1, and T2 indicate that there is no presence of salts, however, in T3 (15% HS) the value indicates the presence of salts, but in low quantities, which could affect the growth of the plant and the development of its leaves.

1.2.2 Organic matter

The percentage of organic matter is shown in Table 15.

Table 15. Differences in the organic matter variable.

Organic matter (%)				
Treatment		**Initial**	**Final**	**Diff**
T0	0% HS	19,37	20,82	+1,45
T1	5% HS	20,03	19,34	-0,69
T2	10% HS	17,91	21,24	+3,33
T3	15% HS	17,23	17,92	+0,69

Note: HS = blood meal; "-" = decrease; "+" = increase; Diff = difference.

Source: Own elaboration, 2022

The results show a decrease in T1 (5% HS) and an increase in the organic matter despite not containing biofertilizer; consequently, T3 (10% HS) obtained an increase of 3.33% of OM, this being the highest value at the end of the experiment. The data show high values of OM in the soil of all our treatments because its optimum value should contain a range of 3-5%.

With a high amount of OM there will be the mineralization of more Nitrogen (N), as well as favoring the microstructure of the soil and the development of edaphic microfauna (21). In this case, the high amount of OM in the soil caused the weeds to grow rapidly, causing damage to the crop because the soil quality was not appropriate as it contained values exceeding those recommended.

1.3 Macronutrients

1.3.1 Nitrogen

For the nitrogen variable expressed in ppm (Table 17).

Table 16. Differences in the nitrate variable of the soil samples.

NH_4 (ppm)				
Treatment		**Initial**	**Final**	**Diff**
T0	0% HS	65,6	127,3	+61,7
T1	5% HS	91,2	66,8	-24,4
T2	10% HS	77,2	73,8	-3,4
T3	15% HS	66,1	141,8	+75,7

Note: HS = blood meal; "-" = decrease; "+" = increase; Diff = difference.

Source: Peñaranda, Jennifer, 2022

The nitrogen content in the soil presented significant increases between variables. Thus, T0 and T3 with an increase in their concentrations, unlike T1 and T2; taking into account Table 16 on the variation in the organic matter content, there is evidence that increasing this variable causes an increase in the nitrogen content in the soil. The high nitrogen content in plants can delay plant maturation and cause susceptibility to diseases, which may correspond to fungi or pests, affecting the quality of the crop (22). Therefore, containing a high nitrogen content in the soil will affect the crop; however, it can be seen that by applying a concentration of 5% and 10% of blood meal, this value decreases, thus trying to improve the soil, compared to high concentrations of 15%, which causes an increase similar to what occurred naturally, as demonstrated at T0.

1.3.2 Iron

The iron content in the soil is presented in ppm, detailed in Table 17.

Table 17. Differences in the iron variable of the soil samples.

Fe (ppm)				
Treatment		**Initial**	**Final**	**Diff**
T0	0% HS	213,7	234,90	+21,2
T1	5% HS	203,80	230,80	+27
T2	10% HS	185,90	195	+9,1
T3	15% HS	193,90	237,60	+43,7

Note: HS = blood meal; "-" = decrease; "+" = increase; Diff = difference.

Source: Peñaranda, Jennifer, 2022

The concentration of iron in the soil presented values outside the optimum range of 20-40 ppm; the high values are presented in the two periods before and after the end of the experiment. T3 (15%) showed a higher increase of 43.7 ppm. It is important to remember that a lower pH value will result in greater iron solubility, which can cause serious toxicity problems. (23).

Conclusions

The effect of the biofertilizer on the soil of the lettuce crop with the addition of 15% blood meal showed a greater difference in the values of electrical conductivity, iron, Nitrogen and pH, generating greater acidity in the soil. However, adding 5% blood meal as a nutrient component showed favorable results in height, the number of leaves and coloration in the lettuce samples analyzed during the first 25 days.

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