

Development of passion fruit (*Passiflora edulis* L.) grown in soil amended with different limestone sources

Desarrollo de maracuyá (*Passiflora edulis* L.) cultivado en suelo enmendado con diferentes fuentes de caliza

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ABSTRACT

Keywords:

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Acid soils need to be corrected through the application of limestone; in this context, the use of mussel shell ash constitutes an ecologically viable alternative. The study was conducted to evaluate the initial development of passion fruit grown in soil amended with limestone and golden mussel shell ash. The experiment was carried out at the Educational Foundation of Andradina, located in Andradina, São Paulo. The experimental design was completely randomized in a 2x3 factorial scheme, where the first factor was composed of correctives: limestone and golden mussel shell ash, interacted with half, one, and four times the recommended dose of dolomitic limestone, plus one group control with no concealer, carrying out seven treatments with four repetitions, totaling 28 repetitions in plastic pots. The pyrolysis process was effective in producing golden mussel shell ash. The use of golden mussel shell ash can replace dolomitic limestone to increase soil pH. The recommended use of approximately 6.0 t ha⁻¹ of golden mussel shell ash is to provide suitable conditions for passion fruit cultivation. Further studies are recommended to understand the response of plants and soil to the use of alternative fertilizer sources.

RESUMEN

Palabras clave:

Limnoperna fortune
Fisiología vegetal
Ceniza de concha
Mejorador de suelos

Es necesario corregir los suelos ácidos mediante la aplicación de caliza; en este contexto, el uso de ceniza de concha de mejillón constituye una alternativa ecológicamente viable. El estudio se realizó para evaluar el desarrollo inicial del maracuyá cultivado en suelo enmendado con caliza y ceniza de conchas de mejillón dorado. El experimento se llevó a cabo en la Fundación Educacional de Andradina, ubicada en Andradina, São Paulo. El diseño experimental fue completamente aleatorizado en un esquema factorial 2x3, donde el primer factor estuvo compuesto por correctivos: caliza y ceniza de concha de mejillón dorado, interactuando con la mitad, una y cuatro veces la dosis recomendada de caliza dolomítica más un grupo control sin corrector, realizando siete tratamientos con cuatro repeticiones, totalizando 28 repeticiones en macetas plásticas. El proceso de pirólisis resultó eficaz en la producción de ceniza de concha de mejillón dorado. El uso de ceniza de concha de mejillón dorado puede sustituir a la caliza dolomítica para aumentar el pH del suelo. Se recomienda el uso de aproximadamente 6,0 t ha⁻¹ de ceniza de concha de mejillón dorado para proporcionar condiciones adecuadas para el cultivo de maracuyá. Se recomiendan estudios adicionales para comprender la respuesta de las plantas y del suelo al uso de fertilizantes alternativos.

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Among the fruit trees cultivated in Brazil, sour passion fruit (*Passiflora edulis* L.) has stood out as a good income option for small producers in family farming. One of the main economic interests is its use in the production of juice in the food industry due to its juice being very concentrated and containing vitamin C in its composition. With a cultivated area of around 50,000 hectares and production exceeding 700,000 tons, the country stands out as the world's main producer of the fruit. In this way, correcting the soil and correct fertilization will contribute to the nutritional status of the plant and will have good initial development (Lisboa et al. 2021).

The process of soil acidification in tropical regions is a natural phenomenon, where H^+ protons increase the absorption rate of acidifying metal ions such as Al^{3+} , Fe^{2+} , and Mn^{2+} . This fact proves that soil acidification increases the positive charge on soil colloids, and thus causes a reduction in the adsorption of basic cations under the action of static electricity and causes nutrients to be easily leached during precipitation. Thus, correcting soil pH directly influences the action of alkaline and alkaline earth ions such as Ca^{2+} , Mg^{2+} , and K^+ , which are important nutrients for plants and act as buffer substances in the soil solution (Yang et al. 2023; Yang et al. 2022). Obtaining agricultural limestone causes environmental damage due to the extraction of rocks from deposits, and it is a limited source, which makes it necessary to seek natural sources to replace agricultural limestone.

Due to the high demand for limestone and the limited sources of this material, it is important to look for alternatives for liming the soil through the use of mollusc shell powder. The use of ash from golden mussel (*Limnoperna fortunei*) shells as a source of lime-stone can make it a renewable and sustainable source the low cost, in addition to solving the problem with the presence of this mollusk in rivers, fish farms, hydroelectric plants and in the metal structures of ship hulls, as they become encrusted on surfaces and make drainage of these areas difficult, thus increasing maintenance costs and causing economic damage (Ferraz et al. 2021; Anggraini et al. 2023). Other studies have demonstrated the efficiency of using oyster shell powder, where this material is subjected to a thermal modification process at a

temperature of 700 °C and is then sieved through a 300-mesh sieve, and another product can also be obtained through the use of oyster shell powder combined with organic microbial fertilizer (Zheng et al. 2024).

Soil preparation combined with adequate pH correction from a limestone source can provide greater stability in the initial stages of plant development, thus reducing the chance of physiological problems, mainly in the formation of chlorophyll and even causing its degradation, and thus, starts to affect the process of light and liquid photosynthesis (Lauer 2023). With the degradation of chlorophyll, its concentration is reduced and thus influences the release of nicotinamide adenine dinucleotide (NAD^+ hydrogen (H) ($NADH^+$) for the atmospheric carbon fixation process during the formation of glucose in the Calvin cycle, and thus the initial development of the plant becomes compromised.

Cultivation in uncorrected soils with a pH that is not ideal for plant development causes damage to the external and internal tissues of the roots and thus begins to influence the morphological parameters of the leaves, mainly in the formation of epidermis cells. Changes in cell formation can occur due to nutrition (Lisboa et al. 2024), water restrictions (Menezes and Lisboa 2023); exposure to aluminum (Lisboa et al. 2021), which makes it necessary to adapt the chemical attributes of the soil for the cultivation of sour passion fruit.

The study was carried out with the aim of the initial development of passion fruit grown in soil fertilized with limestone and ash from golden mussel shells.

MATERIALS AND METHODS

Obtaining mussel shell ash

Mollusk shells of the golden mussel species (*Limnoperna fortunei*) originating from the Paraná River basin were collected and sent to the laboratory, where they underwent the pyrolysis process with gradual heating from 150 to 600 °C in an oven muffle furnace, and the ash was macerated with the aid of a pestle and mortar, and then sieved through a 2.0 mm mesh.

Experimental conduct and design

The experiment was carried out in 2023 at the Educational Foundation of Andradina, located in

Andradina, São Paulo. The experimental design was completely randomized in a 2x3 factorial scheme, where the first factor was composed of correctives: limestone and golden mussel shell ash, interacted with half, totaling six treatments with doses and sources of corrective agents, one and four times the recommended dose of dolomitic limestone plus one group control with

no concealer, carrying out seven treatments with four repetitions totaling 28 repetitions or plastic pots.

The plastic pots had 6 L⁻¹ and were filled with soil originating from the 0-0.3 m layer classified as hypoferric red oxisol (Embrapa 2025) and presented the following chemical attributes as shown in Table 1.

Table 1. Chemical attributes of the soil at the time of experiment installation.

pH	OM	P	K	Ca	Mg	H+Al	Al	SB	CEC	V	M
CaCl ₂	(g L ⁻¹)	(mg dm ⁻³)	mmol _c L ⁻¹							(%)	(%)
3.9	18	2.0	1.6	5.0	3.0	42	11	9.6	51.6	19	53

OM: Organic matter, SB: Sum of bases; V (%): Base saturation; M (%): Aluminum saturation, CEC: Cation exchange capacity.

The soil was fertilized according to the crop's specific requirements, as reported by Cantarella et al. (2022). Soil correction was carried out 11 days before planting the passion fruit seedlings, where the doses applied were 0, 2.0, 3.0, and 12 t ha⁻¹. Passion fruit seedlings of the species *Passiflora edulis* (L.) were planted with 3±1 definitive leaf, with an average height of 25±2 cm. They were obtained from a commercial nursery located in the Municipality of Adamantina, state of São Paulo. During the experiment, all bags were irrigated until they reached field capacity, and all cultural treatments were carried out. At 45 days after the start of the experiment, the following evaluations were carried out:

Plant development analysis

The height of the plant (HP) was determined using a ruler graduated in millimeters; the initial height of the plant was discounted at the time of planting the seedlings, and the number of leaves (NL) was discounted from the initial number of leaves. The developed aerial part was cut at 5.0 cm from the soil level, and the roots were washed with running water to determine the dry mass of the aerial part and roots (DMAP and DMR), which were obtained through drying in a circulation oven and air renewal at a constant temperature of 65 °C until they reached constant weight.

Analysis of chlorophyll A and B and organic nitrogen content

The first fully expanded leaf was selected from the apex of the plant, where the chlorophyll A and B contents

were determined (ChlA and ChlB (μmol m⁻²)), through direct reading using the chlorofiLOG device, Falker® brand, where the values were in SPAD index (Parry et al. 2014) and subsequently converted into absolute concentrations of pigments as described by Chang and Troughton (1972). The Norg expressed in g kg⁻¹ of dry mass was also estimated, using mathematical models for the species, where the Nitrogen concentration presents a correlation with the Spad Index according to Ferreira (2006).

Stomatal analysis

Printing was also performed on the lower or abaxial epidermal side of the fully expanded leaves using cyanoacrylate ester to determine stomatal functionality (EF) and stomatal density (SD) (Castro et al. 2009). For all variables, 10 measurements will be taken per slide and the plots will be represented by the average value obtained from the measurements of each characteristic.

Determination of soil pH in CaCl₂ solution

The pH of the soil was determined in a CaCl₂ solution with a pH adjusted between 5.3±2, where an aliquot of 1.0 g of dry and sieved soil was used, with stirring for 15 minutes, and rest for 30 minutes, and subsequently the pH of the solution was determined using a potentiometer according to the methodology described by Embrapa (2009).

Statistical analysis

For statistical evaluation, the variables were subjected

to normality tests where the Shapiro-Wilk test was used, after complying with the precepts of the test, analysis of variance was performed using the F test ($P < 0.05$) and their means compared using the Scott-Knott at 5% probability (Banzatto and Kronka 2013), for the neutralization efficiency variable, polynomial regression analysis was performed, where the linear, quadratic and cubic models were tested. To identify dependent variables, Pearson's correlation analysis ($P < 0.05$) was performed and presented as a colored heatmap. In

addition, a principal component analysis (PCA) was performed following Lisboa et al. (2024) procedures, using the statistical program R (R Core Team 2019).

RESULTS AND DISCUSSION

No statistical difference was observed in plant height (PH) when limestone and golden mussel shell ash were used; however, a statistical difference was found only in the dose factor of soil amendments used, as shown in Table 2.

Table 2. Average values of pH of the soil, height of the plant (HP), number of leaves (NL), and dry mass of the aerial part and roots (DMAP and DMR) in passion fruit grown with different sources of soil improvers.

Concealers (C)	pH (soil)	HP (cm)	NL	DMAP (g)	DMR (g)
Limestone	5.56	86.35	21.18	10.10	7.87
MSA	5.40	79.78	20.56	10.58	7.99
p-value	0.0541 ^{ns}	0.1365 ^{ns}	0.7626 ^{ns}	0.4922 ^{ns}	0.8734 ^{ns}
Dose t ha⁻¹ (D)					
AC (Zero)	3.90 ^d	64.87 ^c	19.25 ^a	8.12 ^b	8.83 ^a
2.0	5.23 ^c	89.28 ^b	22.12 ^a	12.38 ^a	7.14 ^a
3.0	6.25 ^b	110.28 ^a	21.75 ^a	11.86 ^a	7.72 ^a
12	6.55 ^a	67.84 ^c	20.37 ^a	9.01 ^b	8.03 ^a
p-value	0.0001 ^{**}	0.0001 ^{**}	0.7433 ^{ns}	0.0003 ^{**}	0.4700 ^{ns}
p-value CxD	0.4156 ^{ns}	0.0577 ^{ns}	0.7065 ^{ns}	0.9312 ^{ns}	0.0692 ^{ns}
CV%	3.97	14.53	27.71	18.83	26.90
OA	5.48	83.07	20.87	10.34	7.93

AC = Absence of concealer; MSA = Mussel shell ash. OA: Overall average. CV: Coefficient of variation. ** = Significant at the 1% probability level ($P < 0.01$); * = Significant at the 5% probability level ($0.01 \leq P < 0.05$); Ns = Not significant ($P > 0.05$). The means followed by the same letter do not differ statistically. The Scott-Knott method was applied at the level of 5% probability.

A statistical difference was observed only in the soil amendment dose factor, where the maximum point was 7.83 t ha⁻¹ as shown in Figure 1.

It is essential to correct the soil pH to raise it to the ideal potential of 6.5, where calcium carbonate (CaCO₃) is a mineral with extensive natural existence and can be categorized as biological or mineral depending on the source (Zheng et al. 2024). The main component of oyster shells is CaCO₃, which is slightly soluble in water and alkaline, and can effectively remedy soil acidification in addition to increasing soil base saturation, due to the increase in Ca²⁺ concentration in the soil released by soil amendments

of the lap. Compared with naturally occurring CaCO₃, such as lime and limestone, biologically sourced calcium carbonate, such as oyster shell powder, has a fast formation cycle and is environmentally friendly and renewable (Yang et al. 2023). It is noted that with excess calcium carbonate in the soil, the pH reaches maximum saturation, implying a plateau effect on the hydrogen potential, even with an increase in the concentration of soil improvers, the release of hydroxyls does not increase linearly, showing that other interactions between soil elements, thus unfavorable soil conditions, such as high pH and NaCl, can negatively affect plant growth and survival. Thus, when the pH rises above the ideal, that is, in healthy alkaline soils,

plants show a reduction in the absorption of the element Fe, which affects the development of leaves showing chlorotic spots in younger tissues (Lauer 2023; Lisboa et al. 2024).

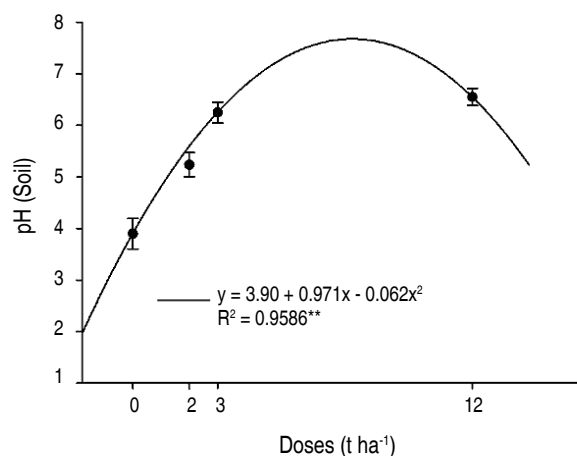


Figure 1. Regression of pH concentration in soil after the use of different soil conditioners.

The main reason for the increase in soil pH value caused by the gray shell of the golden mussel may have been due to CaCO_3 being dissolved in the soil, resulting in a large number of OH^- in the soil and thus causing the pH to rise. The increase in calcium carbonate enhanced CO_2 release during soil microbial activity, with 35% of the released carbon originating from the inorganic carbon

present in the shell, thereby increasing soil respiration (Hu et al. 2023).

A statistical difference was observed only in the effect of the dose factor on plant height (HP), where there was a quadratic response with the maximum point being approximately 6.09 t ha^{-1} as demonstrated in Figure 2.

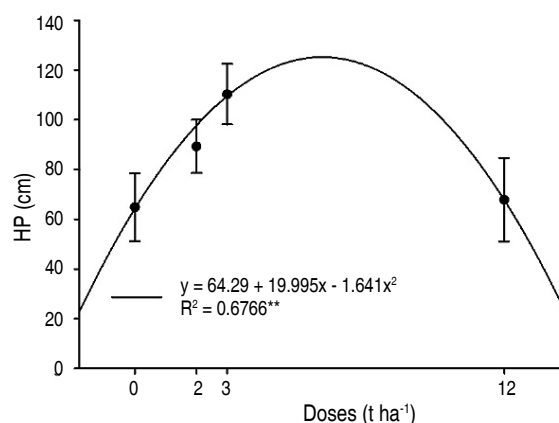


Figure 2. Regression of the height of the plant (HP) of passion fruit grown with different sources of soil improvers.

It is worth highlighting that, regardless of the use of limestone or golden mussel shell ash, they provided the same response for plant height (HP) (Table 1).

It is worth noting that plant height (HP) showed a significant and positive Pearson correlation with dry mass of the shoot (DMAP), as shown in Figure 3.

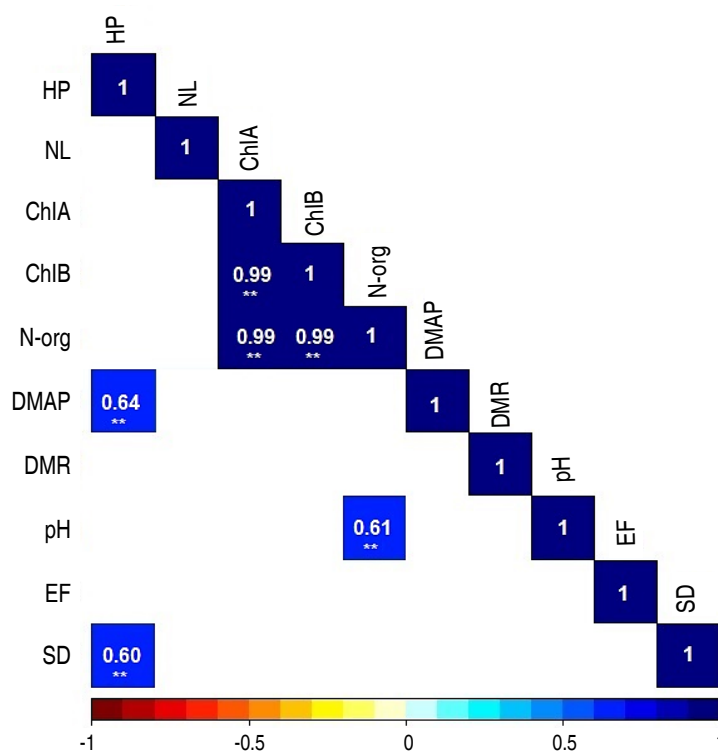


Figure 3. Significant Pearson correlations between the variables evaluated in passion fruit plants grown with different sources and doses of soil improvers.

This difference between plant heights can be well illustrated in Figure 4, where in Figure 4A it is clear that the plant that was grown in the absence of a corrector

showed lower plant development, while this is not observed in the other plants that were grown with the soil improvers.

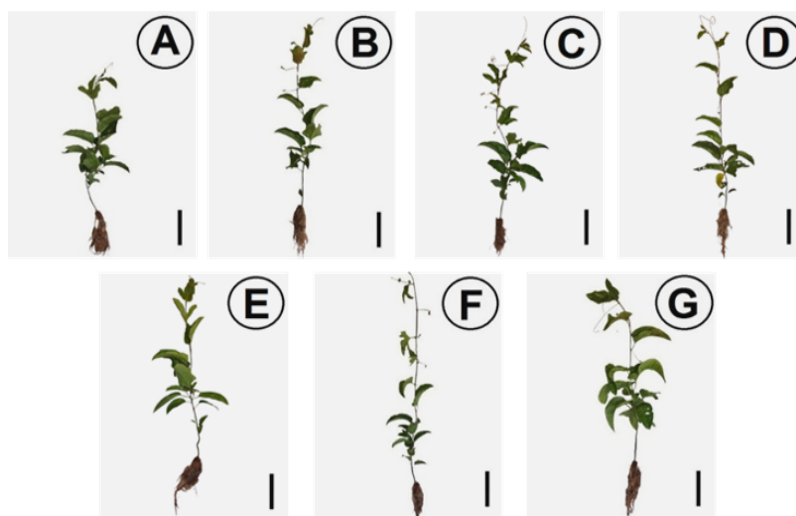


Figure 4. Passion fruit plants grown in soil amended with dolomitic limestone and golden mussel shell powder. Bar = 20 cm. **A.** Absence of corrector; **B.** 2.0 t ha⁻¹ of the dolomitic limestone; **C.** 3.0 t ha⁻¹ of the dolomitic limestone; **D.** 12.0 t ha⁻¹ of the dolomitic limestone; **E.** 2.0 t ha⁻¹ of the mussel shell ash; **F.** 3.0 t ha⁻¹ of the mussel shell ash, and **G.** 12.0 t ha⁻¹ of the mussel shell ash.

Regardless of the use of limestone or shell ash, the plant shows similar responses at plant growth, which demonstrates the similar action of mussel shell ash in altering the chemical properties of the soil, thus, when the soil is amended with mussel powder shells can provide an increase in the availability of nutrients for plants, and thus provided an increase in their height, and dry mass, on average 56.41% as reported by Martial et al. (2023), where showed a correlation between these variables HP with DMAP (Figure 3), so as it increases carbon fixation in the dry mass and assimilation of nutrients, it provides a greater accumulation of dry mass, and it is also worth highlighting that these results can be attributed to the capacity of the

heat-treated shell, which contains mainly calcium oxide and chitin, stimulating the activities of beneficial soil-promoting microorganisms and modifying the physicochemical properties of the soil (Buntaro et al. 2023).

It is notable that the sources of correctives, such as limestone (Red) and golden mussel shell ash (Blue), presented similar contributions to each other as shown in Figure 5A, but it can be observed that the golden mussel shell ash source presented a broader contribution in relation to dolomitic limestone. It is also observed that the main variables are in the positive quadrant, thus confirming the correlation between them (Figure 5B).

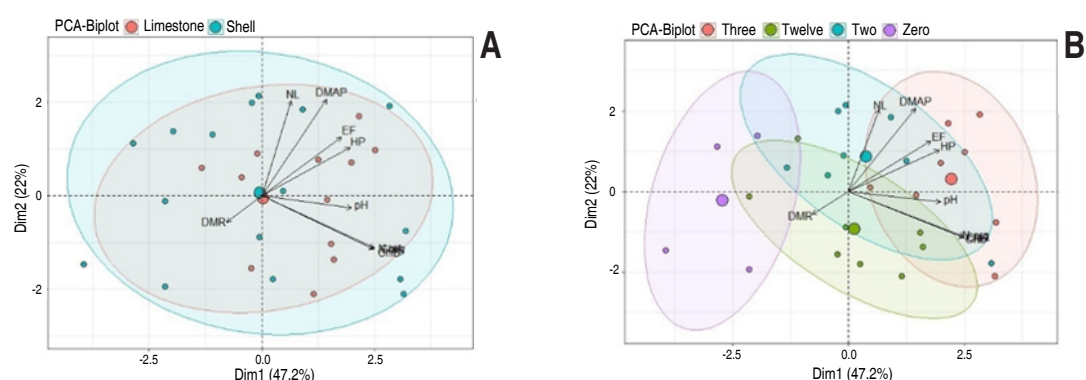


Figure 5. Principal component analysis (PCA) of the contributions of the factors on the response variables. **A.** Contribution of limestones; and **B.** Contribution of limestone doses.

The absence (0 t ha^{-1} - Purple) of amendments presented a smaller contribution to the response of the variables, where its area was plotted in the negative region on the left as seen in Figure 5B, while the concentration of 3.0 t ha^{-1} (Red) of correctives presented a more positive contribution to the right, while the use of 12 t ha^{-1} (Green) did not contribute to the evaluated parameters satisfactorily, showing that high concentrations of soil amendments can influence the availability of nutrients for the plant, and consequently its development.

No statistical difference was observed for the number of leaves (NL) regardless of the type and dose of soil improvers. However, a statistical difference was observed in isolation only for doses of soil improvers for the dry mass of the aerial part (DMAP) as shown in Table 2 and presented a quadratic response in the doses of soil improvers, with a maximum point of approximately 6.09 t ha^{-1} as shown

in Figure 6, and no statistical difference was observed between the factors for root dry mass (DMR) (Table 2).

The lack of difference between the sources of correctives shows the efficiency of shell ash that can replace dolomitic limestone, as these replacements provided an equal dry mass of plants, while the need to correct the soil pH is essential to guarantee a better plant development. It is worth highlighting that the presence of shell dust in the soil, in addition to ensuring larger plants (Figure 4), can also provide the activation of specific genes in the defense of plants against disease attacks, improving plant health (Martial et al. 2023). When a plant has a higher rate of carbon assimilation in its dry mass, it starts to be reflected in the height of the plant, which is reflected in taller plants (Menezes and Lisboa 2023), thus showing a positive correlation between these two variables as demonstrated in Figure 3.

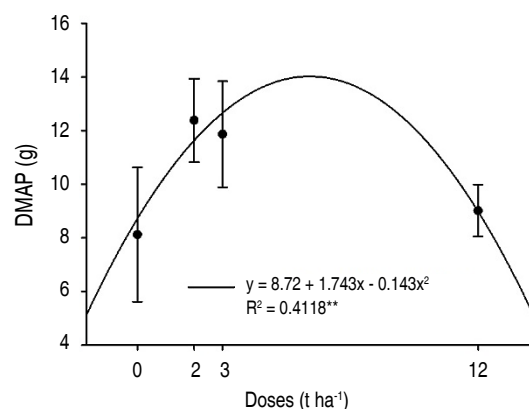


Figure 6. Regression of the dry mass of the aerial part (DMAP) of passion fruit cultivated with different sources of soil improvers.

A significant interaction was observed between types of correctors and the doses used in the concentrations of chlorophylls A and B, as shown in Table 3.

Regardless of the type of soil improver, both presented the same general averages for the concentrations

of chlorophylls A and B, where the maximum point was approximately 8.11 t ha⁻¹ for the use of limestone, respectively in the concentrations of chlorophyll A and B and for the use of golden mussel shell ash, the maximum points were 6.33 t ha⁻¹, respectively, as shown in Figure 7.

Table 3. Average values of chlorophylls A and B (ChIA and ChIB (μmol m⁻²)), organic nitrogen (N (g kg⁻¹)), stomatal functionality (SF), and stomatal density (SD (no./mm²)) in passion fruit grown with different sources of soil improvers.

Concealers (C)	ChIA	ChIB	N	SF	SD
Limestone	356.26	118.75	0.376	1.82	156.56
MSA	351.50	117.16	0.372	2.01	145.00
p-value	0.8761 ^{ns}	0.8761 ^{ns}	0.8094 ^{ns}	0.1118 ^{ns}	0.2273 ^{ns}
Dose t ha ⁻¹ (D)					
AC (Zero)	240.69 ^b	80.23 ^b	0.309 ^b	1.43 ^b	122.50 ^b
2.0	398.15 ^a	132.71 ^a	0.400 ^a	2.13 ^a	160.62 ^a
3.0	396.68 ^a	132.22 ^a	0.399 ^a	2.42 ^a	182.50 ^a
12	380.01 ^a	126.67 ^a	0.389 ^a	1.67 ^b	137.50 ^b
p-value	0.0026 ^{**}	0.0026 ^{**}	0.0005 ^{**}	0.0001 ^{**}	0.0007 ^{**}
p-value CxD	0.0144 [*]	0.0144 [*]	0.0140 [*]	0.0405 [*]	0.0120 [*]
CV (%)	24.14	24.14	12.69	16.69	17.50
OA	353.88	117.96	3.74	1.92	150.78

AC = Absence of concealer; MSA = Mussel shell ash. OA: Overall average. CV: Coefficient of variation. ** = Significant at the 1% probability level ($P < 0.01$); * = Significant at the 5% probability level ($0.01 \leq P < 0.05$); Ns = Not significant ($P \geq 0.05$). The means followed by the same letter do not differ statistically. The Scott-Knott method was applied at the level of 5% probability.

When the plant is grown in unfavorable pH conditions, the absorption of macro nutrients such as magnesium and nitrogen are negatively influenced, as in acidic soils these nutrients are not available to the plant (Hartemink and Barrow 2023; Marschner and Rengel 2023), and

as previously mentioned, that in alkaline soils the availability of micronutrients is compromised, thus highlighting the importance of Mg and Fe for the proper growth and development of plants, as magnesium is part of the composition of the molecule chlorophyll, while Fe

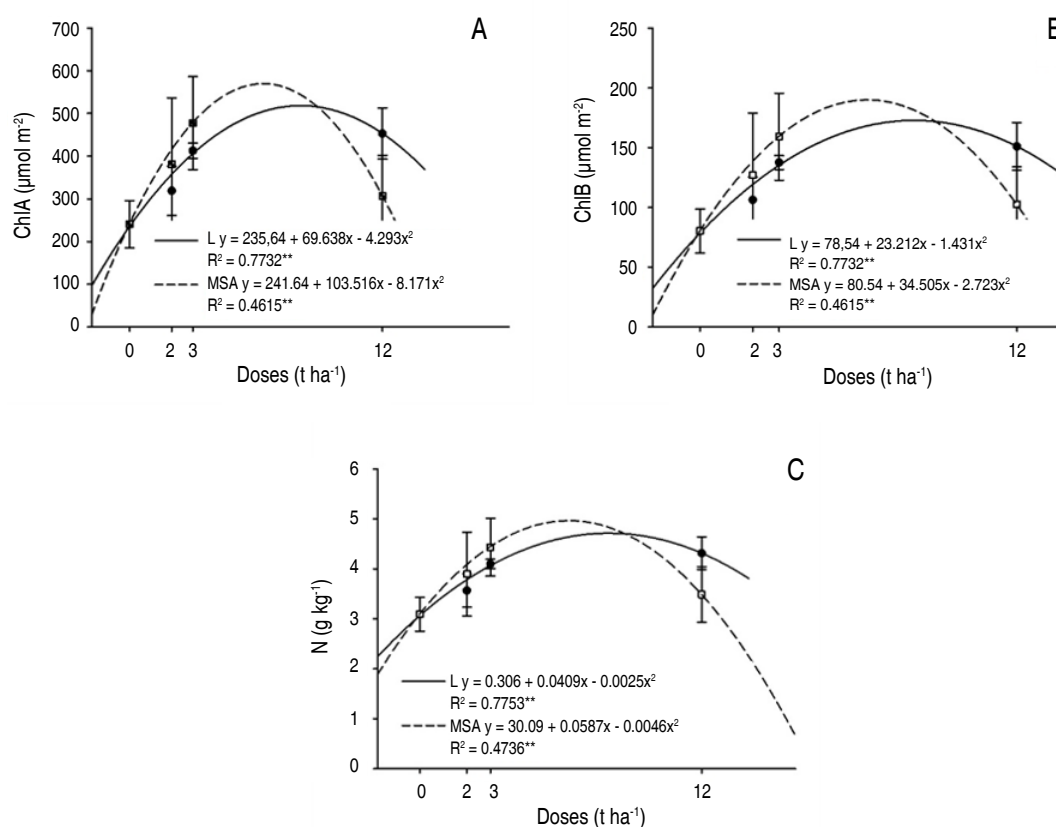


Figure 7. Regression of chlorophylls A and B (ChIA and ChIB) and organic nitrogen (N) in passion fruit grown with different sources of soil improvers. L = Limestone and MSA = Mussel shell ash.

is involved in the structure of ferredoxin in the chloroplast photosynthetic system (Lisboa et al. 2024).

In the N (nitrogen) characteristic, a significant interaction was observed between the types of soil improvers and the applied doses, where the two improvers showed quadratic responses with maximum points of approximately 8.18 t ha⁻¹ of limestone and 6.38 t ha⁻¹ golden mussel shell ash, as shown in Figure 7C.

With the increase in the concentration of chlorophyll in the leaves, the nitrogen content also increases, as the chlorophyll molecule has in its composition a magnesium atom in the center and linked with four nitrogen atoms. This demonstrates the correlation between the concentration of chlorophyll and organic nitrogen present in passion fruit leaves (Figure 3). It is worth noting that nitrogen plays a fundamental role in the synthesis of photosynthetic products and is an essential component of chlorophyll, proteins, amino

acids, and photosynthetic enzymes in plants (Yang et al. 2022). With the low concentration of nitrogen, it changes the carbon fixation capacity and thus negatively reflects the photosynthesis of plants, and thus becomes one of the most important elements in plant growth (Gao et al. 2022). When the corrector concentration passes the maximum point, the concentration of chlorophyll and organic nitrogen is reduced, as the high saturation of soil pH (Figure 1) negatively influences plant development (Figures 2 and 6).

An interaction was observed between the soil corrective factor and the doses in stomatal functionality (SF), where the maximum points were 6.40 t ha⁻¹ of dolomitic limestone and 6.20 t ha⁻¹ of mussel shell ash-golden, as shown in Figure 8A. Likewise, in stomatal density, there was an interaction between the factors, where the responses were quadratic with maximum points of 7.30 t ha⁻¹ of dolomitic limestone and 5.75 t ha⁻¹ of mussel shell ash-golden highlighted in Figure 8B.

The change in the chemical attributes of the soil guarantees better absorption of nutrients by plants due to the adequate regulation of soil pH, as macro nutrients become available in soil solutions (Barrow and Hartemink 2023; Hartemink and Barrow 2023; Marschner and Rengel 2023), when the plant is well nourished, its metabolism occurs with greater efficiency and ensures greater diffusion of CO₂ to the carboxylation

site in net photosynthesis, this occurs due to the improvement in the opening in the ostiole, which results in greater stomatal functionality (SF) and thus starts to influence stomatal conductance and CO₂ diffusion in the mesophyll (Han et al. 2023). Further studies are needed regarding the use of golden mussel shell ash, seeking answers regarding soil fertility and chemistry, as well as its effects on the microbiota of the soil.

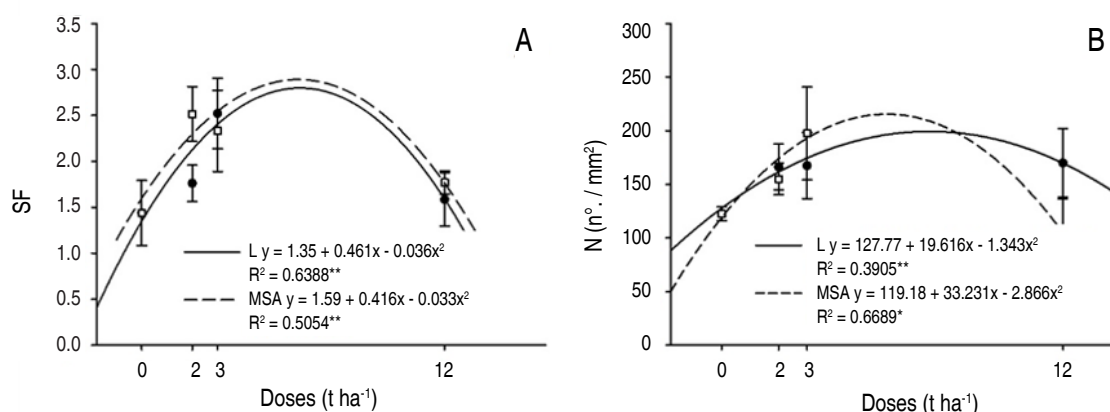


Figure 8: Regression of stomatal functionality (SF) and stomatal density (SD) in passion fruit grown with different sources of soil improvers. L = Limestone and MSA = Mussel shell ash.

CONCLUSION

The pyrolysis process was effective in producing golden mussel shell ash. This material can be used as an alternative to dolomitic limestone to increase soil pH. An application rate of approximately 6.0 t ha⁻¹ of golden mussel shell ash is recommended to establish suitable soil conditions for passion fruit cultivation. Further studies are required to deepen the understanding of plant and soil responses to the use of alternative fertilizer sources.

CONFLICT OF INTERESTS

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 paper.

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