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CÂMPUS DE ILHA SOLTEIRA

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PENNISETUM GLAUCUM AS A PHYTOREMEDIATOR: DEVELOPMENT AND ECOTOXICOLOGICAL RESPONSES TO TEBUTHIURON, THIAMETHOXAM AND/OR VINASSE IN SOIL

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Tese apresentada à Faculdade de Engenharia de Ilha Solteira – Unesp como parte dos requisitos para obtenção do título de Doutor em Agronomia.

Prof. Dr. Paulo Renato Matos Lopes
Orientador

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DEDICATION

I dedicate this work to those who have always been by my side with unconditional love, silent strength, and constant support:

To my grandmother, for being the foundation of wisdom and tenderness that shaped my essence together with my mother;

To my mother, an example of courage, resilience, and dedication, my greatest inspiration;

To my wife, my partner, who stood by me even during the hardest days, offering understanding and patience;

To my son, the reason for my daily effort and my hope for a better world, may he grow up knowing that all of this was also for him;

To my brother, with whom I share not only blood but also dreams, struggles, and the journey of life;

And to my great friend Alessander, who was my strongest supporter when I entered the academic field.

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“I have learned that success is to be measured not so much by the position that one has reached in life as by the obstacles which he has overcome while trying to succeed."

Booker T. Washington

ABSTRACT

The intensive use of persistent pesticides in agriculture poses significant environmental challenges, particularly for soil health and biodiversity. This study integrates findings from three complementary investigations to evaluate the environmental behavior of the insecticide thiamethoxam and the herbicide tebuthiuron, their interactions with vinasse (a byproduct of sugarcane processing), and the phytoremediation potential of *Pennisetum glaucum* (millet). The first component provides a comprehensive overview of thiamethoxam's ecotoxicological impact and presents biological strategies for mitigation, including phytoremediation and microbial degradation. The second experimental study demonstrates that the combined application of vinasse and *P. glaucum* significantly reduces soil ecotoxicity, particularly during the early stages of plant development, as assessed by bioassays using *Lactuca sativa*. The third study applies machine learning regression models with SHAP interpretation to predict the biometric responses of *P. glaucum* under different contamination scenarios. Results highlight that plant development time is the most influential variable, while vinasse contributes positively to growth. In contrast, tebuthiuron and thiamethoxam exhibit neutral to negative effects. This interdisciplinary approach, merging environmental toxicology, phytoremediation, and predictive modeling, supports the use of *P. glaucum* as a sustainable solution for restoring pesticide-contaminated soils, particularly in sugarcane production systems.

Keywords: machine learning; phytoremediation; SHAP; soil ecotoxicity; thiamethoxam; tebuthiuron, vinasse.

RESUMO

O uso intensivo de pesticidas persistentes na agricultura impõe desafios ambientais significativos, especialmente para a saúde do solo e a biodiversidade. Este estudo integra resultados de três investigações complementares para avaliar o comportamento ambiental do inseticida thiamethoxam e do herbicida tebuthiuron, suas interações com a vinhaça (subproduto do processamento da cana-de-açúcar) e o potencial de fitorremediação de *Pennisetum glaucum* (milheto). O primeiro componente oferece uma visão abrangente do impacto ecotoxicológico do thiamethoxam e apresenta estratégias biológicas para mitigação, incluindo fitorremediação e degradação microbiana. O segundo estudo experimental demonstra que a aplicação combinada de vinhaça e *P. glaucum* reduz significativamente a ecotoxicidade do solo, especialmente durante as primeiras fases do desenvolvimento vegetal, avaliada por bioensaios com *Lactuca sativa*. O terceiro estudo utiliza modelos de regressão de aprendizado de máquina com interpretação SHAP para prever as respostas biométricas de *P. glaucum* em diferentes cenários de contaminação. Os resultados destacam que o tempo de desenvolvimento da planta é a variável mais influente, enquanto a vinhaça contribui positivamente para o crescimento. Em contraste, tebuthiuron e thiamethoxam apresentam efeitos neutros a negativos. Esta abordagem interdisciplinar, que combina toxicologia ambiental, fitorremediação e modelagem preditiva, reforça o uso de *P. glaucum* como uma solução sustentável para a recuperação de solos contaminados por pesticidas, especialmente em sistemas de produção de cana-de-açúcar.

Palavras-chave: aprendizado de máquina; fitorremediação; SHAP; ecotoxicidade do solo; thiamethoxam; tebuthiuron; vinhaça.

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1. PRESENTATION

The intensive application of pesticides such as tebuthiuron (TBT) and thiamethoxam (TMX) in agricultural systems, especially sugarcane cultivation, raises significant environmental concerns due to their chemical persistence, mobility in soil, and toxicity to non-target organisms including pollinators and soil microorganisms. This contamination adversely affects soil health, microbial biodiversity, and crop productivity, highlighting the urgent need for sustainable remediation strategies. Phytoremediation, which employs plants to remove, degrade, or immobilize soil contaminants, offers an eco-friendly and cost-effective solution. Pearl millet (*Pennisetum glaucum*) is particularly promising because of its extensive root biomass, tolerance to pesticide-contaminated soils, and capacity to improve soil quality.

Vinasse, a nutrient- and organic matter-rich byproduct of ethanol production, can enhance microbial activity and promote pesticide degradation in soil. Combined with millet cultivation, vinasse serves as a soil conditioner and microbial stimulant, reducing ecotoxicological impacts. Ecotoxicological bioassays using *Lactuca sativa* seeds showed improved germination and root growth in soils treated with millet and vinasse. Early soil sampling, conducted shortly after sowing, revealed higher germination indices, indicative of rapid soil health recovery driven by phytoremediation.

To further explore the effects of contaminants and treatments on millet growth, this study applied machine learning regression models. Nine algorithms including Random Forest, Support Vector Machines, XGBoost and Gaussian Process Regression were tested to predict shoot and root biomass as well as chlorophyll content under varying soil conditions. The Shapley Additive Explanations (SHAP) method enabled the interpretation of variable contributions, showing that time after sowing was the strongest predictor, followed by the positive influence of vinasse, while TBT and TMX had neutral to negative effects. These results underscore the importance of modeling multiple interacting factors to understand plant responses to contaminant stress.

The present thesis is structured in three interconnected chapters that build a coherent narrative from theoretical foundations to experimental validation and methodological advancement. Chapter 1, accepted as a book chapter in *Neonicotinoids in the Environment* (Springer, 2024), provides a comprehensive literature review on the environmental impact of thiamethoxam and discusses biological mitigation strategies such as phytoremediation and microbial biodegradation. This chapter establishes the theoretical basis for understanding the persistence of thiamethoxam in soil, its ecological effects, particularly toxicity to pollinators, and the necessity for sustainable remediation approaches, framing the rationale for the research.

Chapter 2, published in the *Journal of Agriculture and Food Research* (2024), reports an experimental study assessing the ecotoxicity of soils contaminated with tebuthiuron, thiamethoxam and vinasse. Using *Pennisetum glaucum* as the phytoremediator and *Lactuca sativa* seeds as bioindicators, the study demonstrates that pearl millet significantly reduces soil toxicity, especially when combined with vinasse which acts as a soil conditioner. Decision tree analysis highlighted the critical influence of cultivation time and vinasse presence on remediation effectiveness. This chapter validates the practical application of strategies introduced in Chapter 1 and underpinned the Qualifying General Exam.

Chapter 3, currently under preparation for submission, advances the methodology by incorporating predictive machine learning models to analyze millet development under varying soil conditions. Using regression algorithms and SHAP interpretation, this chapter identifies growth time as the most influential factor on plant biomass, while vinasse positively affects growth and pesticides exert neutral or negative impacts. The work emphasizes the limitations of isolated predictors and the benefits of integrated, data-driven approaches for interpreting complex ecological data. This chapter aligns with the central theme of the thesis by proposing evidence-based solutions to mitigate thiamethoxam's environmental impacts.

Together, these chapters present a logical progression from theoretical understanding to experimental evidence and methodological innovation, underscoring the role of phytoremediation and computational modeling in promoting sustainable management of pesticide-contaminated soils. Future research directions include investigating soil microbiota contributions to pesticide degradation, validating findings at the field scale, and employing sensor technologies and remote monitoring to support precision agriculture and soil health management.

2. CHAPTER I

UNDERSTANDING THE ENVIRONMENTAL IMPACT OF THIAMETHOXAM AND IMPLEMENTING BIOLOGICAL MITIGATION STRATEGIES

FRIAS, Yanca Araujo[1]; CRUZ, Victor Hugo[1]; MOREIRA, Bruno Rafael de Almeida[2]; PINCERATO, Giuliano Mikael Tonelo[1]; LOPES, Paulo Renato Matos[1]. Understanding the environmental impact of thiamethoxam and implementing biological mitigation strategies. In: SINGH, R.; SINGH, V. K.; KUMAR, A.; TRIPATHI, S.; BHADOURIA, R. (Org.). *Sustainable landscape planning and natural resources management*. 1 . ed. Cham: Springer Nature Switzerland, 2024. p. 163–170. DOI: http://dx.doi.org/10.1007/978-3-031-45343-4_12

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2.1. ABSTRACT

Thiamethoxam is a second-generation neonicotinoid insecticide characterized by high systemic activity and versatile applications, including foliar, granular and seed treatments. Belonging to the thianicotinyl subclass, it exhibits a broad spectrum of action, effectively controlling a wide range of economically important pests. Despite its recognized efficacy in pest management, its chemical and biological properties confer the potential for environmental impacts that cannot be overlooked. The magnitude of these impacts is influenced by multiple factors, including environmental conditions and agricultural practices. Within this context, bioremediation emerges as a sustainable, cost-effective and environmentally sound approach to reducing the persistence of neonicotinoids such as thiamethoxam in agricultural ecosystems. Scientific evidence has demonstrated promising results using plants and microorganisms as remediation agents capable of decreasing insecticide concentrations in soil and mitigating its adverse effects. This chapter provides an integrated analysis of the environmental impacts of thiamethoxam on agroecosystems, with emphasis on the application of biological strategies to mitigate its effects, aiming to reduce environmental damage and enhance the agronomic quality of soils. The perspectives discussed here offer

relevant insights for sustainable agricultural practices and the responsible use of neonicotinoid insecticides.

Keywords: ecosystem health; food security; microbial biodegradation; phytoremediation; soil health; sustainable agricultural development.

2.2. INTRODUCTION

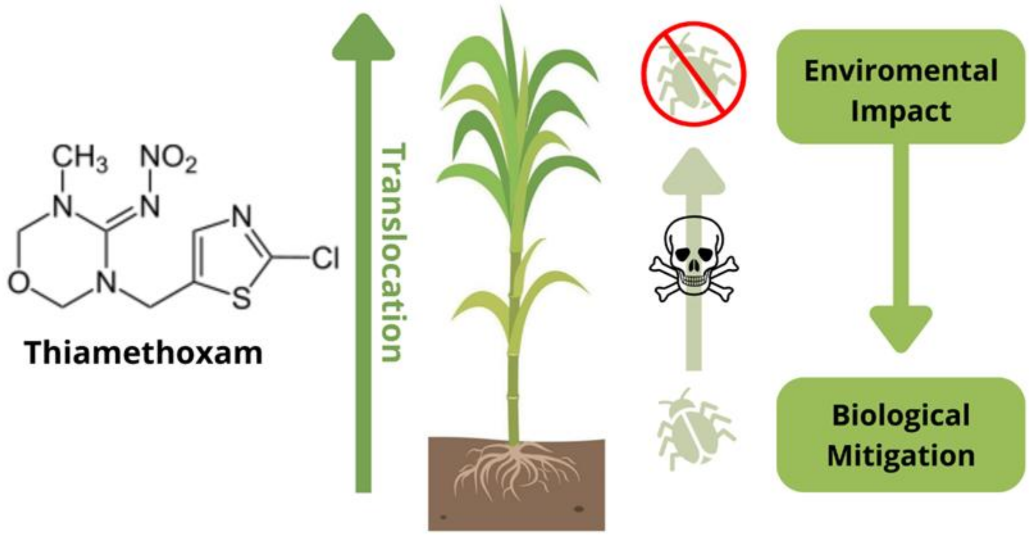
Thiamethoxam, a neonicotinoid insecticide, plays a significant and widely adopted role in modern agriculture. It has been employed to control a diverse range of agricultural pests, including aphids, whiteflies, thrips, and leafhoppers (Barbosa *et al*., 2022). Since its introduction in the 1990s, thiamethoxam has become one of the most extensively used insecticides worldwide (Maienfisch *et al*., 2001). The effectiveness of thiamethoxam is based on its mode of action as a neurotoxin that disrupts the nervous system of insects. This property renders it highly effective against various crop pests such as aphids, thrips, and beetles. In addition, its systemic nature allows it to translocate throughout the plant, from roots to leaves, enabling comprehensive pest control across different plant tissues (Barbosa *et al*., 2022).

Thiamethoxam is applied through multiple methods, including foliar spraying, seed treatment, and soil application, offering considerable versatility to farmers seeking efficient crop protection and enhanced yields (Barbosa *et al*., 2022). In Brazil, it is commercialized under brand names such as Cruiser® 350 FS and Actara® 250 WG. Its use spans several crops, including barley, beans, corn, cotton, pasture, peanuts, rice, sorghum, soybeans, sunflowers, and wheat—primarily as seed treatments. It is also applied to beans, citrus, coffee, cotton, pineapple, potatoes, and wheat through foliar or soil drip applications, according to Syngenta product information (2022, 2023). The product Actara® 750 SG is notably used in sugarcane cultivation, reflecting its diverse applications (Syngenta, 2022; 2023).

Despite its agronomic benefits, thiamethoxam use raises important regulatory and environmental concerns. The indiscriminate application of pesticides can lead to significant negative impacts on ecosystems, biodiversity, and human health. These environmental challenges involve not only effects on target pests but also unintended harm to non-target organisms, including essential pollinators (Serrão *et al*., 2022; Li *et al*., 2023). Moreover, soil contamination represents a critical issue associated with thiamethoxam residues, as reported by Li *et al*., (2022) and Nantes *et al*., (2022).

In response to these challenges, phytoremediation has emerged as a promising strategy to mitigate the environmental impact of thiamethoxam and other chemical pollutants. Phytoremediation exploits the ability of plants to remediate soil, water, and air by removing, degrading, or stabilizing contaminants (Yadav *et al*., 2022). Specifically, this approach holds potential to reduce soil and water contamination caused by thiamethoxam. This chapter explores the effects of thiamethoxam on agroecosystems and emphasizes biological mitigation strategies. It highlights bioremediation as a sustainable, cost-effective, and environmentally sound solution that leverages the roles of plants and microorganisms to decrease the persistence of thiamethoxam in agricultural soils (Figure 1). The discussion aims to contribute to reducing environmental damage and improving soil quality, thus supporting sustainable agricultural practices and the responsible use of neonicotinoid insecticides.

Figure 1 - Thiamethoxam in agricultural soils in pest control and environmental consequences.



2.3. UNDERSTANDING THIAMETHOXAM

Thiamethoxam, a key member of the neonicotinoid insecticide family, was introduced in 1991 as a chemically distinctive compound with notable insecticidal properties (Maienfisch *et al*., 2001). Initially marketed under the brand names Actara® for foliar and soil treatments and Cruiser® for seed treatments, it is currently present in twenty-five commercial products in Brazil alone (Brazil, 2023). Chemically defined as 3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine, with the molecular formula $C_8H_{10}ClN_5O_3S$, its structure notably contributes to environmental persistence. The five-membered thiazolyl ring includes a highly electron-withdrawing nitro group that renders the compound resistant to degradation by environmental agents. This stability complicates microbial bioremediation due to the molecule's complexity. Furthermore, the nitroguanidine moiety, essential for insecticidal activity, may exert toxicity on soil microorganisms by disrupting metabolic pathways, thereby reducing microbial diversity and biodegradation efficiency (NCBI, 2023).

The systemic nature of thiamethoxam, facilitated by the nitroguanidine group, enables its absorption through plant roots and translocation throughout tissues. While this characteristic enhances pest control effectiveness, it poses challenges for phytoremediation, as plants can accumulate thiamethoxam and potentially become sources of recontamination rather than pollutant removal agents (Wei *et al*., 2023; Liu *et al*., 2023). Thiamethoxam's insecticidal mode of action involves binding to nicotinic acetylcholine receptors (nAChRs) in insects, causing overstimulation and paralysis. It acts as a full agonist at afferent interneuron/giant cercal synapses and may bind to mixed nicotinic/muscarinic receptors (Nauen *et al*., 2003).

A notable metabolite of thiamethoxam is thiamethoxam-dm, which has a higher affinity for the imidacloprid binding site but is less potent as an insecticide. Both thiamethoxam and its metabolites, including clothianidin, coexist in natural environments, contributing to cumulative insecticidal toxicity. Clothianidin targets nAChR subtypes that are both sensitive and insensitive to imidacloprid (Nauen *et al*., 2003; Simon-Delso *et al*., 2015).

The success of thiamethoxam as a widely used insecticide stems from its versatile application methods, favorable safety profile, low application rates, prolonged residual activity, and alignment with integrated pest management programs (Li *et al*., 2018b). Despite replacing older, more harmful insecticides such as organochlorines, organophosphates, and pyrethroids, its extensive use raises environmental and toxicological concerns. Thus, a thorough understanding of thiamethoxam's environmental residues is critical for assessing and mitigating its ecological risks (Wei *et al*., 2023).

2.3.1. Environmental impacts of thiamethoxam

The environmental consequences of pesticide contamination are closely related to their physical and chemical properties. Water solubility, in particular, plays a critical role in the dispersion of pesticides into environmental compartments such as rivers, watercourses, and groundwater (Dellamatrice and Monteiro, 2014). The Food and Agriculture Organization (FAO, 2023) manual on data requirements for environmental fate studies has been instrumental in guiding the evaluation of such compounds, focusing on factors essential for establishing Maximum Residue Limits (MRLs). Regarding thiamethoxam, supervised residue trials include seed treatments, roots, tubers, and rice, considering parameters such as aerobic soil degradation, soil photolysis, hydrolysis, and crop rotation to assess its environmental behavior (Table 1).

Table 1 - Chemical properties of the pure active ingredient (FAO, 2023)

Property	**Result**
Melting point	139.1 °C
Vapor pressure	2.7 x 10-9 Pa at 20 °C
Solubility in water	4.1 g/L
Octanol/water Partition coefficient	log Pow = -0.13
Hydrolysis rate	pH 9 at 25 °C
Dissociation constant in water (Kw)	pH range of 2 to 12

Source: Own authorship.

Neonicotinoids, including thiamethoxam, have been identified by numerous studies as major contributors to the global decline of pollinators such as bees and bird populations (Tosi; Nieh, 2017; Serrão *et al*., 2022). Thiamethoxam’s systemic nature allows it to permeate all plant tissues and persist in environmental matrices that bees and other pollinators utilize, including nectar, pollen, guttation droplets, and water runoff. Its high affinity binding to insect nicotinic acetylcholine receptors leads to various sublethal effects on bees and colonies even at low concentrations (Tosi; Nieh, 2017).

Although introduced as alternatives to more toxic insecticides like organophosphates and carbamates, neonicotinoids have demonstrated persistent residues in the environment, contaminating drinking water and crops. This persistence raises concerns about potential bioaccumulation in higher trophic levels (Gautam; Dubey, 2023). The extensive use of thiamethoxam has resulted in its widespread distribution across different environmental compartments. Its fate depends on chemical properties and interactions with ecosystem components, including water solubility, adsorption to soil and sediments, rates of environmental input and removal, and physical-chemical and biological degradation processes (Gautam; Dubey, 2023).

A comprehensive understanding of thiamethoxam's environmental fate, particularly regarding remediation, is essential for effective insecticide removal. This process involves diverse transformation mechanisms, including biotic interactions mediated by microorganisms and plants, as well as abiotic physical-chemical processes. These insights are fundamental to developing strategies that mitigate thiamethoxam contamination and reduce its ecological impact.

2.3.2. Phytoremediation: harnessing nature's cleanup

Phytoremediation is an eco-friendly and promising approach that leverages plants to mitigate environmental pollution caused by organic and inorganic contaminants. This technique depends on several factors, including soil composition, the plants' ability to uptake and transform pollutants, and the synergistic interactions with diverse microorganisms within the ecosystem, all collaborating to reduce environmental damage (Yadav *et al*., 2018). Specifically, in the context of neonicotinoids such as thiamethoxam, phytoremediation exploits plants' remarkable capacity to absorb, extract, immobilize, or enzymatically degrade these pesticides.

The mechanisms involved in neonicotinoid phytoremediation can be broadly divided into two categories: plant-driven processes and microorganism-assisted biodegradation. Plant-driven mechanisms include phytoextraction, whereby contaminants are absorbed from the growth medium through roots and accumulated in harvestable tissues, and rhizofiltration, where roots adsorb or precipitate pollutants. Complementarily, rhizosphere microorganisms play a crucial role in biodegradation by metabolizing neonicotinoids, enhancing overall remediation efficiency through this mutualistic relationship (Wei *et al*., 2023). For instance, Zhan *et al*., (2021) isolated an endophytic bacterial strain capable of degrading thiamethoxam from wild plants and successfully inoculated it into rice, significantly improving remediation capacity.

Despite the increasing research on pesticide phytoremediation, studies focusing explicitly on neonicotinoids remain relatively scarce. Plant roots provide both nutrients and habitats for microbial communities involved in neonicotinoid degradation, strengthening the symbiotic interaction that boosts phytoremediation performance

(Wei *et al*., 2023). Phytoremediation presents multiple advantages such as safety, cost-effectiveness, in situ application, and environmental aesthetics, as it preserves native flora and fauna. However, its success depends on the plant's tolerance to contaminants, as high pesticide concentrations can inhibit growth and remediation potential (Jia *et al*., 2023). Additional challenges include long remediation times and the need for appropriate disposal of contaminated plant biomass.

Del Buono *et al*. (2020) categorize phytoremediation technologies based on the bioremediation mechanism involved: phytoextraction (uptake and translocation to harvestable parts), phytovolatilization (release into the atmosphere), phytostabilization (immobilization of pollutants in soil to prevent leaching), phytodegradation (enzymatic transformation of organic pollutants by plants), and phytostimulation or rhizodegradation (enhancement of microbial biodegradation via root exudates). Selecting the optimal phytoremediation strategy and plant species requires a case-by-case evaluation considering pollutant characteristics and remediation objectives.

2.3.3. Thiamethoxam phytoremediation: insights from recent studies

Phytoremediation remains a relatively unexplored yet promising strategy for mitigating thiamethoxam contamination in the environment. Recent research has begun to elucidate the capacity of specific plant species to absorb, translocate, and degrade this neonicotinoid, providing valuable insights into their potential role in environmental remediation.

Li *et al.* (2018b) investigated *Brassica rapa* var. *perviridis*, revealing that neonicotinoids, including thiamethoxam, predominantly accumulate in the shoots, with the metabolite clotianidina (M-CLO) more abundant in shoots than in roots or soil. Complementing these findings, Liu *et al.* (2021) evaluated nine plant species typical of constructed wetlands over 28 days, observing effective removal of six neonicotinoids. Among these, *Cyperus alternifolius* and *Cyperus papyrus* exhibited superior performance, driven primarily by biodegradation and plant-based accumulation mechanisms. Further expanding the scope, Aragão (2023) demonstrated that *Canavalia ensiformis* and *Mucuna pruriens* possess phytoremediation potential in thiamethoxam-contaminated soils, with thiamethoxam presence unexpectedly enhancing plant height, a phenomenon supported by Barbosa *et al.* (2022), who reported positive effects on seed germination and growth.

Studies by Wang *et al.* (2019) detailed the absorption, translocation, and subcellular distribution of thiamethoxam in *Brassica rapa* var. *chinensis*. The insecticide was found to translocate extensively from roots to shoots, with the soluble cell fraction identified as the primary storage site. More recently, Liu *et al.* (2023) conducted an extensive investigation into *Cyperus papyrus*, revealing that roots absorb multiple neonicotinoids and their metabolites, which are then translocated to aerial parts, mainly shoots. This dynamic phytoremediation process led to progressive decreases in pesticide and metabolite concentrations over time.

Collectively, these studies deepen our understanding of plant-thiamethoxam interactions and highlight phytoremediation as a viable and sustainable strategy for addressing thiamethoxam pollution. While challenges remain, including phytotoxicity and remediation efficiency, this body of research motivates further exploration into harnessing the natural detoxifying abilities of plants for environmental restoration.

2.3.4. Microbial biodegradation: a complementary approach

While phytoremediation offers a promising avenue for mitigating thiamethoxam contamination, recent research has underscored the essential role of microbial biodegradation in addressing the environmental impacts of neonicotinoids. Microbial processes contribute significantly to the natural attenuation of thiamethoxam and similar pesticides, enhancing their breakdown and detoxification. Nantes *et al.* (2022) described a notable interaction between thiamethoxam and vinasse, a residual liquid from the sugarcane industry, where vinasse presence exerted an antagonistic effect on thiamethoxam, resulting in reduced soil respiration. This effect is attributed to biostimulation, whereby organic substances such as vinasse stimulate microbial activity, thereby promoting pesticide biodegradation. This finding highlights the potential of organic amendments to activate indigenous microbial communities in contaminated soils.

Bioaugmentation is an emerging remediation technique that involves the intentional introduction of microorganisms possessing specialized metabolic abilities to accelerate the degradation of harmful compounds into less toxic or inert products. Lopes *et al.* (2022) demonstrated the efficacy of bioaugmentation in enhancing the remediation of environments contaminated by neonicotinoids, including thiamethoxam. Their findings emphasize the potential of leveraging specific microbial strains as effective agents for improving biodegradation and restoring affected ecosystems.

Environmental factors, particularly temperature, play a crucial role in determining the fate of thiamethoxam in soil and aquatic environments. Pavlidis *et al.* (2021) and Li *et al.* (2018a) explored how temperature variations influence the degradation and transformation rates of neonicotinoids. A thorough understanding of the interplay between temperature and microbial activity is essential for optimizing remediation strategies, especially in regions with fluctuating climate conditions. Collectively, these studies on microbial biodegradation—encompassing natural attenuation, biostimulation, and bioaugmentation—provide a comprehensive framework to address thiamethoxam and related neonicotinoid contamination and equip us with valuable tools to mitigate their ecological impacts.

2.4. CONCLUSION

In the context of complex environmental challenges influenced by climate, temperature, and the specific properties of neonicotinoids, it is essential to respond effectively. Thiamethoxam, a widely used insecticide in agriculture, poses ecological concerns due to its high water solubility, persistence, and potential environmental impacts. Phytoremediation appears as a promising strategy, with recent studies identifying plant species capable of absorbing, translocating, and degrading thiamethoxam. The cooperation between plants and rhizosphere microbiota plays a central role in improving phytoremediation efficiency. In addition, microbial biodegradation and the influence of temperature on contaminant reduction offer complementary approaches.

The adoption of responsible and sustainable agricultural practices is crucial to reduce the environmental footprint of pesticides, particularly thiamethoxam. Understanding the complex effects of these chemicals and developing effective mitigation methods are fundamental steps to preserve ecosystem health, biodiversity, and food security. In pursuit of a sustainable future, phytoremediation together with other innovative strategies offers a viable path to address the challenges associated with thiamethoxam use in agriculture.

Striving to balance agricultural productivity with ecological preservation requires commitment to responsible pesticide use, rigorous research, and the effective application of solutions that guide us toward sustainable coexistence with nature.

2.5. CONSIDERATIONS

The bioremediation of thiamethoxam is a complex and evolving field that requires the integration of various scientific disciplines, innovative techniques, and robust regulatory frameworks to address both the environmental and human health aspects of pesticide contamination. The following sections outline the advancements, limitations, challenges, and potential future directions for research and development, providing a comprehensive framework for addressing the challenges and advancing the field of thiamethoxam bioremediation.

2.5.1. Advancements

Phytoremediation techniques have progressed notably, with significant emphasis on plant selection. The process involves identifying and cultivating specific species capable of high thiamethoxam uptake. These plants function as natural filters within contaminated environments, absorbing and accumulating the pesticide. Such an approach may transform certain plants into *hyperaccumulators* capable of efficiently remediating thiamethoxam-contaminated sites. Genetic modification further enhances phytoremediation capacity by introducing genes that encode enzymes responsible for breaking down thiamethoxam and other neonicotinoids. This bioengineering aims to optimize the natural detoxification potential of plants, rendering them more effective at metabolizing the pesticide. The practical application of phytoremediation techniques through real-world case studies has been instrumental in evaluating their sustainability and effectiveness. These investigations provide crucial insights for scaling up phytoremediation strategies in various contaminated settings.

Regarding microbial biodegradation, the isolation and characterization of naturally occurring microorganisms such as bacteria and fungi that can degrade thiamethoxam are fundamental. Researchers explore diverse environments to identify unique microbial strains with potent biodegradation abilities. Elucidating the enzymatic pathways involved in microbial degradation of thiamethoxam and its metabolites is essential to advance this field. Understanding these intricate mechanisms enables the design of more efficient microbial biodegradation strategies. Additionally, genetic engineering techniques improve microbial capabilities by introducing genes that code for enzymes capable of degrading thiamethoxam. Through genome modification, tailored and highly effective microbial agents for pesticide remediation are being developed.

2.5.2. Limitations

The persistence of thiamethoxam and its breakdown products in environmental matrices such as soil, water, and plant tissues poses a significant challenge. Knowledge of the environmental fate and duration of these residues is crucial for devising effective and sustainable remediation approaches. Moreover, the potential accumulation and biomagnification of thiamethoxam residues through food chains raise concerns about adverse ecological effects and risks to human health.

Regulatory challenges further complicate the implementation of bioremediation techniques. Approval processes are often complex and lengthy, demanding rigorous scientific evidence of safety and efficacy prior to large-scale application. This necessitates strategies to streamline regulatory pathways for faster deployment of bioremediation solutions. Furthermore, regulatory standards related to safety, efficacy, and environmental impact must continuously adapt to keep pace with bioremediation advancements. International harmonization of regulations is another critical aspect, facilitating global acceptance and consistent guidelines for bioremediation techniques. Such harmonization could expedite regulatory approvals and promote wider adoption.

2.5.3. Potential future directions

Sustainable contaminant management requires attention to emerging compounds produced during thiamethoxam degradation, which may pose new environmental risks. Identifying these contaminants is vital to anticipate future challenges and maintain the relevance and effectiveness of remediation methods. Advanced multi-omics approaches including genomics, proteomics, and metabolomics are enhancing understanding of bioremediation processes. These technologies offer comprehensive insights into the interactions between microorganisms, plants, and thiamethoxam, thereby informing more effective remediation strategies. Addressing the complexity of thiamethoxam remediation necessitates interdisciplinary collaboration among ecologists, geneticists, microbiologists, and environmental engineers. This collective expertise is key to developing holistic and innovative solutions.

Research on microbial isolation and engineering remains a priority. Ongoing exploration in various environments aims to discover novel microbial strains with unique thiamethoxam-degrading capabilities, which could significantly enhance bioremediation efficiency. The application of cutting-edge gene editing tools such as

CRISPR-Cas9 promises precise modifications of microbial genomes to further improve degradation performance. Moreover, developing microbial consortia where different microorganisms collaborate synergistically to degrade thiamethoxam and its byproducts offers an eco-friendly and sustainable remediation approach.

Finally, addressing regulatory challenges will depend on effective stakeholder engagement among scientists, regulatory bodies, and industry representatives. Such collaboration is crucial for developing adaptive, practical regulatory frameworks that facilitate bioremediation adoption while ensuring safety and efficacy. Comprehensive risk assessment strategies are needed to evaluate the safety of bioremediation practices and guide regulatory decisions. Encouraging standardization of protocols and best practices will support regulatory compliance and consistency across regions, ultimately enhancing the effectiveness and reliability of thiamethoxam bioremediation efforts.

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3. CHAPTER II

PENNISETUM GLAUCUM IN REDUCING ECOTOXICITY IN SOIL WITH TEBUTHIURON, THIAMETHOXAM AND VINASSE

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3.1. ABSTRACT

This study aimed to evaluate the ecotoxicological effects of soil containing the herbicide tebuthiuron (TBT), the insecticide thiamethoxam (TMX), vinasse (VIN), and *Pennisetum glaucum* cultivation. The experiment was conducted under greenhouse conditions following a completely randomized design with five replicates. Treatments consisted of soil applications of tebuthiuron (1000 g ha^{-1} a.i.), thiamethoxam (330 g ha^{-1} a.i.), and vinasse (113 m^3 ha^{-1}), with or without *P. glaucum* cultivation. Soil monitoring extended up to 50 days after sowing (DAS), assessing ecotoxicological potential through bioassays using *Lactuca sativa* seeds as test organisms. Seed germination and root growth were used to calculate the germination index (GI) relative to the negative control prepared with water. The results demonstrated that *P. glaucum* exerted a beneficial effect on soil health by mitigating the ecotoxicological impacts of TBT and TMX. The timing of soil sampling proved critical, as earlier collections after sowing correlated with higher germination rates and faster soil health recovery. Additionally, vinasse reduced the ecotoxicological impact of pesticides, while the presence of tebuthiuron caused harmful effects on the test organisms. The interaction between the growth period of *P. glaucum* and soil sampling time significantly influenced the ecotoxicity bioassays, emphasizing the importance of early intervention.

Consequently, *P. glaucum* and vinasse represent promising components for phytoremediation strategies aimed at sustainable remediation of pesticide-contaminated agricultural soils, suggesting avenues for further research and development.

Keywords: ecotoxicology; *Lactuca sativa*; pesticides; phytoremediation; soil health.

3.2. INTRODUCTION

Ecotoxicology is a critical field of study focused on understanding the impact of contaminants on organisms within their natural environments, with the overarching goal of safeguarding the integrity and functioning of ecosystems. This is typically accomplished by examining the effects of these chemicals on individual species, using selected test organisms, and then extrapolating these findings to estimate safe concentrations for entire populations and communities. In ecotoxicological risk assessment, these derived safe levels are compared with predicted or measured exposure levels to evaluate potential risks to exposed ecosystems, providing essential insights for environmental protection and sustainability (van Gestel, 2012).

Therefore, ecotoxicological bioassays play an essential role in understanding the environmental impacts of chemicals used in agriculture. Effective pesticide management is a pivotal aspect of ecotoxicity assessments of agricultural soils, given the potential for these chemicals to impact soil health and ecosystem functions (Shahid; Khan, 2022).

Regarding sugarcane cultivation, tebuthiuron is an herbicide that acts by inhibiting photosystem II (Brazil, 2023) and presents extended half-life about 360 to 450 days (Blanco; Oliveira, 1987), which contributes to its persistence in the environment and potential for long-term ecological effects. Another critical factor is its high solubility in water (2,500 mg L^{-1} at 25 °C) that enhances mobility through the soil profile (Rodrigues; Almeida, 2018; Mancuso *et al*., 2011). The herbicide's octanol-water partition coefficient (log Kow) of 1.79 further indicates its tendency to remain in the aqueous phase rather than binding strongly to soil particles, facilitating its movement and potential contamination of groundwater (Rodrigues; Almeida, 2018; Mancuso *et al*., 2011). Moreover, tebuthiuron's notable affinity for soil particles rich in organic matter exacerbates its environmental impact. Hence, this organic matter can act as a reservoir, prolonging the herbicide's presence in the soil and its potential for runoff into nearby water bodies, thereby affecting aquatic ecosystems (Teixeira *et al*., 2018; Mendes *et al*., 2021; Guimarães *et al*., 2022). These factors collectively underscore the need for rigorous ecotoxicological evaluations of tebuthiuron and other agrochemicals to manage their environmental impact effectively and mitigate risks to soil and water systems.

Another critical compound in sugarcane cultivation is thiamethoxam, a neonicotinoid insecticide with considerable environmental implications due to its high mobility in the soil (Syngenta, 2023). Thiamethoxam is characterized by a relatively low molar mass of 291.72 g mol^{-1} and exhibits high water solubility of 4,100 mg L^{-1} at 25 °C. Its low octanol-water partition coefficient (0.74 at pH 6.8) indicates that it is more likely to remain in the aqueous phase rather than partitioning into soil organic matter, which facilitates its movement through soil profiles (Carvalho *et al*., 2013). This property enhances thiamethoxam's potential for leaching into deeper soil layers and, consequently, its potential to contaminate groundwater resources.

The average half-life of thiamethoxam is approximately 268.7 days, though this value can vary significantly depending on soil type, environmental conditions, and microbial activity (Brazil, 2019). This variation underscores the necessity of understanding thiamethoxam's environmental dynamics to predict its persistence and mobility accurately. Prolonged soil residence times can lead to sustained environmental exposure and potential risks to non-target organisms, including beneficial insects and plants. Furthermore, the high mobility and persistence of thiamethoxam raise concerns about its cumulative effects on soil health and ecosystem functioning, highlighting the need for comprehensive management strategies to mitigate its impact.

In addition to the application of pesticides in conventional sugarcane management, the practice of fertigation with vinasse has become widespread. Thus, this byproduct of ethanol production is commonly utilized as a beneficial residue disposal method (Souza *et al*., 2015; Prado *et al*., 2017; Soto *et al*., 2017; Pinto and Araujo, 2019). When applied correctly, vinasse can enhance soil fertility and improve crop productivity by providing essential nutrients and organic matter. However, its application is not without risks. When vinasse is combined with other substances such as tebuthiuron and thiamethoxam, it can introduce additional environmental concerns (Nantes *et al*., 2022; Ogura *et al*., 2022; Silva *et al*., 2021; Frias *et al*., 2023). The interaction of these substances can potentially lead to compounded contamination effects, impacting soil health and increasing the risk of leaching into groundwater.

In response to these challenges, phytoremediation has emerged as a promising approach for managing soil contamination by using plants to remove, degrade, or immobilize pollutants from the soil, offering a sustainable solution for environmental decontamination (Yadav *et al*., 2018; Kafle *et al*., 2022). This technique has shown significant efficacy in treating soils contaminated with pesticides, including those associated with sugarcane cultivation. The effectiveness of phytoremediation is attributed to its multidisciplinary approach, which combines agronomic and environmental benefits. Various plant species have been identified as capable of absorbing or degrading contaminants, thus mitigating the adverse effects of pesticide residues and improving soil quality (Pires *et al*., 2003; Pires *et al*., 2006; Pires *et al*., 2008; Mendes *et al*., 2020; Mendes *et al*., 2021; Ferreira *et al*., 2021; Conciani *et al*., 2023). This approach not only addresses the immediate contamination issues but also contributes to long-term soil health and ecosystem sustainability.

Assessing the ecotoxicological potential of soils contaminated with pesticides is essential for devising sustainable environmental recovery strategies. This assessment is crucial for identifying the impact of pesticides on soil health and for developing effective mitigation measures. Among the various methods available for monitoring soil ecotoxicity, the use of *Lactuca sativa* seeds as test organisms is particularly prevalent. This method is favored for its simplicity, robust physiological characteristics, and high sensitivity to contaminants, making it a reliable bioindicator for detecting toxic effects (Gonçalves *et al*., 2016). Numerous studies underscore the significance of these bioindicators in experimental protocols aimed at evaluating the ecotoxicological potential of pesticide-contaminated soils (Chelinho *et al*., 2014; Leitão *et al*., 2014; Ferreira *et al*., 2021; Neves *et al*., 2021; Lima *et al*., 2022; Nantes *et al*., 2022; Frias *et al*., 2023). These studies highlight how *Lactuca sativa* serves as a practical and effective tool for assessing the impact of various contaminants, providing valuable insights into soil toxicity and helping to guide remediation efforts.

Understanding the environmental behavior of pesticides within agricultural soils is critical for developing strategies to mitigate their negative impacts on agroecosystems. This involves studying how pesticides interact with soil components, their persistence, and their potential to leach into groundwater. Therefore, this study aims to investigate the ecotoxicological potential of soils contaminated with the herbicide tebuthiuron, the insecticide thiamethoxam, and vinasse, both individually and in combination with the cultivation of *Pennisetum glaucum* (L.) R. Brown. By examining these factors, the original research seeks to provide a comprehensive assessment of how these substances affect soil health and explore potential methods for improving soil remediation and management practices, based on the innovative decision tree structure models.

3.3. MATERIAL AND METHODS

3.3.1. Experimental location and material

We conducted the experiment under greenhouse conditions from January through May 2023 at 21°28′57″S and 51°31′58″W – Dracena, Brazil. The temperature and air humidity were 23.6 °C and 87%, respectively.

The soil (Ultisol) collected up to 0.20 m depth from Dracena-SP (Brazil) consisted of 79% sand, 14% clay, and 6.5% silt. Soil characterization is shown in Table 2. It was amended with lime to increase the sum of bases to 70%, following Cantarella *et al.* (2022).

Table 2 - Characterization of soil for standard textural and physicochemical properties

Property	Unit	Analytical quantity
Phosphorus	mg dm^{-3}	1
Organic matter	g dm^{-3}	7
Potassium	mmol$_c$ dm^{-3}	0.6
Calcium		1
Magnesium		9
Potential acidity		18
Sum of bases		10.6
Cationic exchange capacity		28.6
Bases Saturation	%	37
Sand		79.5
Clay		14
Silt		6.5
pH		4.2

Source: Own authorship.

The herbicide tebuthiuron (TBT) was applied as Combine® 500 SC (Dow AgroSciences – 1000 g ha^{-1} active ingredient [a.i.]), and the insecticide thiamethoxam (TMX) as Actara® 750SG (Syngenta Crop Protection – 330 g ha^{-1} a.i.). Vinasse was obtained from a full-scale sugar-energy plant in Dracena, Brazil. The seed cultivars used were BRS 1501 for *Pennisetum glaucum* (75% germination rate, 95% purity) and Itapuã Super for *Lactuca sativa* (90% germination rate, 100% purity), both sourced from an agricultural cooperative.

3.3.2. Experimental design

Pesticide dosages followed the manufacturer's leaflet according to the soil characteristics. The recommended volume of vinasse adhered to regulations commonly used in sugarcane crops for cost-effective chemical weed suppression and environmentally secure fertigation (CETESB, 2015).

The experimental design, presented in Table 2, used 4.0 L pots for 16 treatments with four replications each, based on the presence or absence of the factors: thiamethoxam (TMX), tebuthiuron (TBT), vinasse (VIN), and millet (*Pennisetum glaucum*) cultivation.

TBT and TMX were applied to experimental units via a laboratory device equipped with XR 11002 flat nozzles spaced every 0.5 m over a 1.0 m bar for broadcast spraying. The configured sprayer released pesticides at 200 kPa, 0.65 L min^{-1}, 5 km h^{-1}, and 0.75 m height above the pots.

Vinasse was applied a day after the pesticide application. Seven days later, seeds of *P. glaucum* were sown into the pots (3.0 g per experimental unit). On 10^{th} day after sowing (10 DAS), we thinned some seedlings to ensure the growth of a single robust individual plant. All experimental units received daily irrigation via automatic micro-sprinklers at 6 mm·h^{-1} for 1 h.

Table 3 - Factorial tests for the potential phytoremediation of TBT and TMX by *Pennisetum glaucum* in soil with vinasse

Test*	Factor A Thiamethoxam	Factor B Tebuthiuron	Factor C Vinasse	Factor D *P. glaucum*
1	Standard dose	Standard dose	Industrial recommendation	Presence
2	No application			
3	Standard dose	No application		
4	No application			
5	Standard dose	Standard dose	No application**	
6	No application			
7	Standard dose	No application		
8	No application			
9	Standard dose	Standard dose	Industrial recommendation	Absence
10	No application			
11	Standard dose	No application		
12	No application			
13	Standard dose	Standard dose	No application**	
14	No application			
15	Standard dose	No application		
16	No application			

Note: *Every testing condition comprised four replicates to reduce systematic (non-random) errors and ensure the reproducibility of the study
**Application of water to establish control.
Source: Own authorship.

3.3.3. Ecotoxicological bioassay

The ecotoxicological bioassay used lettuce seeds (*Lactuca sativa*) as the test organism, a well-established experimental protocol in the literature (Sobrero and Ronco, 2004) and widely applied in studies demonstrating sensitivity to the analyzed pesticides (Ferreira *et al.*, 2021; Lima *et al.*, 2022; Nantes *et al.*, 2022; Frias *et al.*, 2023).

Ecotoxicity was evaluated using 25.0 g of soil from experimental units at five plant development stages: 0 (initial without *Pennisetum glaucum*), 10, 20, 30, and 40 days after sowing (DAS). This soil amount was introduced into glass tubes containing 100 mL of ultrapure water to prepare analytical solutions, according to NBR 10.006 (ABNT, 2004).

Bioassays were conducted with *L. sativa* seeds incubated at 20°C ± 2°C for 120 hours in the dark, following Sobrero and Ronco (2004). Germination rate and seedling development, measured by hypocotyl and root elongation (≥ 0.1 mm), were quantified.

Positive (PC) and negative (NC) controls were prepared using 0.05 M zinc sulfate, which inhibits seed germination, and deionized water, respectively, to verify sensitivity. Additionally, the Germination Index (GI) was calculated based on seed germination (%G) and root elongation (%R).

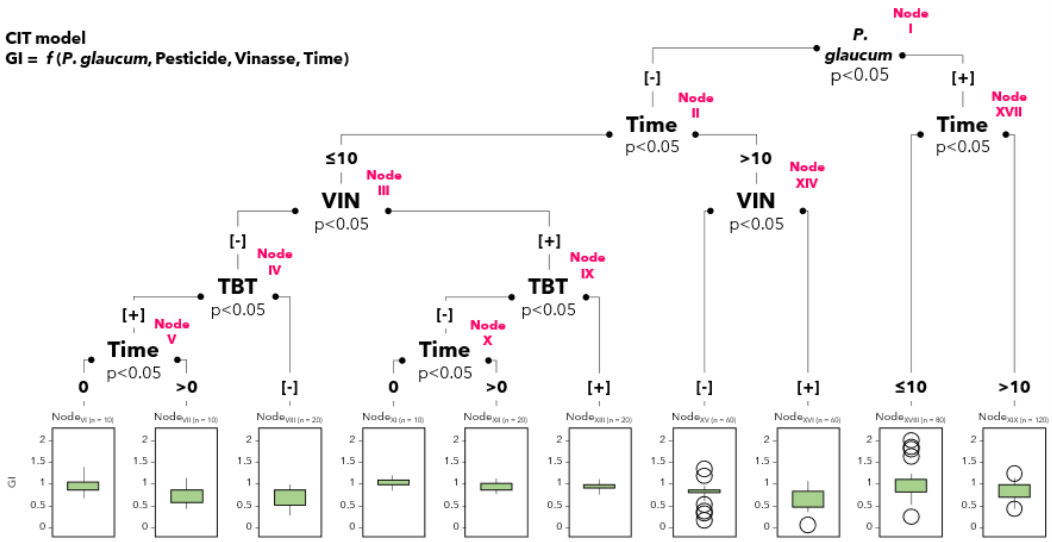
3.3.4. Statistical analysis

A decision tree approach was applied to the ecotoxicological data to model the effects of tebuthiuron, thiamethoxam, and vinasse on the development of *Pennisetum glaucum* and *Lactuca sativa*. To enhance clarity for readers, decision tree diagrams were used instead of numerical tables, facilitating the identification and interpretation of variations in morphometric and developmental traits. All analyses were performed using R software, with the *partykit* and *dplyr* packages employed for statistical calculations and graphical visualization (R Core Team, 2023).

3.4. RESULTS AND DISCUSSION

Decision tree structures in Figures 2, 3, and 4 illustrate the impact of interactions among tebuthiuron (TBT), thiamethoxam (TMX), vinasse (VIN), and *Pennisetum glaucum* in soil on the test organism (*Lactuca sativa*), offering a visual representation of these relationships. The data used to create the decision tree structures are included in the supplementary material.

Figure 2 - Decision-tree structure for germination index of *L. sativa* in ecotoxicity bioassays of soil containing tebuthiuron-thiamethoxam-vinasse associations



Source: Own authorship

In Figure 2, *Pennisetum glaucum* (Node I) exerts a multifaceted influence on the germination index as it fosters favorable conditions and emerges as a promising phytoremediation agent. This underscores *P. glaucum*'s pivotal role in enhancing soil health achieved through mechanisms such as the release of root exudates and remediation actions, which may include phytoextraction and phytoaccumulation (Teófilo *et al.*, 2020; Mendes *et al.*, 2021; Lopes *et al.*, 2022). These actions collectively mitigate the ecotoxicological impact of TBT and TMX. Ferreira *et al.* (2021) similarly described how this species played a significant role in supporting plant growth and development and observed no significant physiological anomalies despite the presence of tebuthiuron in soil.

Temporal factors (Nodes II and XVII), particularly the timing of soil sampling, appear as critical drivers of germination outcomes and reflect in soil health dynamics. Soil sampling conducted after 10 DAS of *P. glaucum* growth (time > 0) results in reduced germination index values, possibly due to evolving soil conditions that may impede phytoremediation. Conversely, early sampling (time = 0) yields higher germination index, which indicate rapid soil health improvement due to *P. glaucum* presence and its potential to alleviate ecotoxicological stress.

Considering these findings, Nantes *et al.* (2022) conducted a study on natural attenuation to mitigate the presence of tebuthiuron and thiamethoxam in the soil. They observed that both pesticides can be microbiologically mineralized into CO2, with vinasse having a catalytic effect by providing an additional energy source for the microbiota. Our results corroborate them as the vinasse introduction probably facilitated pesticide biodegradation, subsequently leading to a higher germination index of *Lactuca sativa* seeds.

Thus, vinasse addition to the soil introduces variability in outcomes (Nodes III and XIV) with significant ecotoxicological implications. In its absence, the germination index range remains relatively narrow (0.75 to 0.8). However, this range includes outliers below 0.6 and above 1, hinting at potential ecotoxicological consequences. The vinasse widens the germination index range (0.5 to 0.8), whose incorporation in soil probably stimulates microbial activity, enhances soil health, and consequently reduces the ecotoxicological impact of TBT and TMX. Recent studies by Nantes *et al.* (2022) and Lima *et al.* (2022) evidenced that microbial respiration yielded positive results in the presence of both TBT and TMX, while vinasse also played a notable role in enhancing the biological process. The authors clearly emphasized that vinasse acted similarly to a readily metabolizable organic energy source for microorganisms.

Regarding tebuthiuron, its presence or absence significantly shapes germination outcomes and offers direct insights into ecotoxicology (Nodes IV and IX). In absence of this herbicide, germination index range of 0.5 to 0.8 indicates improved soil health. Conversely, its application narrows the range (0.900 to 0.975) potentially due to ecotoxicological effects on soil.

The intricate interplay between soil sampling timing and *P. glaucum* cultivation profoundly impacts both the germination index and soil health with notable ecotoxicological implications as shown by Nodes V, X, XI and XII (Figure 2). Prolonged cultivation with *P. glaucum* (time > 0) yields a germination index range of 0.85 to 0.975, signifying ecotoxicological improvements. Early-stage cultivation with *P. glaucum* (time = 0) results in even higher values (0.90 to 1.05), which emphasizes the ecotoxicological significance of early intervention in soil remediation.

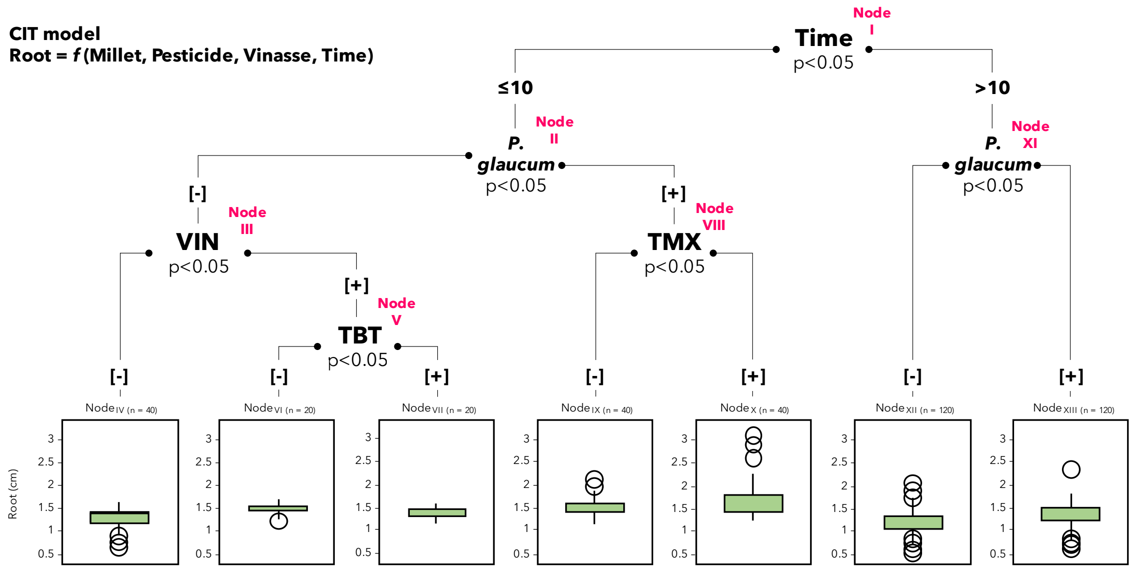
The presence of outliers underscores the inherent ecotoxicological complexity within phytoremediation processes (Nodes XV, XVI, XVIII, XIX). These exceptional data points deviate significantly from expected trends, warranting in-depth investigation to identify the ecotoxicological factors contributing to such deviations. This may involve unique ecotoxicological responses in specific microenvironments or soil conditions, underscoring the necessity for a holistic approach to phytoremediation.

In seedling development analysis, Figures 3 and 4 show the impact of pesticides and vinasse associations in soil with *P. glaucum* according to *Lactuca sativa*'s root and hypocotyl development, respectively.

Regarding time (Node I), it is noteworthy that hypocotyl growth was significantly limited in the presence of *P. glaucum* and without vinasse, whose results reach only 1.0 cm with several outliers after 10 DAS of *P. glaucum* development. On the other hand, vinasse addition in evaluation performed after 30 days reflected in approximately 1.5 cm hypocotyl length. There was a slight decrease in vinasse absence (Node XXI), which suggests that this residue provides plants with the necessary nutrients to cope with the stress caused by TMX and TBT.

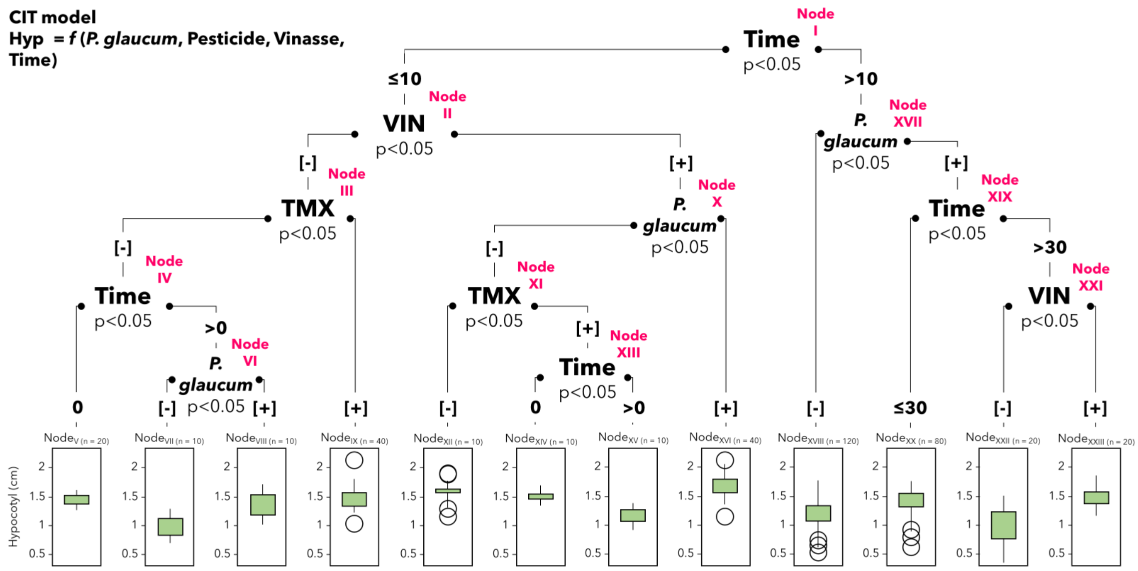
A similar pattern can be seen within 10 days when vinasse is present (Node II). Hypocotyl elongation was greater than 1.5 cm, thus indicating that vinasse provided adequate nutrition to *P. glaucum* (Node X), potentially mitigating TBT and TMX from soil.

Figure 3 - Decision-tree structure for root development of *Lactuca sativa* in ecotoxicity bioassays of soil containing tebuthiuron-thiamethoxam-vinasse associations



Source: Own authorship.

Figure 4 - Decision-tree structure for hypocotyl development of Lactuca sativa in ecotoxicity bioassays of soil containing tebuthiuron-thiamethoxam-vinasse associations.



Source: Own authorship.

In this context, Decision Trees are powerful visual representations used in data analysis and decision-making. When exploring them to select sustainable treatments in agroecosystems, they provide clarity in representing treatment options, considering variables like soil conditions, costs, and sustainability. They help identify the most promising options, facilitating an informed and objective choice. Their flexibility allows adaptations based on new data and environmental changes, ensuring a dynamic and balanced approach to agricultural and environmental management.

Moreover, future research should delve into mechanistic studies to elucidate the specific ecotoxicological pathways through which these factors interact, thereby influencing soil health and ecotoxicological responses. Another crucial factor to be detailed in future investigations in agricultural soils is the role of the microbiota in plant development and pesticide degradation. This knowledge will further refine remediation approaches for pesticide-contaminated soils, ensuring a more sustainable and environmentally friendly remediation process.

3.5. CONCLUSION

The complex interplay of various factors influenced the ecotoxicity potential to test-organism (*Lactuca sativa*) and to soil health: *Pennisetum glaucum* cultivation, vinasse addition, TBT and TMX contamination, and soil sampling timing. The findings underscore the pivotal role of *P. glaucum* in enhancing soil health and mitigating the ecotoxicological impact of TBT and/or TMX.

Furthermore, vinasse can act as a catalyst for mitigating ecotoxicological consequences caused by these pesticides. The absence of TBT improved soil health and resulted in wider germination index ranges for *L. sativa*, while its presence narrows the range due to ecotoxicological effects. The timing of soil sampling is critical, with early sampling yielding higher germination index values, reflecting the rapid improvement in soil health facilitated by *P. glaucum*.

Finally, based on the results that revealed the impact of tebuthiuron and thiamethoxam on the soil, the millet cultivation showed environmental benefits in reducing terrestrial ecotoxicity. This phytoremediation potential, combined with its agronomic benefits as a species used as green manure, highlights its practical use in sugarcane reform. Therefore, this study helps the sustainable management of this crop in an area with a history of pesticide application.

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4. CHAPTER III

PREDICTIVE MODELING OF MILLET DEVELOPMENT UNDER DIFFERENT SOIL CONDITIONS: A REGRESSION APPROACH WITH SHAP INTERPRETATION

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4.1. ABSTRACT

Soil contamination by persistent agrochemicals, such as tebuthiuron (TB) and thiamethoxam (TX), combined with the application of organic by-products like vinasse (VN), presents significant challenges for plant development and environmental sustainability in intensive agricultural systems. This study aimed to evaluate the biometric responses of *Pennisetum glaucum* (pearl millet) cultivated in soils with varying combinations of TB, TX, and VN, using predictive regression modeling techniques. A greenhouse experiment was conducted under controlled conditions over 20 and 60 days after sowing (DAS). Then, nine regression algorithms were applied to model shoot and root dry biomass and chlorophyll content. Shapley Additive Explanations (SHAP) values were employed to interpret the contribution of each predictive variable. Results showed that plant development time was the most influential predictor for all biometric variables, especially in models like “gpr” and “rf.” However, even the best-performing models showed limited predictive capacity when time was excluded, with R^2 values below 0.15. VN had a generally positive impact on plant growth, while TB and TX showed neutral or negative effects. The SHAP analysis emphasized the need to consider variable interactions and temporal dynamics when predicting plant responses under multiple stress factors. This work highlights the limitations of isolated predictors and the value of integrating machine learning with phytoremediation strategies to support sustainable soil management in agriculture.

Keywords: Machine learning; tebuthiuron; thiamethoxam; vinasse.

4.2. INTRODUCTION

Soil contamination by pesticides is one of the foremost environmental challenges associated with modern agriculture, particularly in intensive cropping systems such as sugarcane production. Compounds like tebuthiuron (TB), a substituted urea herbicide, and thiamethoxam (TX), a neonicotinoid insecticide, are widely used for weed and pest control. These substances are characterized by their chemical persistence in the environment, variable soil mobility, and potential toxicity to non-target organisms, including soil microorganisms, edaphic fauna, and cultivated plants (Ferreira *et al*., 2024; Serrão *et al*., 2022). Additionally, the application of agroindustrial by-products such as vinasse, which is rich in organic matter and mineral salts (Balakrishnan, 2024), can alter soil physicochemical properties, influencing the bioavailability and fate of these pesticides, thus exacerbating environmental impacts (Ferreira *et al*., 2024).

The detrimental effects of pesticide contamination manifest not only through the reduction of microbial biodiversity but also through the disruption of biochemical processes essential for soil fertility, such as nutrient cycling and soil structure maintenance, ultimately impairing the development and productivity of subsequent crops (Meena *et al*., 2020). In response to these challenges, phytoremediation, the use of plants to remediate contaminated soils, has gained attention as a sustainable, cost-effective, and environmentally friendly alternative (Yadav *et al*., 2018). Species such as pearl millet (*Pennisetum glaucum*) exhibit significant root biomass production (Serafim *et al*., 2024) and high tolerance to herbicide persistence in soil (Frias *et al*., 2024), making them promising candidates for remediation strategies.

Assessing the biometric responses of these plants under varying contamination levels requires robust analytical approaches capable of capturing the complexity and heterogeneity of experimental data. Traditional methods often prove inadequate when confronted with the multidimensionality and non-linearity inherent to biological processes. Consequently, machine learning (ML) models have emerged as powerful tools for data analysis and prediction in ecology and agronomy (Wagas *et al*., 2025; Aderele *et al*., 2025). Techniques such as random forest regression, support vector regression, and artificial neural networks have been successfully applied to model plant responses to environmental pesticide contamination, identify key predictive variables, and forecast plant performance under diverse conditions (Anandhi; Iyapparaja, 2024). For example, Ali *et al*., (2024) utilized neural networks to model physiological responses of crops exposed to stress, highlighting these models' capacity to handle complex interactions and noisy data.

Therefore, this study aims to apply and compare various machine learning regression models to describe the biometric responses of pearl millet cultivated in soils contaminated with tebuthiuron, thiamethoxam, and vinasse. By integrating advanced data analysis techniques with sustainable phytoremediation approaches, this work seeks to contribute to the development of more effective and environmentally responsible strategies for managing contaminated soils in intensive agricultural systems.

4.3. MATERIAL AND METHODS

4.3.1. Experimental location and material

The experiment was performed under greenhouse conditions from January through May 2023 at 21°28′57″S and 51°31′58″W – Dracena, Brazil. The average temperature and air humidity were 23.6°C and 87%, respectively.

The soil (Ultisol) collected up to 0.20 m depth from Dracena consisted of 79% sand, 14% clay, and 6.5% silt. According to the soil characteristics in Table 4, the parameters for plant development were corrected by applying lime to increase the base saturation until it reached 70% (Cantarella *et al*., 2022). Soil samples for physicochemical analysis were obtained by collecting multiple subsamples at random points within the greenhouse, from the upper layer (0–20 cm) after removing surface debris. The subsamples were homogenized, air-dried, and sieved through a 2 mm mesh. Chemical properties (phosphorus, potassium, calcium, magnesium, organic matter, pH) were determined using the standard methods adopted by Embrapa: phosphorus and potassium via Mehlich-1 extraction, calcium and magnesium via KCl 1 M extraction, organic matter via Walkley-Black, and pH in $CaCl_2$ solution. All procedures followed Brazilian soil analysis recommendations.

The herbicide tebuthiuron (TB) was used as Combine® 500 SC (Dow AgroSciences – 1000 g ha^{-1} active ingredient [a.i.]) and the insecticide thiamethoxam (TX) as Actara® 750SG (Syngenta Crop Protection – 330 g ha^{-1} a.i.). Vinasse was obtained from a sugarcane energy plant in Dracena, Brazil.

Finally, the seeds of *Pennisetum glaucum* were BRS 1501 (75% germination rate and 95% purity)

Table 4 - Characterization of soil for standard textural and physicochemical properties

Property	Unit	Analytical quantity
Phosphorus	mg dm^{-3}	1
Organic matter	g dm^{-3}	7
Potassium	$mmol_c$ dm^{-3}	0.6
Calcium		1
Magnesium		9
Potential acidity		18
Sum of bases		10.6
Cationic exchange capacity		28.6
Bases Saturation	%	37
Sand		79.5
Clay		14
Silt		6.5
pH		4.2

Source: Own authorship.

4.3.2. Experimental design

Pesticide dosages followed the manufacturer's leaflet according to the recommendations for the soil characteristics. The recommended volume of vinasse followed regulations commonly used in sugarcane crops for cost-effective chemical suppression of weeds and environmentally secure fertigation (Cetesb, 2015).

Thus, the experimental design used 4.0 L pots for 16 tests with four replications each, based on the presence or absence of the factors: thiamethoxam (TX), tebuthiuron (TB), vinasse (VN), and pearl millet (*P. glaucum*) cultivation (Table 5).

Pesticides TB and TX were applied via a laboratory device, which provided a suite of XR 11002 flat nozzles every 0.5 m along a 1.0 m bar for broadcast spraying. A configured sprayer released pesticides at 200 kPa, 0.65 L min^{-1}, 5 km·h^{-1}, and 0.75 m height above the top of the pots. Prior to application, the spraying apparatus was calibrated for flow rate and spray pattern uniformity. Pesticide distribution was verified using catch containers across the pot array. All applications were conducted indoors under controlled temperature and humidity, minimizing environmental drift. Pots were arranged to ensure equal exposure, and the operator wore appropriate PPE.

Vinasse was applied one day after pesticide application. Seven days later, seeds of *P. glaucum* were sown into the pots (3.0 g per experimental unit). On the 10th day after sowing (10 DAS), some seedlings were thinned to ensure the growth of a single robust individual plant. All experimental units received daily irrigation via automatic micro-sprinklers at 6 mm h^{-1} for 1 hour.

Table 5 - Factorial tests on the composition of soil samples

Test*	Factor A Thiamethoxam	Factor B Tebuthiuron	Factor C Vinasse	Factor D *P. glaucum*
1	Standard dose	Standard dose	Industrial recommendation	Presence
2	No application			
3	Standard dose	No application		
4	No application			
5	Standard dose	Standard dose	No application**	
6	No application			
7	Standard dose	No application		
8	No application			
9	Standard dose	Standard dose	Industrial recommendation	Absence
10	No application			
11	Standard dose	No application		
12	No application			
13	Standard dose	Standard dose	No application**	
14	No application			
15	Standard dose	No application		
16	No application			

Note: *Every testing condition comprised four replicates to reduce systematic (non-random) errors and ensure the reproducibility of the study
**Application of water to establish control.
Source: Own authorship.

4.3.3. Monitoring plant development

Several parameters monitored the growth of *P. glaucum* to assess the physiological responses of the plants to different soil samples until 60 DAS. Chlorophyll content in the leaves was determined using a portable chlorophyll meter at 20 and 60 DAS (ClorofiLOG®, model CFL 1030; Falker Automação Agrícola SS, Brazil), which provides rapid and non-destructive readings. This instrument allows for real-time assessment of chlorophyll levels, enabling accurate monitoring of plant physiological status under varying conditions.

Fresh and dry biomass (g) were quantified to evaluate plant growth at 20 and 60 DAS. After completing all other measurements, the shoot and root parts of the plants were weighed to determine fresh biomass. These samples were then dried in a forced-air oven at 65 °C for 72 hours and reweighed to obtain dry biomass. This rigorous protocol provided detailed insights into how the different treatments, such as pesticide exposure, vinasse application, and their combinations, affected plant growth and resource allocation.

4.3.4. Statistical analysis

A predictive analysis was conducted using regression algorithms. The independent variables included time (DAS) and, indirectly, the effects of soil conditions with TB, TX, and/or VN. The data were organized into 50 subsets through repeated sampling (bootstrapping), and each model was trained and evaluated on each subset, allowing for an aggregated statistical analysis of errors and model fit quality.

The nine models tested included: conditional regression tree (ctree), boosting (gbm and xgb), penalized regressions (lasso and ridge), classical linear regression (lm), random forest (rf), support vector machine (svm), and Gaussian process regression (gpr). The evaluation metrics used were Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and the coefficient of determination (R^2).

Given the complexity of biological systems and the multidimensional nature of the data involved, the application of multiple regression algorithms enables the comparison of linear and nonlinear approaches, allowing for a more robust assessment of the relationships between variables (Kuhn and Johnson, 2013; Molnar, 2022).

To compare the predictive performance of the applied machine learning models, the SHAP (Shapley Additive Explanations) approach was employed to interpret the importance of the predictor variables. SHAP is based on Shapley values theory and enables quantification of the individual contribution of each variable to the model's prediction by considering all possible combinations of feature interactions (Molnar, 2022; Wang *et al*., 2025).

The analysis was performed using the R programming language, supported by the caret, mlr, and ggplot2 packages.

All biometric and soil data, including replicate, treatment, factor presence, and time point were compiled in a long-format spreadsheet, with each row representing an individual experimental measurement. Quantitative variables were standardized, and categorical factors encoded appropriately. Bootstrapped model training and validation used 80/20 data splits. All data processing, modeling, and evaluation took place in R, ensuring reproducibility.

4.4. RESULTS AND DISCUSSION

For each of the variables monitored in the development of *Pennisetum glaucum* across different soil samples, a general modeling approach was applied using various regression models, followed by the use of SHAP to explain the individual contribution of each predictor variable (TB, TX, VN, and time) to the variation in the analyses. This allowed the identification of the most influential factors in the predictive performance of the models under each scenario. Consequently, the most important variable in the study was identified within the regression models.

The models were evaluated based on the coefficients of determination (R^2), mean absolute error (MAE), and root mean square error (RMSE), considering 50 data subsets for each modeling approach.

In this context, Figure 5 presents the regression prediction for root dry biomass, influenced by the plant’s development time.

The coefficients of determination (R^2) were low across all tested models, indicating that development time alone was not sufficient to explain the observed variability in root dry biomass. The "gpr" model showed the best performance in terms of variance explanation, with $R^2 = 0.146$, followed by "lm" with $R^2 = 0.129$ and "ridge" with $R^2 = 0.128$. The "rf" model achieved an R^2 of 0.124 but stood out for presenting the lowest mean absolute error (MAE = 1055.841), suggesting good point accuracy in the predictions.

The “svm” model yielded the lowest MAE (879.803), but with a markedly low R^2 (0.050), highlighting its limitation in capturing broader trends within the dataset. Models such as “gbm” and “xgb” exhibited the poorest performance, with R^2 values of 0.046 and 0.036, respectively, and high error rates, indicating low predictive power under these experimental conditions.

Figure 5 - Regression results for Root Dry Biomass, considering plant development time

Note: *Scatter plots with trend lines (in red) representing the relationship between observed and predicted values from different regression models (ctree, gbm, gpr, lasso, lm, rf, ridge, svm, xgb). Each point corresponds to an individual prediction from one of the 50 datasets. RMSE, MAE, and R^2 metrics are indicated in each panel.
Source: Own authorship.

These results indicate that, despite the application of machine learning techniques, the models' ability to predict *pearl millet* root dry biomass over time in contaminated soil was limited. No model achieved an R^2 above 0.15, demonstrating that development time alone is not a sufficient predictor to capture the complexity of the physiological responses of millet exposed to TB, TX, and/or VN.

The superior performance of the “gpr” and “rf” models may be attributed to their ability to handle non-linear data and irregular distributions (Rasmussen, 2004; Saha *et al*., 2023; Mushagalusa *et al*., 2024), typical characteristics of experiments involving multiple environmental stress factors. The “gpr” model, in particular, incorporates uncertainties associated with predictions, making it a promising approach for scenarios with high data noise (Rasmussen, 2004).

Figure 6 presents the SHAP model for improved interpretability of the most important variable influencing root dry biomass. The colors represent the intensity of the original variable values, transitioning from blue (low values) to red (high values), while the X-axis indicates the magnitude and direction of each variable's contribution to the prediction.

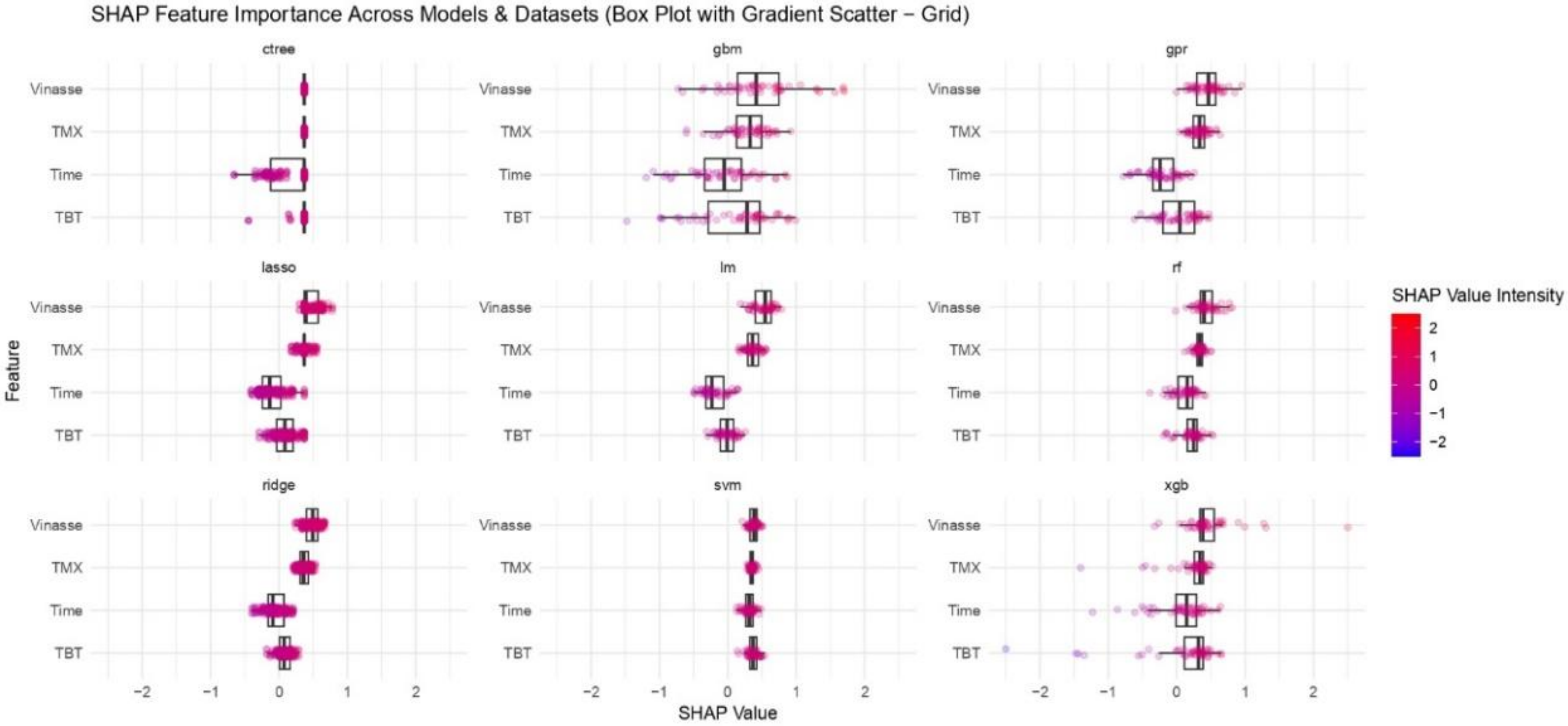
The SHAP analysis including the time variable revealed notable changes in the relative contribution of the predictors (Figure 6). Time became the dominant predictive factor, showing the highest and most widely distributed SHAP values across the models. In contrast, the applied pesticides maintained a secondary influence, suggesting a possible cumulative or delayed residual effect, as observed by Ferreira *et al*., (2024), who studied the residual activity and sorption of TB in different soils. The authors observed that TB's residual activity decreases over time, with no toxicity detected 360 days after application in all soils studied, but higher sorption occurs in soils with higher organic carbon and clay contents, as the VN in this work.

The relevance of VN as a predictive variable was preserved, although with less relative importance compared to time, possibly due to its role as a soil conditioner rather than a direct toxic contaminant.

Thus, the interaction between the compounds and the soil affects nutrient availability, microbial activity, and root absorption, resulting in growth variations that are not easily explained by time alone. Previous studies have shown that herbicides such as TB exhibit high persistence in the soil and can compromise the root system of non-target plants for extended periods, which may explain the variability observed in root dry biomass (Pires *et al*., 2005). Similarly, vinasse can act as a soil conditioner (Ferreira *et al*., 2022) or, depending on the dose, as a source of stress (Frias *et al*., 2023).

When the variable “plant development time” was removed from the regression models for root dry biomass (Figure 7), an even greater decline in the predictive performance of the algorithms was observed.

Figure 6 - SHAP values for predicting Root Dry biomass of *Pennisetum glaucum*, considering plant development time



Note: *Boxplots with colored scatter display the SHAP values assigned to each explanatory variable (VN, TB, TX, and Time) across different models. The colors represent the magnitude of the original feature values.
Source: Own authorship.

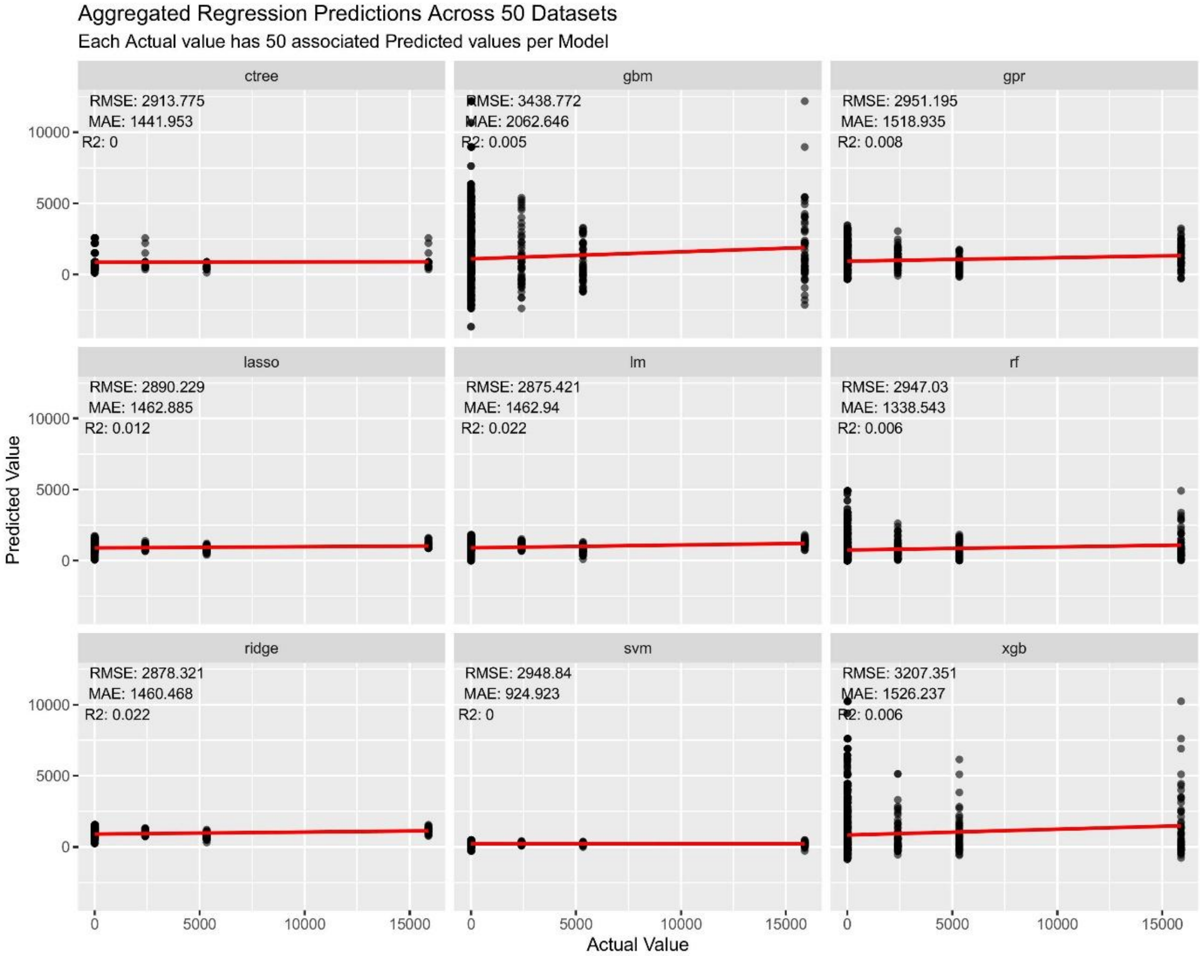
No model exceeded a coefficient of determination (R^2) of 0.022, indicating an almost negligible explanation of the variability in root dry biomass. The “lm” (0.022), “ridge” (0.022), and “lasso” (0.012) models yielded the highest R^2 values, although still extremely low, with RMSE values ranging from 2875 to 2890 g/plant. These results suggest a slight advantage of these models in detecting minimal patterns in the data. In contrast, the “rf,” “gpr,” and “xgb” models also performed poorly, with R^2 values between zero and 0.008, confirming their limited modeling capacity with the available data. Even the “rf” model, which presented a relatively higher MAE (1338.5 g), failed to explain the variance in the data. The “svm” model achieved the lowest MAE (924.92 g), but with a null R^2, reinforcing that its predictions may be centered around the mean without capturing actual variations in root biomass. Finally, the “gbm” model was the least suitable in terms of absolute error (MAE = 2062.6 g), with a negligible R^2 (0.005), indicating its inadequacy for the data pattern.

The absence of time as a predictor variable substantially compromised the performance of the models. This is expected, as time is directly associated with the dynamics of root growth (Kou *et al*., 2025). Ignoring this factor implies assuming that all plant developmental stages equally impact root dry biomass, which is biologically inaccurate.

In addition, the compounds present in the soil (TB, TX, and VN) affect the plant in dynamic and cumulative ways, with their effects varying according to exposure time, phenological stage, and the intensity of chemical stress. Therefore, excluding time removes an essential component in explaining the plant’s response to the environment.

The interpretability analysis using SHAP (Figure 8) reinforced this finding. The distribution of SHAP values was concentrated and showed low variability among the applied residues when the time variable was excluded. Although vinasse exhibited a higher relative contribution in most models, the effects of TB and TX were also relevant in models such as “rf” and “xgb.” However, the low amplitude of SHAP values confirms the limited predictive influence of these variables when isolated from the time factor.

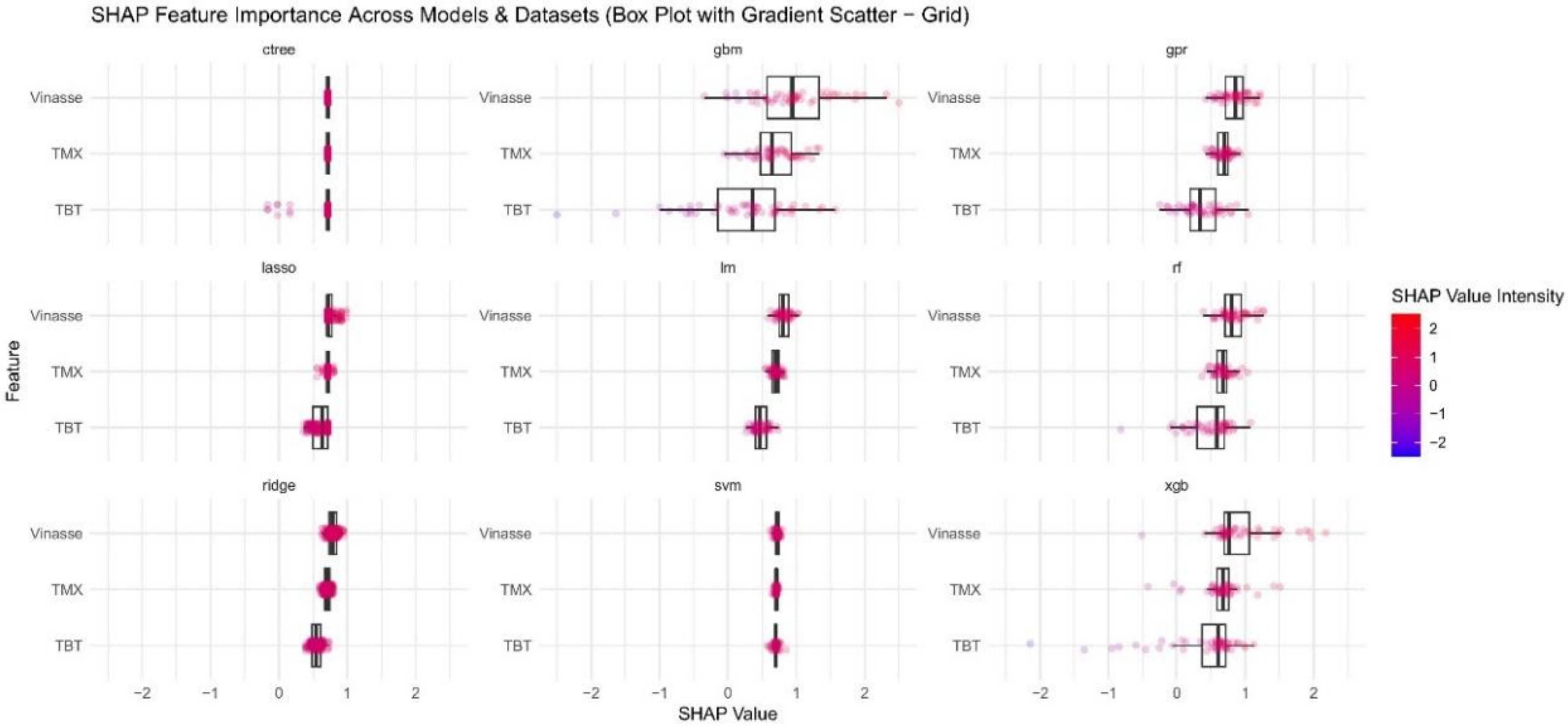
Figure 7 - Regression results for Root Dry Biomass, excluding plant development time



Note: *Scatter plots with trend lines (in red) representing the relationship between observed and predicted values from different regression models (ctree, gbm, gpr, lasso, lm, rf, ridge, svm, xgb). Each point corresponds to an individual prediction from one of the 50 datasets. RMSE, MAE, and R^2 metrics are indicated in each panel.
Source: Own authorship.

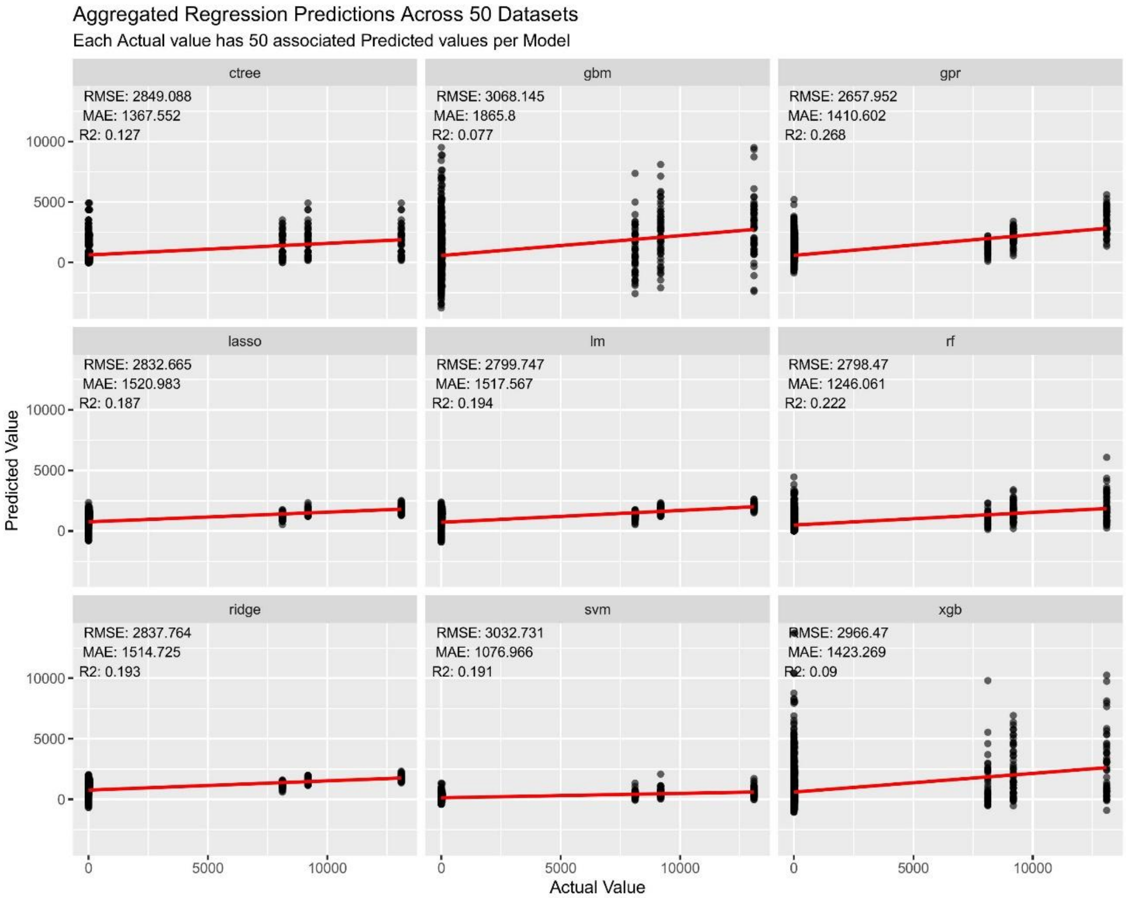
Additionally, the predictive analysis of shoot dry biomass of *Pennisetum glaucum* under the influence of development time showed significant variations in performance among the tested regression models (Figure 9).

Figure 8 - SHAP values for predicting Root Dry Biomass of *Pennisetum glaucum*, excluding plant development time



Note: *Boxplots with colored scatter display the SHAP values assigned to each explanatory variable (VN, TB, TX, and Time) across different models. The colors represent the magnitude of the original feature values.
Source: Own authorship.

Figure 9 - Regression results for Shoot Dry Biomass, with plant development time



Note: *Scatter plots with trend lines (in red) representing the relationship between observed and predicted values from different regression models (ctree, gbm, gpr, lasso, lm, rf, ridge, svm, xgb). Each point corresponds to an individual prediction from one of the 50 datasets. RMSE, MAE, and R^2 metrics are indicated in each panel.
Source: Own authorship

Among the algorithms evaluated, the “gpr” model demonstrated the best overall performance, with R^2 = 0.268, RMSE = 2657.95, and MAE = 1410.60, suggesting a relatively superior ability to capture response variability as a function of the predictor variables. Following that, the “rf” and “lm” models also stood out, with R^2 values of 0.222 and 0.194, respectively, both showing satisfactory performance compared to the other algorithms.

Penalized linear models, such as “lasso” and “ridge,” showed similar performances, with R^2 values of 0.187 and 0.193, respectively, reinforcing that the inclusion of plant development time as a predictor variable slightly improved the performance of these methods.

The “gbm” and “xgb” models, although widely used for their robustness in regression problems (Huber *et al*., 2022), showed inferior performance, with R^2 values of 0.077 and 0.090, respectively, and high RMSE values (> 2900).

The “svm” model, although presenting the lowest MAE among all models (1076.97), did not show strong explanatory power (R^2 = 0.191), indicating that its predictions are close to the overall mean but with low explained variability.

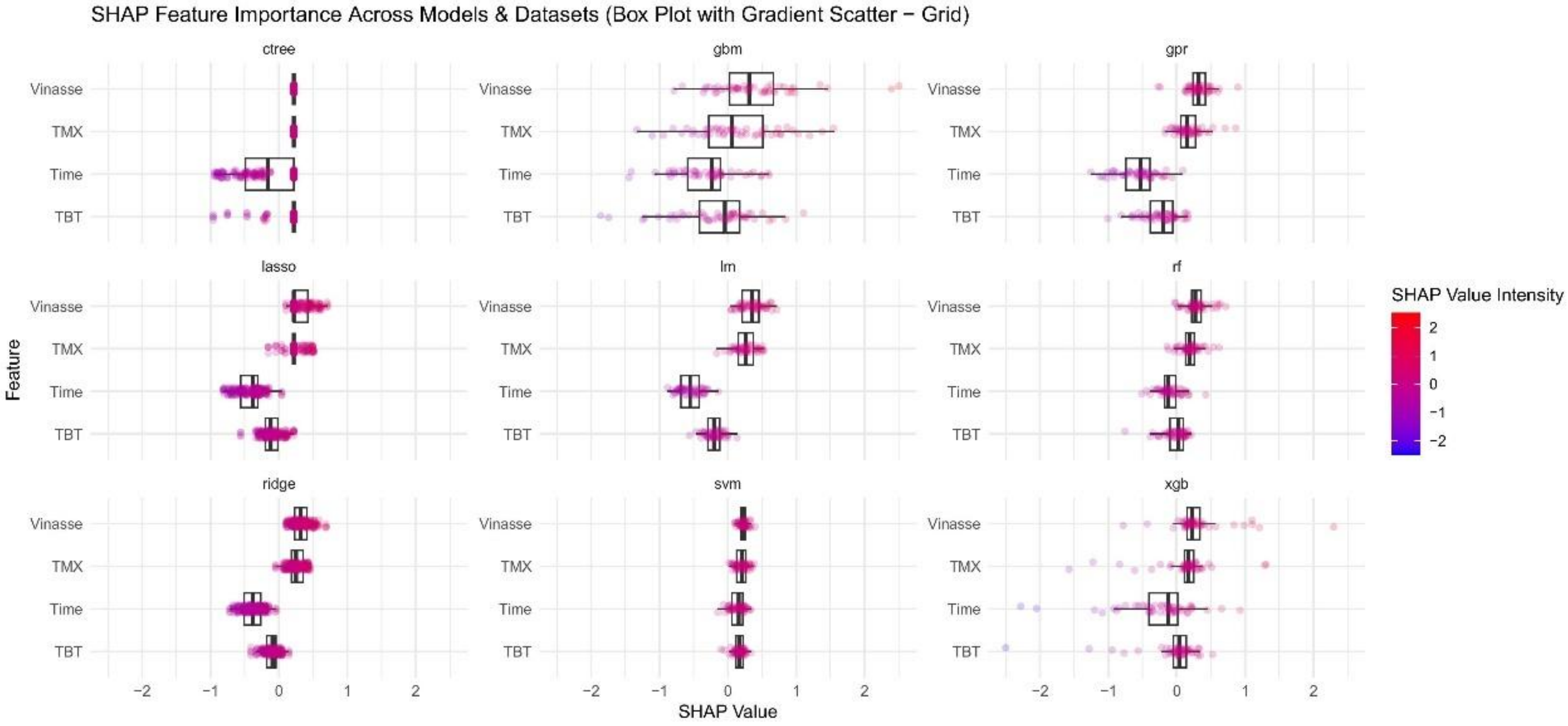
The results suggest that including time as an explanatory variable contributed to improved model fitting, as evidenced by the overall increase in coefficients of determination compared to the analyses without this variable. Furthermore, it is noteworthy that the models' ability to represent shoot biomass growth is directly related to the complexity of interactions between the pesticides and the soil environment over time.

Among all the variables evaluated, plant development time had the greatest positive impact on shoot dry biomass prediction, particularly in the “lasso,” “ridge,” “lm,” and “gpr” models. In these models, the SHAP values (Figure 10) associated with time show a broader distribution centered around positive values, indicating that increased cultivation time is robustly associated with greater shoot biomass.

The VN variable also showed considerable importance, with positive effects in the “lm,” “lasso,” “ridge,” and “gbm” models, suggesting that the presence of this residue may have contributed to greater nutrient availability and, consequently, promoted shoot growth. Similar findings were reported by Ferreira *et al*. (2022), who observed that the presence of vinasse contributed to biomass development of *Mucuna pruriens* in soil containing TB.

The pesticides exhibited varied effects, generally associated with SHAP values that were negative or showed minimal positive contribution across the analyzed models. TB, in particular, presented SHAP values concentrated near zero or negative in models such as “xgb,” “lm,” “lasso,” and “gpr,” indicating its inhibitory or negligible effect on shoot biomass gain. TX, although showing some variability, had a more discreet and less consistent contribution among the models.

Figure 10 - SHAP values for predicting Shoot Dry Biomass of *Pennisetum glaucum*, considering plant development time



Note: *Boxplots with colored scatter display the SHAP values assigned to each explanatory variable (VN, TB, TX, and Time) across different models. The colors represent the magnitude of the original feature values.
Source: Own authorship.

It is important to highlight that the best-performing predictive models, such as “gpr” and “rf,” also most effectively captured the combined influence of time, VN, and TB variables. These models visually demonstrated a clear pattern of separation among SHAP values, indicating greater sensitivity to the complex interactions between soil factors and plant development.

Thus, the SHAP analysis results reinforce the hypothesis that plant development time is the primary determinant of *Pennisetum glaucum* shoot biomass in soils containing organic residues and pesticides, followed by VN as a potentially growth-promoting factor, while the evaluated pesticides tended to exert negative or neutral effects on the response variable.

The results without including plant development time as a predictor (Figure 11) demonstrate limited predictive performance across all models, with R^2 values below 0.04, indicating that less than 4% of the variability in dry biomass was explained by the isolated environmental variables. The “ridge” model showed the best performance (RMSE = 2984.93; MAE = 1631.82; R^2 = 0.035), followed by the “lm” model with RMSE = 2978.44 and R^2 = 0.034. Nevertheless, these values indicate low predictive accuracy, suggesting that the included factors, although relevant, are insufficient to explain the complexity of accumulated shoot biomass.

Thus, in the absence of the temporal factor, *Pennisetum glaucum* shoot dry biomass cannot be adequately explained solely by VN, TB, and TX. The absence of time compromises the modeling of the physiological process of plant growth, which is inherently dynamic.

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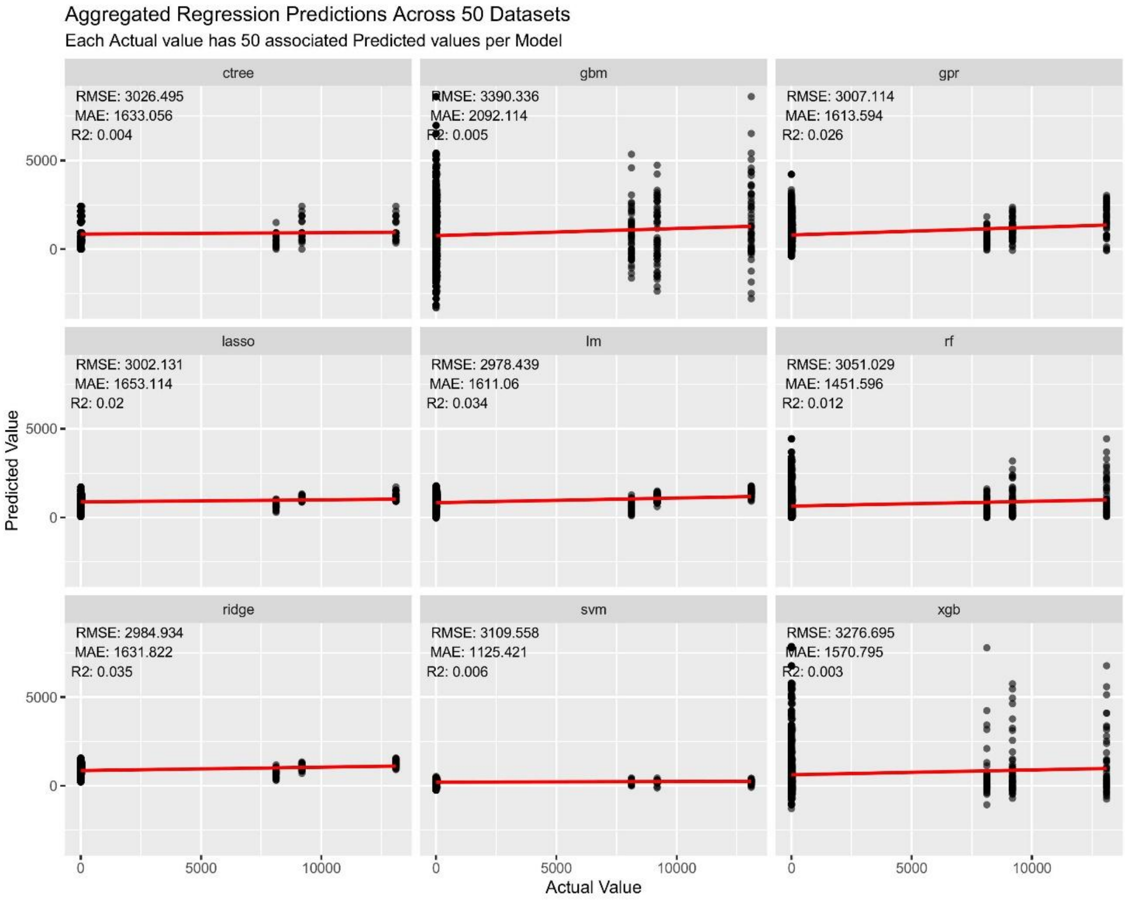
The SHAP analysis provided explanations on the individual contribution of each variable in the predictive models (Figure 12). Overall, TB showed the greatest negative influence on dry biomass in the most responsive models. In the “rf,” “gbm,” and “xgb” models, TB exhibited a wider dispersion of SHAP values with negative intensity, indicating a more consistent deleterious effect on *P. glaucum* growth.

Figure 11 - Regression results for Shoot Dry Biomass, excluding plant development time

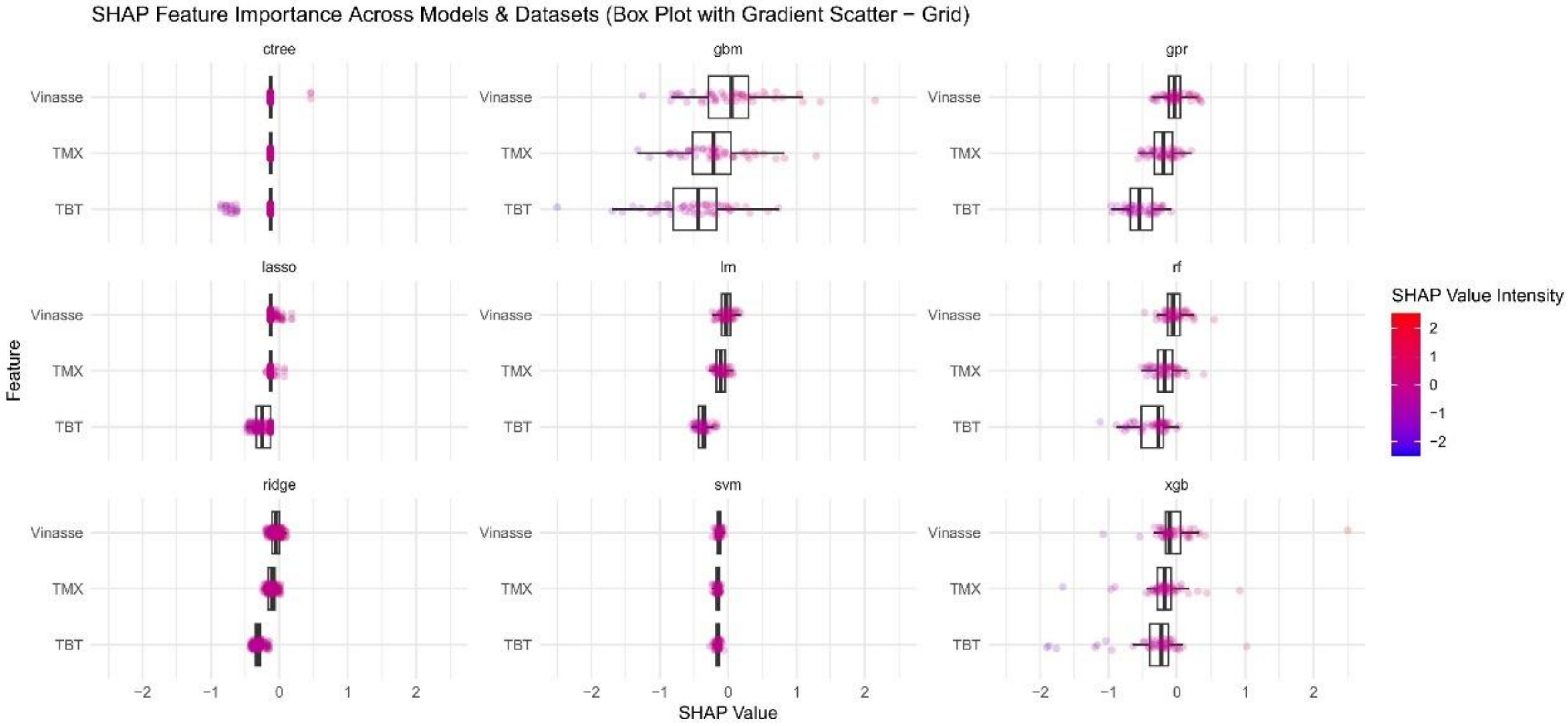


Note: *Scatter plots with trend lines (in red) representing the relationship between observed and predicted values from different regression models (ctree, gbm, gpr, lasso, lm, rf, ridge, svm, xgb). Each point corresponds to an individual prediction from one of the 50 datasets. RMSE, MAE, and R² metrics are indicated in each panel.
Source: Own authorship.

VN showed a predominantly positive contribution in several models (especially “gbm,” “gpr,” and “xgb”), supporting its potential role as a soil conditioner or organic fertilizer. TX demonstrated intermediate variation among the models, with a less pronounced impact compared to TB and VN. In the linear models, the influence of all three factors was more subdued, with little variation in SHAP values, reflecting their limitation in capturing complex interactions.

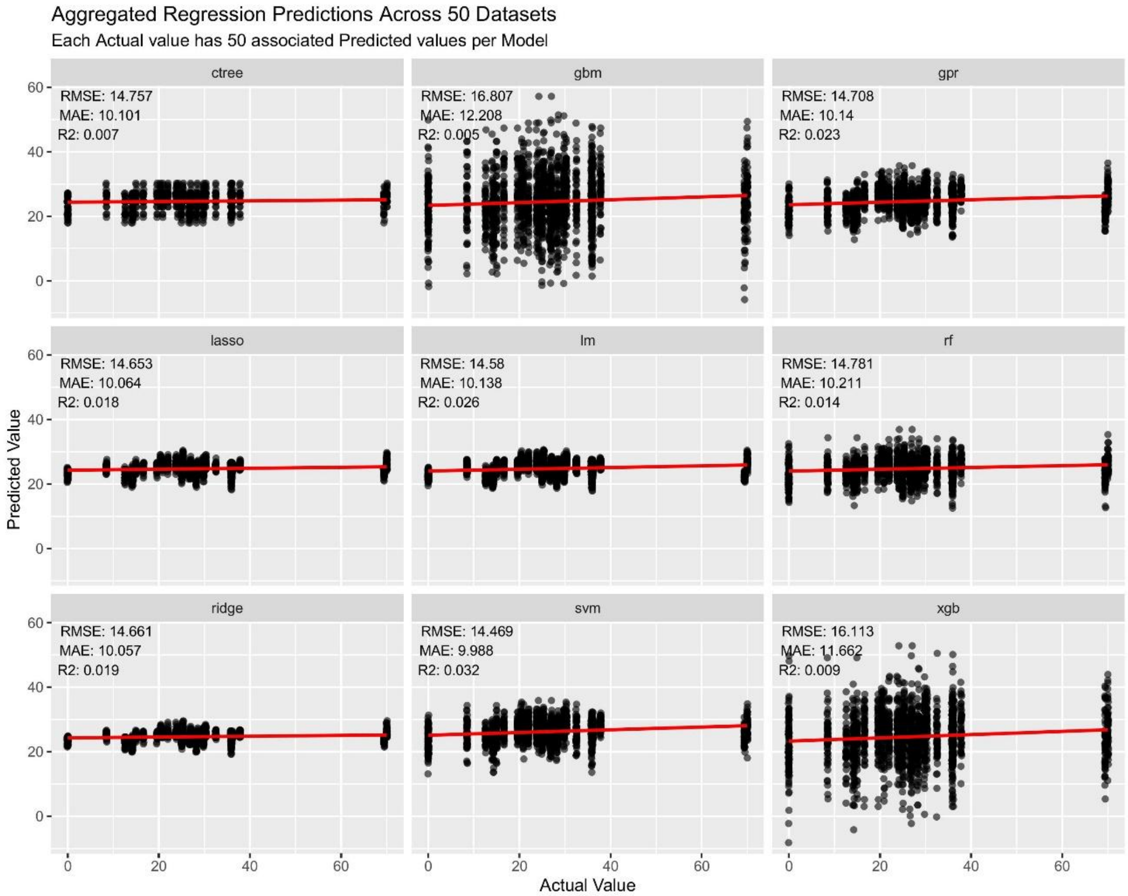
Figure 13 presents the regression results for the leaf chlorophyll index, influenced by plant development time.

Figure 12 - SHAP values for predicting Shoot Dry Biomass of *Pennisetum glaucum*, excluding plant development time



Note: *Boxplots with colored scatter display the SHAP values assigned to each explanatory variable (VN, TB, TX, and Time) across different models. The colors represent the magnitude of the original feature values.
Source: Own authorship.

Figure 13 - Regression results for Leaf Chlorophyll Index, with plant development time

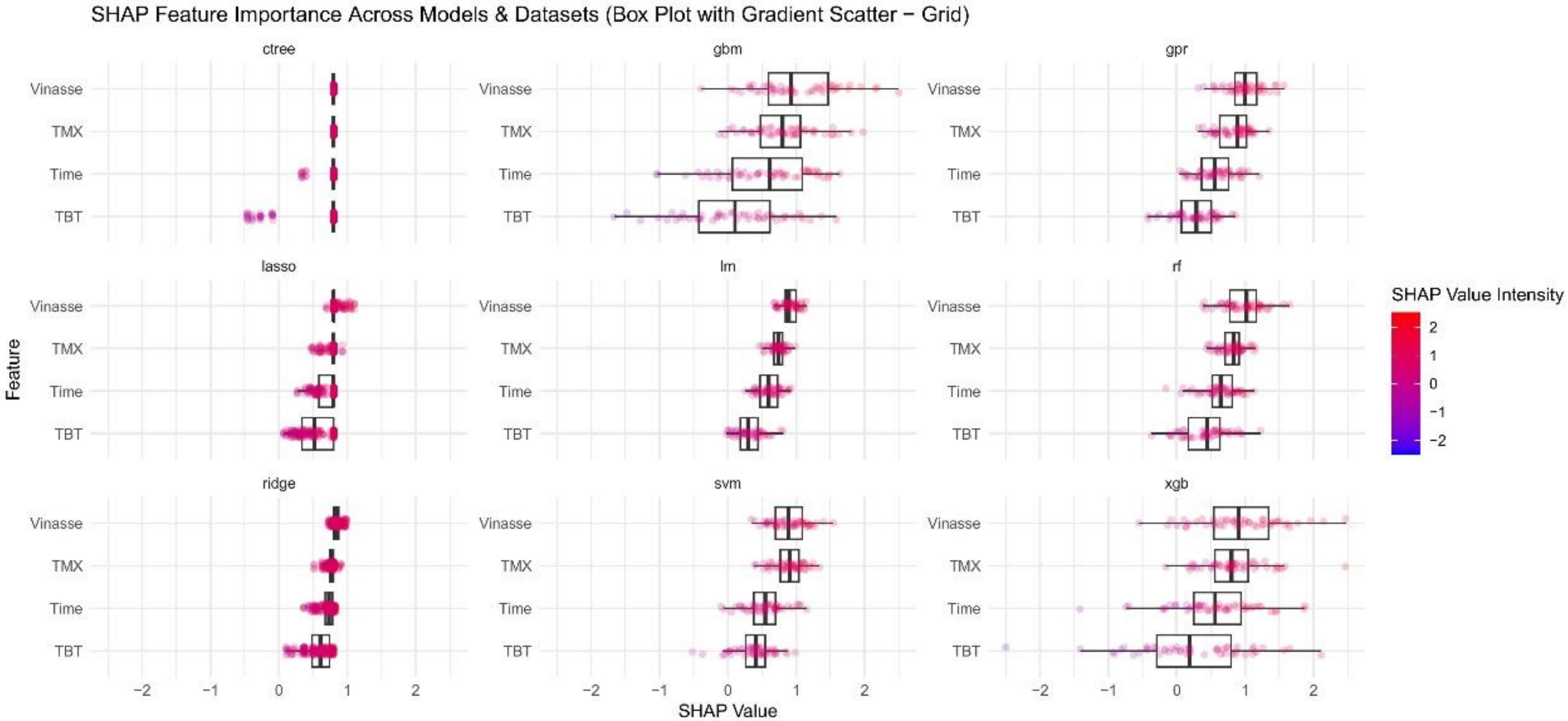


Note: *Scatter plots with trend lines (in red) representing the relationship between observed and predicted values from different regression models (ctree, gbm, gpr, lasso, lm, rf, ridge, svm, xgb). Each point corresponds to an individual prediction from one of the 50 datasets. RMSE, MAE, and R^2 metrics are indicated in each panel.
Source: Own authorship.

The best-performing model was "svm," showing an RMSE of 14.469, MAE of 9.988, and R^2 of 0.032, suggesting a slight ability to capture patterns between the input variables and plant development time. The linear models ("lm," "ridge," "lasso") also demonstrated similar performance, with RMSE and MAE values close to each other and low coefficients of determination (R^2 between 0.018 and 0.026), indicating low explained variability.

Tree-based models such as "ctree," "rf," "gbm," and "xgb" did not outperform the linear models, with "xgb" performing the worst among all, showing an MAE of 11.662 and an R^2 of only 0.009. The low R^2 observed across all models suggests that the tested factors have limited influence on the development time of *Pennisetum glaucum*.

Figure 14 - SHAP values for predicting Leaf Chlorophyll Index of *Pennisetum glaucum*, considering plant development time



Note: *Boxplots with colored scatter display the SHAP values assigned to each explanatory variable (VN, TB, TX, and Time) across different models. The colors represent the magnitude of the original feature values.
Source: Own authorship.

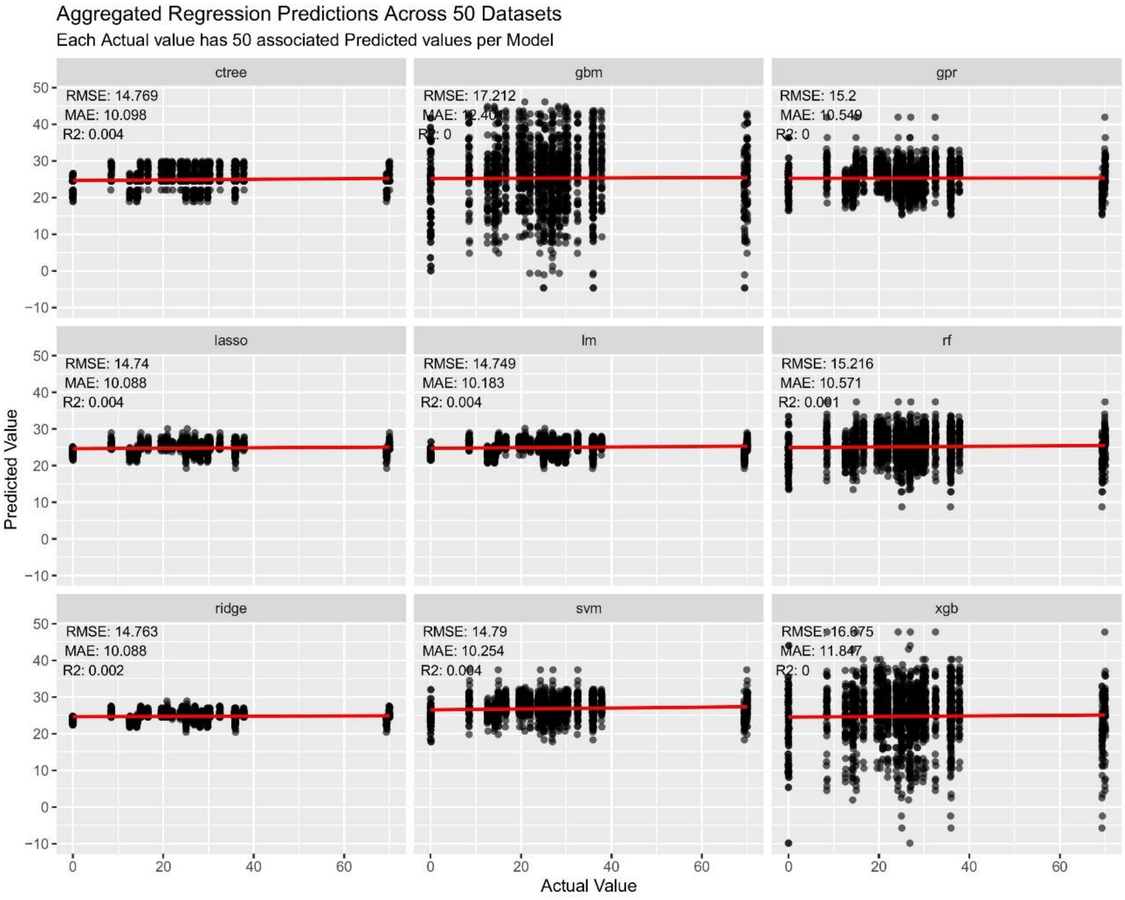
The SHAP analysis (Figure 14) showed that no variable exhibited clear and consistent dominance across all models, reinforcing the predictive analysis results that indicated low explanatory power (low R^2). However, the “gbm,” “rf,” and “xgb” models suggested that all variables had modest and relatively balanced contributions to the prediction, with slight emphasis on VN and TB. Additionally, the “svm” model displayed greater dispersion in values, with time and TB appearing with slightly higher importance. In the linear models and “gpr,” SHAP values showed less variability, indicating that these models assigned more consistent weights to all variables. Finally, the “ctree” model presented near-zero SHAP values for all variables, consistent with its previously observed poor predictive performance.

Figure 15 presents the results for the Leaf Chlorophyll Index, excluding the variable “plant development time.” All models exhibited extremely limited performance, with R^2 values ranging from 0.000 to 0.004, indicating that practically no variation in the response variable could be explained by the models. RMSE values ranged between 14.74 and 17.21, and MAEs between 10.088 and 12.40, suggesting a high mean error rate in the predictions.

Compared to the scenario where development time was included as an explanatory variable (Figure 13), it is observed that, despite modest R^2 values, the models were slightly more effective. This indicates that the duration of exposure to inputs and the environment plays a fundamental role in crop response, acting as a mediator of physiological effects.

Figure 16 presents the distribution of SHAP values for the tested models, allowing the observation of the variation in the importance of each variable independently. TB showed the greatest variability in SHAP values across several models, especially those based on tree algorithms such as “gbm,” “rf,” and “xgb,” suggesting that its influence is more sensitive to context or interactions with other variables, even though its average contribution was not high. In some cases, its impact was negative (SHAP values < 0), which may indicate a possible inhibitory effect of the herbicide on the response variable. VN and TX showed modest and relatively consistent positive contributions across most models, with less dispersion in SHAP values. The “lm,” “lasso,” “ridge,” and “svm” models highlighted both variables as slightly more relevant than TB, although with low magnitudes close to zero. In the “xgb” model, a wide dispersion of SHAP values was observed, with TB presenting negative contributions at various points, suggesting possible toxicity at high concentrations. The "ctree" model once again showed virtually null SHAP values, corroborating its limited predictive capacity as previously indicated in the regression analysis.

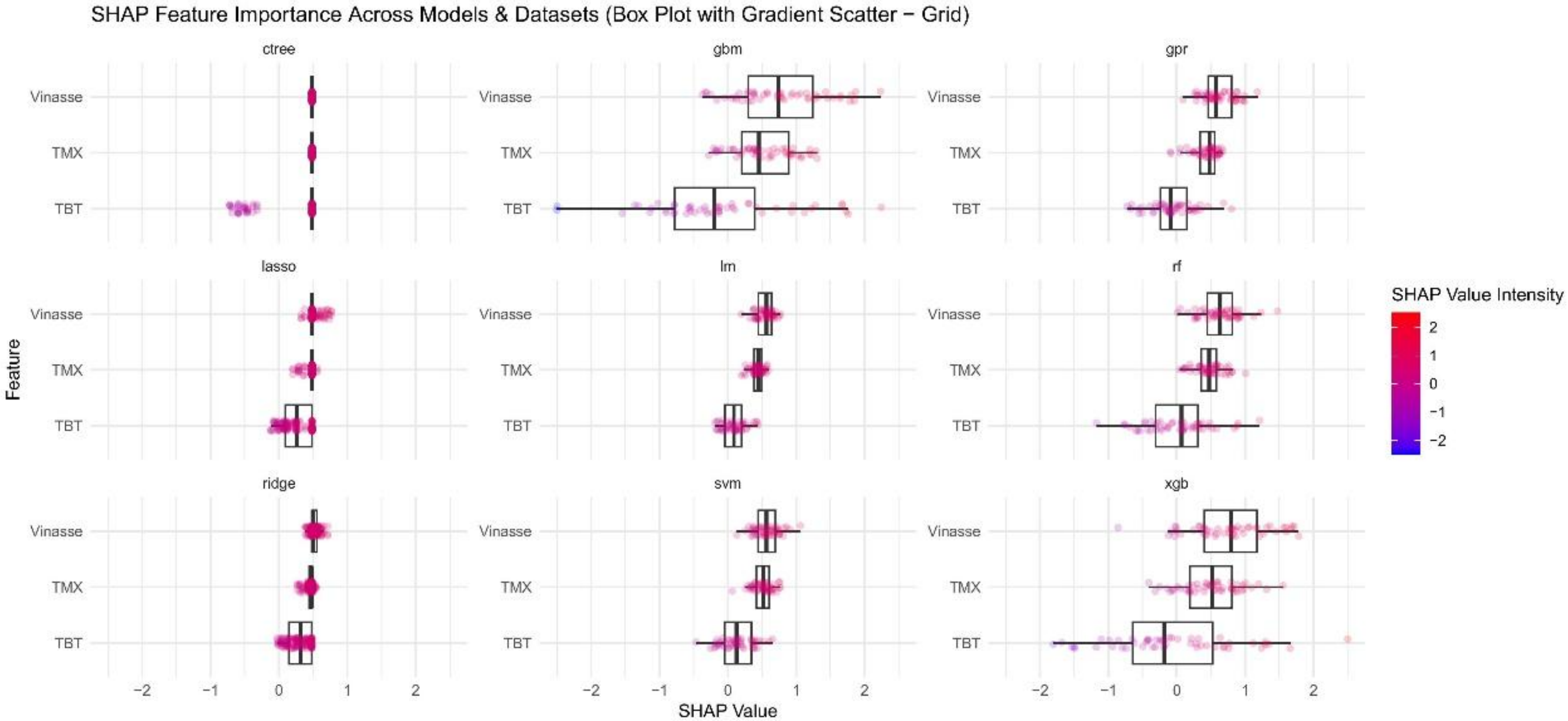
Figure 15 - Regression results for Leaf Chlorophyll Index, excluding plant development time



Note: *Scatter plots with trend lines (in red) representing the relationship between observed and predicted values from different regression models (ctree, gbm, gpr, lasso, lm, rf, ridge, svm, xgb). Each point corresponds to an individual prediction from one of the 50 datasets. RMSE, MAE, and R² metrics are indicated in each panel.
Source: Own authorship.

Despite some differences among the models, no single variable proved dominant, and most SHAP values remained close to zero, indicating low explainability. These results complement the previous regression analysis, which also revealed low coefficients of determination (R^2), even for complex models. Therefore, the absence of time as a predictive variable substantially reduces the models' ability to capture system dynamics, reinforcing the hypothesis that soil treatment effects are modulated over time or depend on interactions with variables not included in the models. It is important to acknowledge that the experimental design employed only four replicates per treatment, which is standard in greenhouse studies but may limit the statistical power and the reliability of the regression models. The reduced replication could partially explain the low R^2 values obtained and the limited capacity to detect subtle biological responses to soil contamination. Increasing the number of replicates in future studies is recommended to improve model robustness.

Figure 16 - SHAP values for predicting Leaf Chlorophyll Index of *Pennisetum glaucum*, excluding plant development time



Note: *Boxplots with colored scatter display the SHAP values assigned to each explanatory variable (VN, TB, TX, and Time) across different models. The colors represent the magnitude of the original feature values.
Source: Own authorship

4.5. CONCLUSION

This study demonstrated that plant development time was the most influential factor in predicting root and shoot dry biomass, as well as the chlorophyll index of *Pennisetum glaucum* cultivated in soils containing tebuthiuron, thiamethoxam, and vinasse. Among the regression models tested, Gaussian Process Regression and Random Forest exhibited the best predictive performance when time was included as an explanatory variable, albeit with limited explanatory power. The exclusion of time resulted in a sharp decline in model accuracy, confirming that millet's physiological responses are inherently dynamic and cannot be sufficiently explained by soil conditions alone.

Vinasse generally exerted a positive effect, suggesting its role as a soil conditioner, whereas tebuthiuron and thiamethoxam showed neutral or negative impacts, with context-dependent variations. Shapley Additive Explanations analysis reinforced that while time was the primary determinant of growth, interactions between agroindustrial residues and pesticides introduced nonlinear complexities that traditional linear models failed to capture.

The findings indicate that predicting *Pennisetum glaucum* development in contaminated soils requires approaches that account not only for the presence of pollutants but also for their interaction with temporal and edaphic factors. Machine learning techniques combined with Shapley Additive Explanations interpretability proved useful for identifying patterns but also revealed limitations in modeling subtle physiological responses such as chlorophyll content. Future studies should explore additional variables, including microbial activity and nutrient dynamics, to enhance predictive capability in multi-stress scenarios.

In summary, this work underscores the critical role of temporal dynamics in phytotoxicity assessments and highlights vinasse's potential as a mitigator of chemical stress, while emphasizing the need for more robust models to address the complexity of pesticide-impacted agricultural systems.

These modeling insights suggest that, with larger and well-characterized datasets, regression approaches can serve as practical support for decision-making in contaminated agricultural soils, especially to optimize the use of organic amendments like vinasse for mitigating pesticide effects and enhancing phytoremediation outcomes.

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5. FINAL CONCLUSION

This doctoral thesis addressed the environmental impacts caused by soil contamination with tebuthiuron and thiamethoxam, and evaluated sustainable remediation strategies, focusing on phytoremediation using *Pennisetum glaucum*, commonly known as pearl millet, along with the use of vinasse as a soil conditioner. Through an interdisciplinary methodology that combined literature review, controlled experiments, and predictive modeling, the results demonstrated that the interaction between living organisms and physical-chemical factors is a determining aspect for the effectiveness of soil remediation, providing insights for more sustainable agricultural management.

The first chapter synthesized existing knowledge about the ecotoxicological effects of thiamethoxam, emphasizing its persistence in soil, toxicity to organisms that are not the intended targets, and its potential to accumulate in the environment. The systematic review pointed out that biological methods, such as phytoremediation and microbial biodegradation, constitute viable approaches to reduce the environmental impacts of this insecticide, especially in sugarcane cultivation systems where its use is common. The cooperative relationship between plants and rhizosphere microorganisms was identified as essential for transforming neonicotinoids into less harmful compounds.

In the second chapter, greenhouse experiments confirmed the potential of pearl millet to reduce ecotoxicity in soils contaminated with tebuthiuron and thiamethoxam, whether applied separately or in combination with vinasse. Bioassays conducted with lettuce seeds showed that the presence of pearl millet significantly increased germination rates and root growth, particularly in the early stages of development between ten and twenty days after sowing. Vinasse functioned as a mitigating agent by stimulating microbial activity, which contributed to decreasing the toxicity of the pesticides. Analysis based on decision trees highlighted that the duration of cultivation and the interaction between the studied factors were decisive for the efficiency of the remediation process, reinforcing the importance of temporal aspects in decontamination practices.

The third chapter provided further understanding of pearl millet's biometric responses under chemical stress by applying machine learning models and Shapley additive explanation analysis. Among the tested algorithms, random forest and

Gaussian process regression models identified the growth time of the plants as the most influential variable for predicting root and shoot dry biomass. Vinasse was generally associated with positive effects on growth, while tebuthiuron and thiamethoxam showed neutral to negative effects depending on their interactions with other factors. The Shapley additive explanation analysis demonstrated that models aiming to predict plant development must include temporal dynamics and interactions between contaminants to adequately represent the physiological responses observed, underscoring the limitations of simpler, linear models.

Taken together, the findings support phytoremediation using pearl millet as an effective approach for recovering soils affected by pesticide contamination, particularly when integrated with the use of vinasse. Vinasse not only reduces the toxicity of agrochemicals but also improves the physical and biological characteristics of the soil, enhancing the remediation capacity of pearl millet. Nevertheless, the results also indicate the need for further studies to deepen the understanding of the microbial processes involved in the degradation of tebuthiuron and thiamethoxam, including the role of microbial communities and specific enzymes. It is also necessary to validate the results in field conditions that consider climatic and soil variability over larger areas. Incorporating real-time monitoring technologies, such as sensor systems and multispectral imaging, may improve the accuracy of predictive models. Moreover, investigations should assess the environmental fate of metabolites generated during phytoremediation to ensure that no residual toxicity remains.

In summary, this thesis advances the scientific knowledge about the impacts of tebuthiuron and thiamethoxam in agricultural ecosystems and offers practical alternatives for their mitigation. The integration of phytoremediation, organic soil amendment, and computational modeling presents a sustainable framework for managing contaminated sites, balancing agricultural production with environmental preservation. The findings highlight the importance of promoting remediation practices supported by scientific evidence, which contribute to the security of food systems and the health of ecosystems in the context of intensified agricultural activity.

6. APPENDIX A

Publication of Chapter I

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Understanding the Environmental Impact of Thiamethoxam and Implementing Biological Mitigation Strategies

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Abstract

Thiamethoxam stands as a significant addition to the second generation of neonicotinoids, distinguished by its impressive systemic properties. It is a versatile compound suitable for foliar, granular, and seed treatments, and offers a promising solution for safeguarding crops. As a member of the thiannicotinyls subclass, thiamethoxam exhibits broad-spectrum insecticidal properties, effectively controlling a variety of economically significant pests. Nonetheless, the efficacy of thiamethoxam in pest management is indisputable; we cannot disregard the potential environmental ramifications associated with its unique characteristics. The extent of this impact depends on the interplay of various environmental conditions and agricultural practices. Given these considerations, the adoption of bioremediation emerges as a sustainable, cost-effective, and ecologically friendly alternative for mitigating the environmental persistence of neonicotinoids, including thiamethoxam. Sufficient research has unveiled promising results that leverage plants and microorganisms as crucial components in addressing this challenge, with the primary aim of diminishing thiamethoxam's environmental presence in agricultural soils. Within this context, our chapter endeavors to explore the profound impact of thiamethoxam on agroecosystems, with a specific emphasis on the application of biological strategies to ameliorate these effects. By addressing it, we contribute not only to the reduction of environmental damage but also to the enhancement of overall soil agronomic quality. The implications presented in this chapter bear significant relevance to sustainable agricultural practices and the responsible use of neonicotinoid insecticides.

Keywords

Ecosystem health • Food security • Microbial biodegradation • Phytoremediation • Soil health • Sustainable agricultural development

1 Introduction

Thiamethoxam, a member of the neonicotinoid family, occupies a prominent and widely utilized position in the realm of agriculture as an insecticide. This chemical tool has been harnessed to combat a diverse array of agricultural pests, including aphids, whiteflies, thrips, and leafhoppers, as highlighted by Barbosa et al. (2022) in their recent study. Since its inception in the 1990s, thiamethoxam has ascended to become one of the most prevalent insecticides globally, a fact underscored by the findings of Maienfisch et al. (2001).

The effectiveness of thiamethoxam in pest control is underpinned by its unique mode of action, functioning as a neurotoxin for insects. This disruption of their nervous systems renders them a potent adversary against a multitude of agricultural pests, including aphids, thrips, beetles, and other crop-damaging insects. Furthermore, its systemic properties grant it the capacity to permeate the entire plant,

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7. APPENDIX B

Publication of Chapter II

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Pennisetum glaucum in reducing ecotoxicity in soil with tebuthiuron, thiamethoxam and vinasse

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ABSTRACT

The purpose of this study was to evaluate the ecotoxicological effects on soils containing associations of the herbicide tebuthiuron (TBT), the insecticide thiamethoxam (TMX), vinasse (VIN), and millet (*Pennisetum glaucum*) cultivation. We conducted the experiment under greenhouse conditions using a completely randomized design with five replications. Treatments consisted in soil application of tebuthiuron (1000 g ha^{-1} a. i), thiamethoxam (330 g ha^{-1} a. i), vinasse (113 m^3 ha^{-1}) with or without *P. glaucum* cultivation. The monitoring extended to 50 days after sowing (DAS) and evaluated the ecotoxicological potential from *Lactuca sativa* seeds as test-organism. Seed germination and root development determined the germination index (GI) concerning the negative control, which was prepared with water. Thus, *P. glaucum* highlighted a beneficial influence on soil health, thereby mitigating the ecotoxicological effects of TBT and TMX. Results emphasized the importance of the timing in soil sample collection, where early collections after plant seeding result in higher germination rates and rapid soil health improvements. Furthermore, vinasse reduced the ecotoxicological impact of pesticides and TBT presence presented harmful effects to test-organism. The interaction between *P. glaucum* growth period and soil sampling had a profound impact on ecotoxicity bioassays, which highlighted the importance of early intervention. Therefore, *P. glaucum* and vinasse can be valuable components in effective phytoremediation strategies, pointing towards future research and enhancement of approaches for sustainable treatment strategies of agricultural soils with pesticides.

1. Introduction

Ecotoxicology is a critical field of study focused on understanding the impact of contaminants on organisms within their natural environments, with the overarching goal of safeguarding the integrity and functioning of ecosystems. This is typically accomplished by examining the effects of these chemicals on individual species, using selected test organisms, and then extrapolating these findings to estimate safe concentrations for entire populations and communities. In ecotoxicological risk assessment, these derived safe levels are compared with predicted or measured exposure levels to evaluate potential risks to exposed ecosystems, providing essential insights for environmental protection and sustainability [1].

Therefore, ecotoxicological bioassays play an essential role in understanding the environmental impacts of chemicals used in agriculture. Effective pesticide management is a pivotal aspect of ecotoxicity assessments of agricultural soils, given the potential for these chemicals to impact soil health and ecosystem functions [2].

Regarding sugarcane cultivation, tebuthiuron is a herbicide that acts by inhibiting photosystem II [3] and presents extended half-life about 360–450 days [4], which contributes to its persistence in the environment and potential for long-term ecological effects. Another critical factor is its high solubility in water (2500 mg L^{-1} at 25 °C) that enhances mobility through the soil profile [5,6]. The herbicide's octanol-water partition coefficient (log Kow) of 1.79 further indicates its tendency to remain in the aqueous phase rather than binding strongly to soil particles, facilitating its movement and potential contamination of groundwater [5,6]. Moreover, tebuthiuron's notable affinity for soil particles

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***Pennisetum glaucum* as a phytoremediator: development and ecotoxicological responses to tebuthiuron, thiamethoxam and/or vinasse in soil**

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