Physiological effects of *Acmella ciliata* essential oil on root development of *Nicotiana tabacum*

Efectos fisiológicos del aceite esencial de *Acmella ciliata* sobre el desarrollo radical de *Nicotiana tabacum*

Lizeth Daniela Méndez-Grateron[1], Luz Yineth Ortiz-Rojas[1], and Giovanni Chaves-Bedoya[1*]

ABSTRACT

In this study, the essential oil of *Acmella ciliata*, characterized by the presence of significant amounts of spilanthol, α-phellandrene epoxide, and carvotanacetone, was assessed for its influence on the growth of *Nicotiana tabacum* cv. Xanthi, with the focus primarily on root hair density and primary root length. Following its extraction through microwave-assisted hydrodistillation, the oil was stored at 4°C in amber vials, distinguishable by its unique yellowish-reddish hue, with a refractive index of 1.3478 and a density of 0.847 g cm^{-3}. Among the various dilutions evaluated, the undiluted oil (T3) and the dilution 1.5:0.5 (Oil:EtOH) (T6) demonstrated the most prominent effects. The T3 and T6 treatments markedly enhanced root hair numbers, with T6 additionally promoving root length compared to other treatments. Considering the presence of bioactive alkamides such as spilanthol in the oil, these compounds may have contributed to the observed root growth modulation. When compared against the positive control, affinin, *Acmella ciliata* essential oil displayed a more pronounced effect on root hair proliferation, while affinin predominantly boosted primary root elongation. The findings highlight the differential effects of the essential oil on specific plant growth parameters.

Key words: alkamides, root elongation, root hairs, bioactivity.

RESUMEN

En esta investigación, el aceite esencial de *Acmella ciliata*, caracterizado por la presencia de compuestos significativos como el espilantol, el epóxido de α-felandreno y la carvotanacetona, fue evaluado por su influencia en el crecimiento de *Nicotiana tabacum* cv. Xanthi, centrándose principalmente sobre la densidad de pelos radicales y la longitud de la raíz primaria. Luego de la extracción mediante hidrodestilación asistida por microondas, el aceite se almacenó a 4°C en viales de ámbar y se distinguió por su único matiz amarillo-rojizo, con un índice de refracción de 1.3478 y una densidad de 0.847 g cm^{-3}. Entre las diversas diluciones evaluadas, el aceite sin diluir (T3) y la dilución 1.5:0.5 (Aceite:EtOH) (T6) demostraron los efectos más prominentes. Los tratamientos T3 y T6 aumentaron notablemente el número de pelos radicales, con T6 además promoviendo la longitud de la raíz de manera comparable a otros tratamientos. Considerando la presencia detectada de alcamidas bioactivas como el espilantol en el aceite esencial, es plausible que contribuyeran a la modulación observada del crecimiento de la raíz. En comparación con el control positivo, afinina, el aceite esencial de *Acmella ciliata* mostró un efecto más pronunciado en la proliferación de pelos radiculares, mientras que la afinina impulsó predominantemente la elongación de la raíz primaria. Los hallazgos destacan los efectos diferenciales del aceite esencial en parámetros específicos del crecimiento vegetal.

Palabras clave: alcamidas, elongación radical, pelos radicales, bioactividad.

Introduction

Roots are vital for a plant's nutrient and water uptake, as well as storage and plant anchorage. For plants to grow, they must uptake from the soil 14 essential mineral elements, as well as silicon beneficial for certain plants like rice, which shows yield improvement with increased doses of silicon (Tajima, 2021). Plant roots adapt for better acquisition of elements, influenced by agricultural practices aimed at enhancing yield and quality. Moreover, optimizing root systems can lessen environmental impacts by reducing nutrient loss, thus preventing potential water pollution from substances like nitrate. Enhanced root development aids in efficient nutrient capture, linking root functionality directly to environmental and crop health (Tajima, 2021).

Seeking sustainable strategies to enhance agricultural productivity and crop resilience to environmental stresses is a critical challenge in modern agriculture. Among various approaches, the exploitation of natural compounds that stimulate root growth and development emerges as a promising pathway to optimize nutrient and water absorption,

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[1] Fitobiomol Research Group, Francisco de Paula Santander University, Cúcuta (Colombia).
[*] Corresponding author: gchavesb@ufps.edu.co

crucial for plant vigor and yield. In this context, *Acmella ciliata*, known for its rich composition of compounds such as alkamides, including spilanthol, stands out for its potential to positively influence root architecture. Previous studies have suggested that manipulating root biology with phytohormonal compounds can lead to significant improvements in crop yield by facilitating more efficient nutrient and water uptake (Bano *et al*., 2022). However, significant gaps remain in our understanding of how specific natural compounds can be harnessed for this purpose.

Our grasp of plant roots, the unseen segment of plants, is notably limited compared to our understanding of their aerial counterparts. The roles roots play in supporting and nourishing the plants, alongside their complex communication mechanisms, are still shrouded in mystery. Efforts to enhance root traits are often overshadowed by the challenges in studying them within their natural soil habitat. However, the exploration of root biology is essential for advancing agricultural practices and environmental conservation (Ryan *et al*., 2016). As we begin to unravel the complexities of root systems, we set the stage for breakthroughs in food security and sustainability. This involves tapping into the vast yet unexplored potential of roots to bolster plant health and crop yields. Therefore, it is important to understand the factors that affect root growth and development and how these factors can be manipulated to optimize nutrient and water absorption (Li *et al*., 2016).

Phytohormones, as plant growth regulators, play a pivotal role in orchestrating the growth and development of plants by influencing root architecture and function. Plant hormones, including auxins, cytokinins, gibberellins, ethylene, abscisic acid and other compounds, mediate a wide array of physiological processes that directly or indirectly modulate root proliferation, elongation, and branching. In agriculture, harnessing the power of phytohormones presents a promising avenue for elevating crop productivity. Application of these hormones can stimulate root growth in a targeted manner, thereby optimizing the plant's ability to absorb essential nutrients and water from the soil. This enhanced root system not only supports the plant physical structure but also plays a critical role in the efficient utilization of soil resources, potentially leading to significant improvements in crop yield and quality (Mukherjee *et al*., 2022)

Bridging the gap between the phytohormone effects on root development and the insights from plant extracts, like *Moringa oleifera*, which enhance root formation in *Arabidopsis thaliana* (Ortiz-Rojas *et al*., 2017), unfolds a multifaceted aspect of plant biology. This synergy reveals a wider lens through which the impact of both synthetic and natural compounds on plant growth is appreciated. The meticulous research into phytohormones elucidates the complex molecular and physiological frameworks facilitating root growth and stress adaptation (Wang & Komatsu, 2022).

Acmella ciliata (Kunth) Cassini (Fig. 1), also known as *Spilanthes ciliata* Kunth and belonging to the Asteraceae family, is a tropical herb native to South America. Studies conducted on this species identify the presence of various alkamides (Silveira *et al*., 2016). Alkamides are a class of natural compounds found in a variety of plant species. These compounds, which are chemical messengers, have been studied for their potential role as plant growth regulators (Greger, 2016). *Acmella ciliata* is a plant species that is known for its production of spilanthol (Silveira *et al*., 2016).

FIGURE 1. *Acmella ciliata* (Kunth) Cass.

In this study, we aimed to understand the impact of *Acmella ciliata* essential oil, obtained from the department Norte de Santander (Colombia), on root growth and development in *Nicotiana tabacum* cv. Xanthi. The decision to use *Nicotiana tabacum* cv. Xanthi was based on its well-established role as a versatile model organism in plant science (Kurotani *et al*., 2023). This cultivar was specifically chosen for its consistent growth patterns and well-characterized genetic makeup, making it a robust subject for assessing the essential oil influence on root architecture. The use of cv. Xanthi facilitates a thorough exploration of the bioactive properties of *Acmella ciliata* essential oil and lays a robust foundation for applying these insights to broader agricultural contexts, potentially enhancing the growth of other crops and plant species.

The findings obtained from this research may lay the groundwork for further exploration into the utilization of such natural bio-stimulants in agricultural environments to optimize nutrient and water absorption.

Materials and methods

Plant material

The plant material was collected manually from Vereda Iscala, Chinácota, department Norte de Santander, located at 7.46837° N and 72.55574° W at an altitude of 2547 m a.s.l. After collection, the material was placed in a paper envelope and stored in a -20°C freezer at the Fitobiomol Research Laboratory, Francisco de Paula Santander University, Cúcuta.

Extraction and chemical analysis of essential oil from *Acmella ciliata*

Microwave-assisted extraction of essential oil from *Acmella ciliata* was carried out starting with the plant material collected and dried for 7 d at room temperature. Subsequently, 300 g of the dried whole plant (stem, leaves, and flowers) was placed in a microwave reactor, mixed with 250 ml of distilled water, and heated at 75% power for 30 min, divided into three cycles of 10 min each. The resulting essential oil was collected in a Clevenger trap for subsequent analysis. Following extraction, water traces within the oil were efficiently removed using a Pasteur column filled with anhydrous sodium sulfate. Further purification was achieved through the use of a 0.45 µm silica gel filter, ensuring oil purity (Torres *et al*., 2007).

The initial verification of alkamides in the *Acmella* essential oil was conducted through thin-layer chromatography, using affinin as a standard. To gain a more comprehensive understanding of the oil's composition and to confirm the presence of specific compounds, the sample was subsequently subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

The GC-MS analysis of the essential oil extracted from *Acmella ciliata* utilized an AT 6890 Series Plus gas chromatograph integrated with an MSD 5973 mass spectrometer (Agilent Technologies, Santa Clara, USA). The chromatographic separation was achieved on a DB-5MS column (5%-phenyl-poly(methylsiloxane)) with dimensions of 60 m x 0.23 mm x 0.25 µm. The sample introduction was performed using the Solid-Phase Microextraction (SPME) method in Split mode (30:1). The mass spectral data obtained were compared with reference spectra from the Adams, Wiley, and NIST databases to identify the compounds present.

Organoleptic and physical characterization of the essential oil

Following extraction, the essential oil was analyzed for its organoleptic characteristics and physical properties. The estimation of color parameters of the *Acmella ciliata* essential oil was made according to the geometry of the CIELAB system, which is based on color saturation; color brightness (L = 0 means black; L = 100 means white); chroma (a*), where positive values correspond to red and negative values to green; and color tone (b*), where positive values correspond to yellow and negative values refer to blue (Dini *et al*., 2019). Three replicates were performed per sample. The values obtained from a* and b* determined the hue angle, giving the red color attributes of each oil sample analyzed.

Physical parameters of oil, such as the refractive index and density, were measured using a digital Abbe refractometer and a pycnometer, respectively.

Seed germination and early growth conditions for *Nicotiana tabacum* L. cv. Xanthi

Nicotiana tabacum L. cv. Xanthi seeds were surface sterilized and germinated in peat. Fifteen days after germination the seedlings were passed to Petri dishes with MS medium to a plant growth chamber. The temperature in the growth chamber was maintained at 25 ± 2°C, the photoperiod cycle was 16/8 h of light/darkness, and the air humidity was 60% (Jalil *et al*., 2019). The pH was adjusted to 5.7 after the addition of agar. The Petri dishes containing the germinating seeds were incubated vertically to promote root growth on the surface of the medium and measure the growth of the primary root.

Effect of *Acmella ciliata* essential oil on *Nicotiana tabacum* root growth

The bioactivity of *Acmella ciliata* essential oil on the root growth of *Nicotiana tabacum* seedlings was assessed through a set of treatments following a completely randomized design. The statistical analysis was conducted using ANOVA, with further multiple comparisons made using Tukey's method. All analyses were performed in the SAS software package, with a 95% confidence level maintained.

In the study, 10 *Nicotiana tabacum* plants, each 8 d post-germination, were exposed to various treatments, including controls and various dilutions (v/v) of the essential oil, as detailed in Table 1. For each treatment, 60 µl of the solution was applied. The total study population comprised 180 plants. Parameters such as the growth of the primary root, development of lateral roots, and root hair density were meticulously recorded using a precision ruler for growth measurements in millimeters, and in-depth observations were made using a Stemi DV4 binocular stereomicroscope by Zeiss.

TABLE 1. Treatment descriptions.

Treatment ID	Description
T1	Positive control (affinin)
T2	Negative control (EtOH)
T3	Undiluted essential oil
T4	Dilution 0.5:1.5 (Oil:EtOH)
T5	Dilution 1:1 (Oil:EtOH)
T6	Dilution 1.5:0.5 (Oil:EtOH)

EtOH refers to ethanol. Oil:EtOH indicates v/v dilutions of *Acmella ciliata* essential oil and ethanol.

Results and discussion

Extraction and storage of essential oil

There are various methodologies for the extraction of essential oils, such as steam distillation, organic solvent extraction, microwave-assisted distillation, microwave hydrodiffusion and gravity, high-pressure solvent extraction, supercritical CO_2 extraction, ultrasonic extraction, and solvent-free microwave extraction (Okoh *et al*., 2010). In this study, the microwave-assisted hydrodistillation method was utilized for the extraction of the essential oil from *Acmella ciliata*. This technique was chosen for its ability to provide an extraction free of organic solvents, with water being used as the extracting medium, ensuring a purer product without the residues that organic solvents might leave behind. Furthermore, this method offers enhanced preservation of natural compounds and carries additional environmental and safety benefits (Lucchesi *et al*., 2007).

Following the extraction and purification process, a total volume of 12 ml of *Acmella ciliata* essential oil was obtained. To ensure the preservation of the oil metabolites and prevent their degradation, the oil was stored in amber vials at 4°C (Toplan *et al*., 2022).

Organoleptic and physical characterization of the essential oil

The yellowish-reddish coloration of the *Acmella ciliata* essential oil, characterized using the CIELAB model (Luo, 2015), reflects a complex interaction of its chemical components. The essential oil displayed a distinct yellowish-reddish hue. A hue* angle of 28°05' and a lightness of 33.3 were recorded (Tab. 2).

TABLE 2. Physicochemical colorimetry parameter of the essential oil from *Acmella ciliata* to determine its heterogeneity.

Essential oil	Average ± standard deviation
L (Lightness)	33.3 ± 0.165
a (green/red)	0.360 ± 0.175
b (blue/yellow)	0.220 ± 0.090
Hue angle*	28°05´ ± 5.232
Saturation	345.667 ± 62.132

*Hue angle = arctan (b/a).

The primary compounds identified in the oil, α-phellandrene epoxide, carvotanacetone, and spilanthol, may play a significant role in this distinctive coloration. Although the direct impact of each compound on the oil's coloration may not be fully established, the literature suggests that variations in concentration and the presence of certain chemical compounds can influence the optical properties of essential oils (Khayyat, 2018). For instance, spilanthol, known for its bioactive properties, could contribute to the yellow color of the oil (Barbosa *et al*., 2016).

While the exact relationship between these compounds and the essential oil coloration requires further studies, this observation aligns with previous studies indicating that the coloration of essential oils can be indicative of their chemical compositions (Sadgrove *et al*., 2022).

In addition to color characterization, the refractive index of the essential oil was determined to be 1.3478. This is key information for a better understanding of the composition and characteristics of the oil (Tab. 3). The refractive index and density are critical metrics that can influence how the oil interacts in various applications (Ispiryan *et al*., 2023).

TABLE 3. Physical characteristics of the essential oil from the leaves and flowers of *Acmella ciliata.*

Physical property	Average ± standard deviation
Refractive index	1.3478 ± 0.00
Density (g cm^{-3})	0.847 ± 0.013

GC-MS profiling of *Acmella ciliata* essential oil

The essential oil from *Acmella ciliata* was profiled using gas chromatography-mass spectrometry (GC-MS). The GC-MS chromatogram indicated the presence and relative abundance of compounds in the form of peaks over time (Fig. 2).

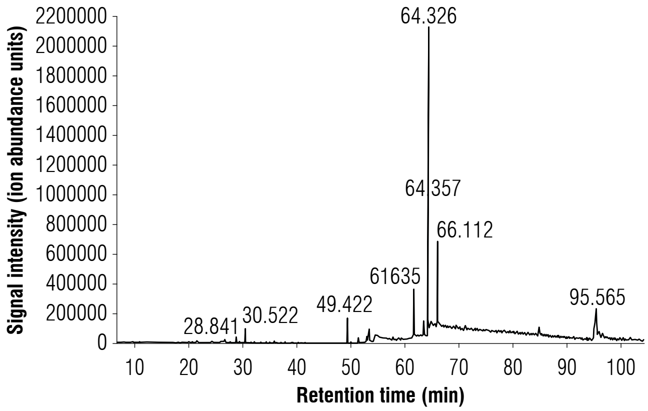

FIGURE 2. GC-MS chromatogram of *Acmella ciliata* essential oil. X-axis: retention time (min), indicating the elapsed time from sample injection to the elution of components through the chromatographic column, Y-axis: signal intensity (ion abundance units), reflecting the amount of each component detected by the mass spectrometer. Peaks represent the various compounds present in the sample, with their height proportional to the compound's concentration.

The profiling of *Acmella ciliata* essential oil via Gas Chromatography-Mass Spectrometry (GC-MS) provided a detailed snapshot of its chemical landscape, revealing α-phellandrene epoxide, carvotanacetone, and spilanthol as its major constituents (Tab. 4).

TABLE 4. Major compounds identified in the essential oil of *Acmella ciliata* by Gas Chromatography-Mass Spectrometry (GC-MS).

RT (min)	Tentative identification	Relative amount (%)
28.84	α-phellandrene epoxide	0.8
30.52	Carvotanacetone	1.9
49.42	Spilanthol	3.1

RT: retention time.

a-phellandrene epoxide and carvotanacetone, though present in smaller amounts than spilanthol, represented an intricate part of the oil's bioactivity spectrum. The chemical diversity observed in the chromatogram underscores the multifaceted role essential oils can play in modulating plant physiological processes. For instance, α-phellandrene epoxide's antimicrobial properties (Iscan *et al.*, 2012) could suggest a protective role for plants against pathogenic soil microorganisms, benefiting root health and development.

Spilanthol (Fig. 3), accounting for 3.1% of the *Acmella ciliata* essential oil and identified by its molecular formula $C_{14}H_{23}NO$ with a molar mass of 221.339, was notably prominent in the oil composition. This aligns with its well-documented bioactivity, underscoring spilanthol's pivotal role in the therapeutic and biological properties attributed to the essential oil. Spilanthol has been shown to exert a variety of effects, including analgesic, neuroprotective, antioxidant, antimutagenic, anticancer, anti-inflammatory, antimicrobial, larvicidal, and insecticidal actions (Barbosa *et al.,* 2016). These diverse biological activities highlight spilanthol's potential influence beyond analgesic and anti-inflammatory effects in animals. While the exact mechanisms through which spilanthol might influence root development in plants are not yet clear, it is plausible to consider that, akin to its effects in animals, spilanthol could moderate stress responses in plants. This could lead to enhanced root growth under stress conditions, although further research is required to fully understand these effects in the context of root development in *Nicotiana tabacum*. The existing evidence, including the documented range of bioactivities of spilanthol, provides a strong basis for future studies to explore the role of spilanthol and similar compounds in modulating plant physiology.

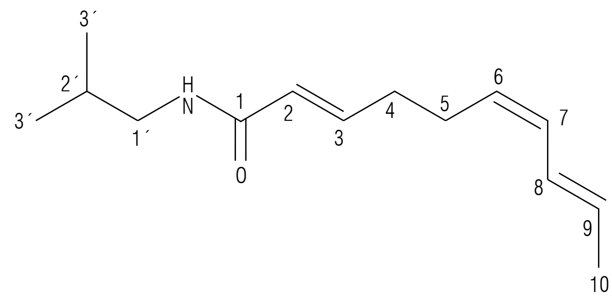

FIGURE 3. Chemical structure of spilanthol, a prominent alkamide found in *Acmella ciliata*.

Future research could explore the synergistic effects of these compounds, examining how combinations, rather than single constituents, influence plant development.

Effect of *Acmella ciliata* essential oil on the density of root hairs and length of the main root in *Nicotiana tabacum*

Prior literature findings have highlighted the presence of alkamides in *Acmella ciliata* (Rios-Chavez *et al.*, 2003; Greger, 2016; Silveira *et al.*, 2016). The identification of spilanthol offers a direct link between the observed bioactivity in the root growth of *Nicotiana tabacum* cv. Xanthi and the presence of this particular alkamide in the essential oil. Spilanthol has a range of properties, from providing oral pain relief to exhibiting antibacterial effects. In cosmetics, it is an ingredient in anti-wrinkle products, while culinary traditions include plants rich in spilanthol for their distinctive flavor (Barbosa *et al.*, 2016). There is also evidence pointing towards its potential in mosquito control, which can be vital in combating certain tropical diseases. Furthermore, emerging studies hint at its possible anti-cancer properties (Barbosa *et al*., 2016).

The essential oil from *Acmella ciliata* exerted a considerable influence on the number of root hairs and the primary root length of *Nicotiana tabacum* seedlings. The General Linear Model (GLM) analyses for the dependent variables number of hairs and root length in *Nicotiana tabacum* revealed statistically significant differences across treatments (Fig. S1). For the number of root hairs, the model yielded a highly significant p-value of less than 0.0001, indicating that substantial variability in root hair count can be attributed to the treatment effects. This is further corroborated by an F-value of 9.37, suggesting that at least one treatment is significantly different from the others in influencing hair count. The analysis for root length also showed statistical significance with a p-value of 0.0174, although with a smaller F-value of 3.07 (Fig. S1). This suggests that treatments have a discernible but less pronounced impact on root length as compared to root hair count. These findings underscore the differential influence of the treatments on various aspects of root physiology in *Nicotiana tabacum*.

Treatments T6 and T3 resulted in the highest average number of root hairs (Fig. 4), with no statistical differences between them based on the Tukey´s test (Fig. S2). The reported number of root hairs represents an average derived from the nine replicates. Although spilanthol is a component in this oil, the potential synergistic effects of all compounds in the oil cannot be overlooked. The observed effects are likely linked to the presence of these alkamides, as suggested by their documented bioactive properties in *Acmella* sp. and other plant species (Ramírez-Chávez *et al*., 2004). This assertion is further reinforced by the documented bioactive properties of alkamides in *Acmella* sp. (Elufioye *et al*., 2020).

For primary root length, the model is also significant (Fig. S1). The Tukey's Honestly Significant Difference (HSD) test reveals that, for the number of root hairs, treatment T6 had

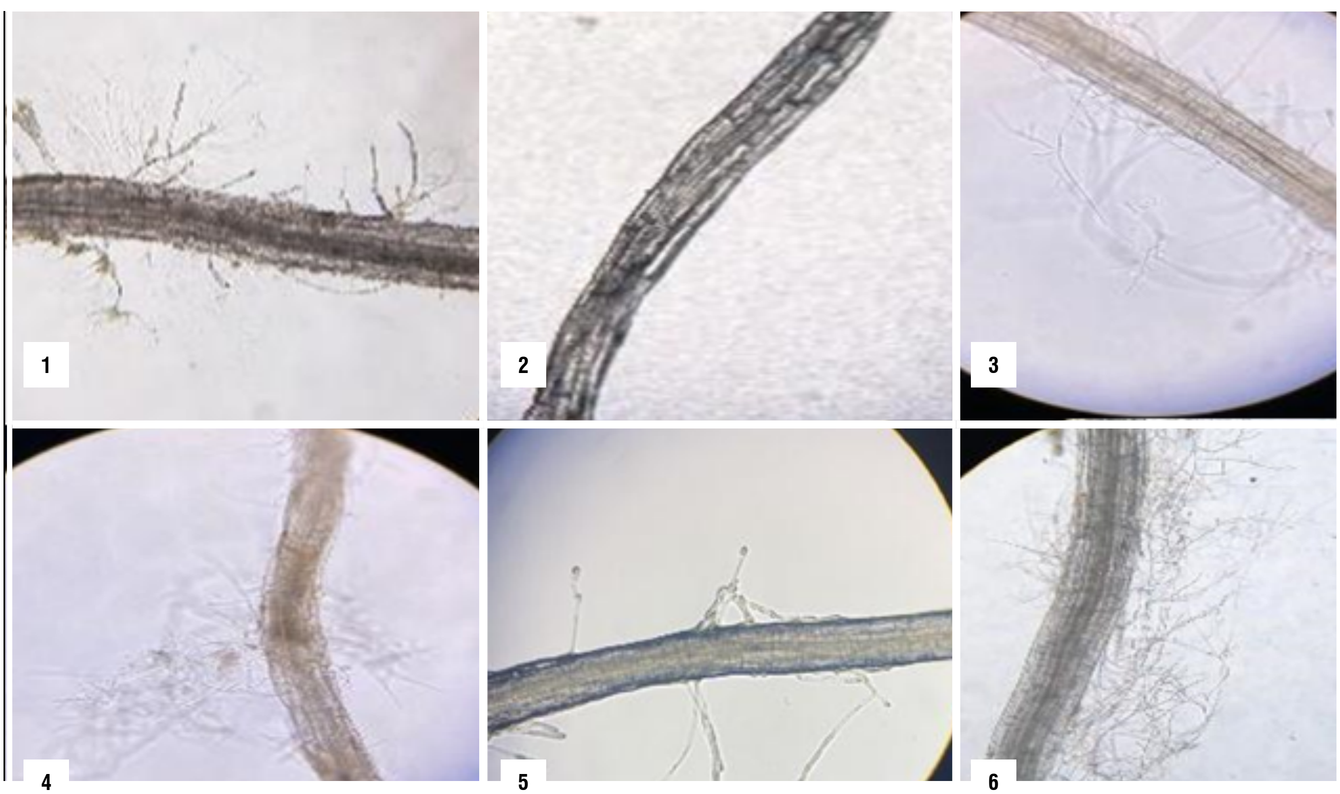

FIGURE 4. The effect of various treatments of essential oil extracted from *Acmella ciliata* on root hair density in *Nicotiana tabacum* plants. T1: Positive control (affinin), T2: Negative control (EtOH), T3: Undiluted essential oil, T4: Dilution 0.5:1.5 (Oil:EtOH), T5: Dilution 1:1 (Oil:EtOH), T6: Dilution 1.5:0.5 (Oil:EtOH). The number of root hairs is the average from nine replicates, counted within the visual field of a microscope using a 10X ocular and a 10X objective, resulting in a total magnification of 100X. EtOH refers to ethanol. Oil:EtOH indicates v/v dilutions of *Acmella ciliata* essential oil and ethanol.

the highest mean count, categorizing it in group A along with treatments T3 and T5, without statistically significant differences between these treatments (Fig. S2). Despite having the highest mean, the similarity with treatments T3 and T5 suggests that the essential oil concentration does not linearly increase the number of hairs. Regarding root length, treatment T6 resulted in the highest average root length. However, no significant differences were found between the treatments, indicating a more uniform response pattern in terms of primary root length among the different treatments (Fig. S2). Notably, although treatment T6 was the most effective for root hair growth, it was the least effective in increasing primary root length.

In the positive control group (treatment T1) using affinin, there was a notable increase of the average primary root length, while the effect on growth of root hairs was comparatively minimal. This suggests that alkamides like affinin could preferably increase primary root growth rather than the growth of root hairs. As bioactive compounds, alkamides have been recognized to influence plant growth and development (Ramírez-Chávez *et al*., 2004). Comparing their effects with those of other treatments is important, offering a standard to evaluate the efficacy of various compounds or relative to these bioactive agents. Significantly, alkamides are natural plant products with a wide array of biological activities across multiple phyla. They exhibit properties with antifungal, antibacterial, antimalarial, and insecticidal effects, among others (Buitimea-Cantúa *et al*., 2020). Furthermore, they play roles in plant growth promotion and defense gene induction, and even interact with intercellular signaling molecules in organisms ranging from humans to bacteria and plants (Buitimea-Cantúa *et al*., 2020).

The results suggest that treatment effectiveness can vary depending on the plant growth characteristic being analyzed. For instance, a treatment that is effective in promoting one growth attribute might not be as effective for another. Given that treatment T6 was most effective for root hair growth but least effective for elongation of primary root, it is evident that the impacts of the treatments can be complex. This implies that when selecting treatments to enhance plant growth, it is essential to determine which growth features are most desired for the specific crop scenario. For example, in crops like lettuce where rapid nutrient absorption is essential due to their short growth cycle, treatment T6, which promotes root hair growth, might be more advantageous. Conversely, for deep-rooted plants like maize, which require deeper rooting for stability and water access from deeper soil horizons, a treatment with affinin (T1: positive control), which increases primary root length, could be more appropriate. The findings from this research not only offer the basis for further exploration into the utilization of natural bio-stimulants to optimize nutrient and water absorption but also emphasize the potential of plants like *Acmella*, often considered weeds, as sources for biologically active compounds. Given the multifunctional nature of alkamides, their applications could extend beyond crop cultivation, including a broader spectrum of their application in botanical and agricultural research.

Conclusion

This study showed a significant effect of the essential oil extracted from *Acmella ciliata* on the density of root hairs and the length of the main root of *Nicotiana tabacum*. This effect suggests that the compounds present in the oil, potentially alkamides, play a crucial role in promoting root growth. The study establishes strong evidence that the alkamides present in the *Acmella ciliata* oil have bioactive effects on the growth of other plant species.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

LDMG: methodology, writing of original draft; LYOR: supervision, formal analysis, data curation. GCB: conceptualization, methodology, writing. All authors reviewed the final version of the manuscript.

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SUPPLEMENTARY FIGURE 1. Results of GLM analysis (in Spanish) for root growth of *Nicotiana tabacum* plants treated with *Acmella ciliata* essential oil. Top panel: number of root hairs, bottom panel: root length (cm).

Sistema SAS

Procedimiento GLM

Variable dependiente: pelos

Fuente	DF	Suma de cuadrados	Cuadrado de la media	F-Valor	Pr > F
Modelo	5	12783.42593	2556.68519	9.37	<.0001
Error	48	13097.55556	272.86574		
Total correcto	53	25880.98148			

R-cuadrado	Coef Var	Raiz MSE	pelos Media
0.493931	75.02162	16.51865	22.01852

Fuente	DF	Tipo I SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	12783.42593	2556.68519	9.37	<.0001

Fuente	DF	Tipo III SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	12783.42593	2556.68519	9.37	<.0001

Procedimiento GLM

Variable dependiente: long

Fuente	DF	Suma de cuadrados	Cuadrado de la media	F-Valor	Pr > F
Modelo	5	18.14814815	3.62962963	3.07	0.0174
Error	48	56.66666667	1.18055556		
Total correcto	53	74.81481481			

R-cuadrado	Coef Var	Raiz MSE	long Media
0.242574	34.51342	1.086534	3.148148

Fuente	DF	Tipo I SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	18.14814815	3.62962963	3.07	0.0174

Fuente	DF	Tipo III SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	18.14814815	3.62962963	3.07	0.0174

SUPPLEMENTARY FIGURE 2. Results of the Tukey's HSD test (in Spanish) for root growth of *Nicotiana tabacum* plants treated with *Acmella ciliata* essential oil . Top panel: number of root hairs, bottom panel: root length (cm).

Sistema SAS

Procedimiento GLM

Prueba del rango estudentizado de Tukey (HSD) para pelos

NOTA: Este test controla el índice de error experimentwise de tipo I, pero normalmente tiene un índice de error de tipo II más elevado que REGWQ.

Alfa	0.05
Error de grados de libertad	48
Error de cuadrado medio	272.8657
Valor crítico del rango estudentizado	4.19724
Diferencia significativa mínima	23.111

Medias con la misma letra no son significativamente diferentes.

Tukey Agrupamiento	Media	N	trat
A	43.556	9	6
B A	38.667	9	3
B A C	24.556	9	5
B C	17.000	9	4
C	6.444	9	1
C	1.889	9	2

Sistema SAS

Procedimiento GLM

Prueba del rango estudentizado de Tukey (HSD) para long

NOTA: Este test controla el índice de error experimentwise de tipo I, pero normalmente tiene un índice de error de tipo II más elevado que REGWQ.

Alfa	0.05
Error de grados de libertad	48
Error de cuadrado medio	1.180556
Valor crítico del rango estudentizado	4.19724
Diferencia significativa mínima	1.5201

Medias con la misma letra no son significativamente diferentes.

Tukey Agrupamiento	Media	N	trat
A	3.8889	9	5
B A	3.5556	9	1
B A	3.4444	9	4
B A	3.1111	9	2
B A	2.7778	9	3
B	2.1111	9	6