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Stomatal Density and its Relationship with Yield of Radish (Raphanus Sativus L.) Fertilized with Biol Produced from Sugar Cane Residues

Dante D. Cruz Nieto[a], Ronald F. Rodriguez Espinoza[b,*], José A. Legua Cárdenas[a], Juan M. Ipanaqué Roña[a], Ronald L. Ramos Pacheco[a], Joaquin J. Abarca Rodriguez[a], Delicias E. Natividad Huasupoma[a], Fredesvindo Fernández Herrera[a], Helen A. Zapata del Solar[a]

[a]Universidad Nacional José Faustino Sánchez Carrión, Huacho, Perú
[b]Universidad Autónoma del Perú, Lima, Perú
ronaldferd@gmail.com

The impact of agro-industrial waste can be reduced by using it in crop fertilization. The aim of this study was to evaluate the stomatal density and yield of radish (Raphanus Sativus L.) fertilized with biol at different concentrations, produced from sugar cane residues. A completely randomized blocks design statistical model was used, which consisted of 5 tests with 3 repetitions for each one, being T1 the control trial, and T2, T3, T4 and T5 the treatments using 2, 3, 4 and 5 L of biol in 200 L of water respectively. In terms of crop physical characteristics, the T5 treatment excelled in plant length, equatorial diameter, plant weight, as well as yield with 12.71 t/ha. Likewise, in the chemical analysis of the radish leaves, the T5 treatment showed an increase in K, Ca, Zn and MN, while the T2 did it in N, P and Cu, the T3 in Fe and the T4 in Mg. In terms of stomatal density, T5 stood out with 122 stomatal/mm^2. Based on this, it is concluded that the increase in the dose of biol influences the increase in nutrient and stomatal density and hence the yield of the radish crop.

1. Introduction

The indiscriminate use of chemical fertilizers has contributed to the deterioration of the biological, physical and chemical properties of the soil, resulting in the loss of its productive capacity (Camelo-Rusinque et al., 2017). On the other hand, organic waste, as suggested by Díaz (2011), has a strong impact on the environment, polluting the air, soil and water, especially due to the presence of a high content of organic matter, minerals, recalcitrant organic compounds, heavy metals, phytotoxins, among others. The sugar industry is an important activity in a country's economy, but in many cases the waste it generates is not properly managed. Among the effluents generated in this type of industry, vinasse has a high content of organic matter, together with nutrients such as nitrogen, phosphorus and potassium, as well as minerals such as zinc, iron, manganese and others (De Lima and De Souza, 2014), reason why, measures to prevent, minimize and control pollution caused by sugar mills must be put in place (Dominguez-Manjarrez et al., 2014).
Thus, due to this increase in waste from the sugar industry, it is necessary to give them an added value in order to use them as a sustainable resource; Therefore, the alternative of elaborating an organic fertilizer such as biol, which is used to improve plant nutrition and increase crop yields, is proposed. This constitutes an alternative for small farmers, who, by using organic residues from sugar cane waste, can improve their crop yields and at the same time help their economy by no longer consuming commercial fertilisers. Furthermore, it should be noted that this would be achieved without depleting soil quality, making its application a means to support both long-term food security and environmental preservation (Cen et al., 2020).
Therefore, the aim of the present research work was to analyse the relationship between stomatal density and yield of radish fertilized with biol produced from sugar cane residues.

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2. Materials and methods

The study was carried out in the village of Medio Mundo, located in the district of Végueta, province of Huaura in the Lima region, Perú. This place, located on the coast of the Pacific Ocean with an altitude of 19 m and with geographical coordinates: Latitude: -11.0233, Longitude: -77.6442, 11° 1′ 24″ South, 77° 38′ 39″ West. In addition, it has an average temperature that ranges between 19 °C and 22 °C and a relative humidity of between 77 - 85 %.

2.1 Study factor

The application of the doses of biol for the radish crop was made considering the analysis made of the soil and the organic fertilizer, which is shown in table 1, where T1 corresponds to the control test. For T2, T3, T4, and T5, 2 L, 3 L, 4 L, and 5 L of biol were used for each 200 L of water, respectively. These dosage values are similar to those recommended by Tencio (2017), who used a dosage of 3 L of biol per 200 L of water for vegetable crops.

Table 1: Biol dose applied to the radish crop

Treatment	L of biol/200 L of water
T1	0
T2	2
T3	3
T4	4
T5	5

2.2 Procedures

In the preparation of the biol, first of all, the compost was prepared, which was composed of 30 % guinea pig guano, 30 % dry grass, 20 % bagasse and 20 % vinasse, which, after mixing, was left to compost for 112 days. Subsequently, 20 kg of this compost was taken and 25 L of water was added, the mixture was covered and left to ferment for 20 days, thus obtaining the biol.
Before sowing radish, the soil was prepared in the conventional way, that is, in the same way as the farmers in the area. The experimental area was then delimited and 5 treatments with 3 replications were carried out.
Sowing was carried out uniformly in all demonstration plots with a spacing of 0.10 m between plants and 0.60 m between furrows, where after 20 days the doses were applied only once as detailed in table 1. After application of the doses, the physical characteristics of the radish crop were evaluated until harvest and the data obtained were processed by analysis of variance and Duncan's test.
The radish was harvested 30 days after planting. During harvest, leaf samples were taken from each treatment and taken for evaluation of the corresponding parameters. Soil, biol and foliar analyses were carried out at the National Institute for Agricultural Research (INIA) located in Huaral, Peru. Finally, stomatal density was determined using a Thermo Fisher Scientific scanning electron microscope at the National University José Faustino Sánchez Carrión (Figure 1).

Figure 1: Thermo Fisher Scientific scanning electron microscope

2.3 Statistical analysis

The data obtained, from sowing to harvest, were processed through an analysis of variance, comparing the result with the F values of Fisher's distribution at 5 % error, which allowed to determine if there was a significant effect of the biol dose on the characteristics of the radish crop. The components of the analysis of variance for the experiment are detailed in Table 2.

To determine whether any of the treatments had significance, i.e., whether there was an effect of the biol doses on the physical characteristics of the radish crop, a Duncan's multiple test was performed. This was used to determine which treatment stood out in relation to the others and how they differed from each other.

Table 2: Analysis of variance for the completely randomized blocks design (Lind et al., 2012).

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatments	SST	K - 1	SST/(k - 1) = MST	MST/MSE
Blocks	SSB	b -1	SSB/(b - 1) = MSB	MSB/MSE
Error	SSE	(k - 1)(b - 1)	SSE/(k - 1)(b - 1) = MSE	
total	SS Total	n -1		

3. Results and discussion

The results of the soil analysis are detailed in table 3. It was determined that the electrical conductivity corresponds to a non-saline soil, the pH is slightly alkaline, low concentration of organic matter, nitrogen, potassium and high concentration of phosphorus according to the values of Prialé (2016). As for the exchangeable cation elements such as calcium, magnesium, sodium and potassium are within the average values according to McKean (1993). The value of the cation exchange capacity is low, taking into account Valencia et al. (2018), who state that the natural soil has a value of 9.9 cmol.kg^{-1}, justified by the high presence of kaolinite, whose CEC values vary between 3 and 15 cmol.kg^{-1}.

Table 3: Soil analysis of the experimental area

E.C. 1:2.5	pH	O.M.	N	P	K	$CaCO_3$	Interchangeable cations (mEq/100 g soil)				CEC
mS/cm		% mass	% mass	ppm	ppm	% mass	Ca	Mg	Na	K	
0.74	8.1	0.51	0.03	17.10	115	5.73	5.96	1.77	0.09	0.28	8.08

ppm: Parts per million.
CEC: Cation exchange capacity.
OM: Organic matter.
EC: Electrical conductivity.

According to the results obtained from the foliar fertilizer or biol, which are detailed in table 4, it can be seen that the organic matter, nitrogen, phosphorus, potassium, calcium and magnesium are found in higher concentrations in relation to the conventional foliar fertilizer made from leguminous plant residues, cereals, kitchen waste, cattle guano, guinea pig and sugar cane residues such as molasses (Alvarez, 2010). A comparison with the findings of this author also shows that iron and manganese are found in higher concentrations, while copper has a low concentration. It should be noted that the concentrations of these nutrients efficiently influence many biochemical reactions, which contributes to a higher yield and quality of the radish crop. In this respect, Chojnacka et al. (2020) mention that the use of biowaste is a practical solution to recover valuable components from fertilizers.

Table 4: Chemical analysis of foliar fertilizer (biol)

E.C. 1:5	pH 1:2.5	Humidity	O.M.	N	P_2O_5	K_2O	CaO	MgO	C/N	Fe	Zn	Cu	Mn
mS/cm		%	%	%	%	%	%	%	%	ppm	ppm	ppm	ppm
4.30	7.68	99.25	99.25	1.82	0.96	0.67	6.18	0.62	13.24	3.465	1.17	0.01	0.13

With respect to the results of the physical characteristics of the radish crop shown in table 5, it can be seen that as the doses of biol increased, the architecture of the plant was strengthened, increasing the yield and improving the quality of the fruit in T5, which differs by 24.86 % with respect to the control sample. This is due to the fact that, by increasing the dose, the quantities of nitrogen, phosphorus, potassium and other nutrients added also increased, which favours the development of the plant, which is in agreement with what was stated by Cando and Malca (2016), who mention that the biol obtained from livestock manure, water and organic waste is an organic fertilizer with excellent results in terms of yield and nutrients.

Table 5: Results of the physical characteristics of the radish crop

Treatment	Dose (L of bio/200 L water/ha)	Plant length (cm)	Equatorial diameter (mm)	Total weight of plant (g)	Commercial yield (t/ha)
T_5	5	32.41 a	38.53 a	37.26 a	12.71 a
T_4	4	32.05 a	37.11 a	35.56 a	12.02 ab
T_3	3	31.36 a	36.26 a	33.43 a	11.15 ab
T_2	2	30.44 a	34.75 a	31.86 a	10.38 ab
T_1	0	29.17 a	32.82 a	28.82 a	9.55 b
Significance		**	**	**	**
CV		12.14	11.62	28.22	13.14

CV: Coefficient of variation.

The chemical analysis of radish leaves is detailed in table 6, showing that as the dose of biol is increased up to treatment T5, the concentrations of potassium, calcium, zinc and manganese increase, but the concentrations of nitrogen, phosphorus, magnesium, copper and iron decrease; this variation occurs mainly because these elements are involved in biochemical reactions such as photosynthesis, carbohydrate formation and thus influence the yield. This is supported by Restrepo-Correa et al. (2017), who state that phosphorus, nitrogen, iron and potassium are necessary compounds for plant growth and development, furthermore, the nutritional value of radish leaves far exceeds the corresponding value for the roots, with calcium being the most abundant mineral (Goyeneche et al., 2015).

Table 6: Chemical analysis of radish leaves per treatment

Percentage	Treatments				
(%)	T_1	T_2	T_3	T_4	T_5
N	3.47	4.03	3.47	3.30	3.36
P	0.63	0.57	0.51	0.56	0.54
K	0.72	0.79	0.81	0.84	0.97
Ca	3.31	3.47	3.44	3.27	3.67
Mg	0.18	0.28	0.43	0.67	0.20
Na					
parts per million (ppm)	6.45	14.15	5.05	7.63	10.85
Cu	2051.80	1787.80	2382.08	1724.40	1664.62
Fe	108.18	112.53	116.66	108.89	126.13
Zn	186.75	193.31	186.54	170.20	195.42

The stomata present on the leaves of radish (Raphanus Sativus L.) were photographed using a Thermo Fisher Scientific scanning electron microscope are shown in figure 2.
Stomatal morphology is a key phenotypic trait for the analysis of plant response under various environmental stresses such as drought, salinity, etc. (Bhugra et al., 2018). The quantification of stomata for each of the treatments is shown in table 7, where it is observed that, as the doses of biol increase, the stomatal density increases, with the T5 treatment standing out with a density of 917 stomata/mm^2, so there is a direct relationship between the increase in nutrients contained in the biol and the increase in the quantity of stomata in the plant. Considering that the stomata play an important role in the exchange of gases and water in the leaves (Li et al., 2022), these nutrients influence the efficiency of biochemical reactions such as biosynthesis and evapotranspiration, taking into account that stomata are pores that are present in high densities in the leaves and are responsible for the control of gas exchange and transpiration of the plant allowing the assimilation of CO_2 and the output of O_2 and maintaining a good water ratio in the plant (Larriva, 2006 and Fernández et al., 2015).

Table 7: Stomatal density of radish (Raphanus Sativus L.)

Evaluation	Treatments				
	T_1	T_2	T_3	T_4	T_5
Number of stomata /mm^2	93	107	100	114	122
Stomatal density (Number of stomata /mm^2)	699	805	752	857	917
stomata index %	30.90	42.63	34.48	43.18	40.40

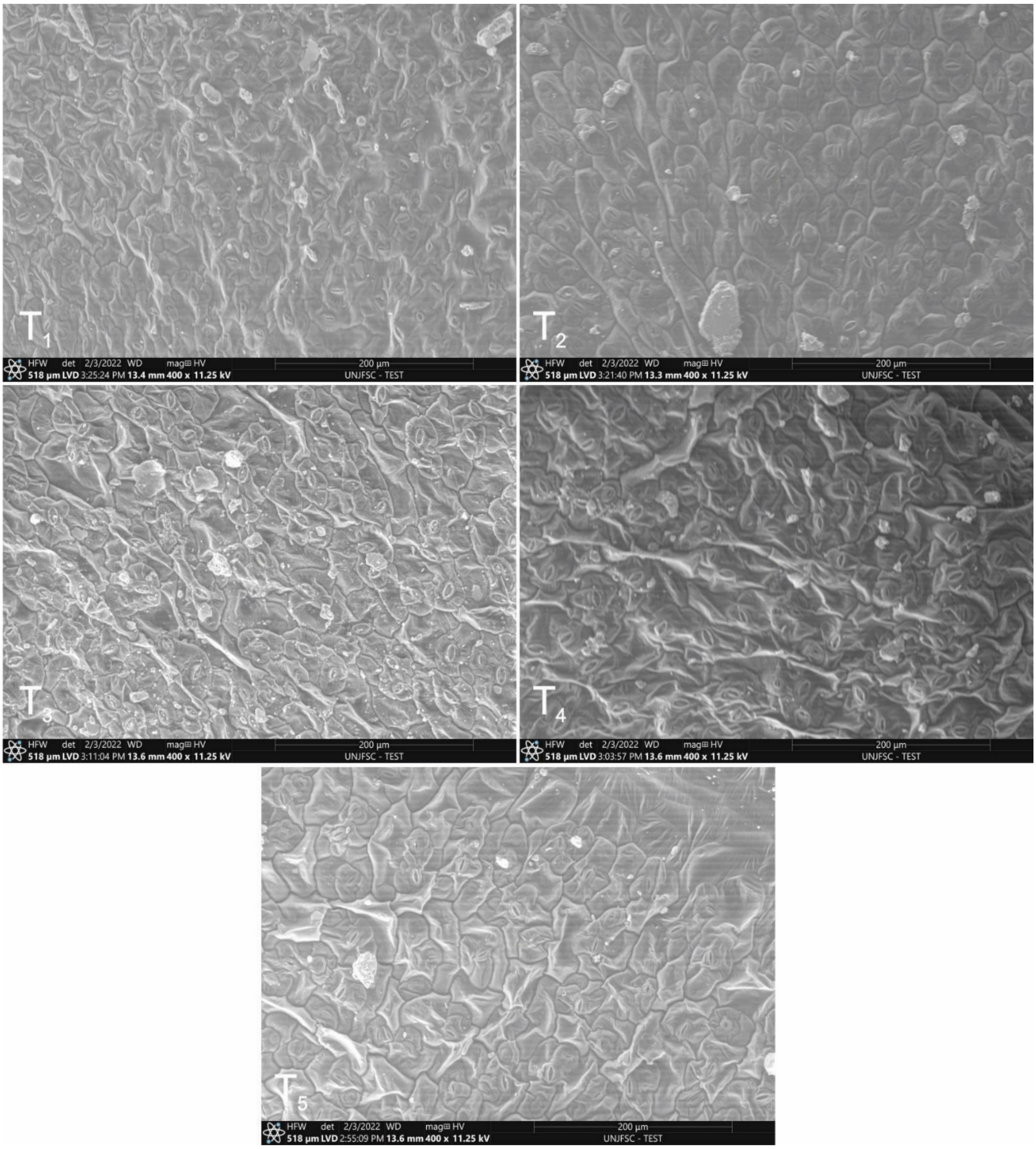

Figure 2: Micrographs of the surface of stomata on leaves of the five treatments.

4. Conclusions

The highest stomata density corresponds to the highest dose of biol, which belongs to treatment T5, which was prepared with 5 L of biol in 200 L of water. Furthermore, this increase in stomatal density is directly related to the increase in the yield of the radish crop (Raphanus Sativus L.), differing by 24.86 % with respect to the control sample, which is due to the greater number of stomata, which, together with the high concentration of nutrients such as nitrogen, potassium, calcium, zinc and manganese in this treatment, influences the biochemical processes of the plant.

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