



Vinasse as a bioherbicide and biofertilizer: A sustainable alternative for organic ecological

La vinaza como bioherbicida y biofertilizante: Una alternativa sostenible para la agricultura ecológica

Authors

*Washington Agapo Guzmán Pa-

✉ washington_guzman_mga@espam.edu.ec



Ever Darío Morales Avendaño

✉ edmorales@espam.edu.ec



Escuela Superior Politécnica Agropecuaria de
Manabí Manuel Félix López – ESPAM MFL.

Abstract

Stillage, a by-product of bioethanol, has great potential as a biofertilizer and herbicide in organic farming. This study evaluated the effect of untreated and pretreated vinasse with CO_3Ca , NaHCO_3 and $\text{Ca}(\text{OH})_2$ in 1 m² plots, using a completely randomized design with four replications. The vinasse was pretreated to adjust its pH and different doses were applied, measuring variables such as plant height, number of leaves and foliar burns. The results showed that treatments with NaHCO_3 and $\text{Ca}(\text{OH})_2$ reduced electrical conductivity (EC) by more than 99%, reaching values close to 13.64 $\mu\text{S}\cdot\text{cm}^{-1}$, compared to the control of 6,420 $\mu\text{S}\cdot\text{cm}^{-1}$. BOD₅ decreased to 5,000 mg·L⁻¹, while COD increased to 155.250 mg·L⁻¹. The CO_3Ca treatment promoted remarkable plant growth, with an average height of 32.25 ± 1.12 cm at 23 days, demonstrating its effect as a biofertilizer. In weed control, untreated vinasse caused up to 80% burndown on *Microtea debilis* and 100% on *Cyanthillium cinereum*. Treatments with NaHCO_3 and $\text{Ca}(\text{OH})_2$ showed herbicidal effects ranging from 5 to 60% on ten different species. In conclusion, vinasse treated with CO_3Ca favors biofertility, whereas vinasse combined with NaHCO_3 and $\text{Ca}(\text{OH})_2$ is more effective as a herbicide.

Keywords: agro-industrial sustainability, weed control, waste management.

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Resumen

La vinaza, un subproducto del bioetanol, tiene un gran potencial como biofertilizante y herbicida en la agricultura ecológica. Este estudio evaluó el efecto de vinaza no tratada y pretratada con CO_3Ca , NaHCO_3 y $\text{Ca}(\text{OH})_2$ en parcelas de 1 m², utilizando un diseño completamente al azar con cuatro repeticiones. La vinaza fue pretratada para ajustar su pH y se aplicaron distintas dosis, midiendo variables como la altura de las plantas, número de hojas y quemaduras foliares. Los resultados mostraron que los tratamientos con NaHCO_3 y $\text{Ca}(\text{OH})_2$ redujeron la conductividad eléctrica (CE) en más del 99%, alcanzando valores cercanos a 13,64 $\mu\text{S}\cdot\text{cm}^{-1}$, frente al testigo de 6.420 $\mu\text{S}\cdot\text{cm}^{-1}$. La DBO₅ disminuyó a 5.000 mg·L⁻¹, mientras que la DQO aumentó a 155,250 mg·L⁻¹. El tratamiento con CO_3Ca promovió un notable crecimiento vegetal, con una altura promedio de 32,25 ± 1,12 cm a los 23 días, demostrando su efecto como biofertilizante. En el control de malezas, la vinaza no tratada causó hasta un 80% de quemaduras en *Microtea debilis* y un 100% en *Cyanthillium cinereum*. Los tratamientos con NaHCO_3 y $\text{Ca}(\text{OH})_2$ mostraron efectos herbicidas de entre un 5 y 60% en 10 especies diferentes. En conclusión, la vinaza tratada con CO_3Ca favorece la biofertilidad, mientras que la vinaza combinada con NaHCO_3 y $\text{Ca}(\text{OH})_2$ es más efectiva como herbicida.

Palabras clave: sostenibilidad agroindustrial, control de malezas, manejo de residuos.



Introduction

Conventional agriculture is based on the intensive use of agrochemicals, which has raised environmental and social concerns due to soil degradation, water pollution, and adverse effects on biodiversity and human health (Tiwari, 2023). In the face of these challenges, organic agriculture emerges as a sustainable alternative that promotes the efficient use of natural resources and the reduction of chemical inputs, prioritizing practices that maintain soil fertility and ecosystem balance (Beltrán y Bernal, 2022). One of the fundamental challenges in this system is weed control and the maintenance of productivity without resorting to synthetic products (Koskey et al., 2021).

In this context, vinasse, as a liquid by-product generated during the distillation of artisanal alcohol, has attracted interest for its agricultural potential; this is due to the fact that its chemical composition—rich in organic matter, essential minerals, and phenolic compounds—gives it the ability to inhibit the growth of certain plants and improve soil fertility (González et al., 2021; Luz et al., 2021). Nevertheless, its indiscriminate use can generate negative environmental impacts due to its high chemical oxygen demand (COD), biochemical oxygen demand (BOD5), electrical conductivity (EC), and acidic pH, which can affect soil quality and water sources (Zielińska et al., 2021).

The utilization of vinasse in organic agriculture contributes to the circular economy by reusing an agro-industrial waste that would otherwise represent an environmental problem (Torres et al., 2022; Rachman et al., 2023). Its application has been shown to improve soil structure, increase microbial activity, and enhance nutrient content in crops such as sugarcane and maize (Otoya et al., 2023). However, to maximize its benefits and minimize its adverse effects, it is necessary to subject it to prior physicochemical treatments that modulate its properties and reduce its phytotoxicity (Duarte et al., 2023). Among other treatments, the combination of vinasse with plant growth-promoting bacteria has been reported in irrigated crops, whose application may contribute to food security through nutrient recycling for agriculture (Soares et al., 2024).

In the area where the research has been conducted, the daily accumulation of vinasse during the artisanal alcohol production season is placed in improvised sites and is even discharged into estuaries, which causes severe environmental impacts for the environment and the community in general.

For this reason, a previous study has been carried out on the application of vinasse to establish a protocol that optimizes its

use as a fertilizer and/or herbicide, which will make it possible to reduce its environmental impact through its utilization and thus decrease the high daily volumes that are drained without control, and therefore contribute to the development of accessible treatment and utilization technologies to transform this environmental liability into a sustainable resource.

Previous research has evaluated strategies to modify the composition of vinasse and optimize its use in agriculture. In an *in vitro* and *ex situ* bioassay, the application of sodium bicarbonate (NaHCO_3) reduced COD and EC at different pH levels (4, 5, 6, and 7), modulating its herbicidal impact and limiting it to a foliar effect of 5-20% across various weed species. This suggests the possibility of adjusting its properties to improve its performance as both a biofertilizer and a bioherbicide (Morales et al., 2023). Nevertheless, further studies are needed to explore other compounds that may enhance its effectiveness as both a herbicide and a fertilizer, optimizing its application under real agricultural condition.

Materials and methods

The study was conducted in the Politécnico Forest of the Escuela Superior Politécnica Agropecuaria de Manabí “Manuel Félix López,” located in the El Limón sector, Calceta parish, Manabí Province, Ecuador. The selected sites exhibited a dry tropical climate and well-drained sandy loam soils with low stoniness and no significant limitations for agricultural use. (Cárdenas y Vélez, 2024).

The research followed an experimental and exploratory approach to evaluate the effect of different physicochemical treatments applied to vinasse. A quantitative method was used, focusing on the measurement of plant morphological variables and physicochemical parameters. Additionally, this was complemented by a qualitative analysis that allowed for the interpretation of numerical data based on observations of vinasse behavior as a biofertilizer and bioherbicide. This methodological combination provided a comprehensive evaluation of the treatments, offering a more complete perspective on their effects on weed control and plant development (Gao y Su, 2024).

Experimental design

A completely randomized design (CRD) was used with five treatments and four replications, resulting in a total of 20 experimental units. Each experimental unit consisted of 1 m² plots, delimited with wooden frames in an open-field setting. The treatments were randomly assigned to the plots to ensure homogeneity and minimize any bias, and were designated as

shown in table 1. The microplots contained different species and populations of wild plants under *in vivo* and *in situ* conditions, which allowed for observation of the treatments' effects on vegetation in its natural environment. The replications were randomly placed to evaluate both the fertilizer and herbicide effects, ensuring that external variables did not influence the experimental results.

Tabla 1. Treatment description and nomenclature.

Nomenclature	Treatment
T1	Untreated vinasse (VC)
T2	Vinasse + Sodium bicarbonate (NaHCO_3)
T3	Vinasse + Calcium hydroxide (Ca(OH)_2)
T4	Vinasse + Calcium carbonate (CaCO_3)

Experimental management

Vinasse collection and pretreatment

The vinasse samples were collected as a byproduct of the distillation of the fermented substrate used in the production of artisanal alcohol (currincho) in the Agua Fría sector, located in the Junín canton, Manabí Province. In accordance with the storage regulations established by the Ecuadorian Standards Institute (2013), the byproduct was stored in 20-L plastic containers to ensure its preservation prior to subsequent treatment and experimental application.

Before its experimental application, the vinasse underwent pH adjustment through the controlled addition of 15 g of CaCO_3 , NaHCO_3 , or Ca(OH)_2 per 1000 mL of fresh vinasse, with the objective of reaching a pH between 6 and 6.5. Each compound served a specific purpose: CaCO_3 facilitated the precipitation of soluble compounds; NaHCO_3 reduced acidity without altering other physicochemical properties; and Ca(OH)_2 increased the alkalinity of the solution, promoting the solubility of essential nutrients for its later use as a biofertilizer and bioherbicide (Toscano et al., 2022).

Physicochemical characterization of the vinasse

The physicochemical characterization of the vinasse was performed once, after the pretreatment, to evaluate changes in parameters such as electrical conductivity, chemical oxygen demand (COD), biological oxygen demand (BOD_5), and salinity. The analyses were conducted in the laboratory using specific methods: electrical conductivity was measured with a conductivity meter following the protocol of Bamba et al. (2021); COD was determined by dichromate oxidation according to ASTM Standard D1252 (Rawajfeh, 2021); BOD_5 was assessed through incubation at 20 °C for 5 days in accordance with EPA methodology; and salinity was measured through direct conductivity using a salinity meter. These measurements allowed the detection of changes in the physicochemical properties of the treated vinasse, confirming that it met the optimal conditions for its application as a biofertilizer and bioherbicide.

Treatment application of biofertilizers

The biofertilizer treatments were applied to previously weeded and conditioned plots, using the natural soil of the area to replicate real agricultural conditions. This approach allowed for the evaluation of plant responses in a context more representative of field development, as suggested by Hází et al. (2023) and Anggraini et al. (2024), who emphasize the importance of using natural substrates in biofertilizer assessment. Each experimental unit received 200 mL of the corresponding solution on three occasions, applied every 7 days, ensuring a uniform distribution of the treatment.

The morphological variables used to evaluate the biofertilizer effect were plant height and number of leaves, which were measured on days 9, 16, and 23 of vegetative growth. These variables were selected due to their ability to reflect the effects of the treatments on plant growth, vigor, and health, as noted by Atero et al. (2024), who recommend their use for assessing the impact of biofertilizers in early developmental stages. In addition, a botanical identification of the emerging species in each plot was carried out using botanical guides to assign the corresponding scientific names. This approach not only allowed for the observation of differential species responses to the treatments but also enriched the analysis by providing information on the effectiveness of the biofertilizer across different species, as suggested by Calcan et al. (2022).

Treatment application of bioherbicide

Unlike the biofertilizer assay, in this evaluation the vegetation in the experimental area was not altered, preserving intact the non-edible wild species present at the site. This approach allowed for the analysis of the treatment's impact on the preexisting vegetation under natural conditions, avoiding biases associated with the removal of competition or modifications to the ecosystem, as suggested by Repajić et al. (2021). The treatment solution was applied foliarly in three applications of 50 mL each, every 7 days, on days of emergence (16, 20, and 27), ensuring homogeneous distribution in order to assess the direct foliar effect of the treatment.

The variables analyzed included the percentage of foliar burn and the final plant height. The percentage of foliar burn was considered the primary indicator, as it reflected the visual and physiological damage caused by the herbicide, manifested by the presence of dry or necrotic areas on the leaves—a key criterion for determining the effectiveness of the treatment in controlling unwanted vegetation (Barroso et al., 2022; Kaur et al., 2023). Final plant height, measured on day 27, allowed the identification of potential secondary effects on growth, considering that an effective herbicide should inhibit plant development.

To classify the level of foliar damage, a percentage scale adapted from Vieira et al. (2022) was used, establishing the following categories: no damage (0%), resistant (5%), sublethal (6-90%), and lethal (100%). This classification facilitated comparison



among treatments and provided objective criteria to evaluate burn severity, enabling an accurate interpretation of the bioherbicide's effectiveness.

Statistical analysis

The data obtained from the biofertilizer and herbicide effects were analyzed using an analysis of variance (ANOVA) to determine the existence of significant differences among treatments. Before performing this analysis, the assumptions of normality and homogeneity of variances were verified using the Shapiro-Wilk test and Levene's test, respectively. Variables that did not meet these assumptions were adjusted using a Log_{10} data transformation. Subsequently, Tukey's multiple comparison test ($P \leq 0.05$) was applied to identify specific differences among groups. The analysis was conducted using InfoStat (2020), ensuring a rigorous statistical evaluation consistent with the objectives of the study.

Results and discussion

The additives applied to the vinasse— CaCO_3 , NaHCO_3 , and Ca(OH)_2 —significantly reduced its electrical conductivity (EC) and salinity, indicating a substantial improvement in its quality (table 2). A reduction greater than 99% in EC was observed for all treatments, with average values of $13.64 \mu\text{S}\cdot\text{cm}^{-1}$ for the CaCO_3 treatment, $15.71 \mu\text{S}\cdot\text{cm}^{-1}$ for NaHCO_3 , and $12.75 \mu\text{S}\cdot\text{cm}^{-1}$ for Ca(OH)_2 . This reduction was crucial for preventing salt accumulation in the soil—an essential condition for avoiding salinization and preserving fertility—which is consistent with the findings reported by Cedeño et al. (2024).

Table 2. Physicochemical characteristics of the treatments.

Treatment	Average EC ($\mu\text{S}\cdot\text{cm}^{-1}$)	Average salinity (%)	Average pH	EC Reduction (%)
Untreated Vinasse	$6,420 \pm 9.13$	0.34 ± 0.008	4 ± 0.09	-
CaCO_3	13.64 ± 0.08	0.6 ± 0.008	6 ± 0.08	99.82
NaHCO_3	15.71 ± 0.09	0.9 ± 0.014	6 ± 0.08	99.70
$(\text{OH})_2\text{Ca}$	12.75 ± 0.13	0.7 ± 0.008	5 ± 0.08	99.80

Regarding salinity, although the values increased slightly compared with untreated vinasse, they remained within acceptable limits for agricultural applications, with averages ranging from 0.6 to 0.9. The average pH reached optimal values for agricultural soils—6.00 in the treatments with CaCO_3 and NaHCO_3 —favoring the neutralization of the vinasse's initial acidity. In contrast, the treatment with Ca(OH)_2 showed an average pH of 5.0, slightly below the desired range, which may require dosage adjustments (Hirzel et al., 2021).

The notable removal of EC, exceeding 99%, was attributed to the precipitation of insoluble salts, primarily carbonates and bicarbonates, formed during the process. Soukaina et al. (2022) reported similar decreases, with reductions of 96.82% in industrial effluents treated with NaHCO_3 , demonstrating that the formation and precipitation of salts—mainly carbonates and bicarbonates—was the dominant mechanism. Although still effective, this behavior may require adjustments in its application, as suggested by Medina et al. (2024), who emphasize that its efficacy depends on the specific conditions of the medium, particularly in complex matrices such as vinasse.

The application of NaHCO_3 and Ca(OH)_2 reduced BOD_5 from 10,000 to 5,000 $\text{mg}\cdot\text{L}^{-1}$ —equivalent to a 50% decrease (figure 1)—indicating a significant improvement in the biodegradation of the organic matter present in the vinasse. This result suggests that the increased alkalinity favored the oxidation of simple organic compounds, enhancing the biodegradability of the effluent. This behavior aligns with the mechanism proposed by Mensah et al. (2022), who reported that hydroxyl ions generated in alkaline environments promote the breakdown of organic compounds.

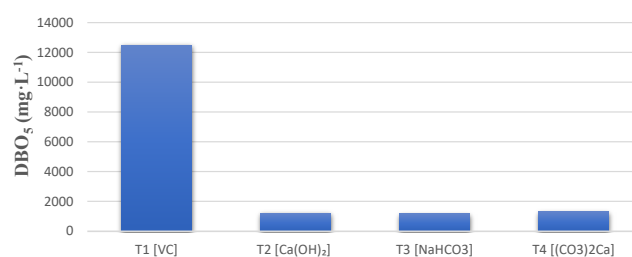


Figure 1. DBO_5 ($\text{mg}\cdot\text{L}^{-1}$) measure in treatments.

However, in the case of COD (figure 2), a reduction of 20.58% was observed with NaHCO_3 , 13.04% with Ca(OH)_2 , and only 6.04% with $(\text{CO}_3)_2\text{Ca}$ derived from eggshell. This result appears to be attributed to the solubilization of organic compounds from the eggshell (González et al., 2019), which increases the organic load without being easily biodegradable (Zielińska et al., 2021). Similarly, these authors highlighted that alkaline pretreatment with NaOH solubilized compatible organic compounds, increasing dissolved carbon, proteins, and carbohydrates.

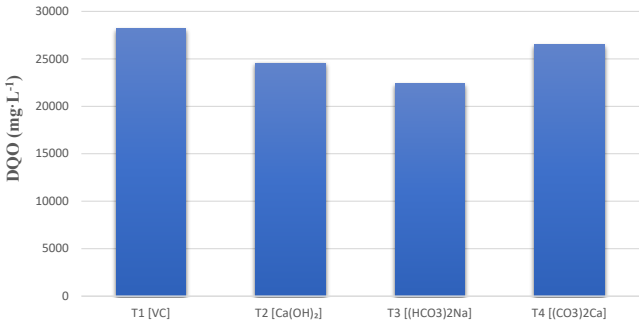


Figure 2. DQO (mg·L⁻¹) measure in the treatments.

Biofertilizer effect

The analysis of variance (ANOVA) showed no statistically significant differences ($P>0.05$) among the evaluated treatments in terms of plant height and number of leaves. This result aligns with the findings of Pino et al. (2022) and Torres et al. (2022), who suggest that variability in the effects of vinasse may prevent significant differences when key environmental factors—such as soil type, moisture, and nutrient levels—are not controlled.

Despite the lack of statistical significance, the descriptive results revealed certain trends in plant responses to the treatments. As shown in table 3, in most cases (T2, T3, and T4), there was a positive relationship between plant height and number of leaves, suggesting a possible correlation between the two variables (Aditiawati et al., 2021). However, the growth rate was not uniform, indicating that factors such as treatment type and evaluation time influenced plant development.

In terms of average height, treatment T1 (vinasse + CaCO₃) showed a notable increase, reaching 32.25 ± 6.83 cm on day 23. This result reflects a positive interaction between vinasse and calcium carbonate, in which vinasse acted as a nutrient-rich source—providing nitrogen, phosphorus, and potassium, all essential for plant growth—while calcium strengthened cell structure and enhanced phosphorus uptake (Weng et al., 2022). Previous studies have shown that vinasse significantly improves growth in crops such as soybean and cauliflower, supporting its effectiveness as a biofertilizer (González et al., 2018; Ma’rufah et al., 2020). Moreover, vinasse can partially replace conventional fertilizers, covering up to 50% of nitrogen, 40% of phosphorus, and 100% of potassium requirements, making it an efficient and sustainable nutrient source (Mahmoud et al., 2019).

On the other hand, the control treatment (T5) with water, although it showed an average height of 31.75 ± 6.83 cm on day 23, exhibited more uniform development starting on day 16.

Table 3. Wild plant growth during testing.

Treatment	Average height (cm)			Number of leaves			Identified species
	9	16	Días 23	9	16	23	
T1 (Vinasse + CO ₃ Ca)	16.25	17.75	32.25	22.25	23.50	28.75	<i>Rauvolfia tetraphylla</i> <i>Streblus asper</i> <i>Heliotropium indicum</i> <i>H. indicum</i>
T2 (vinasse + NaHCO ₃)	11.25	13.75	16.75	10.75	13.75	19.00	<i>Tabernanthe sp.</i> <i>Terminalia catappa</i>
T3 (vinasse + Ca(OH) ₂)	15.50	23.50	26.00	16.50	24.50	25.75	<i>Allamanda cathartica</i> <i>Heritiera sp.</i> <i>Nicotiana tabacum</i>
T4 (Pure vinasse)	9.75	12.75	20.50	10.75	11.75	25.25	<i>H. angiospermum</i> <i>Picconia excelsa</i> , <i>Blumea balsamifera</i>
T5 (Water-control)	13.25	27.25	31.75	19.00	27.75	34.50	<i>H. angiospermum</i> <i>Acroceras sp.</i> <i>Streblus</i>

This behavior highlights the importance of water in fundamental processes such as photosynthesis, nutrient transport, and cell turgor, which explains the stable plant growth observed without the application of additional fertilizers (Mahajan et al., 2024). However, the fact that the control presented growth comparable to the vinasse treatment does not imply that vinasse provided no benefits. The use of vinasse in T1 not only promoted plant growth in terms of height but also improved overall plant nutrition, which may be crucial for long-term plant health and their ability to withstand adverse conditions. In fact, according to Ma’rufah et al. (2020), vinasse application also supplies compounds that enhance soil quality and nutrient retention, which could have positive impacts on future growth cycles.

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enhance soil quality and nutrient retention, which could have positive impacts on future growth cycles.

This behavior can be explained by the variability in vinasse effectiveness, as its performance as a biofertilizer depends on factors such as the treated species and soil conditions. Bridhikitti et al. (2023) emphasized that its effectiveness is influenced by soil type, pretreatment processes, and environmental conditions. Likewise, Pino et al. (2022) and Zamarreño et al. (2022) suggested that vinasse derived from sugarcane plant tissues contains phyto-regulators that contribute to plant growth. Nevertheless, its application must be adjusted to the specific conditions of the environment to maximize its benefits.

Figure 2 highlighted the remarkable response of *Heliotropium angiospermum* in treatment T5 (control), reaching a maximum height of 60 cm and 70 leaves by day 23. This confirmed the growth potential of this species under optimal conditions and without the influence of vinasse, aligning with the findings of Knauf et al. (2021), who argued that plants in their natural state efficiently take advantage of available resources when not exposed to external sources of organic matter.

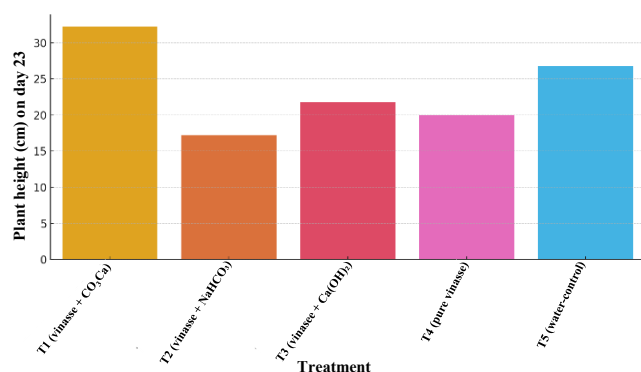


Figure 3. Plant height at day 23 per species and treatment.

The vinasse treatments showed differentiated effects on height growth depending on the type of amendment used. In T1 (vinasse + CaCO_3), species such as *Rauvolfia tetraphylla* and *H. indicum* reached notable heights, with averages exceeding $38 \text{ cm} \pm 2.5$, supporting the hypothesis proposed by Stephen et al. (2024) regarding the positive synergy between vinasse and calcium carbonate sources. In contrast, treatments T2 (vinasse + NaHCO_3) and T3 (vinasse + Ca(OH)_2) exhibited more heterogeneous responses, with lower average heights, which could be explained by the limited availability of micronutrients such as iron and manganese, as suggested by Sagwal et al. (2023). Treatment T4, which used vinasse without alkaline addition, produced

notable responses especially in *H. angiospermum*, suggesting that in certain species the stimulating effect of vinasse did not require pH adjustments or additional chemical synergies. These results confirm that the combination of vinasse with certain amendments enhances plant growth depending on the species and the formulation used.

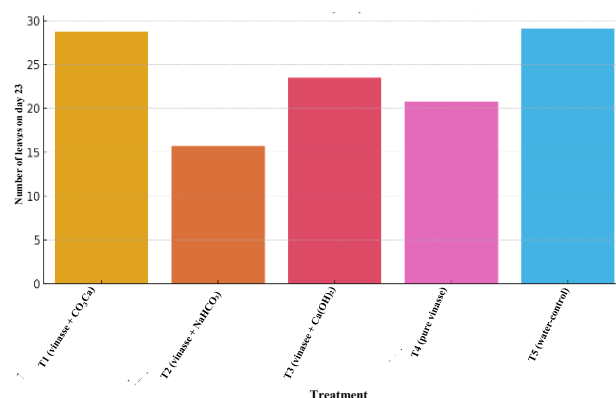


Figure 4. Number of leaves in day 23 per species and treatment.

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Herbicide effect

The analysis of variance (ANOVA) used to evaluate the herbicidal effect through the percentage of foliar burn and plant growth yielded clear results. Regarding the percentage of foliar burn, the p-value of 0.2655 indicated that there were no significant differences among treatments, suggesting that

the herbicides did not cause notable effects on foliar damage. The treatment means were similar (between 0.29 and 0.65), and the low standard deviation of 0.15 reflected little variability in the response, confirming the absence of a significant impact on burn levels. However, this may indicate high variability among species and a lack of homogeneity in treatment response, as previously documented in studies on genetic variability in plants (Chuchert et al., 2022).

With respect to plant height—which in this context was interpreted as an inverse indicator of herbicidal effect (lower height implying greater efficacy)—the *p*-value of 0.0021 showed that there were significant differences among treatments. Tukey's multiple comparison test (table 4) revealed that the treatment with untreated vinasse (T4) presented the greatest heights (20.25 cm), suggesting that this treatment had the least herbicidal effect. In contrast, the treatments combining vinasse with NaHCO_3 (T2) and Ca(OH)_2 (T3) showed lower heights (10.50 cm and 10.75 cm), indicating a stronger herbicidal effect. The standard deviation of 1.56 for height reflected greater variability in plant responses compared with foliar burns, suggesting that the effect on growth was more pronounced.

Table 4. Height comparison during testing days.

Treatment	Median	n	E.E.	Statistical Group
T4 (pure vinasse)	20.25	4	1.56	A
T5 (water-control)	16.25	4	1.56	A B
T1 (vinasse + CO_3Ca)	12.75	4	1.56	B
T3 (vinasse + Ca(OH)_2)	10.75	4	1.56	B
T2 (vinasse + NaHCO_3)	10.50	4	1.56	B

Test: Tukey, **Alfa** = 0.05, **DMS** = 6.81212,

S.E.= Standard error.

Error: 9.7333, **gl:** 15

Statistically, the treatments in which vinasse was combined with CO_3Ca , Ca(OH)_2 , and NaHCO_3 demonstrated greater herbicidal effectiveness, reducing plant growth more consistently. These results are consistent with those reported by Luz et al. (2021), who noted that vinasse fractions exhibited phytotoxic activity against weeds.

Despite the statistical evidence for growth reduction, the descriptive analysis of the percentage of foliar burns and its relationship with plant height revealed interesting trends. A higher presence of plants with sublethal burns was observed, suggesting a cumulative effect on foliar structure and on the development of the analyzed species. As shown in figure 3, the interaction between foliar damage and plant growth appears to follow a consistent pattern, which could indicate, according to Chen et al. (2021), a physiological response mechanism to the stress induced by the treatments.

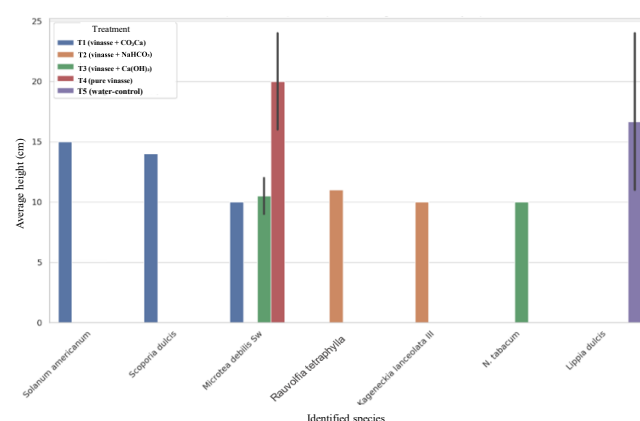


Figura 5. Average height of burned plants based on the treatment and species.

Treatment T1 (vinasse + CaCO_3) affected *Solanum americanum*, which showed an average height of 15 cm and 70% foliar burn, indicating high susceptibility. In *Scoparia dulcis*, which reached an average height of 14 cm and 40% foliar burn, greater resistance was observed. Meanwhile, *Microtea debilis* reached 10 cm in height with 50% foliar burn, demonstrating a moderate level of injury (Table 5). This damage pattern is consistent with the findings reported by Hamouzová et al. (2023), who indicated that calcium-based compounds increased herbicidal efficacy in sensitive plants due to alterations in foliar pH, a trend also observed in the results of this study.

Table 5. Plants with sublethal burns (6-90%).

Treatment	Average height (cm)	Percentage of burns (%)	Identified species
T1 (vinasse + CO_3Ca)	15	70	<i>Solanum americanum</i>
T1 (vinasse + CO_3Ca)	14	40	<i>Scoparia dulcis</i>
T1 (vinasse + CO_3Ca)	10	50	<i>Microtea debilis</i>
T2 (vinasse + NaHCO_3)	11	47	<i>Rauwolfia tetraphylla</i>
T2 (vinasse + NaHCO_3)	10	52	<i>Kageneckia lanceolata III</i>
T3 (vinasse + Ca(OH)_2)	12	60	<i>M. debilis</i>
T3 (vinasse + Ca(OH)_2)	10	40	<i>N. tabacum</i>
T3 (vinasse + Ca(OH)_2)	9	25	<i>M. debilis</i>
T4 (untreated vinasse)	16	80	<i>M. debilis</i>
T4 (untreated vinasse)	24	50	<i>M. debilis</i>

Treatment T2 (vinasse + NaHCO_3) caused 47% foliar burn in *R. tetraphylla*, with an average height of 11 cm, while *K. lanceolata III* showed 52% burn and a height of 10 cm. These results indicate moderate injury in both species, possibly due to cellular dehydration from osmotic stress caused by bicarbonates, as noted

by Azevedo et al. (2024). In treatment T3 (vinasse + $\text{Ca}(\text{OH})_2$), *M. debilis* showed marked differences in the level of injury, with burns of 60 and 25% and heights of 12 cm and 9 cm, respectively. Similarly, *Nicotiana tabacum* recorded 40% foliar burn and a height of 10 cm, demonstrating intermediate susceptibility. According to Ferreira et al. (2021), such effects may be related to a thicker cuticle in certain species, which reduces treatment penetration; this could explain the lower impact observed in *N. tabacum* and part of the variation found in *M. debilis*.

Treatment T4 (untreated vinasse) produced contrasting effects in *M. debilis*, which exhibited foliar burns of 80% and 50%, with heights of 16 cm and 24 cm, respectively. Despite the presence of significant damage, the plants showed compensatory growth, suggesting the activation of post-stress recovery mechanisms. This finding is consistent with what was proposed by Bridhikitti et al. (2023), who reported that plants activate recovery mechanisms following severe stress, including drought, metal toxicity, and salinity. During drought stress, chloroplasts play a crucial role in triggering protective responses and repair mechanisms after rehydration (Chen et al., 2021).

Finally, treatment T5 (water-control) applied to *L. dulcis* resulted in heights of 15, 24, and 11 cm, with foliar burns of 70%, 40%, and 50%, respectively. Although this treatment did not involve herbicidal compounds, the presence of burns under controlled conditions may suggest a possible influence of environmental factors or prior predisposition (table 6). While vinasse is valued for its capacity to improve fertigation, excessive application can lead to soil salinization and toxicity, negatively affecting plant health (Yin et al., 2019). Nevertheless, Portocarrero et al. (2023) reported that its long-term use in sugarcane fields has shown benefits, such as increased dissipation of the herbicides atrazine and ametryn by 45 and 33%, respectively, compared with conventional fertilization systems, suggesting its potential to enhance the sustainability of agricultural soils.

Tabla 6. Plants with lethal burns (100%).

Treatment	Average height (cm)	Identified species
T1 (vinasse + CaCO_3)	12	<i>Cyanthillium cinereum</i>
T4 (untreated vinasse)	20	<i>Marsdenia</i> sp.

Plants that experienced 100% foliar burn were classified as lethally affected, exhibiting a complete collapse of their foliar structure. In treatment T1 (vinasse + CaCO_3), *Cyanthillium cinereum* presented an average height of 12 cm, indicating considerable lethal damage. This result is consistent with previous research showing that vinasse, due to its high concentration of nutrients and organic compounds, can alter soil pH, affect nutrient uptake,

and induce extreme physiological stress in plants (Luz et al., 2020). In comparison, treatment T4, with untreated vinasse, caused lethal damage in *Marsdenia*, which, despite reaching an average height of 20 cm, exhibited a total loss of foliar structure, indicating that untreated vinasse is equally phytotoxic, although with a lower impact on plant height compared with T1. These findings align with prior studies documenting the high toxicity of vinasse to various plant species. For example, Brito et al. (2024) reported that high concentrations can be toxic to some aquatic plants, although certain species such as *Eichhornia crassipes* showed tolerance to vinasse concentrations of 10-20%.

On the other hand, treatment T5, with water (control), showed that *Richardia scabra* reached an average height of 15 cm, without immediate lethal effects but with a slight reduction in growth. This result highlights the importance of additional factors—such as competition for resources and environmental conditions—that also influence plant development, as suggested by studies analyzing water stress and soil dynamics in the presence of organic nutrients (Parise et al., 2021; Rehling et al., 2021). Comparison with these studies showed that, although vinasse has strong potential as an herbicide, its effectiveness and the resulting damage depend on the applied dose, the type of vinasse, and the specific tolerance of each species.

Table 7. Resistant plants (0%-5%).

Treatment	Percentage of burns	Identified Species
T2 (vinasse + NaHCO_3)	5	<i>Symphyotrichum subulatum</i>
T4 (untreated vinasse)	2	<i>Microtea debilis</i>

In contrast to lethally affected plants, the resistant species exhibited minimal foliar burn. These species demonstrated a high tolerance to the treatments applied. In this category, *Symphyotrichum subulatum* (T2, vinasse + NaHCO_3) showed 5% foliar burn, while *M. debilis* (T4, untreated vinasse) presented only 2% burn. These results suggest that vinasse, both in combination with sodium bicarbonate and in its untreated form, was less effective in these specific species, indicating significant variability in plant responses to the herbicide treatments. The tolerance observed in these species may be related to physiological or biochemical mechanisms that enable them to withstand the effects of the compounds present in vinasse without experiencing substantial damage to their foliar structure. Herbicide resistance in plants involves complex physiological and biochemical mechanisms, and selectivity can occur through physical factors or physiological processes that affect the retention, penetration, movement, and detoxification of herbicides (Gwatidzo et al., 2023).

Conclusion

The treatment of vinasse with CaCO_3 , NaHCO_3 , and Ca(OH)_2 significantly improves its quality by reducing electrical conductivity by more than 99% and keeping salinity within acceptable agricultural limits. Furthermore, adjusting pH to values closer to neutrality and reducing BOD_5 suggest that these treatments can mitigate the environmental impact of vinasse and make it more viable for agricultural use.

Vinasse treated with CaCO_3 showed a more pronounced biofertilizer effect, promoting the growth of certain species such as *H. angiospermum*, *R. tetraphylla*, and *H. indicum*. However, performance varied depending on the treatment and the species evaluated, indicating that its effectiveness as a biofertilizer depends on specific factors such as soil composition and plant characteristics.

Vinasse combined with calcium-based compounds, particularly NaHCO_3 and Ca(OH)_2 , exhibits a significant herbicidal effect by reducing plant growth (T1 and T4). However, its impact on foliar burn varies by species, revealing differences in susceptibility and resistance mechanisms. Although some treatments caused lethal effects in certain plants, others showed tolerance, underscoring the need to adjust dosages and evaluate selectivity to ensure effective and sustainable use as a bioherbicide.

Conflict of interest

The authors declare that they have no conflicts of interest regarding this publication at any stage.

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Author contribution statement according to CRediT

Washington Agapo Guzmán Paredes: conceptualization, investigation, data curation, writing – original draft..

Ever Darío Morales Avendaño: conceptualization, methodology, supervision, writing – review and editing.