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# Sources and Doses of Phosphorus in the Production of Red-Leaf Lettuce in an Organic Farming System

**Bruno Rocha Tamelini<sup>1</sup>**

<https://orcid.org/0000-0001-9762-8459>

**Emanuele Possas de Souza<sup>1\*</sup>**

<https://orcid.org/0000-0002-8039-2176>

**Thatiane Nepomuceno Alves<sup>1</sup>**

<https://orcid.org/0000-0002-9077-9104>

**Antonio Ismael Inácio Cardoso<sup>1</sup>**

<https://orcid.org/0000-0003-3251-9491>

<sup>1</sup>Universidade Estadual Paulista, Faculdade de Ciências Agrônomicas, Departamento de Produção Vegetal, Botucatu, São Paulo, Brasil.

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\*Correspondence: [emanuele.possas@unesp.br](mailto:emanuele.possas@unesp.br); Tel.: +55-18-996188830 (E.P.S.)

## HIGHLIGHTS

- Bone Meal results in plants with higher circumference and dry matter weight.
- Yoorin Thermophosphate promotes greater phosphorus accumulation than Bone Meal.
- Phosphorus doses above the recommended result in an increase of the attributes.

**Abstract:** Lettuce is one of the most consumed vegetables in the world, with significant global economic relevance. Furthermore, the demand for organic products today is considerably higher than in the past, causing changes in the production methods, such as the sources of fertilizer. Several nutrients are limiting for the development of lettuce when deficient in the soil, among them phosphorus stands out. Thus, the purpose of this study was to evaluate the effect of two organic sources of phosphorus at different doses on the production of red-leaf lettuce in an organic farming system. The plants used were from the cultivar Scarlet (Sakata<sup>®</sup>), transplanted and placed in 12 L plastic pots in a greenhouse, with 10 treatments being carried out in a 2 x 5 factorial scheme. Yoorin Thermophosphate and Bone Meal were tested at doses of 0, 200, 400, 600 and 800 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>. The experimental design was in randomized blocks, with four replications. Bone Meal resulted in plants with higher dry matter weight and plant circumference but lower values for plant height and leaf width. Doses above the recommended (400 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> according to the literature for soils with low phosphorus content: 6 mg dm<sup>-3</sup>), regardless of the source, resulted in an increase in the values of the characteristics evaluated, but, with a decrease in the increases as high was the dose, except for the number of leaves where the increase was linear. Yoorin Thermophosphate resulted in plants with higher phosphorus content.

**Keywords:** *Lactuca sativa*; organic production; phosphate fertilization; yield.

## INTRODUCTION

Lettuce is the leafy vegetable with the highest volume of production and consumption in Brazil. It is a rich source of phenolic acids and flavonoids, such as quercetin, glycosides and caffeic acid derivatives, particularly in the red/purple varieties. Other important elements found are iron (Fe), zinc (Zn), calcium (Ca), phosphorus (P), magnesium (Mg), manganese (Mn) and potassium (K) and other health-promoting bioactive compounds [1].

In addition to its importance in nutritional terms, it also plays a key role in the socioeconomic sphere. This is due to the fact that its production represents an important source of employment and income throughout the production chain. In addition, family farming is responsible for most of the production, requiring the employment of a substantial number of people [2].

As with other vegetables, lettuce production has increased under the organic system and plant nutrition is crucial in this production system to obtain healthy plants and good yield [3]. Many nutrients are presented as limiting factors for the development of lettuce when deficient in the soil, among them phosphorus stands out. Phosphorus is a necessary element for plant growth and plays an important role in a wide variety of metabolic processes within plants. Phosphorus deficiency is a limiting factor in agricultural production, second only to nitrogen in importance [4,5].

Phosphorus is one of greatest soil fertility issues in Brazil, as most of its soils are not only deficient in this mineral, but also extremely fixative of it. As phosphorus is a finite and natural resource, optimizing its usage is essential for a more sustainable agriculture [6].

The main sources of phosphorus in fertilization are single and triple superphosphates. However, these cannot be used in the organic farming system [6]. Rock phosphates, reactive phosphates and thermophosphates are some sources of P allowed for organic cultivation [7].

Among the options of phosphate fertilizers allowed in the organic system are Yoorin Thermophosphate and Bone Meal. These sources have as their main characteristic the slower dissolution of P in relation to soluble phosphate sources, conferring an immediate and/or residual effect on subsequent cultivations.

Bone meal is a byproduct of the slaughter of animals in slaughterhouses and if not used, it would be considered a waste to be discarded. It has low solubility in water and good solubility in weak acids, which results in the release of P more slowly, reducing its fixation in the soil [8].

Although these products are used by producers in the organic farming system, there is little scientific information about their use, mainly Bone Meal. In addition, because they release P more slowly, they may not make the nutrient available in time to be used by the plant, particularly in species with a short cycle like lettuce. Due to these factors, the objective of this study was to evaluate the influence of doses of Yoorin Thermophosphate and Bone Meal fertilizers on the production of red-leaf lettuce in an organic farming system.

## MATERIAL AND METHODS

The research was carried out in the experimental area of the Plant Production Department, Horticulture sector, belonging to the College of Agriculture (FCA) - São Paulo State University "Júlio de Mesquita Filho" (UNESP), located in the municipality of Botucatu, SP (22° 58' 11" S and 48° 23' 56" W and altitude of 870 m), in a protected environment, arch-type agricultural greenhouse, measuring 7 x 20 m and a ceiling height of 2.5 m, covered with a low-density polyethylene film of 150µm, the sides being closed with anti-aphid screen.

The plants were grown in plastic pots with a capacity of 12 L. A low natural fertility soil was used, which was analyzed and the following results were obtained: pH<sub>(CaCl<sub>2</sub>)</sub> = 4.7; organic matter = 5 g dm<sup>-3</sup>; P<sub>resin</sub> = 6 mg dm<sup>-3</sup>; H+Al = 17 mmol<sub>c</sub> dm<sup>-3</sup>; K = 0.9 mmol<sub>c</sub> dm<sup>-3</sup>; Ca = 9 mmol<sub>c</sub> dm<sup>-3</sup>; Mg = 3 mmol<sub>c</sub> dm<sup>-3</sup>; SB = 13 mmol<sub>c</sub> dm<sup>-3</sup>; CEC = 30 mmol<sub>c</sub> dm<sup>-3</sup>; V = 44 %. Corrections were made at first to increase base saturation to 80%, as reported by Raij and co-authors [9].

Ten treatments were studied, in a 2 x 5 factorial scheme. The first factor consisted of two fertilizers (Yoorin Magnesian Thermophosphate and Bone Meal) and the second of five doses of phosphorus applied before planting (0, 200, 400, 600 and 800 kg ha<sup>-1</sup> de P<sub>2</sub>O<sub>5</sub>). The chosen doses are proportional to 0, 50%, 100%, 150% and 200% of the recommended dose [9], in which for lettuce in these soil conditions it is recommended 400 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>. The experimental design was in randomized blocks, with four repetitions. The pots were placed at the spacing 1.0 x 0.5 m. Each experimental plot consisted of three pots, with one plant per pot.

The chemical characteristics of the fertilizers used were determined, obtaining the following values for Yoorin Thermophosphate: P<sub>2</sub>O<sub>5</sub>, Ca and Mg (%) = 17.0; 19.0; 8.0, respectively. For the Bone Meal the values

were: N, P, K, Ca, Mg, S, Humidity and Organic Matter (%): 0.96; 13.66; 0.70; 8.81; 0.65; 4.0; 14.0; 11.0, respectively, and Na, Cu, Fe, Mn, Zn (mg kg<sup>-1</sup>) = 3.41; 82.0; 19.08; 414.0; 279.0, respectively.

The red-leaf lettuce cultivar used was Scarlet (Sakata®). The seedlings were produced in flexible plastic trays with 200 cells and were transplanted on October 22, 2021.

The water supply was given by drip irrigation twice a day, according to the needs of the crop, using layflat hose with a flow of 1.6 L h<sup>-1</sup> and drippers spaced at 0.50 m. For topdressing fertilization Horn Meal was used. (N, P, K, Ca, Mg, S (%) = 14.59; 0.08; 0.11; 0.25; 0.04; 1.33, respectively; B, Cu, Mn, Zn, Fe (mg kg<sup>-1</sup>) = 1.33; 5.2; 66; 30; 585; 90, respectively). 2g per plant (pot) of Hoof and Horn Meal were applied 15 days after transplanting (DAT) the seedlings. It was not necessary to control pests and diseases and weeds were removed manually from the pots.

The plants were harvested on November 26, 2021, at 35 DAT, and the characteristics of plant height (cm) were evaluated: measured with a graduated ruler, from the stalk at soil level of the plant to the end of the leaf; number of leaves per plant: manual count of all leaves on the plant; length and width of the leaves (cm): all the leaves were measured with a graduated ruler, from the longitudinal and transverse ends and the average per leaf was obtained; head diameter (cm): measured with a graduated tape; head circumference: measured with a graduated tape; weight of fresh and dry matter of the plant (leaves + stem) (g plant<sup>-1</sup>): the freshly harvested plant was weighed on a semi-analytical scale, with a precision of 0.1g, then the leaves and stem were placed in paper bags and dried in a Marconi MA35 model drying oven, with forced air circulation and a temperature of 65°C to obtain a constant weight for weighing on a semi-analytical scale with a precision of 0.1g and macronutrient contents (g kg<sup>-1</sup>): N, P, K, Ca, Mg, S were accounted through chemical analysis of the aerial part according to the methodology of Malavolta and co-authors [10].

Experimental data were subjected to analysis of variance and, in case of significant effect, according to the F test, regression analysis was performed to verify the effects of phosphorus doses (P<sub>2</sub>O<sub>5</sub>) in the characteristics evaluated. To compare the sources, the Tukey test at 5% probability was used using the Sisvar software, version 5.8 [11].

## RESULTS AND DISCUSSION

The interaction between the factors was significant for the head diameter trait, while the doses factor was significant for all evaluated traits and the sources differed among themselves for plant height, leaf width and length, plant dry weight and head circumference.

Comparing the sources, it is observed that the use of Bone Meal resulted in higher values for leaf length, total dry weight and head circumference (Table 1) and head diameter, only at the dose of 400 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> (Table 2). On the other hand, the use of Yoorin Thermophosphate resulted in greater height of plants and width of leaves (Table 1), while for number of leaves and fresh weight there was no difference between the sources (Table 1).

**Table 1.** Means of plant height (PHE), number of leaves per plant (NL), leaf width (LW) and length (LL), plant fresh (PF) and dry (PD) weight and head circumference (HC) according to the source of phosphorus

Source	PHE (cm)	NL	LW (cm)	LL (cm)	PF (g planta <sup>-1</sup> )	PD (g planta <sup>-1</sup> )	HC (cm)
Bone Meal	13.3b	12.2a	13.3b	13.6a	134a	9.06a	82.5a
Yoorin Thermophosphate	14.5a	12.7a	14.5a	12.6b	122a	7.97b	75.5b

Means followed by the same letter, in the columns, do not differ from each other according to Tukey's test at 5% probability.

**Table 2.** Means of head diameter values as a function of sources for each dose of phosphorus.

Source	Doses (kg ha <sup>-1</sup> of P <sub>2</sub> O <sub>5</sub> )				
	0	200	400	600	800
	cm				
Bone Meal	14.0a	24.5a	30.0a	30.8a	32.0a
Yoorin Thermophosphate	14.0a	25.0a	23.3b	28.3a	30.5a

Means followed by the same letter, in the columns, do not differ from each other according to Tukey's test at 5% probability.

For most of the characteristics, data fit to the quadratic model was obtained (Figures 1 to 8), except for the number of leaves, where a linear effect was obtained (Figure 2). For the characteristics in which adjustment to the quadratic model was obtained, an increase in values was observed the higher the dose of

phosphorus ( $P_2O_5$ ) without reaching a maximum point within the range studied, and as the dose increases, the increases in values are smaller and smaller, with stabilization in the values in the two highest doses. Only for the number of leaves the increase was linear, which means continuous and uniform the higher the dose of phosphorus, going from approximately 9 leaves per plant in the absence of phosphate fertilizers (dose 0) to about 16 leaves per plant at the highest dose ( $800 \text{ kg ha}^{-1}$  of  $P_2O_5$ ) (Figure 2). For all characteristics analyzed, the difference between the absence of phosphorus in the planting fertilization (dose 0) and the highest dose ( $800 \text{ kg ha}^{-1}$  of  $P_2O_5$ ) were high, obtaining increases of 51% for plant height (Figure 1), 77% for number of leaves (Figure 2), 118% for leaf width (Figure 3), 59% for leaf length (Figure 4), 114% for head diameter (Figure 7) and 117% for head circumference (Figura 8). However, the greatest increases were observed for fresh and dry weight, 471% and 486% (Figures 5 and 6), respectively.

Therefore, significant gains were obtained for all evaluated characteristics when organic phosphate fertilization was used, regardless of whether it was Yoorin Thermophosphate or Bone Meal, confirming the importance of phosphate fertilization in lettuce production.

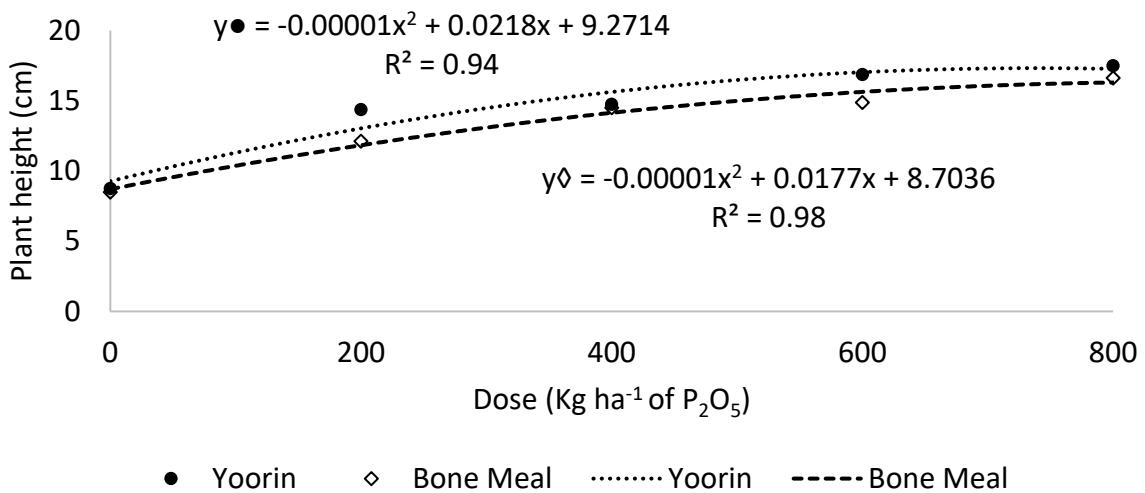


Figure 1. Plant height according to doses and sources of phosphorus ( $P_2O_5$ ).

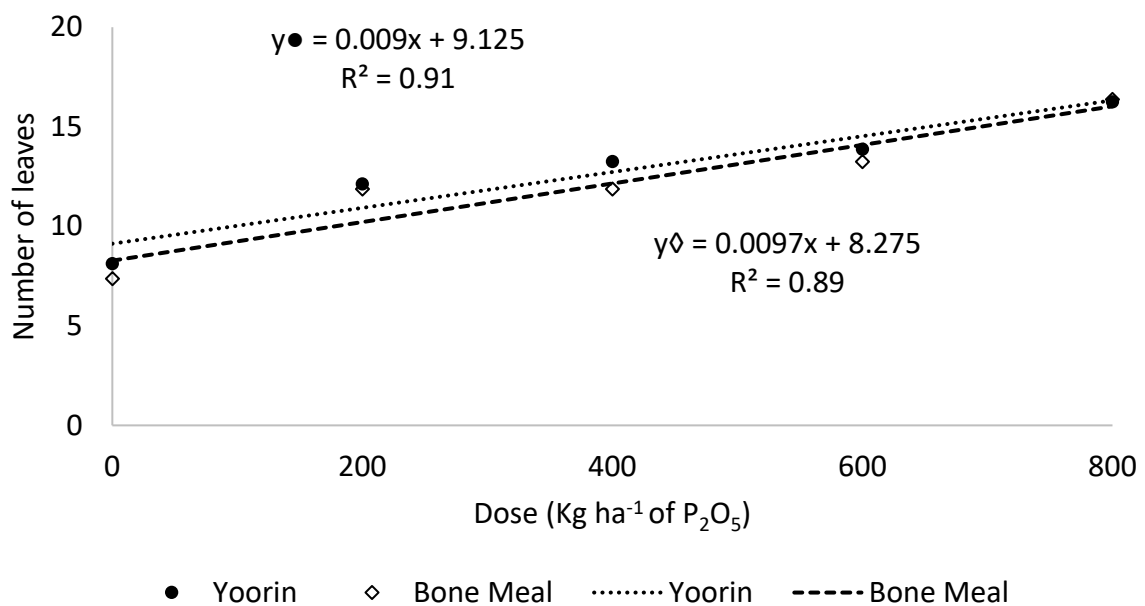
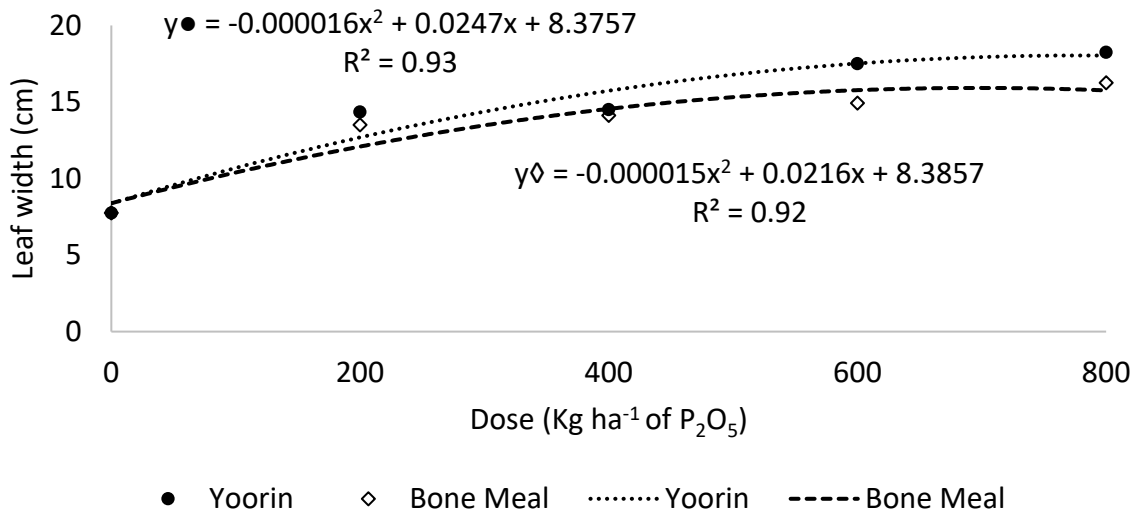
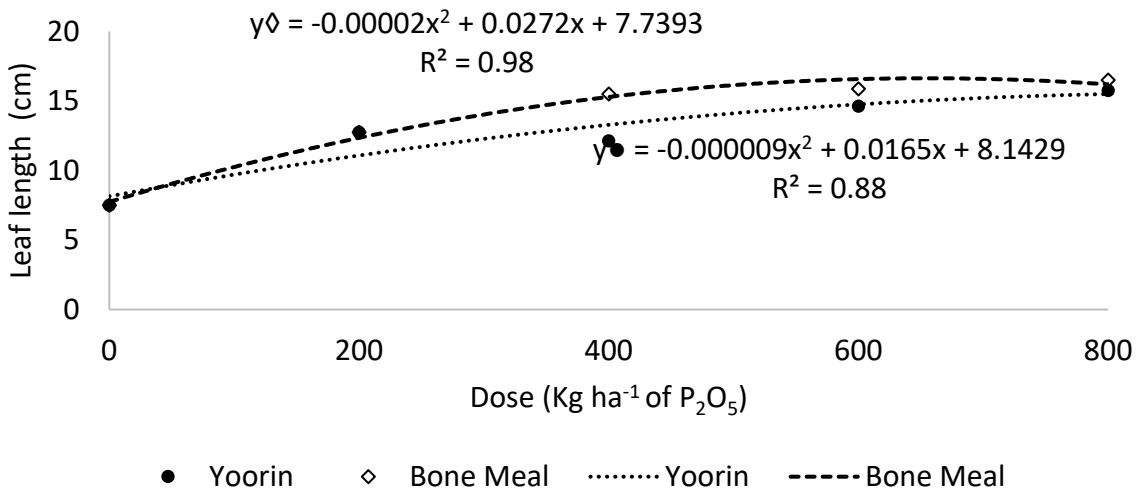


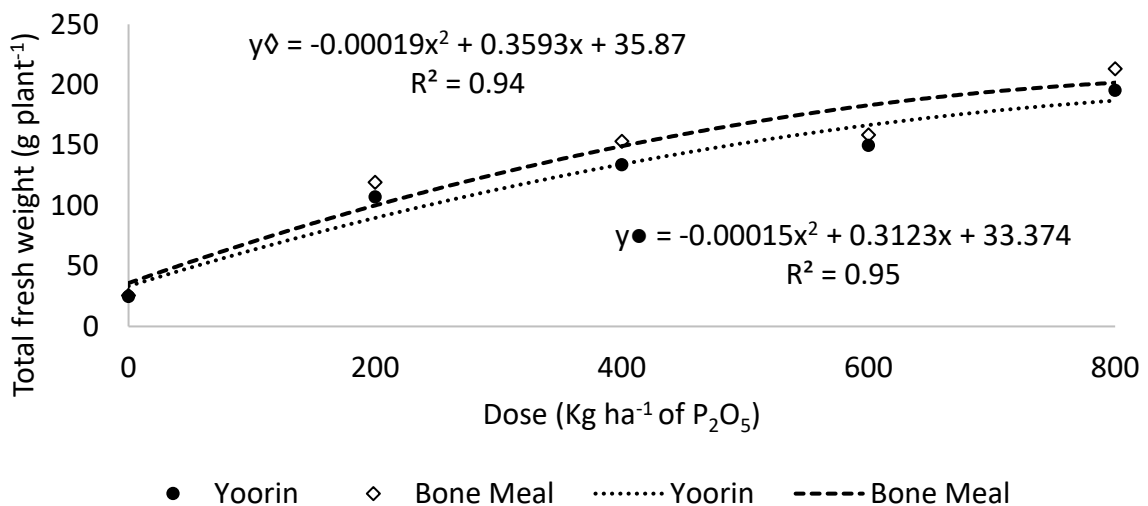
Figure 2. Number of leaves per plant according to doses and sources of phosphorus ( $P_2O_5$ ).



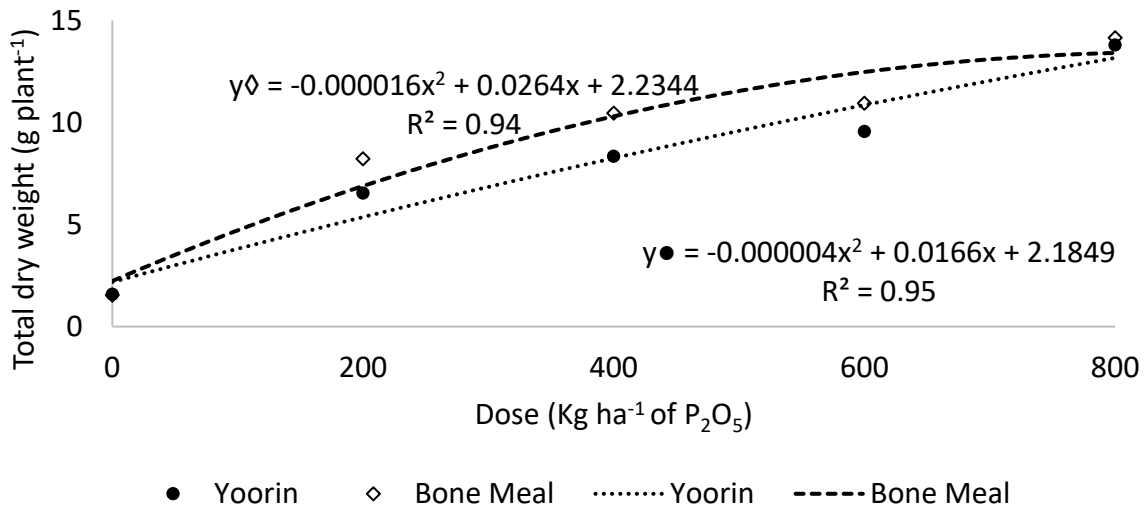
**Figure 3.** Leaf width according to doses and sources of phosphorus (P<sub>2</sub>O<sub>5</sub>).



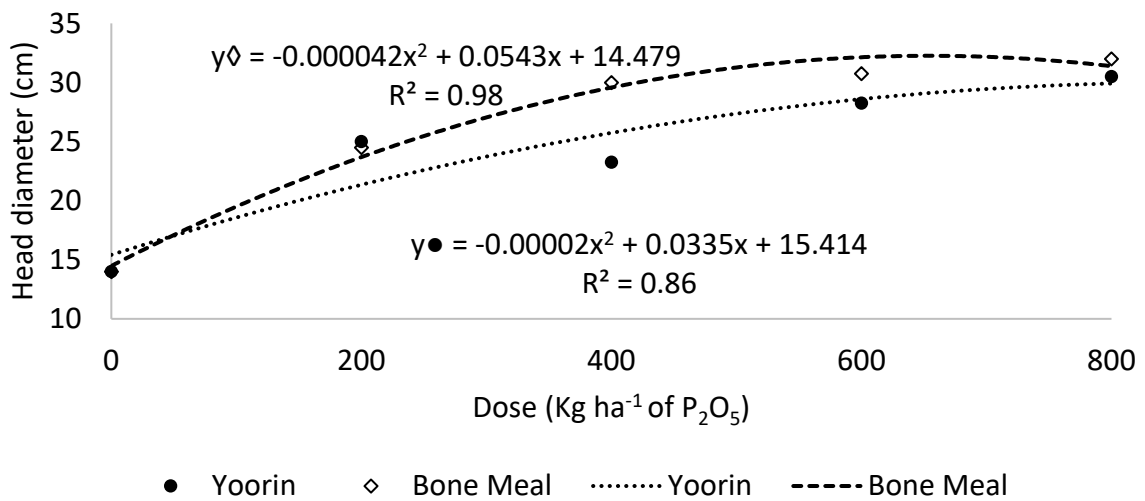
**Figure 4.** Leaf length according to doses and sources of phosphorus (P<sub>2</sub>O<sub>5</sub>).



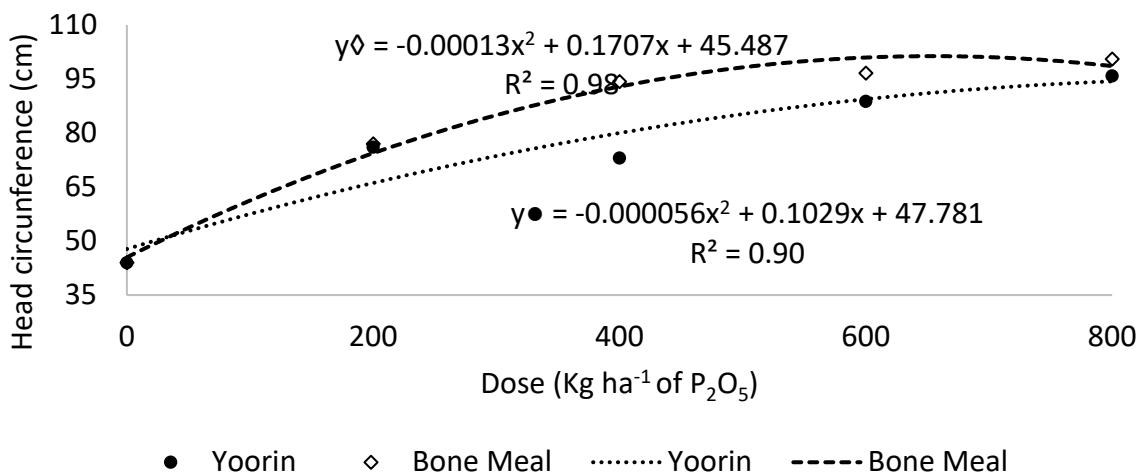
**Figure 5.** Total fresh weight according to doses and sources of phosphorus (P<sub>2</sub>O<sub>5</sub>).



**Figure 6.** Total dry weight according to doses and sources of phosphorus ( $P_2O_5$ ).



**Figure 7.** Head diameter according to doses and sources of phosphorus ( $P_2O_5$ ).



**Figure 8.** Head circumference according to doses and sources of phosphorus ( $P_2O_5$ ).

Lana and co-authors [12], in a study of fertilization with different sources of phosphorus in lettuce production, also concluded that the lack of phosphorus results in a decrease in productivity and plant diameter, in addition to lower values for all variables associated with plant development, demonstrating that lettuce needs this nutrient in the soil. Kano and co-authors [13] also obtained increases in lettuce production of up to 172% of fresh weight with the use of triple superphosphate at a dose of 733kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, dose close to the maximum evaluated in the present study, although sources with slower release were used in the present study.

Phosphorus from some organic sources, such as bone meal, is only available to plants in more acidic soils, with pH ≤ 7 [14]. However, the excessive use of this source can cause an increase in soil pH, due to the presence of Ca in its composition, which in high amounts of bone meal in the soil, could affect the availability of phosphorus for plants. For some characteristics, such as diameter and head circumference, a small reduction was estimated at the highest dose, which corresponds to 100% above the recommended dose, showing the deleterious effect of excess phosphorus applied to the soil, even with less soluble organic sources. Researching three lettuce cultivars of different varietal types, Cecílio Filho and co-authors [15] reported increased production up to the highest dose studied (300 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>), even the soil being initially rich in P (136 mg dm<sup>-3</sup>). However, in the present study, higher doses were studied (up to 800 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>), the sources were poorly soluble and the soil was low in phosphorus levels (6 mg dm<sup>-3</sup>).

According to Cecílio Filho and co-authors [16], it is common to find soils with high levels of phosphorus in areas where vegetables are grown, most of them with a short cycle and with high doses of P applied in each season. As a result, throughout the cycles, it may no longer be necessary to apply phosphorus or, more likely, it may only be necessary to replace the amount absorbed by the plant and exported with the harvest.

The recommended dose in Boletim Técnico 100 from IAC [9] is 400 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>. However, increases in production were obtained with doses higher than this. It can be assumed that this recommendation may have to be revised or, more likely, because this is a recommendation that considers only soluble sources of phosphorus, when using less soluble sources, doses should probably be higher, mainly due to the short cycle of lettuce that remained only 35 days in the field after planting. Cardoso and co-authors [6] observed that the estimated dose for maximum production in broccoli was lower when using triple superphosphate (soluble source) compared to thermophosphate (less soluble source).

Another factor that may influence the responses of Bone Meal to phosphate fertilization is the fact that to obtain it, the thermal processing method and the temperature at which the carcasses are incinerated can affect the solubility and availability of bioapatite, a valuable source of P<sub>2</sub>O<sub>5</sub> for plants [17].

The same can be said about Yoorin Thermophosphate. As stated by Cardoso and co-authors [6], Yoorin can also act as a pH neutralizing agent in the soil, due to the presence of silicate in its constitution (10%), having phosphorus bound together with magnesium, reducing its fixation in the soil, helpi

ng the absorption by the plants, not only for the phosphorus, but of other minerals essential to its full development. For lettuce, Büll and co-authors [18] noticed that Yoorin increased the concentration of phosphorus in the aerial parts of the plant and neutralized the acidity in the soil.

As for the macronutrient, the descending order of the contents in the red-leaf lettuce leaves was K>N>Ca>Mg>P>S (Table 3). Kano and co-authors [19] found the same order of accumulation of macronutrients described in this research for the green-leaf lettuce cultivar Verônica, however, the plant was intended for seed production. Regarding commercial consumption, a similar order was found by Kano and co-authors [20] also for the cultivar Verônica, with inversion of the elements phosphorus and magnesium, being K>N>Ca>P>Mg>S.

Cantarella and co-authors [21] define that adequate macronutrient contents in lettuce leaves are 30-50 (N), 3-7 (P), 50-80 (K), 15-25 (Ca), 4-6 (Mg) and 2-4 g kg<sup>-1</sup> (S). Although the nitrogen and sulfur contents of this research are lower, these are in accordance with the average values found by Palavicini and co-authors [22] for red-leaf lettuce, being 20.01 (N) and 0.92 g kg<sup>-1</sup> (S).

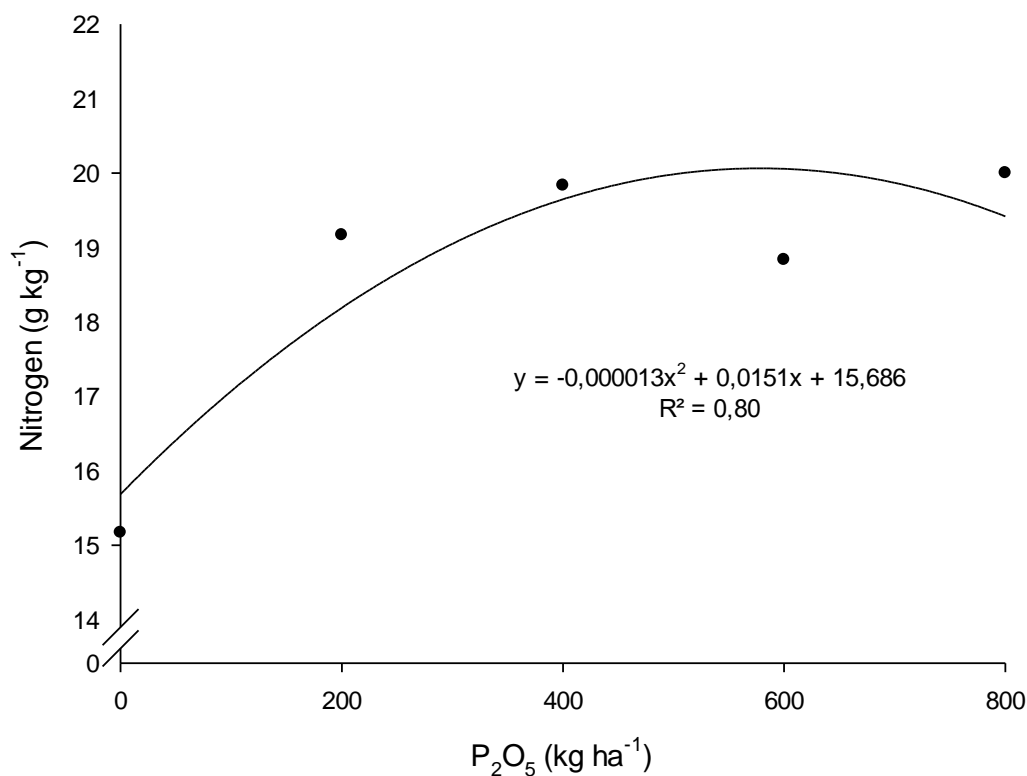
The highest values for nitrogen were found when Bone Meal was used, while for phosphorus, potassium and magnesium the highest content was for Yoorin Thermophosphate (Table 3).

The maximum dose of P<sub>2</sub>O<sub>5</sub> in which the highest nitrogen content was obtained in the plants was 580.8 kg ha<sup>-1</sup>, resulting in 20.1 g kg<sup>-1</sup> of N, or 171.15 mg plant<sup>-1</sup>. Higher doses resulted in a decline in nitrogen content (Figure 9). On the other hand, Kano and co-authors [20] found a linear response for the nitrogen content with maximum accumulation of 361.95 mg plant<sup>-1</sup> using 800 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

**Table 3.** Contents of macronutrients present in the dry matter of the lettuce plant produced according to phosphorus sources and doses.

	N	P	K	Ca	Mg	S
	-----g kg <sup>-1</sup> -----					
Bone Meal	19.73a	2.28b	55.33b	16.27a	4.97b	1.21a
Yoorin Thermophosphate	17.47b	3.13a	50.27a	16.40a	5.89a	1.13a

Means followed by different letters in the column differ according to Tukey test. ( $p < 0,05$ ).

**Figure 9.** Nitrogen content present in the dry matter of the lettuce plant produced according to phosphorus doses.

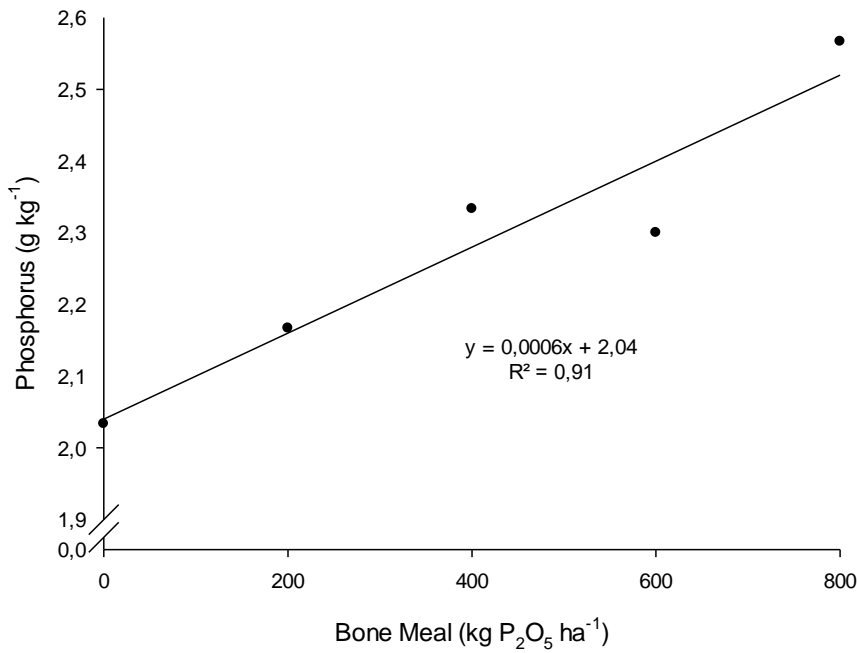
Regarding phosphorus, for both fertilizers, there is a tendency for a linear increase in phosphorus content in dry weight with increasing doses (Figures 10 and 11). Fertilization with Yoorin Thermophosphate provided the highest levels of phosphorus in all doses tested compared to the use of Bone Meal. The same linear increase effect was observed for the magnesium content, but this behavior was representative only for Yoorin Thermophosphate (Figure 11), which is a fertilizer that also contains Mg in large amounts (8%). The use of this fertilizer stood out in comparison to Bone Meal for the doses of 200, 600 and 800 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> providing the highest levels of magnesium in red-leaf lettuce plants (Table 4).

Researches testing the same doses of phosphorus fertilization of the present study demonstrated an increase in the accumulation of phosphorus with the increase in the dose of P<sub>2</sub>O<sub>5</sub> for the Verônica cultivar [20].

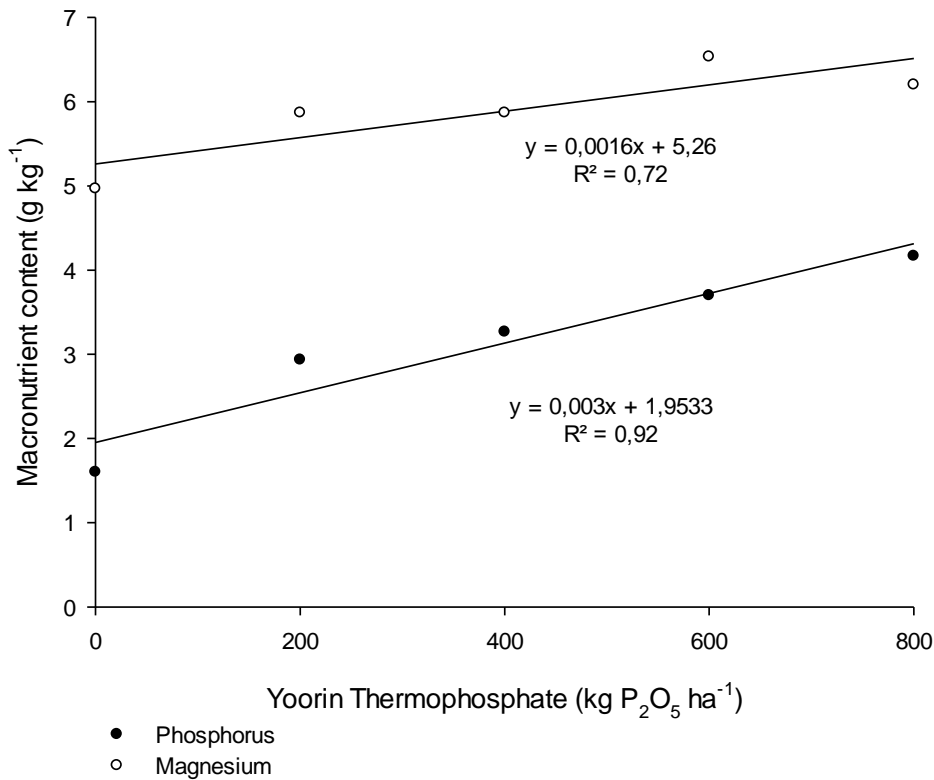
It is expected that the higher the dose of phosphate fertilizer, the greater the availability of this nutrient (up to a certain limit) to the plants and, consequently, the greater the content in the leaves of the plants. Prado [23] reports that in P deficiency, plants lack energy, which compromises biosynthetic processes. There is a great commitment to the vegetative development of the plant and also of the roots, which further reduces the phosphorus absorption capacity, therefore, it is explained that the lower the P<sub>2</sub>O<sub>5</sub> dose is, the lower is its accumulation.

The higher Mg content in plants with the use of Yoorin Thermophosphate is due to the fact that this fertilizer has a high concentration of this nutrient, compared to Bone Meal (Table 4), while Bone Meal has a greater amount of N and S, favoring an increase in the contents of these nutrients compared to Yoorin Thermophosphate.





**Figure 10.** Phosphorus content present in the dry matter of lettuce produced with different doses of Bone Meal.



**Figure 11.** Phosphorus and magnesium content present in the dry matter of lettuce produced with different doses of Yoorin Thermophosphate.

**Table 4.** Magnesium content present in the dry matter of lettuce according to the source of phosphorus for each dose of phosphorus (P<sub>2</sub>O<sub>5</sub>).

Source	Doses (kg ha <sup>-1</sup> de P <sub>2</sub> O <sub>5</sub> )				
	0	200	400	600	800
	g kg <sup>-1</sup>				
Bone Meal	4.93a	5.07b	5.23a	4.73b	4.90b
Yoorin Thermophosphate	4.97a	5.87a	5.87a	6.53a	6.20a

Means followed by different letters in the column differ according to Tukey test. (p < 0,05).

**CONCLUSION**

It is concluded that for the production of the Scarlet cultivar in a soil poor in phosphorus, the dose to be applied of a slower release phosphate fertilizer should be higher than the recommendation made for inorganic phosphates, considering that crops such as lettuce require nutrients in a short space of time.

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