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Activated charcoal mitigating the effects of indaziflam on the germination of restoration species

Carvão ativado mitigando os efeitos do indaziflam na germinação de espécies restauradoras

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ABSTRACT

Brazil's extensive forest resources, covering 4.5 million km^2 including the Amazon and Atlantic Rainforest, play crucial roles in biodiversity, climate regulation, and socio-economic benefits. Human activities, such as introducing exotic species, threaten these ecosystems, necessitating strategies like selective herbicide use. Indaziflam, effective against invasive grasses with low soil mobility, shows promise for forest restoration. The objective of this study was to evaluate the role of activated charcoal in mitigating the phytotoxic effects of indaziflam on the germination and early establishment of nine restoration species: *Peltophorum dubium*, *Croton floribundus*, *Handroanthus vellosoi*, *Handroanthus impetiginosus*, *Platypodium elegans*, *Guazuma ulmifolia*, *Senna multijuga*, *Canavalia ensiformis*, and *Cajanus cajan*. The greenhouse study was carried out under a randomized complete block design with ten replications per treatment. Each species was evaluated as an independent experiment, conducted twice at different times of the year, to ensure reproducibility. Treatments included activated charcoal mixed with soil, indaziflam herbicide, and their combinations. Results showed that activated charcoal consistently reduced the negative effects of indaziflam, preserving germination and seedling viability across species, even under herbicide stress. These findings confirm that activated charcoal mitigates indaziflam's phytotoxicity, enhancing germination and early establishment in forest restoration species, and thus represents a valuable tool for sustainable forest management and biodiversity conservation.

Keywords: *Herbicide; Phytotoxicity; Selectivity; Weeds.*

RESUMO

Os extensos recursos florestais do Brasil, cobrindo 4,5 milhões de km^2, incluindo a Amazônia e a Mata Atlântica, desempenham papéis cruciais na biodiversidade, regulação do clima e benefícios socioeconômicos. Atividades humanas, como a introdução de espécies exóticas, ameaçam esses ecossistemas, exigindo estratégias como o uso seletivo de herbicidas. O indaziflam, eficaz contra gramíneas invasoras e de baixa mobilidade no solo, mostra-se promissor para a restauração florestal. O objetivo deste estudo foi avaliar o papel do carvão ativado na mitigação dos efeitos fitotóxicos do indaziflam sobre a germinação e o estabelecimento inicial de nove espécies utilizadas em restauração florestal: *Peltophorum dubium, Croton floribundus, Handroanthus vellosoi, Handroanthus impetiginosus, Platypodium elegans, Guazuma ulmifolia, Senna multijuga, Canavalia ensiformis* e *Cajanus cajan*. O estudo foi conduzido em casa de vegetação, em delineamento de blocos completos ao acaso, com dez repetições por tratamento. Cada espécie foi avaliada em um experimento independente, conduzido em dois períodos distintos do ano, garantindo a reprodutibilidade dos resultados. Os tratamentos incluíram carvão ativado incorporado ao solo, aplicação do herbicida indaziflam e suas combinações. Os resultados mostraram que o carvão ativado reduziu consistentemente os efeitos negativos do indaziflam, preservando a germinação e a viabilidade das mudas, mesmo sob estresse herbicida. Esses achados confirmam que o carvão ativado mitiga a fitotoxicidade do indaziflam, favorecendo a germinação e o estabelecimento inicial de espécies restauradoras e configurando-se como uma ferramenta valiosa para o manejo florestal sustentável e a conservação da biodiversidade.

Palavras-chave: *Herbicida; Fitotoxicidade; Seletividade; Plantas daninhas.*

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1. INTRODUCTION

Brazil holds extensive forest potential, including representative biomes like the Cerrado, Amazon and Atlantic Rainforest, covering over 4.5 million square kilometers (Pimenta et al., 2020). These ecosystems play crucial ecological roles for biodiversity conservation, climate regulation, and protection of water resources, offering various societal benefits and economic opportunities, including generation of carbon credits (Isernhagen et al., 2009). However, forest remnants and areas to be restored face ongoing challenges from anthropogenic disturbances, such as the invasion of exotic plant species, leading to an imbalance and vulnerability of forests and the Cerrado (Florido, 2015; Pivello, 2011).

In areas for forest restoration, these challenges require comprehensive interventions throughout the initial establishment phase to canopy closure, focusing on controlling invasive species effectively (Brancalion et al., 2019). Restoration efforts necessitate careful planning and execution, especially regarding the control of exotic species, particularly invasive ones, which represent a critical challenge during the operational stage (D'Antonio et al., 2016). Various weed control methods exist, ranging from manual techniques like weeding and mowing to the use of herbicides, which offer operational efficiency (Silva et al., 2009; Brighenti & Oliveira, 2011).

The pre-emergent herbicide indaziflam, known for its distinct features, is commonly used in agricultural and forestry contexts (Ministério da Agricultura, Pecuária e Abastecimento, 2024). With attributes such as low soil mobility, prolonged control duration, and broad-spectrum weed suppression, it serves as a valuable tool for mitigating invasive plant competition and facilitating desired species establishment in forest restoration projects. However, ensuring selectivity requires proper timing of application, precise positioning and doses, also avoiding leaching risks (Gonçalves et al., 2021). This emphasizes the importance of further evaluating safe application strategies to mitigate potential adverse impacts. Despite this consideration, indaziflam's integration into restoration practices holds promise for streamlining operations and enhancing effectiveness, warranting ongoing exploration within controlled frameworks.

One such strategy involves the use of activated charcoal to protect desired species from the effects of herbicides (Clenet et al., 2019; Terry et al., 2021a; Davies et al., 2023). Studies have demonstrated the potential of activated charcoal to mitigate herbicide phytotoxicity, safeguarding germination and early plant development (Rodrigues & Almeida, 2018). Further research aims to validate this approach's effectiveness across different plant species, contributing to more sustainable ecosystem management practices.

The United Nations' Decade on Ecosystem Restoration underscores the urgency of enhancing restoration efforts and proposing environmentally friendly strategies to ensure project success (United Nations, 2019). Effective weed management in forest restoration is pivotal for project success, demanding a combination of approaches and long-term planning with continuous monitoring (Souza, 2023). Collaboration, knowledge exchange, and adoption of best practices globally are essential for addressing ecosystem degradation, meeting agreements, and addressing climate change challenges (Resende & Leles, 2017). Incorporating techniques and products from agriculture and forestry into weed control strategies can significantly contribute to achieving environmental, economic, and social benefits in restoration efforts (Florido et al., 2021).

The motivation for this study arises from the need to confront persistent challenges in forest restoration in Brazil, especially the management of invasive plant species and the careful use of herbicides. Given the ecological and socioeconomic importance of forests, there is a clear need for strategies that reduce herbicide phytotoxicity during establishment.

Accordingly, this study tested whether activated charcoal mitigates the phytotoxic effects of indaziflam on germination and early establishment of nine restoration species under greenhouse conditions. Further implications for field use and operational restoration are beyond the scope of this experiment and require additional studies.

2. MATERIAL AND METHODS

2.1. Study design

This study was conducted in a greenhouse facility, located at 22° 45' 2,247" S e 47° 7' 9,589" W, in Paulínia, São Paulo state, Brazil. This study was composed of nine independent experiments, one for each restoration species. Each experiment was repeated at two different times of the year, from November 18th, 2022 to January 4th, 2023, and from April 12th to May 29th, 2023, respectively, each lasting 47 days. A randomized complete block design was employed, with five replications per treatment in each repetition. Statistical analyses were initially performed considering the two replications separately; since no significant differences were detected between experimental times. The data were pooled, resulting in 10 replications per treatment for each species.

2.2. Soil description

The soil has a medium-texture, with sand content of 584 g dm^{-3}, clay content of 371 g dm^{-3}, silt content of 45 g dm^{-3}, and organic matter content of 8 g dm^{-3}.

2.3. Plant species

Reforestation efforts rely on careful species selection to restore ecosystems and enhance biodiversity. In each experiment, fifteen seeds of key species per plot were used, so in total 150 per species. Species included *Canavalia ensiformis* (feijão-de-porco) and *Cajanus cajan* (feijão-guandu) from the Fabaceae family, used for enriching soil fertility and advancing microclimate environment. Additionally, *Croton floribundus* (capixingui) from the Euphorbiaceae family and *Guazuma ulmifolia* (mutamba) from the Malvaceae family serve as valuable ground cover, protecting soil and promoting other plants establishment. *Handroanthus impetiginosus* (ipê-roxo) from the Bignoniaceae family and *Peltophorum dubium* (canafístula) from the Fabaceae family also contribute significantly to later ground cover. Secondary species such as *Platypodium elegans* (jacarandá-do-campo) and *Handroanthus vellosoi* (ipê-amarelo) aid in ecosystem diversity and natural regeneration. These species play crucial roles in successful reforestation and ecosystem restoration projects.

2.4. Indaziflam pre-emergent herbicide

Indaziflam, an active herbicidal ingredient belonging to the alkylazine chemical class, works by inhibiting cellulose biosynthesis. It possesses low water solubility (0.0028 kg m^{-3} at 20 ºC) and exhibits moderate to low mobility in soil, indicated by a Koc value of < 1,000 mL g^{-1} organic carbon, a pKa of 3.5, and a log Kow of 2.8 at pH 4, 7, or 9. With a soil half-life exceeding 150 days, indaziflam offers flexibility in application timing. Soil physicochemical properties influence its availability and efficacy, with its adsorption by clay colloids and organic matter significantly impacting its behavior in the soil environment.

2.5. Herbicide application

Indaziflam, a pre-emergent herbicide commonly used in forest restoration, was applied at a dose of 75 g active ingredient (a.i.) per hectare according to manufacturer recommendations using a spray chamber. The herbicide was diluted in water and applied uniformly to the planting substrate, which had been lightly moistened beforehand to enhance application quality, immediately after sowing.

2.6. Activated charcoal treatment

Treatments included control (no herbicide), activated charcoal mixed into the surface soil without herbicide application, herbicide application alone, and herbicide application with activated charcoal mixed into the surface soil. The surface soil was represented as 5% of total soil volume. Activated charcoal was incorporated into the planting soil, specifically into the surface layer of the pot, and subsequently mixed with the seeds of each tested species. This methodology was decided based only on whether charcoal could contribute to the objectives, without yet considering on how to use it in real operationalization. Synth brand activated charcoal powder was mixed with the soil at concentrations of 0.6% w/w in proportion to the dry soil in the treatments in which it should be present.

2.7. Experimental conditions

The greenhouse environment was kept under temperatures ranging from 16 to 28°C and a relative humidity ranging from 62 to 91%. The greenhouse was covered with plastic sheeting and enclosed on the sides with fine mesh netting to prevent the entry of aphids. Additionally, irrigation was conducted daily using sprinklers to ensure there was no water deficit throughout the experiment.

2.8. Pot setup and planting

The medium-textured soil was placed in planter-style pots with a 3-liter capacity to suit seeds of diverse sizes from different species. All pots were equipped with drainage holes to prevent waterlogging. They were filled with dry soil, comprising 95% of the total volume. The next day, seeds were counted and mixed with the soil and activated charcoal (Synth brand), with the charcoal amounting to 0.6% of the dry soil mass or only with the dry soil depending on the treatment, ensuring consistent distribution. Individual pots received one or two different species, depending on seed/seedling size, to promote unhindered vegetative growth. The species *Peltophorum dubium*, *Guazuma ulmifolia* and *Senna multijuga* needed dormancy-breaking processes with thermic shock, and *Croton floribundus* needed previous water imbibition, Following substrate preparation, a simulated 5 mm rainfall was applied to all pots, followed by herbicide application after 30 minutes using a single stainless steel flat fan nozzle TP8002EVS calibrated to deliver 200 L of slurry per hectare.

Figure 1 illustrates the experimental procedure, shiwing the difference between treatments and the results obtained; which helps to understand of the observed effects.

2.9. Data collection

Evaluations were carried out 47 days after sowing, based on the number of healthy seedlings relative to the total number of seeds sown at the beginning of each experiment. Seedling vigor (e.g., dry mass) was not assessed, as this exploratory work prioritized germination percentage as the main response variable. Following studies should include biomass and growth parameters to complement the evaluation of activated charcoal effects on restoration species.

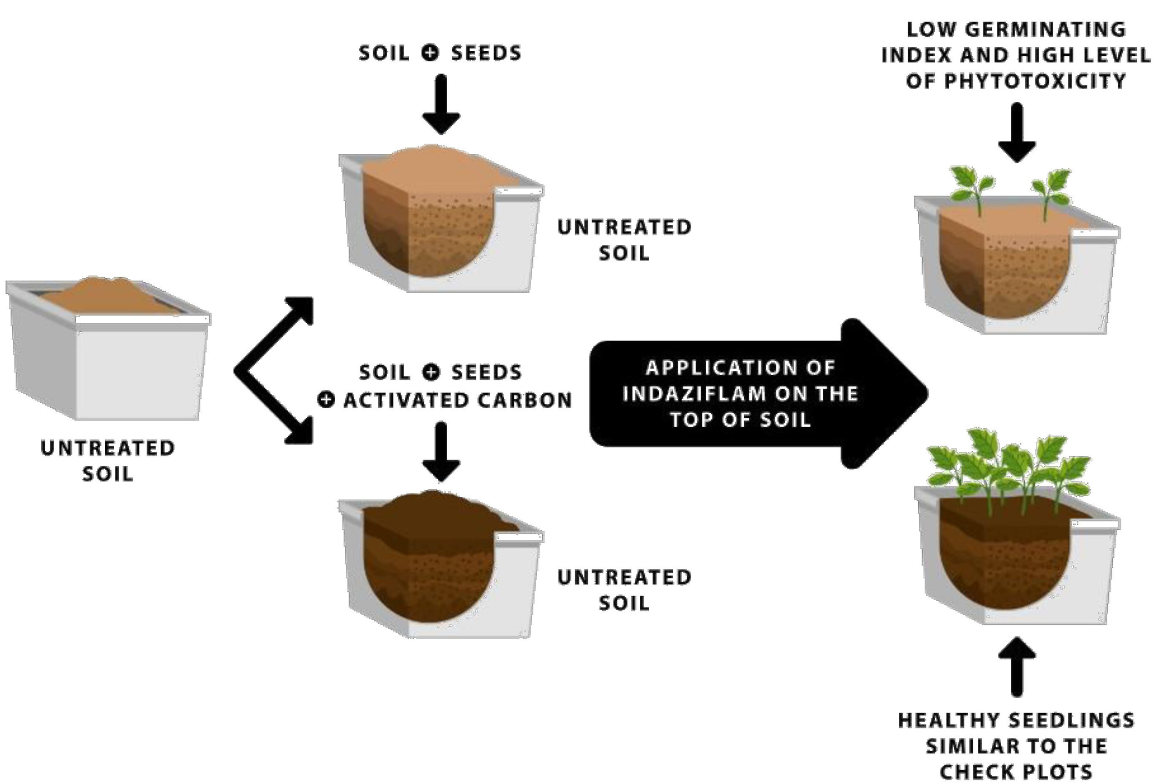

Figure 1. Schematic representation of filling pots with substrates and establishment of forest restoration species in different treatments

2.10. Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using appropriate statistical software to assess treatment effects. Mean separation was performed using Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$.

3. RESULTS

3.1. Effect of activated charcoal on germination

The application of indaziflam on soil resulted in varying levels of phytotoxicity and seed germination, depending on the treatment with activated charcoal. In the experiment, seeds were sown in untreated soil (control) and in soil mixed with activated charcoal. The application of indaziflam on the untreated soil (control) led to a low germination index and a high level of phytotoxicity, as evidenced by poor seedling growth. In contrast, mixing activated charcoal into the soil mitigated the phytotoxic effects of indaziflam, resulting in healthy seedlings, similar to those in the check pots

When analyzing the number of seedlings germinated at 47 days after indaziflam application, in pots filled with medium soil texture with and without activated charcoal, significant differences were found between the treatments that contained activated carbon on the soil surface compared to those that did not, as shown in Table 1. Thus, it is possible to perceive that activated carbon played a significant role for the responses of the plants to the experimental conditions after the application of indaziflam.

Figure 2 shows how these species behaved under the conditions of the study. For the two species of beans and seven forest species frequently used in forest restoration, charcoal activated, if added to the surface layer of soil, provided protection for germination and the initial development of these species against the herbicide effects.

Seedling emergence at 47 days after sowing revealed clear and consistent patterns of species-specific sensitivity to indaziflam and the mitigating effect of activated charcoal (Figure 2; Table 1). Across the nine forest restoration species, three major response groups could be distinguished.

Highly sensitive species with strong charcoal mitigation

Peltophorum dubium, Croton floribundus, Guazuma ulmifolia, Cajanus cajan, and *Senna multijuga* proved extremely vulnerable to indaziflam in the absence of charcoal, with germination reduced to below 10% of control levels, and in some cases (*C. floribundus* and *G. ulmifolia*) complete failure of establishment. The addition of activated charcoal fundamentally changed this scenario: *P. dubium* exhibited 33% more healthy seedlings even under herbicide stress, *C. floribundus* rose to ~76% germination compared with zero in the herbicide-only treatment, and *C. cajan* increased from ~7% to nearly 80%. These results provide robust evidence that charcoal acts as a decisive protective factor for highly sensitive species, effectively determining whether or not successful establishment may occur.

Moderately tolerant species stabilized by charcoal protection

Handroanthus vellosoi and *H. impetiginosus* showed partial tolerance to indaziflam. In the absence of charcoal, *H. impetiginosus* maintained ~19% germination relative to the control, while *H. vellosoi* germination was completely inhibited. When charcoal was incorporated, both species recovered to levels statistically comparable to their controls (above 40%), confirming that charcoal acted as a stabilizer, preventing further loss of viability under herbicide pressure. These findings suggest that, although these species are not as critically dependent on charcoal as the previous group, its presence helps to secure establishment.

Low-germination species with partial mitigation

Platypodium elegans showed inherently low germination potential, with values below 40% across all treatments, reflecting biological constraints under greenhouse conditions. Nevertheless, when exposed to indaziflam without charcoal, germination dropped to ~30% of the control, whereas the addition of charcoal partially counteracted this reduction. Even though absolute rates remained modest, the result proves that charcoal protection extends even to species with a low baseline germination.

Overall, the results demonstrate clearly that activated charcoal consistently mitigates the phytotoxic effects of indaziflam, with the magnitude of response strongly dependent on species' sensitivity. For highly susceptible species, charcoal was essential for survival; for moderately tolerant species, it provided stability; and even for inherently low-germinating species, it conferred measurable protection. These findings reinforce the ecological relevance of integrating charcoal into restoration practices, while simultaneously providing novel insights for species not previously tested under such conditions.

Table 1. F values and coefficient of variation for germination percentage of nine native species under different conditions of activated charcoal application and the herbicide indaziflam.

Source of variation	QMe		
	Germination (%)		
	Peltophorum dubium	***Croton floribundus***	***Handroanthus vellosoi***
Treatment	6865,0504**	11792,5719**	9408,1904**
Residue	280,2472	379,7254	497,8851
VC (%)	44,05	39,50	48,68
	Handroanthus impetiginosus	***Platypodium elegans***	***Guazuma ulmifolia***
Treatment	6925,4389**	1103,3944**	12990,7237**
Residue	547,2849	189,2482	223,3219
VC (%)	44,28	49,42	30,19
	Senna multijuga	***Canavalia ensiformis***	***Cajanus cajan***
Treatment	5357,2207**	6548,8415**	12645,8874**
Residue	319,4805	194,3361	208,3827
VC (%)	51,56	16,40	24,61

**significant at the 1% probability level by the F-test.

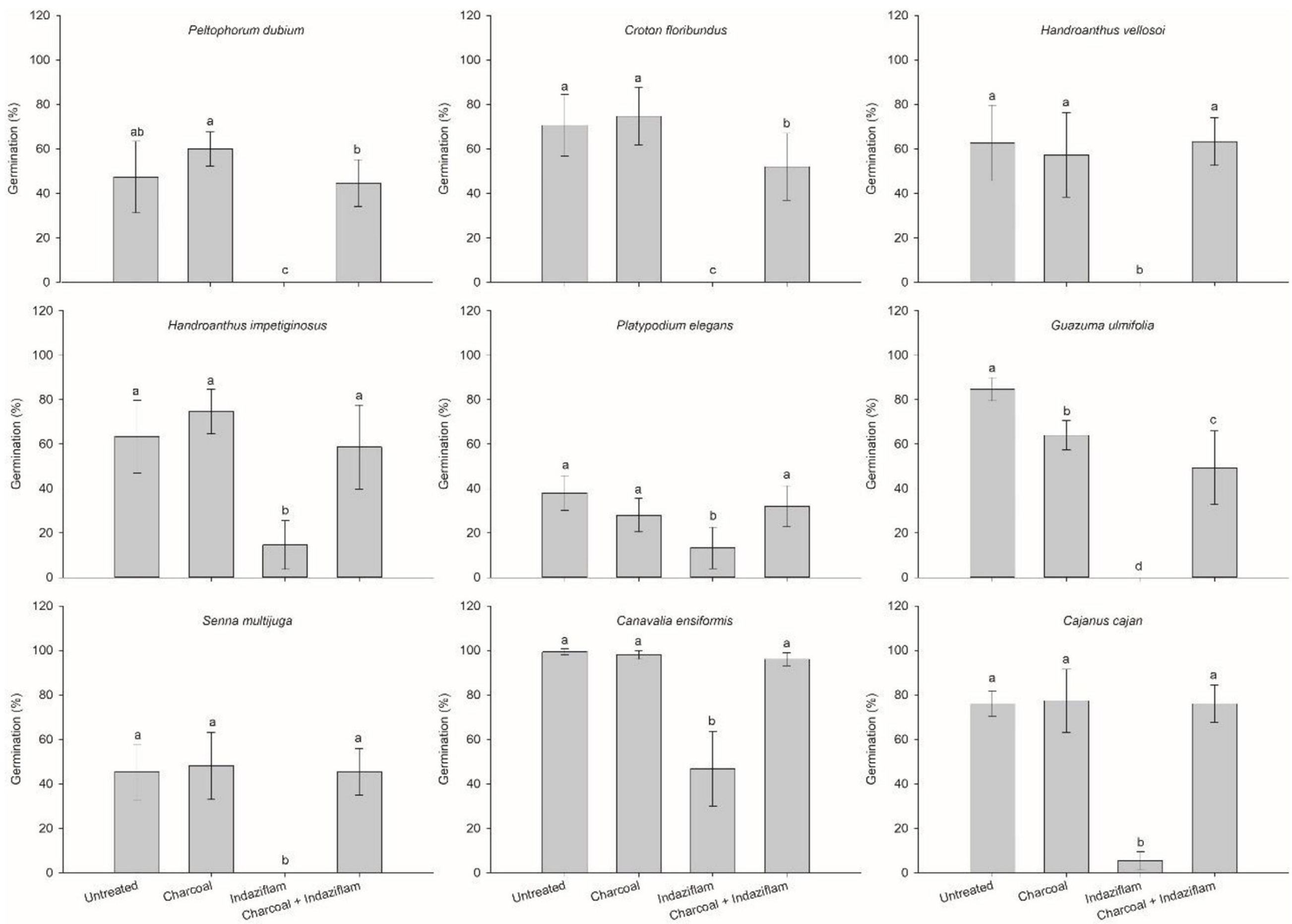

Figure 2. Germination percentage of native species under different conditions of activated charcoal application and exposure to indaziflam. Treatments consisted of untreated control, activated charcoal, and activated charcoal combined with indaziflam. Bars represent mean values ± standard deviation. Different letters (a, b, c) indicate significant differences among treatments within each species, according to Tukey's test (p ≤ 0.05). Treatments sharing the same letter are not significantly different.

4. DISCUSSION

Activated charcoal has emerged as a promising tool in mitigating herbicidal effects and promoting germination and initial growth in various forest restoration species. This discussion delves into the multifaceted aspects surrounding the use of activated charcoal in ecological restoration efforts, considering its efficacy, limitations, and potential avenues for future research and application.

4.1. Efficacy of activated charcoal in herbicide mitigation

The findings presented in this study confirm previous research by Davies et al. (2023), Baughman et al. (2023) and Terry et al. (2021b), indicating the beneficial effects of activated charcoal in counteracting herbicidal impacts on seed germination and seedling establishment. By encapsulating seeds in activated charcoal granules, Davies et al. (2023) explored a novel approach that showed promise in enhancing vegetation restoration efforts. However, as noted by the authors, the effectiveness of this strategy may vary depending on factors such as precipitation and seeding methods, warranting further investigation.

4.2. Challenges and considerations

Despite its potential benefits, the use of activated charcoal in ecological restoration poses several challenges and considerations. Baughman et al. (2023) emphasize the need for effective approaches to ensure the desired vegetation establishment while controlling the spread of invasive species. Additionally, since Burr et al. (1972), and more recently Clenet et al. (2019) underscore the complexity of managing variables such as moisture content and herbicide dosage, which can influence the efficacy of activated charcoal applications. These challenges emphasize the importance of conducting comprehensive studies under diverse conditions to elucidate the optimal application and management strategies for utilizing activated charcoal in restoration practices.

4.3. Promising strategies and future directions

Sebastian et al. (2017) investigated the effectiveness of indaziflam, a cellulose-biosynthesis-inhibiting herbicide, in managing resistance and controlling a broad spectrum of weeds at low application rates, with a focus on both monocotyledons and dicotyledons. Concurrently, Terry et al. (2021b) proposed alternative methods to mitigate the herbicidal effects of indaziflam, such as seed coating with activated charcoal which, when combined with other treatments, shows promise in reducing adverse impacts on desired species. Additionally, insights from Madsen et al. (2014) suggested the use of activated charcoal capsules as a flexible strategy for restoring ecosystems invaded by exotic plants, offering innovative solutions to address challenges posed by invasive species.

4.4. Implications for restoration practice

The results of this study underscore the potential of activated charcoal as a valuable tool in mitigating herbicidal effects and facilitating vegetation restoration. However, it is essential to interpret these findings cautiously. These experiments mimicked a planting hole or strips treatment, differing from certain cited experiments involving seed encapsulation. Moreover, there are limitations for these results due to the short evaluation period for such a greenhouse study and a limited genetic variability in seeds in a controlled condition of greenhouse, making further studies necessary. Nevertheless, the promising outcomes warrant further exploration in field settings to assess the practicality and scalability of activated charcoal and indaziflam in restoration approaches.

Medium-textured soil, utilized in this study, provided a balanced environment conducive to assessing the effectiveness of activated charcoal in neutralizing herbicide effects. This type of soil, characterized by its balanced composition of sand, silt, and clay, offers a representative medium for ecological experiments (Brady & Weil, 2008). The interaction of indaziflam and activated charcoal within medium-textured soil is particularly relevant as it mirrors the conditions found in many degraded landscapes where restoration efforts are necessary.

Several studies support the potential of activated charcoal in mitigating herbicide impacts. For example, research by Singh et al. (2019) demonstrated that activated charcoal could significantly reduce the phytotoxicity of herbicides in contaminated soils, promoting healthier plant growth. Additionally, investigations by Ravit et al. (2007) confirmed the role of activated charcoal in enhancing soil microbial activity and improving overall soil health, further substantiating its application in ecological restoration.

In the context of forest restoration, the use of activated charcoal has shown promise in enhancing soil conditions and promoting tree growth. Studies by Novak et al. (2012) and Lehmann et al. (2006) illustrate that activated charcoal, also known as biochar, can improve soil fertility and water retention, thereby supporting the establishment and growth of forest species. These benefits are particularly crucial in degraded or nutrient-poor soils where traditional restoration efforts might be difficult.

Research by Major et al. (2010) in tropical forest restoration has demonstrated that biochar application can significantly increase tree biomass and soil microbial activity. Similarly, a study by Liang et al. (2006) found that biochar-amended soils had higher nutrient availability and better physical properties, leading to improved seedling survival and growth rates.

In summary, the findings of this research provide valuable insights into the application of activated charcoal in ecological restoration efforts. By elucidating its efficacy in mitigating herbicidal effects and promoting seed germination and seedling establishment, this study contributes to the ongoing discourse on innovative restoration practices. Moving forward, it is imperative to conduct field studies to validate these findings under practical conditions and to develop robust protocols for incorporating activated charcoal into restoration frameworks. The integration of activated charcoal-based strategies has the potential to significantly enhance the success of restoration projects and contribute to the buildup of biodiversity in degraded ecosystems.

This work is exploratory, as it evaluated only one variable, germination percentage, for nine forest restoration species under the effect of indaziflam, with and without activated charcoal. Other parameters, such as seedling vigor, were not assessed and could provide additional insights in future studies. The mitigating property of activated charcoal against herbicide phytotoxicity is well established in the literature; therefore, the contribution of this study lies in the evaluation of these specific species, which had not been previously tested under these conditions.

5. CONCLUSION

The use of activated charcoal proved effective in mitigating the phytotoxic effects of indaziflam, preserving germination and initial growth of nine restoration species under greenhouse conditions. These results confirm its potential as a practical tool to support the success of forest restoration initiatives.

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AUTHOR CONTRIBUTIONS

FGOS: conceptualization, methodology, writing – original draft; EDV and EMK: conceptualization, supervisions writing – review & editing; CAC: writing – review & editing; NCB and RNC: data curation, investigation, formal analysis; JTF and BMPD: data curation, investigation