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Alternative culture formulations for in vitro banana cultivation

Formulações alternativas para cultivo in vitro de banana

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ABSTRACT

The banana plant (*Musa spp.*) is a tropical crop of significant socioeconomic importance, and micropropagation emerges as a viable alternative for large-scale production. This study aimed to evaluate the efficacy of alternative culture media and the use of essential oils in controlling fungal contamination. Four media were tested: tomato-based, fruit and vegetable mixture, NPK fertilizer-based, and Murashige & Skoog (MS). Explants were cultivated under aseptic conditions for 30 days. The tomato-based medium showed the best performance among the alternatives, while MS promoted greater vigor and shoot development. Essential oils of pepper and *Aloe vera* demonstrated 95% and 90% effectiveness in contamination control, respectively. The findings indicate that alternative media, particularly the tomato-based formulation, effectively support the in vitro development of banana plants, offering a sustainable approach with potential for reducing production costs. **Keywords:** *Musa spp.*, *in vitro* cultivation, sustainable agriculture, plant extracts, fungal inhibition.

RESUMO

A bananeira (*Musa* spp.) é uma cultura tropical de grande relevância socioeconômica, e a micropropagação surge como uma alternativa viável para sua produção em larga escala. Este estudo teve como objetivo avaliar a eficácia de meios de cultura alternativos e o uso de óleos essenciais no controle de contaminações fúngicas. Foram testados quatro meios: base de tomate, mistura de frutas e legumes, adubo NPK e Murashige & Skoog (MS). Explantes foram cultivados em ambiente asséptico por 30 dias. O meio à base de tomate apresentou melhor desempenho entre os alternativos, enquanto o MS promoveu maior vigor e desenvolvimento. O uso de óleos essenciais de pimenta e *Aloe vera* demonstrou eficácia de 95% e 90% no controle de contaminações. Os achados indicam que meios alternativos, particularmente o formulado à base de tomate, possibilitam o desenvolvimento *in vitro* de bananeira, oferecendo uma abordagem sustentável com potencial para reduzir custos de produção.

Palavras-chave: Musa spp., cultivo in vitro, agricultura sustentável, extratos vegetais, inibição fúngica.

INTRODUCTION

The banana plant (*Musa spp.*) stands out as one of the primary tropical fruit crops, with significant socioeconomic relevance in various countries. The growing demand for high-quality fruits and the need for varietal renewal drive the search for efficient



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vegetative propagation technologies Wijayani et al. (2023). Micropropagation, a well-established in vitro cultivation technique, is widely used for producing banana seedlings due to its high multiplication potential and ability to maintain genetic uniformity (Guambe et al., 2024; Setyowati et al., 2024). However, large-scale implementation of micropropagation faces challenges, such as high demand for costly inputs, microbial contamination, and a lack of specific protocols for different banana cultivars (Li et al., 2024; Meucci et al., 2024; Saraswathi et al., 2024). In developing countries, these challenges are even more pronounced, limiting small producers' access to this technology.

The hypothesis is that formulating culture media with ingredients such as coconut water, tomato, potato, papaya, watermelon, banana, sugar, and NPK fertilizer, combined with the addition of essential oils from plants like aloe vera and eucalyptus, may significantly reduce production costs, increase process efficiency, and simultaneously control microbial contamination without compromising the quality of the seedlings produced (Table 1).

Table 1 presents the potential nutrients and bioactive compounds present in the alternative ingredients, suggesting they can provide the essential nutrients for

in vitro plant growth and development. Furthermore, essential oils from *Aloe vera*, pepper, and eucalyptus possess proven antifungal and antibacterial properties, which may help control common contaminants in in vitro cultures (Darwish et al., 2023; Singh et al., 2024).

The presence of vitamins, minerals, sugars, amino acids, and other bioactive compounds in these ingredients (Table 1) suggests that they may provide the essential nutrients for in vitro plant growth and development, partially or entirely replacing commercial culture media.

In this context, this study aims to evaluate the feasibility of using alternative culture media composed of locally sourced, low-cost ingredients for banana micropropagation.

MATERIAL AND METHODS

The experiment was conducted in the Biotechnology Laboratory of the Instituto Superior Politécnico de Manica (ISPM), Mozambique.

Preparation of Solutions and Culture Media

Four culture medium treatments were prepared: three alternative formulations (Media I, II, and III) and a conventional control medium (Medium IV). For each of these

Table 1. Potential nutrients and bioactive compounds present in the alternative ingredients for in vitro banana cultivation.

Ingredient	Main Nutrients and Bioactive Compounds	Functions	Ref.	
Coconut water	Cytokinins, auxins, B	Source of vitamins, minerals and	Freestone et al. (2023),	
	vitamins, potassium,	phytohormones; stimulates cell	Lu et al. (2024)	
	magnesium, sugars	division; regulates water balance		
Tomato	Vitamin C, vitamin A,	Source of vitamins, minerals and	Dong et al. (2024),	
	potassium, lycopene	antioxidants; aids plant growth	Dzhos et al. (2024)	
	(antioxidant)	and development		
Potato	Starch, vitamin C,	Source of carbohydrates, vitamins	Behera et al. (2024),	
	potassium	and minerals; provides energy for cell growth	Olivas-Aguirre et al. (2024)	
Papaya	Vitamin C, vitamin	Source of vitamins, minerals and	Cao et al. (2024)	
	A, papain (proteolytic	enzymes; aids in the digestion and		
	enzyme), potassium	absorption of nutrients		
Watermelon	Vitamin C, lycopene,	Source of water, vitamins and	Zia et al. (2025)	
	citrulline (amino acid)	antioxidants; helps hydrate cells		
Banana	Starch, potassium,	Source of carbohydrates,	Souza et al. (2024),	
	vitamin B6	potassium and vitamins; provides energy and essential nutrients	Tan et al. (2024)	
Crystal sugar	Sucrose	Carbon source; provides energy	Guambe et al. (2024),	
		for metabolic processes	Wijayani et al. (2023)	
NPK fertilizer	Nitrogen (N),	Provides essential macronutrients	Yang et al. (2024)	
	phosphorus (P), potassium (K)	for plant growth		
Essential oils (Aloe	Phenolic compounds,	Antifungal and antibacterial	Darwish et al. (2023),	
vera vera, pepper,	terpenes, flavonoids	action; reduction of microbial	Mrvová et al. (2024),	
Eucalyptus sp.)		contamination	Singh et al. (2024)	

four media, two variations were tested: one supplemented with essential oils and a control without essential oils. All media were solidified with agar, and their pH was adjusted to 5.8 ± 0.1 using 0.1 mol/L HCl or NaOH solutions prior to agar addition. Sterilization was carried out in an autoclave at 121 °C for 15 minutes under a pressure of 1 atm.

- i) Medium I (Tomato-based): Five hundred grams (500 g) of ripe tomatoes (Solanum lycopersicum L.) were sanitized, blended, and filtered through a 100-μm mesh sieve. The filtrate was centrifuged at 3000 × *g* for 15 min. For the preparation of 1 L of medium, 200 mL of the supernatant was used, supplemented with 100 mL/L of coconut water. The medium was then divided into two parts: one was supplemented with 5 mL/L of essential oil from Aloe vera or pepper (Capsicum spp.) (Distriol® brand, 100% pure), and the other part served as a control without essential oil. The volume of each was brought to 1 L with distilled water, and 15 g/L of agar-agar (Merck®) was added.
- hundred and fifty grams (250 g) of each ingredient were used: tomato, potato (*Solanum tuberosum* L.), green corn (*Zea mays* L.), papaya (*Carica papaya* L.), and watermelon (*Citrullus lanatus*). The ingredients were sanitized, individually blended (with the addition of 50 mL of distilled water for corn and 25 mL for potato), and filtered. The filtrates were centrifuged as described for Medium I. To compose 1 L of

- the medium, 50 mL of the supernatant from each ingredient (totaling 250 mL) was used, supplemented with 100 mL/L of coconut water. This base was similarly split into two variations: with and without the addition of 5 mL/L of essential oil. The volume was adjusted to 1 L with distilled water, and 15 g/L of agar-agar (Merck®) was added.
- iii) Medium III (NPK Fertilizer-based): Half a gram (0.5 g) of NPK fertilizer (5:10:15 formulation) was dissolved in 100 mL of distilled water. This solution was used to prepare 1 L of medium, supplemented with 100 mL/L of coconut water. As with the other media, it was prepared with and without the addition of 5 mL/L of essential oil. The volume was completed with distilled water, and 15 g/L of agar-agar (Merck®) was added.
- the methodology described by Guambe et al. (2024).

 The media were dispensed into glass jars (40 mL per jar) and subsequently sterilized in an autoclave at 121 °C

iv) (Murashige & Skoog, MS) was prepared according to

jar) and subsequently sterilized in an autoclave at 121 °C for 15 minutes under a pressure of 1 atm. Following sterilization, the jars were hermetically sealed and stored in the dark for 72 hours prior to explant inoculation to confirm the absence of microbial contamination.

Collection, Pre-treatment, Disinfection, Inoculation and Growth of explants

The experimental procedures included the preparation of solutions and culture media, collection, pre-treatment, disinfection of explants, inoculation, and growth (Figure 1).



Figure 1. Steps in the *in vitro* cultivation process: Horn-type explants collected from the family banana plantation (A); Sectioning and washing of explants (B); Disinfection of explants (C); Reduction of explant size for placement in the growth vessel (D); Arrangement of explants for growth in an aseptic environment (E); Shoots and explant growth observed after 30 days of cultivation (F).

The explants were collected from a family production farm. After collection, the material was immediately transported to the laboratory, where it underwent a cleaning process to remove excess soil, roots, and other surface dirt from the rhizome (Figures 1A and 1B). Disinfection was performed with 1% hypochlorite and 70% ethanol (Figure 1C), following the methodology described by Guambe et al. (2024). The horn-type rhizome explants were sectioned under a laminar flow hood, using tweezers and scalpels flamed in 70% ethanol between each handling (Figure 1D). One explant per flask was inoculated in the culture medium (40 mL), and the flasks were sealed (Figure 1E). The cultures were incubated in a growth room under cold fluorescent light (18 W) at a controlled temperature of 24 ± 2 °C, with a photoperiod of 16 hours (Figure 1F).

Data Collection and Analysis

Data collection was performed 15 and 30 days after the *in vitro* introduction of explants. Plant height was measured using a 15 cm ruler inside the laminar flow cabinet. The number of shoots and leaves was obtained through direct counting. The experimental design was completely randomized, with four treatments and 25 replicates (each replicate consisting of one explant). The collected data were subjected to analysis of variance (ANOVA) using the statistical software Jamovi version 2.6. The assumption of normality of residuals was verified by the Shapiro-Wilk test (p > 0.05). When ANOVA

indicated significant effects, means were compared by Tukey's test at a 5% significance level.

RESULTS AND DISCUSSION

The assessment was conducted 15 and 30 days after the *in vitro* introduction of explants. After 15 days of cultivation, the explants in culture medium 1 - Tomatobased, (M1) showed greater vigor, with more pronounced shoot development and a more intense green coloration compared to the alternative media (culture medium 2 - Fruit and Vegetable Mixture (M2) and culture medium 3 - NPK Fertilizer-based (M3)) (Figure 2). However, when compared to the control (culture medium 4 - Murashige & Skoog (M4)), M1 presented inferior results in terms of shoot size, and vigor (Figure 2). At 30 days, the superiority of M1 was confirmed over M2 and M3 regarding shoot development and vigor. No significant differences were observed between M1 and M4 for these parameters.

Buds, Height, and Leaves of Explants

To compare the effects of treatments and evaluation periods, the data for the number of shoots, explant height, and number of leaves were subjected to a univariate analysis of variance (ANOVA). The analysis revealed significant differences (p < 0.05) both between the evaluation periods (15 and 30 days) and among the different culture media for all variables evaluated. A significant interaction between the period and treatment

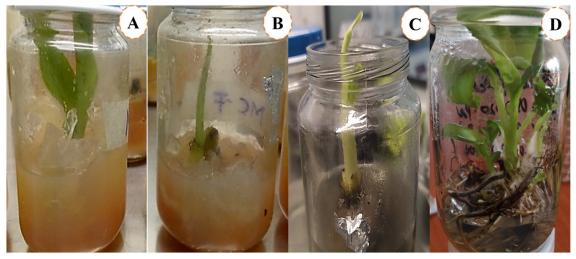


Figure 2. Morphological aspect of banana (*Musa* spp.) explants 30 days after in vitro inoculation in different culture media. (A) Tomato-based medium (M1); (B) Fruit and vegetable mixture-based medium (M2); (C) NPK fertilizer-based medium (M3); (D) Murashige & Skoog medium (MS - Control, M4).

factors was observed only for explant height, indicating that the effect of the culture medium on growth was dependent on the evaluation time. Following significant ANOVA results, means were compared using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level (Table 2).

The results for the buds parameter showed that, at 15 days, treatment M1 demonstrated superior development compared to treatments M2 and M3, which did not show significant statistical differences between them (Figure 3A). After 30 days, a similar trend persisted, with treatment M1 continuing to outperform treatments M2 and M3,

Table 2. Univariate ANOVA of the parameters evaluated in the study.

	Dependent Variable	Sum of Squares	df	Mean Square	F	P
Period	Buds	6.84	1	6.845	8.771	0.003
	Height	33.59	1	33.587	34.934	< .001
	Leaves	8.82	1	8.820	11.302	< .001
Tratment	Buds	103.65	3	34.552	44.273	< .001
	Height	129.76	3	43.253	44.988	< .001
	Leaves	132.66	3	44.220	56.662	< .001
Period*Tratment	Buds	3.54	3	1.178	1.510	0.213
	Height	29.79	3	9.930	10.328	< .001
	Leaves	2.26	3	0.753	0.965	0.410
Residuals	Buds	149.84	192	0.780		
	Height	184.60	192	0.961		
	Leaves	149.84	192	0.780		

Legend: df = Degrees of freedom; F = calculated value of the F-statistic for the test; P = significance level.

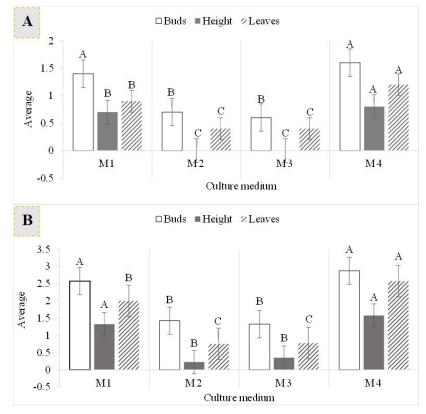


Figure 3. Number of buds, explant height (cm), and number of leaves of banana (Musa spp.) plants cultivated in vitro in different culture media, at 15 (A) and 30 (B) days after inoculation. M1: Tomato-based medium; M2: Fruit and vegetable mixture-based medium; M3: NPK fertilizer-based medium; M4: Murashige & Skoog medium (MS - control). Statistical analyses were performed separately for each response variable and evaluation period. Means followed by the same lowercase letter, within the same variable and period, do not differ statistically from each other by Tukey's test (p > 0.05). Bars represent the standard error of the mean.

which also showed no significant differences from each other (Figure 3B). Treatment M4 demonstrated the highest shoot count at both 15 and 30 days compared to the alternative treatments. However, statistically, there were no significant differences between treatment M4 and treatment M1 at 30 days. At 15 days, height measurements revealed significant differences among treatments, with M1 showing the best performance compared to M2 and M3, which again had no significant differences between them (Figure 3A). This suggests that M1 had a higher growth and development rate than the other treatments. At 30 days, the results remained consistent, with M1 performing the best, followed by treatments M2 and M3, which maintained similar growth rates without significant differences (Figure 3B).

Treatment M4 consistently outperformed the other treatments, displaying the highest growth and development rates among all alternatives. This suggests that M4 achieved the greatest growth and development rate compared to the other treatments. In summary, the results indicate that M1 and M4 exhibited the best performance in height parameters, while treatments M2 and M3 showed similar, lower growth rates than M1 and M4. Regarding leaf count, the results at 15 days indicated that treatment M1 performed the best in terms of leaf number compared to treatments M2 and M3, which did not show significant differences between them. The 30day evaluation showed similar results to those observed at 15 days. Notably, treatment M4 showed no significant differences from treatment M1 at either 15 or 30 days, suggesting that treatment M4 did not significantly affect the leaf count compared to treatment M1 (Figure 3).

These observations suggest that alternative culture media represent a viable option for *in vitro* plant cultivation. Studies like that of Cavalcante et al. (2019) indicate that the addition of specific compounds to culture media can significantly influence buds development and *in vitro* growth rates. Additionally, as reported by Kumar et al. (2024), the presence of organic compounds in alternative media can enhance explant vigor, potentially explaining the superior performance of treatments M1 and M4 in this study.

Flores-Hernández et al. (2017), in evaluating different alternative substrates for *in vitro* cultivation

of orchids, observed that explants in synthetic media exhibited shoot growth comparable to those cultivated on alternative substrates after 35 days. These findings support the viability of alternative media for in vitro plant cultivation. Similarly, Flores-Escobar et al. (2008) reported that Oncidium stramineum Lindl. buds grown in MS medium supplemented with organic extracts, such as coconut water, peptone, activated charcoal, and polyvinylpyrrolidone, reached an average length of 2.05 cm after 90 days—a result comparable to those grown in media with 50% MS salts. Similarly, Silva et al. (2016) investigated substituting analytical-grade mineral salts with commercial fertilizers and analytical-grade sucrose with regular sugar in the micropropagation of Musa cv. Williams. Their results demonstrated that these alternative sources did not compromise explant development, supporting the findings of the present research. Yang et al. (2024) highlighted the increasing use of commercial fertilizers in in vitro culture as a strategy to simplify media preparation and reduce production costs, enabling large-scale plant development at a lower cost.

Working with bananas, Wijayani et al. (2023) observed a significant effect on the shoot length of explants 45 days after establishment in alternative culture media, demonstrating the potential of these substrates to promote in vitro growth. Similarly, Guambe et al. (2024), in studying various concentrations of commercial sucrose in the in vitro cultivation of bananas, observed that Murashige and Skoog (1962) medium with higher sucrose concentrations was the most effective in seedling germination and growth, emerging as a viable option due to its accessibility and lower cost compared to traditional MS medium. These findings reinforce the viability of alternative culture media, particularly in large-scale production contexts where cost reduction is critical.

In contrast to the findings of the present study, Silva et al. (2016) reported no statistically significant differences in leaf count over eight weeks of in vitro growth when using alternative media for *Citrus volkameriana* Ten. Conversely, Meucci et al. (2024), using organic extract-based alternative media for *Limonium latifolium* Lindl., found statistically significant differences in leaf area and fresh and dry mass within the first 30 days of cultivation. In this context, the results of the present study

demonstrate that the use of organic compound mixtures in culture media for *Musa* sp. produced a leaf count similar to that observed in explants grown in synthetic media. Additionally, Wijayani et al. (2023), investigating the effect of cane molasses on the in vitro development of bananas, found that media containing analytical-grade sucrose promoted greater fresh biomass accumulation than those prepared with cane molasses. These results suggest that, while alternative organic compounds may be effective in certain contexts, substituting specific components can significantly impact in vitro plant development.

Contamination Control

The results indicated that pepper essential oil was highly effective in controlling fungal growth, with 95% of the flasks showing no fungal proliferation. Only 5% of the flasks containing this essential oil exhibited small fungal colonies, suggesting significant efficacy in preventing contamination. On the other hand, aloe vera essential oil also proved effective, with 90% of the flasks exhibiting no contamination, and only 10% showing some fungal colonies.

In contrast, the flasks without the application of fungicides showed a higher percentage of contamination, with 85% of the flasks exhibiting fungal presence and only 15% remaining uncontaminated. These findings suggest that the use of essential oils as fungicides represents an effective and safe alternative for contamination control in preservation flasks (Figure 4).

Additionally, the use of essential oils as fungicides may also represent a more environmentally friendly option, as they do not contain aggressive chemical substances that can harm the environment. Essential oils are naturally biodegradable and do not leave toxic residues, making them a more sustainable choice for contamination control.

Permadi et al. (2024), in testing citrus aurantifolia essential oil against the growth of *Colletotrichum gloeosporioides*, observed inhibition of mycelial growth regardless of the oil concentration used, highlighting the fungitoxic potential of this plant extract. Similar results were reported by Singh et al. (2024), who evaluated the fungitoxic activity of oils from three eucalyptus species against *Fusarium oxysporum*, *Botrytis cinerea*, and *Bipolaris sorokiniana*, finding varied rates of mycelial growth inhibition across all tested species and different oil concentrations.

Mrvová et al. (2024) also investigated the effects of essential oils from pepper and Eucalyptus citriodora on Didymella bryoniae, noting complete inhibition of both mycelial growth and conidial germination. Similarly, Darwish et al. (2023) reported a 100% inhibition of spore germination of Colletotrichum gloeosporioides in the presence of essential oils from aloe vera, clove basil, lemongrass, citronella, and guava. Moreover, several studies have documented the effectiveness of essential oils from plants such as aloe vera, pepper, rosemary, basil, mint, and onion in progressively inhibiting the growth of various fungi, including Aspergillus niger, A. flavus, A. ochraceus, and Fusarium spp. (Dong et al., 2024; Nobre and Marques, 2021; Zia et al., 2025). These findings reinforce the fungitoxic potential of essential oils and plant extracts, underscoring their applicability in controlling fungal pathogens in various crops.

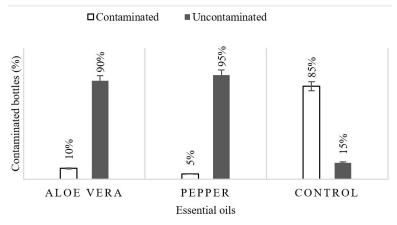


Figure 4. Effect of essential oil on contamination control.

CONCLUSION

The alternative culture media, particularly the tomato-based formulation, effectively support the in vitro development of banana (Musa spp.) explants. This medium promoted significant development of buds, height, and leaf number, achieving parameters statistically equivalent to the conventional MS medium (M4) at 30 days of cultivation. Furthermore, pepper and Aloe vera essential oils (Distriol®) demonstrated a potent fungistatic effect, with contamination control rates exceeding 90%, representing a natural and effective alternative to conventional fungicides. Thus, this work provides robust scientific evidence on the agronomic efficacy of using low-cost local inputs in banana micropropagation. Future studies, including a detailed economic analysis and acclimatization tests, are required to confirm the economic and operational feasibility of this sustainable approach on a large scale.

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