# Alternative use of soil conditioners for cotton cultivation

Uso alternativo de condicionadores de solos para a cultura do algodoeiro

Uso alternativo de acondicionadores de suelo para el cultivo de algodón

Received: 08/08/2023 | Revised: 08/25/2023 | Accepted: 02/21/2024 | Published: 02/25/2024

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#### Abstract

Some production environments may present low pH and high levels of  $Al^{3+}$ , thus making this element a limiting factor in the productivity of the cotton crop, which makes it necessary to use agricultural gypsum as a soil conditioner and even shell dust as sources low-cost alternatives to improve soil chemical attributes. The objective of this work was to know the responses of the use of alternative soil conditioners with aluminum in the cotton crop. The experiment was carried out in August 2022, at Faculdades Integradas Stella Maris (FISMA). The design was completely randomized, with six treatments, namely: no use of soil conditioners, agricultural gypsum (3.0 t ha<sup>-1</sup>); golden mussel shell powder (3.0 t ha<sup>-1</sup>); snail powder (3.0 t ha<sup>-1</sup>); agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail powder (1.5 t ha<sup>-1</sup>) and with four replications, totaling 24 plots or pots. The use of agricultural gypsum at a dose of 1.5 t ha<sup>-1</sup> associated with golden mussel shell powder at a dose of 1.5 t ha<sup>-1</sup> showed better responses in cotton development. The use of agricultural gypsum at a dose of 1.5 t ha<sup>-1</sup> associated with golden mussel shell powder at a dose of 1.5 t ha<sup>-1</sup> improves soil conditions for cultivation. The pyrolysis method at high temperatures to break the crystalline structures of mollusc shells was efficient.

Keywords: Gossypium hirsutum L.; Agricultural gypsum; Shell powder; Golden mussel; Snail.

### Resumo

Alguns ambientes de produção podem apresentar, baixo pH e altos teores de  $Al^{3+}$ , tornando assim pois esse elemento torna um fator limitante na produtividade da cultura do algodoeiro, que tonar necessário o uso de gesso agrícola como condicionador de solo e mesmo pó de conchas como fontes alternativas debaixo custo para melhorar os atributos químicos do solo. O objetivo desse trabalho foi de conhecer as respostas do uso de condicionadores alternativos de solo com alumínio na cultura do algodoeiro. O experimento foi realizado em agosto de 2022, nas Faculdades Integradas Stella Maris (FISMA). O delineamento foi inteiramente casualizado, com seis tratamentos, sendo eles: ausência do uso de condicionadores de solo; gesso agrícola (3,0 t ha<sup>-1</sup>); pó de concha de mexilhão-dourado (3,0 t ha<sup>-1</sup>); pó de caramujo (3,0 t ha<sup>-1</sup>); gesso agrícola (1,5 t ha<sup>-1</sup>) com pó de concha de mexilhão dourado (1,5 t ha<sup>-1</sup>) e gesso agrícola (1,5 t ha<sup>-1</sup>) com pó de caramujo (1,5 t ha<sup>-1</sup>) e com quatro repetições, totalizando 24 parcelas ou vasos. O uso do gesso agrícola na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado na dose de 1,5 t ha<sup>-1</sup> associado com o pó de concha de mexilhão dourado solo para cultivo. O método de pirólise em altas temperaturas para romper as estruturas cristalinas das conchas dos moluscos foi eficiente. **Palavras-chave:** *Gossypium hirsutum* L., Gesso agrícola, Pó de concha, Mexilhão-dourado, Caramujo.

#### Resumen

Algunos ambientes de producción pueden presentar pH bajo y niveles altos de Al<sup>3+</sup>, lo que convierte a este elemento en un factor limitante en la productividad del cultivo de algodón, lo que hace necesario el uso de yeso agrícola como acondicionador del suelo e incluso polvo de concha como fuentes alternativas de bajo costo para mejorar los atributos químicos del suelo. El objetivo de este trabajo fue conocer las respuestas del uso de acondicionadores de suelo alternativos con aluminio en el cultivo de algodón. El experimento se realizó en agosto de 2022, en las Faculdades Integradas Stella Maris (FISMA). El diseño fue completamente al azar, con seis tratamientos, a saber: sin uso de acondicionadores de suelo; yeso agrícola (3,0 t ha<sup>-1</sup>); polvo de concha de mejillón dorado (3,0 t ha<sup>-1</sup>); polvo de caracol (3,0 t ha<sup>-1</sup>); yeso agrícola (1,5 t ha<sup>-1</sup>) con polvo de concha de mejillón dorado (1,5 t ha<sup>-1</sup>) y yeso agrícola (1,5 t ha<sup>-1</sup>) con polvo de caracol (1,5 t ha<sup>-1</sup>) y con cuatro repeticiones, totalizando 24 parcelas o macetas. El uso de yeso agrícola en dosis de 1,5 t ha<sup>-1</sup> asociado a polvo de concha de mejillón dorado en dosis de 1,5 t ha-1 mostró mejores respuestas en el desarrollo del algodón. El uso de yeso agrícola a dosis de 1,5 t ha<sup>-1</sup> asociado a polvo de concha de mejillón dorado a dosis de 1,5 t ha<sup>-1</sup> mejora las condiciones del suelo para el cultivo. El método de pirólisis a altas temperaturas para romper las estructuras cristalinas de las conchas de los moluscos resultó eficiente.

Palabras clave: Gossypium hirsutum L.; Yeso agrícola; Polvo de concha; Mejillón dorado; Caracol.

## **1. Introduction**

Cotton belongs to the Malvaceae family, where Brazil is one of the largest cotton producers in the world, with the largest producing region being in the Midwest region. Some varieties may show greater adaptation in different production environment conditions, therefore, in regions that have high levels of  $Al^{3+}$ , as this element becomes a limiting factor in crop productivity (Wei *et al.*, 2021).

The cotton plant, despite being an adaptable plant in different types of soil, needs medium fertility conditions to express its productive potential. increase in aluminum solubility. The high concentration of aluminum causes direct damage to root formation, which may reflect in damage to the metabolic functions of plants (Rahman et al. 2018).

In the soil solution, in general, the literature points out that less than 5 mmolc dm<sup>-3</sup> of Al<sup>3+</sup> should not be toxic; 5-10 mmolc dm<sup>-3</sup> is probably toxic and 10 mmolc dm<sup>3</sup> is highly likely to be, as species and cultivars within the same species can show very different tolerance to aluminum toxicity (Malavolta, 1980). When seedlings were grown in soils with pH 4.2 and exposed to Al<sup>3+</sup> concentrations of 10 mmol m<sup>-3</sup> of soil, NO<sup>3-</sup> absorption was stimulated, whereas above 44 mmol m<sup>-3</sup> it caused a decrease in the absorption of this nutrient. And a change in the architecture of the roots was also observed, where at lower concentrations there was an elongation of the root, which did not occur at high concentrations of Al<sup>3+</sup> (Rufty et al. 1995).

The choice of genetically improved varieties can be a way to enable the cultivation of cotton in acid soils, however, correctives can be used to neutralize aluminum with the application of agricultural gypsum, where it is used as a soil conditioner, as it increases rapidly the concentration of calcium and sulfate in the subsoil. Because that would be a management strategy, when the increasing costs of inputs are considered, which requires seeking alternative sources of correctives and soil conditioners, in addition to mixing with other sources and concentrations (Lauricella *et al.*, 2020, Luiz *et al.*, 2022).

The objective of this work was to know the responses of the use of alternative soil conditioners with aluminum in the cotton crop.

### 2. Material and Methods

Mollusc shells of the golden mussel (*Limnoperna fortunei*) and snail (*Pomacea megastoma*) species were collected, originating from the Paraná River basin, and sent to the laboratory, where they underwent the pyrolysis process with gradual heating from 150 to 150°C to 600 °C in a muffle furnace, and the ash was macerated with the aid of a pestle and mortar, and then sieved through a 2 mm mesh.

The experiment was carried out in August 2022, at Faculdades Integradas Stella Maris (FISMA), located in the Municipality of Andradina, State of São Paulo. The design was completely randomized, with six treatments, namely: no use of

soil conditioners; agricultural gypsum (3.0 t ha<sup>-1</sup>); golden mussel shell powder (3.0 t ha<sup>-1</sup>); snail powder (3.0 t ha<sup>-1</sup>); agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail powder (1.5 t ha<sup>-1</sup>) and with four replications, totaling 24 plots or pots.

The pots had a volumetric capacity of 12 dm-3 and were filled with soil originating from the 0-0.3 m layer classified as Hypoferric Red Latosol (Embrapa, 2013) and has the following chemical attributes as shown in Table 1.

pН	OM	Р	Κ	Ca	Mg	H+Al	Al	SB	CTC	V%	m%
CaCl <sub>2</sub>	g dm-3	mg dm <sup>-3</sup>	mmol <sub>c</sub> dm <sup>-3</sup>								
3,9	18	2	1,6	5	3	42	11	9,6	51,6	19	53

Table 1 - Soil chemical attributes at the time of installation of the experiment.

OM: Organic matter; SB: Sum of bases; V%: Base saturation; m%: Saturation by aluminum. Source: Authors.

The soil was fertilized according to the requirements of the cotton culture according to Raij *et al.*, (1996). And a seed was sown two inches deep. During the experiment, all pots will be irrigated until they reach field capacity and all cultural treatments will be performed.

At 45 days after sowing, the following were determined: plant height (AP) using a graduated ruler in millimeters, total leaf area (LA) obtained using the Easy Leaf Area® application (Easlon & Bloom, 2014), number of leaves (NL), shoot dry mass (SDM) and root dry mass (RDM) which was obtained through drying in a circulation oven and air renewal at constant temperature of 65°C until reaching constant weight.

The levels of chlorophyll a and b (Chlorine A and Chlorine B -  $\mu$ mol m-2) were determined by reading with the use of the chlorofiLOG device, Falker® brand, given the values of the SPAD index (Parry *et al.*, 2014) and later converted in absolute values of the pigments as described by Chang and Troughton (1972) and the N-org expressed in dag/kg of dry mass was also estimated according to Ferreira (2006).

An impression was also made on the lower or abaxial epidermal face of the first fully expanded leaf from the apex of the plant, where the cyanoacrylate ester was used for the determination of stomatal functionality (FUNE) and stomatal density (DE) (Carlquist, 1975, Castro *et al.*, 2009). For all variables, five measurements were performed per slide, and the plot was represented by the mean.

The following soil chemical attributes were also determined: pH in CaCl<sub>2</sub> and exchangeable aluminum in KCl according to Embrapa (2009). The plots were represented by the average value obtained from the measurements of each characteristic.

The neutralization action of soil conditioners in solution where aliquots of 0.1; 0.2; 0.4 and 0.8 g of conditioners in 100 mL of  $CaCl_2$  solution with initial pH of 4.5, according to methodology adapted from Embrapa (2009).

For statistical evaluation, the variables were submitted to normality tests where the Shapiro-Wilk test was used, after meeting the precepts of the test, the analysis of variance was performed using the F test (p<0.05) and their means were compared using the Test of Tukey at 5% probability (Banzatto & Kronka, 2013), a Pearson correlation was also performed between the development variables and a polynomial regression analysis was performed for soil conditioner concentrations and models were tested, linear, quadratic and cubic, where the R Studio statistical program was used (R Studio Team, 2019).

## 3. Results and Discussion

A statistical difference was observed for plant height (PH), where the treatment with agricultural gypsum (1.5 t ha-1) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) showed higher averages, along with golden mussel shell powder (3.0 t ha<sup>-1</sup>), which implied approximately 35.63% higher than the treatment without soil conditioners, as shown in Figure 1.

Figure 1 - Average values of plant height (PH) of cotton after being cultivated in soils amended with different soil conditioners.



CV (%): 14.90. \*\* - significant at the 1% probability level (p <0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

Due to the chemical properties of golden mussel shells, as it contains calcium carbonate in its composition, this compound has been studied to correct soil acidity, and in addition to providing calcium to the plant, while applied together with agricultural gypsum can also reduced to aluminum toxicity in soils mainly in savanna regions, and thus allows an increase in the availability of nutrients for the plant that can reflect in a greater development of the aerial part of the plant (Hou et al. 2022).

There was a statistical difference in the characteristic leaf area (LA), where again the use of agricultural gypsum (1.5 t  $ha^{-1}$ ) with golden mussel shell powder (1.5 t  $ha^{-1}$ ) showed a higher mean value, with approximately 52.11% higher than the treatment with snail shell powder (3.0 t  $ha^{-1}$ ) which presented the lowest average (Figure 2).

Figure 2 - Mean values of total leaf area (LA) of cotton after being grown in soils corrected with different soil conditioners.



CV (%): 27.88. \*\* - significant at the 1% probability level (p <0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

Calcium carbonate occurs chemical reactions in the soil, being an effective strategy in the correction of acids, leaving residue in the soil associated with ions, in addition to being used in the bioremediation of soils with heavy metals (Hannan *et* 

*al.*, 2021) and thus promoting the absorption of nutrients with greater availability, acting on plant growth in such a way that the leaf area of the plant increases, since the increase in nutrient availability, mainly the calcium present in the mussel shell powder and the sulfur present in agricultural gypsum, brings benefits directly to the metabolism of the plant such as amino acid synthesis, cellular construction and in the leaf area of the plant (Chakraborty *et al.*, 2020).

Again, a statistical difference was observed in the number of leaves (NL), where the use of isolated golden mussel shell powder ( $3.0 \text{ t} \text{ ha}^{-1}$ ) and agricultural plaster ( $1.5 \text{ t} \text{ ha}^{-1}$ ) with golden mussel shell powder ( $1.5 \text{ t} \text{ ha}^{-1}$ ) showed the same mean values, which were approximately 24.24% higher than the use of agricultural gypsum ( $1.5 \text{ t} \text{ ha}^{-1}$ ) with snail shell powder ( $1.5 \text{ t} \text{ ha}^{-1}$ ) wi

Figure 3 - Mean leaf number (LN) values of the cotton plant after being cultivated in soils amended with different soil conditioners.



CV (%): 12.54. \*\* - significant at the 1% probability level (p <0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

A well-nourished plant presents greater development of its organs, where the increase in the number of leaves guarantees a higher photosynthetic rate, as this way the plant guarantees greater assimilation of  $CO_2$  in its dry mass, which can directly reflect on its productivity (Lisboa *et al.*, 2021). In this way, a statistical difference was observed in the dry mass of the shoot dry mass (SDM), where again the treatment with the association of agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) had the highest average, approximately 49.62% higher than the control group with no use of soil conditioners, as shown in Figure 4.

Figure 4 - Average values of shoot dry mass (SDM) of cotton after being cultivated in soils amended with different soil conditioners.



CV (%): 31.59. \*\* - significant at the 1% probability level (p <0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

The use of shell powder as an alternative soil acidity corrective can be an interesting strategy because it has low economic value, and that enhances the availability of macronutrients by correcting soil acidity (Chakraborty *et al.*, 2020; Hannan *et al.*, 2021).

A statistical difference was observed in the root dry mass (RDM) where again the agricultural gypsum juice (1.5 t  $ha^{-1}$ ) with golden mussel shell powder (1.5 t  $ha^{-1}$ ) presented a higher average, implying by approximately 61.90% higher in relation to the non-use of soil conditioner that presented the lowest average, as shown in Figure 5.

Figure 5 - Mean values of root dry mass (RDM) of cotton after being cultivated in soils amended with different soil conditioners.



CV (%): 47.04. \*\* - significant at the 1% probability level (p < 0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

The use of agricultural gypsum as a soil conditioner is already well discussed and confirmed, however, the use associated with alternative sources of calcium carbonates is little studied, which when used in soils with low availability of nutrients or with high aluminum content, can mitigate these deleterious effects of the soil, which enables greater root growth in

depth, in addition to providing calcium levels, an important element in the formation of the middle lamella of the cell wall, reflecting in a good architecture of this organ (Khushboo *et al.*, 2018). These differences in the development of cotton plants when cultivated in different soil conditioners can be clearly seen in Figure 6.

**Figure 6** - Cotton plants after being cultivated under different soil conditioners. A – Absence of the use of soil conditioners; B – Agricultural gypsum (3.0 t ha<sup>-1</sup>); C – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); D – Snail shell powder (3.0 t ha<sup>-1</sup>); E – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and F – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>).





Statistical differences were observed for the contents of chlorophylls A and B, where again the best treatment was the use of agricultural gypsum (1.5 t ha<sup>-1</sup>) associated with golden mussel shell powder, which resulted in approximately 38.83 % higher in relation to the treatment with snail shell powder (3.0 t ha<sup>-1</sup>) which presented a lower mean value, as shown in Figure 7.

**Figure 7** - Average values of chlorophyll A and B (Chloro A and B) of the cotton plant after being cultivated in soils amended with different soil conditioners.



CV (%): 18.87. \*\* - significant at the 1% probability level (p <0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

By increasing the pH of the soil, we will have an improvement in its chemical attributes, which will thus have greater availability of nutrients, which will thus enable plants to be more efficient in their metabolism, especially in the formation of proteins and biosynthesis of the chlorophyll pigment in their leaves (Piao *et al.*, 2022). A significant Pearson correlation was found between the contents of chlorophylls A and B with the leaf area (Figure 8), showing that plants with a higher concentration of chlorophylls provide a greater leaf area in the plant, start to carry out more photosynthesis and thus fix more carbon dioxide, which can provide an increase in the dry mass of the plant, so the use of herbicides that act on the photosystems directly influences the development of the plant, according to the Table 2.

**Figure 8** - Significant Pearson correlations between the analyzed variables in peanut after being cultivated in soil with 2,4D herbicide residue.



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	p-value	y=b + ax	$\mathbb{R}^2$
LA	0,0003**	y = 3,00215770 + 0,16984779 Chloro A	0,4015
LA	0.0003**	y = 3,00215772 + 0,50954336 Chloro B	0,4015
LA	0.0078**	y = -47,653434 + 29,5230467 N-org	0.3870
Chloro B	0.0001**	y = -8,650E-09 + 0,333333333 Chloro A	0.9999
N-org	0.0001**	y = 1,75712388 + 0,00564530x Chloro A	0,9989
N-org	0.0001**	y = 1,75712388 + 0,01693589x Chloro B	0,9989

 Table 2 - Significant linear regressions after Pearson's correlation analysis.

LA = Leaf area; Chloro = Chlorophyll and N-org = Organic nitrogen. Source: Authors.

This increase in the concentration of chlorophyll also guarantees an increase in the nitrogen content (Table 2), a fact that the rate of mineralization of organic nitrogen in humic and fuvic acids varies with the interaction of the pH in the soil that becomes available for the plant (Sherrod *et al.*, 2019, Pérez-Esteban *et al.*, 2019, Karčauskienė et al. 2019), therefore, a statistical difference was observed in the concentration of organic nitrogen (N-org) in cotton leaves, where again the use of agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) had the highest average, which was approximately 23.79% higher than the treatment using snail shell powder (3.0 t ha<sup>-1</sup>), as shown in Figure 9.

Figure 9 - Average values of organic nitrogen (N-org) of the cotton plant after being cultivated in soils amended with different soil conditioners.



CV (%): 10,53. \*\* - significant at the 1% probability level (p <0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

Observing that gypsum with golden mussels may have increased calcium availability in the soil and neutralized the toxic aluminum present in the soil, and thus may have allowed greater absorption of nutrients, which reflected in a better synthesis of chlorophylls, so while the nitrogen makes up the molecule of this pigment, when its concentration increases it also increases the organic nitrogen content in the cells of the cotton leaves (Chang & Troughton, 1972), where nitrogen together with chlorine play important functions in the plant, mainly in the regulation of stomatal opening and formation of stomata (Maron, 2019). And the concentration of organic nitrogen guarantees a greater leaf area, so well-nourished plants have a greater leaf area (Table 2).

A statistical difference was observed in the stomatal density (DEN) where again the use of agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) showed the highest average with approximately 38,08% higher compared to the treatment that did not use any soil conditioner, as shown in Figure 10.

Figure 10 - Mean values of stomatal density (DEN) of cotton after being cultivated in soils amended with different soil conditioners.



CV (%): 15,57. \*\* - significant at the 1% probability level (p <0.01). Means followed by the same letter do not differ statistically. The Scott&Knott method was applied at the level of 5% probability of the event occurring. T1 – No use of soil conditioners; T2 – Agricultural gypsum (3.0 t ha<sup>-1</sup>); T3 – Golden mussel shell powder (3.0 t ha<sup>-1</sup>); T4 – Powdered snail shell (3.0 t ha<sup>-1</sup>); T5 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with golden mussel shell powder (1.5 t ha<sup>-1</sup>) and T6 – Agricultural gypsum (1.5 t ha<sup>-1</sup>) with snail shell powder (1.5 t ha<sup>-1</sup>). Source: Authors.

Plants may change stomatal density when they are under nutritional restriction (Mirjalili *et al.*, 2022; Wang *et al.*, 2018), with water restriction (Hao *et al.*, 2019); salt stress (Pompelli *et al.*, 2021) and even species and varieties (Neves *et al.*, 2022). in greater productivity. The use of soil conditioners presents important responses in raising the pH in aqueous solution as shown in Figure 11, which starts to imply the availability of nutrients for the plant.

Figure 11 - Neutralization action of soil conditioners in CaCl<sub>2</sub> solution with initial pH of 4.5.



C = Limestone; G = Plaster; M = Golden mussel shell powder; CA = Snail shell powder. Source: Authors.

All soil conditioners showed quadratic responses when there was an increase in their doses, whereas the maximum point for limestone was 0.54 g L<sup>-1</sup>, gypsum was 0.53 g L<sup>-1</sup>, mussel was 0.61 g L<sup>-1</sup>, snail was 0.59 g L<sup>-1</sup>, plaster with mussel shell powder was 0.56 g L<sup>-1</sup>, plaster with snail shell powder 0, 55 g L<sup>-1</sup>, as shown in Table 3.

	p-value	$y=c+bx+ax^2$	$\mathbb{R}^2$
С	0.0025**	$y = 3.68066340 + 12.1227336x - 11.0623233x^2$	0.5800
G	0.0001**	$y = 1.69720853 + 22.4765430x - 21.0898992x^2$	0.8154
М	0.0078**	$y = 3.13964679 + 14.0712764x - 11.4771082x^2$	0.6869
CA	0.0096**	$y = 2.67328388 + 17.5862639x - 14.7383066x^2$	0.6646
G+M	0.0001**	$y = 2.27326916 + 25.4844678x - 22.4186178x^2$	0.8873
G+CA	0.0001**	$y = 3.81291864 + 16.8494368x - 15.2999655x^2$	0.7975

Table 3 - Regression analysis of the pH in solution after the use of doses of soil conditioners.

\*\* - significant at the 1% probability level (p <0.01). C = Limestone; G = Plaster; M = Golden mussel shell powder; CA = Snail shell powder. Source: Authors.

The maximum points of all soil conditioners were close, with an overall average of 0.56 g L<sup>-1</sup>. In aqueous solution, the pH neutralization action reaches its maximum point until the equilibrium of solubility of the shell powders, as the anionic forces within the solution started to interfere with the availability of OH<sup>-</sup>, and thus the hydroxyls may have associated with some cations forming other hydroxides such as Ca(OH)<sub>2</sub>, KOH, Mg(OH)<sub>2</sub> and others, and thus, due to their reaction equivalence points in the relationship between H<sup>+</sup> protons and OH<sup>-</sup> anions were not detected in the measurement of the hydrogen ion potential of the solution (Harris, 2005).

A statistical difference was observed in the pH of the soil after the use of soil conditioners, with a general average of 5.9, however, it is worth mentioning that there was a variation in the initial pH (Table 1) which was 3.9, which implied in a difference of approximately 33.89% more, which demonstrates the action of soil conditioners, which can improve cultivation conditions for the cotton crop.

## 4. Conclusions

The use of agricultural gypsum at a dose of  $1.5 \text{ t ha}^{-1}$  associated with golden mussel shell powder at a dose of  $1.5 \text{ t ha}^{-1}$  showed better responses in cotton development.

The use of agricultural gypsum at a dose of 1.5 t ha<sup>-1</sup> associated with golden mussel shell powder at a dose of 1.5 t ha<sup>-1</sup> improves soil conditions for cultivation.

The pyrolysis method at high temperatures to break the crystalline structures of mollusc shells was efficient.

### References

Banzatto, D. A. & Kronka, S. do N. (2013). Experimentação Agrícola. (4a ed.), Funep. 237p.

Carlquist, S. (1975). Ecological strategies of xylem evolution. University of California. 259 p.

- Castro, E. M., Pereira, F. J. & Paiva, R. (2009). Histologia vegetal: estrutura e função de órgãos vegetativos. UFLA, 234p.
- Chakraborty, A., Parveen, S., Chanda, D. K. & Aditya, G. (2020). An insight into the structure, composition and hardness of a biological material: the shell of freshwater mussels. *Rsc Advances*, 10(49), 29543-29554. http://dx.doi.org/10.1039/d0ra04271d
- Chang, F. H. & Troughton, J. H. (1972). Chlorophyll a/b ratios in C3 and C4 plants. Photosynthetica, 6: 57-65.
- Embrapa Empresa Brasileira de Pesquisa Agropecuária. (2009). Manual de análises químicas de solos, plantas e fertilizantes. (2a ed.), 627p.
- Embrapa Empresa Brasileira de Pesquisa Agropecuária. (2013). Sistema brasileiro de classificação de solos. (3a ed.), 353p.

Ferreira, M. M. M., Ferreira, G. B., Fontes, P. C. R. & Dantas, J. P. (2006). Índice SPAD e teor de clorofila no limbo foliar do tomateiro em função de doses de nitrogênio e da adubação orgânica, em duas épocas de cultivo. *Ceres*, 53(305), 83-92.

Hannan, F., Islam, F., Huang, Q., Farooq, M. A., Ayyaz, A., Fang, R., Ali, B., Xie, X. & Zhou, W. (2021). Interactive effects of biochar and mussel shell activated concoctions on immobilization of nickel and their amelioration on the growth of rapeseed in contaminated aged soil. *Chemosphere*, 282, 130897. http://dx.doi.org/10.1016/j.chemosphere.2021.130897

Hao, S., Cao, H., Wang, H. & Pan, X. (2019). The physiological responses of tomato to water stress and re-water in different growth periods. *Scientia Horticulturae*, 249, 143-154. http://dx.doi.org/10.1016/j.scienta.2019.01.045

Harris, D. C. (2005). Análise quimica quantitativa. (6a ed.), Trad. Bonapace. A. P. & Barcia, O. E. LTC Editora. 876p.

Hou, J., Riaz, M., Yan, L., Lu, K. & Jiang, C. (2022). Effect of exogenous l-aspartate nano-calcium on root growth, calcium forms and cell wall metabolism of Brassica napus L. *Nanoimpact*, 27, 1-14. http://dx.doi.org/10.1016/j.impact.2022.100415

Karčauskienė, D., Replienė, R., Ambrazaitienė, D., Mockevičienė, I., Iaudinis, G. & Skuodienė, R. (2019). A complex assessment of mineral fertilizers with humic substances in an agroecosystem of acid soil. *Zemdirbyste-Agriculture*, 106(4), 307-314. http://dx.doi.org/10.13080/z-a.2019.106.039

Khushboo, Bhardwaj, K., Singh, P., Raina, M., Sharma, V. & Kumar, D. (2018). Exogenous application of calcium chloride in wheat genotypes alleviates negative effect of drought stress by modulating antioxidant machinery and enhanced osmolyte accumulation. *In Vitro Cellular & Developmental Biology – Plant.* 54(5), 495-507. http://dx.doi.org/10.1007/s11627-018-9912-3

Lauricella, D., Butterly, C.R., Weng, Z., Clark, G.J., Sale, P.W.G., Li, G. & Tang, C. (2020). Impact of novel materials on alkalinity movement down acid soil profiles when combined with lime. *Journal of Soils and Sediments*, 21(1): 52-62. http://dx.doi.org/10.1007/s11368-020-02747-4

Lisboa, L. A. M., Cavichioli, J. C., Vitorino, R., Figueiredo, P. A. M. & Viana, R. S. (2021). Nutrient suppression in passion fruit species: an approach to leaf development and morphology. *Colloquium Agrariae*, 17(3), 89-102. http://dx.doi.org/10.5747/ca.2021.v17.n3.a443

Lisboa, L. A. M, Dias, G. H. O., Sacco, H. A. A., Padovan, J. V. R., Rodrigues, G. B., Ribeiro, K. B., Silva, G. G., Cardoso, A. S., Pereira, L. B. & Figueiredo, P. A. M. (2021). Urochloa brizantha cultivated in aluminum-toxic soil: changes in plant growth and ultrastructure of root and leaf tissues. *Tropical Grasslands-Forrajes Tropicales*, 9(1), 23-33. http://dx.doi.org/10.17138/tgft(9)23-33

Luiz, M. S., Zanão Junior, L. A., Ribeiro, M. R., Matos, M. A. & Andrade, D. S. (2022). Residual effects of agricultural gypsum on soil chemical and microbiological characteristics. *Soil Use and Management*, 38(4), 1–11. http://dx.doi.org/10.1111/sum.12837

Malavolta, E. (1980). Elementos de nutrição mineral de plantas. Ceres. 251p.

Maron, L. (2019). From foe to friend: the role of chloride as a beneficial macronutrient. The Plant Journal, 99(5), 813-814. http://dx.doi.org/10.1111/tpj.14498

Mirjalili, A., Lebaschi, M. H., Ardakani, M. R., Sharifabad, H. H. & Mirza, M. (2022). Plant density and manure application affected yield and essential oil composition of Bakhtiari savory (*Satureja bachtiarica* Bunge.). *Industrial Crops and Products*, 177, 114516. http://dx.doi.org/10.1016/j.indcrop.2021.114516

Neves, G. F. O., Brito, B. S., Januário, T. V. V., Santos Junior, E. D. & Lisboa, L. A. M. (2022). Morphophysiological and developmental parameters of maize varieties. *Journal of Biotechnology and Biodiversity*, 10(3), 261-271. http://dx.doi.org/10.20873/jbb.uft.cemaf.v10n3.neves

Parry, C., Blonquist Junior, J. M. & Bugbee, B. (2014). In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. *Plant, Cell and Environment*, 37, 2508–2520. https://doi.org/10.1111/pce.12324

Pérez-Esteban, J., Escolástico, C., Sanchis, I., Masaguer, A. & Moliner, A. (2019). Effects of pH Conditions and Application Rates of Commercial Humic Substances on Cu and Zn Mobility in Anthropogenic Mine Soils. *Sustainability*, 11(18), 4844. http://dx.doi.org/10.3390/su11184844

Piao, J., Che, W., Li, X., Li, X., Zhang, C., Wang, Q., Jin, F. & Hua, S. (2022). Application of peanut shell biochar increases rice yield in saline-alkali paddy fields by regulating leaf ion concentrations and photosynthesis rate. *Plant and Soil*, 27, 1-8. http://dx.doi.org/10.1007/s11104-022-05767-w

Pompelli, M. F., Ferreira, P. P. B., Chaves, A. R. M., Figueiredo, R. C. B. Q., Martins, A. O., Jarma-Orozco, A., Bhatt, A., Batista-Silva, W., Endres, L. & Araújo, W. L. (2021). Physiological, metabolic, and stomatal adjustments in response to salt stress in Jatropha curcas. *Plant Physiology and Biochemistry*, 168, 116-127. http://dx.doi.org/10.1016/j.plaphy.2021.09.039

R Studio Team. (2019). RStudio: Integrated Development for R. RStudio, Inc. http://www.rstudio.com/

Raij, B., Cantarella, H., Quaggio, J. A. & Furlani, A. M. C. (1996). Recomendações de adubação e calagem para o Estado de São Paulo. (2a ed.), IAC, 285p.

Rufty, T. W., Mackown, C. T., Lazof, D. B. & Carter, T. E. (1995). Effects of aluminium on nitrate uptake and assimilation. *Plant, Cell and Environment*, 18(11), 1325-1331. http://dx.doi.org/10.1111/j.1365-3040.1995.tb00192.x

Sathee, L. & Jain, V. (2021). Interaction of elevated  $CO_2$  and form of nitrogen nutrition alters leaf abaxial and adaxial epidermal and stomatal anatomy of wheat seedlings. *Protoplasma*, 259(3), 703-716. http://dx.doi.org/10.1007/s00709-021-01692-4

Sherrod, L. A., Vigil, M. F. & Stewart, C. E. Do. (2019). Fulvic, Humic, and Humin Carbon Fractions Represent Meaningful Biological, Physical, and Chemical Carbon Pools? *Journal of Environmental Quality*, 48(6), 1587-1593. http://dx.doi.org/10.2134/jeq2019.03.0104

Wang, C., Wu, S., Tankari, M., Zhang, X., Li, L., Gong, D., Hao, W., Zhang, Y., Mei, X., & Wang, Y. (2018). Stomatal aperture rather than nitrogen nutrition determined water use efficiency of tomato plants under nitrogen fertigation. *Agricultural Water Management*, 209, 94-101. http://dx.doi.org/10.1016/j.agwat.2018.07.020

Wei, Y., Han, R., Xie, Y., Jiang, C. & Yu, Y. (2021). Recent Advances in Understanding Mechanisms of Plant Tolerance and Response to Aluminum Toxicity. *Sustainability*, 13(4), 1782. http://dx.doi.org/10.3390/su13041782